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CONTENT

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Gordan S. KARAMAN[1]

FURTHER DISCOVERY OF NEW OR PARTIALLY KNOWN TAXA OF THE GENUS *NIPHARGUS* SCHIÖDTE, 1849 (FAM. NIPHARGIDAE) IN GREECE (CONTRIBUTION TO THE KNOWLEDGE OF THE AMPHIPODA 302)

SUMMARY

The new subterranean subspecies of the genus *Niphargus* Schiödte, 1849 (Amphipoda Gammaridea, Niphargidae), *N. (Orniphargus) lourensis skirosi*, ssp. n. is described and figured from the underground waters of Skiros Island in the Aegean Sea (Greece), taxon rather similar to the nominal species *N.(Orniphargus) lourensis* Fišer et al. 2006 described and known based on one single specimen from Epirus region of Greece only.

Niphargus (*Orniphargus*) *lindbergi* S. Karaman 1956, described and known based on single specimen from Attica region of Greece only (Draconera Cave), is established in Thessaly region also [spring on main road Lamia-Larissa (=Laritza) in Central Greece], and new taxonomical data of this species from both localities are presented. The presented data put some more lights on the taxonomical position of these taxa. The mentioned taxa, *Niphargus lourensis*, *skirosi* and *lindbergi* belong to the subgenus *Orniphargus* S. Kar. 1950.

Keywords: Subterranean waters, taxonomy, Amphipoda, *Niphargus*, *Orniphargus*, *lourensis*, *skirosi*, *lindbergi*, Greece.

INTRODUCTION

The freshwater fauna of Amphipoda in Greece has been intensively studied by various scientists, and numerous new species of this group have been discovered and described. The investigations of the subterranean waters in Greece with prevalently calcareous rocks and karstic phenomena, showed the high richness of subterranean endemic genera and species, especially within the family Niphargidae.

Because of hard sampling and often scarce number of collected specimens, some new species were described based on single male or female specimen (*Niphargus lindbergi* S. Karaman 1956; *N. lourensis* Fišer et al. 2006, *N. cimbalus* G. Karaman 2017, *N. rhodi* S. Karaman 1950c, etc.]. The recent discovery of numerous new taxa request much more detailed description of taxonomical characters of each species, and new redescription of these taxa based on the same or other localities is necessary to understand the taxonomical position of these species and its relations to other *Niphargus* taxa.

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Note: The author declare that he has no conflicts of interest. Authorship Form signed online.

Discovery of *N. lindbergi* in one new locality (Thessaly), urged us to partially redescribe this species known from Draconera Cave (Attica) only. Thanks to Prof Dr Giuseppe Pesce from the University of Aquilla, who sent us collected samples of Amphipoda from Greece accompanied by some physical and chemical parameters (Pesce & Maggi 1983), we have possibility to study the samples of *Niphargus* from Skiros Island in Greece.

The specimens from Skiros Island are rather similar to *N. lourensis*, but based on present known taxonomical characters of *N. lourensis*, not identic with later, considering the specimens from Skiros as a distinct new subspecies, *N. lourensis skirosi*, ssp. n.

MATERIAL AND METHODS

The studied material of amphipods has been collected by hand-net in the wells and springs and preserved in the 70% ethanol. The specimens were dissected using a WILD M20 microscope and drawn using camera lucida attachment. All appendages were temporarily submersed in the mixture of glycerin and water for study and drawing. The body-length of examined specimens was measured from tip of head to end of telson using camera lucida. All illustrations were inked manually. After the end of the study, the dissected body-parts were submerged in Liquid of Faure and covered by cover glass for final preservation. Some body-parts of holotype were drawn directly from holotype-slides.

Some morphological terminology and seta formulae follows Karaman`s terminology (G. Karaman, 1969) for the last mandibular palpus [A= A-setae on outer face; B = B-setae on inner face; C = additional setae on outer face; D = lateral marginal D-setae; E = distal long E-setae] and for propodus of gnathopods 1 and 2 (G. Karaman, 2012) [S = corner S-spine; L = lateral slender serrate L-spines; M = facial M-setae; R = subcorner R-spine on inner face]. Terms “setae” and “spines” are used based on shape, not origin.

All investigations are based on provided morphological, ecological and zoogeographical investigations and data.

TAXONOMICAL PART

Family NIPHARGIDAE

NIPHARGUS (*ORNIPHARGUS*) *LOURENSIS SKIROSI* ssp. n.

Figures 1-6

Niphargus sp. Pesce & Maggi 1983: 34.

MATERIAL EXAMINED: GREECE:

G-212= Skiros island, Molos, 50 m from the coast, 2 wells, water temperature 15.2°C, pH 7.3, 1.7.1980, 2 exp. (leg. G. Pesce);

G-213.= Skiros island, Molos, radar station, 2 wells, water temperature 13.3°C, pH 7.1, 1.7.1980, 2 exp. (leg. G. Pesce) (locus typicus);

G-216= Skiros island, Road Linaria-Skiros, 6 km from Skiros city, 2 wells, pH 7.1, water temperature 15.0°C, 2.7.1980, 3 exp. (leg. G. Pesce);

G-217= Skiros island, Aspous, 2 wells, along the road Linaria-Skiros, water temperature 15.1°C, pH 7.1, 2.7.1980, 1 exp. (leg. G. Pesce);

G-218= Skiros island, Aspous, 2 wells, along the road Linaria-Skiros, water temperature 15.1°C, pH7.1, 2.7.1980, 1 exp. (leg. G. Pesce);

G-222= Skiros Island, along road Linaria-Skiros, 2 km. from Linaria, water temperature 15.1°C, pH 7.1, 3.7.1980, 4 exp. (leg. G. Pesce).

DIAGNOSIS:

The subspecies *skirosi* is rather similar to *Niphargus lourensis lourensis*, but maxilla 1 inner plate is with 2-3 setae, outer plate with 6 spines bearing 1 lateral tooth, only mesial spine with 3-5 teeth, palpus is hardly longer, with 5-7 setae; urosomal segment 1 on each dorsolateral side with one seta; urosomal segment 2 on each dorsolateral side with one strong spine. Coxa 4 is unlobed. Gnathopods 1-2 with trapezoid propodus having rather inclined palm and dactylus with a row of setae along outer margin. Propodus of gnathopod 2 with L-spines sitting partially behind corner S-spine or sitting laterally of S-spine.

Dactylus of pereopods 3-7 at inner margin with one spine near basis of the nail; article 2 of pereopods 5-7 without distinct ventroposterior lobe. Epimeral plates are distinctly angular. Pleopods 1-3 with 2 retinacula, peduncles scarcely setose. Uropod 1 with equal rami. Uropod 3 moderately slender, with short distal article of outer ramus. Lobes of telson are mainly with 4 short distal spines; lateral and facial spines are absent.

DESCRIPTION: Male 8.0 (from G-213):

Body moderately slender, head with short rostrum and short subrounded lateral cephalic lobes (fig. 3E), eyes absent. Metasomal segments 1-3 with 3-4 dorsoposterior marginal setae (fig. 1H). Urosomal segment 1 on each dorsolateral side with one slender seta; urosomal segment 2 on each dorsolateral side with one strong spine; urosomal segment 3 naked. Urosomal segment 1 at each ventroposterior corner with one spine near basis of uropod 1 peduncle (fig. 3G).

Epimeral plates are distinctly angular. Epimeral plate 1 is provided with slightly concave ventral margin and convex posterior margin bearing 3-4 setae, ventroposterior corner is with stronger seta (fig. 1H). Epimeral plate 2 is with slightly convex ventral margin bearing 3 subventral spines, posterior slightly convex margin bearing 4-5 short setae, ventroposterior corner is marked by spine-like seta (fig. 1H). Epimeral plate 3 is with poorly convex ventral margin bearing 4 subventral spines, ventroposterior corner is well marked by spine-like seta, posterior margin is poorly convex bearing 3-4 short setae (fig. 1H).

Antenna 1 reaching nearly half of body-length; peduncular articles 1-3 progressively shorter (ratio: 48:40:27), scarcely setose (fig. 1A); main flagellum is scarcely setose, consisting of 14 articles (most of them with one aesthetasc) (fig. 1B). Accessory flagellum is 2-articulated (fig. 1A), not reaching half of peduncular article 3.

Antenna 2 is moderately slender; peduncular article 3 with one ventral bunch of longer setae; peduncular article 4 is hardly longer than article 5 (ratio: 75:71), at ventral margin with 2 bunches of setae (the longest setae are remarkably longer than diameter of article itself, along dorsal margin with several bunches of short setae; article 5 at ventral margin with 4 bunches of setae (the longest setae slightly exceeding diameter of article itself), along dorsal margin with several bunches of short setae. Flagellum is slightly longer than last peduncular article and consisting of 8 moderately slender articles (fig. 1C); antennal gland cone short (fig. 1C).

Mouthparts: Labrum is broader than long, with almost poorly concave distal margin (fig. 1D). Labium is much broader than long, with small well developed inner lobes and entire broad outer lobes (fig. 2A).

Mandible is with triturative molar. Right mandible: incisor with 4 teeth, lacinia mobilis bifurcate, with several teeth, accompanied by 6 rakers (fig. 5A). Left mandible: incisor with 5 teeth, lacinia mobilis with 4 teeth accompanied by 6 rakers. Palpus 3-articulated: first article is naked, second article is provided with 11-12 strong setae; article 3 is falciform, longer than article 2 (ratio: 75:66), provided with nearly 20 D-setae and 5 long distal E-setae, on outer face appear a row of 6 A-setae and one single additional A-seta (fig. 5B), on inner face are attached 4 long single B-setae (fig. 5C).

Maxilla 1: inner plate is with 3 distal setae, outer plate is provided with 7 spines [6 spines are with one lateral tooth, one (mesial) spine is provided with 3-4 lateral teeth] (fig. 1F), palpus is 2-articulated, second article hardly exceeding basis of outer plate-spines and provided with 5 distal setae (fig. 1E).

Maxilla 2: both lobes are almost equal, longer than broad and provided with numerous distal marginal setae only (fig. 3F).

Maxilliped: inner plate is short, with 3 distal pointed spines mixed with several longer setae (fig. 1G); outer plate reaching nearly half of palpus article 2 and provided with nearly 12 short spines and several proximal and distal setae; palpus article 3 along outer margin with 1-2 median setae, palpus article 4 at inner margin with one seta near basis of the nail (fig. 1G).

Coxae 1-4 are relatively short. Coxa 1 is slightly broader than long (ratio: 40:36), with subrounded ventral margin provided with nearly 5 setae (fig. 2B). Coxa 2 is longer than broad (ratio: 55:47), along ventral subrounded margin with 10 setae (fig. 2E). Coxa 3 is longer than broad (ratio: 57:49), with 9 marginal setae (fig. 3A); coxa 4 is slightly longer than broad (ratio: 57:52), with 8 setae along ventral margin, ventroposterior lobe absent, posterior margin is slightly concave (fig. 3C). Coxa 5 is poorly shorter than coxa 4, bilobed, much broader than long (ratio: 60:40) (fig. 4A). Coxa 6 is smaller than coxa 5, bilobed, broader than long (ratio: 50:32) (fig. 4C). Coxa 7 is much broader than long (ratio: 48:20), entire (fig. 4E).

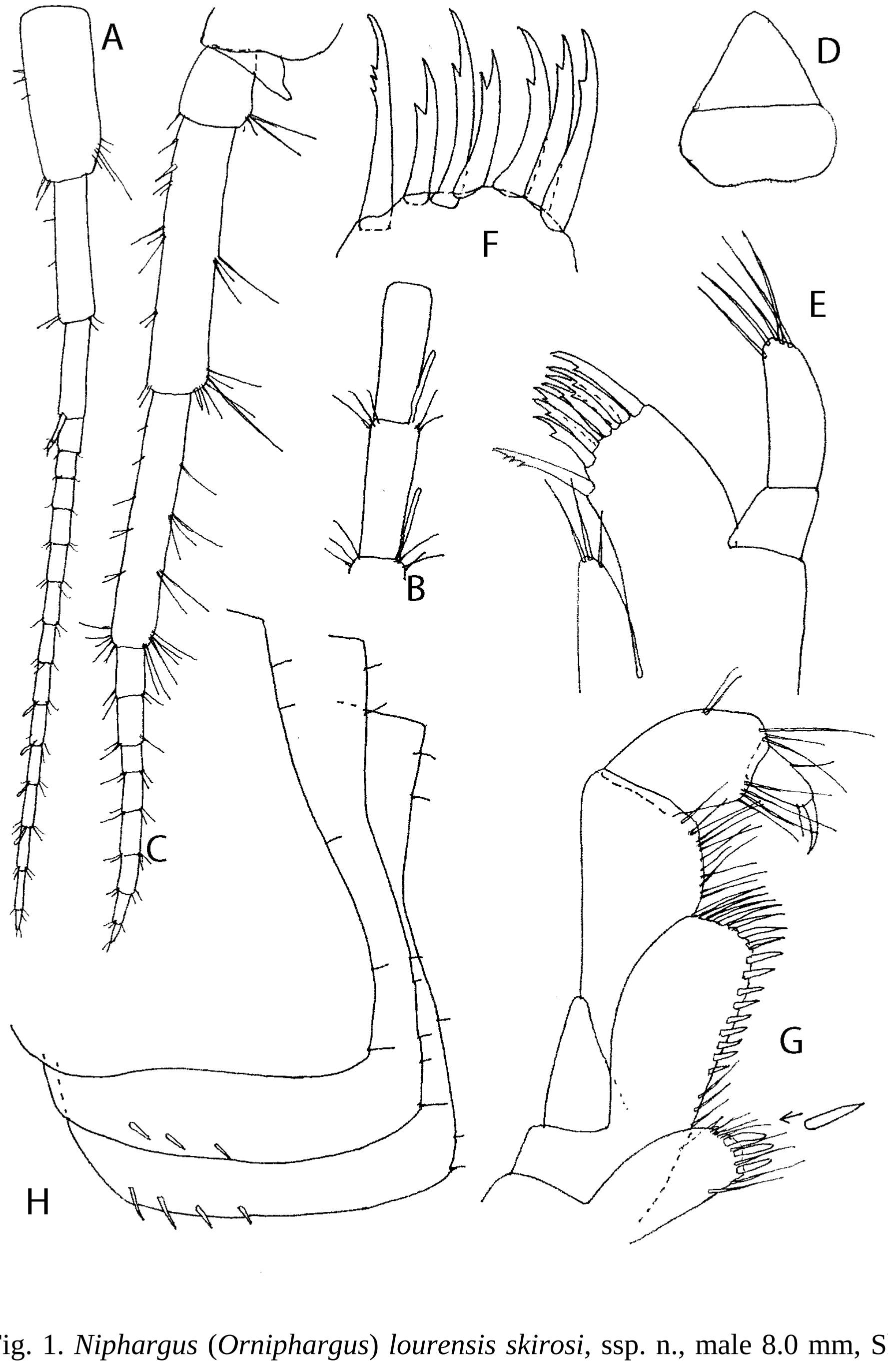

Fig. 1. *Niphargus* (*Orniphargus*) *lourensis skirosi*, ssp. n., male 8.0 mm, Skiros Island, Molos (G-213): A= antenna 1; B= aesthetasc on antenna 1-flagellum; C= antenna 2; D= labrum; E= maxilla 1; F= outer plate of maxilla 1; G= maxilliped; H= epimeral plates 1-3.

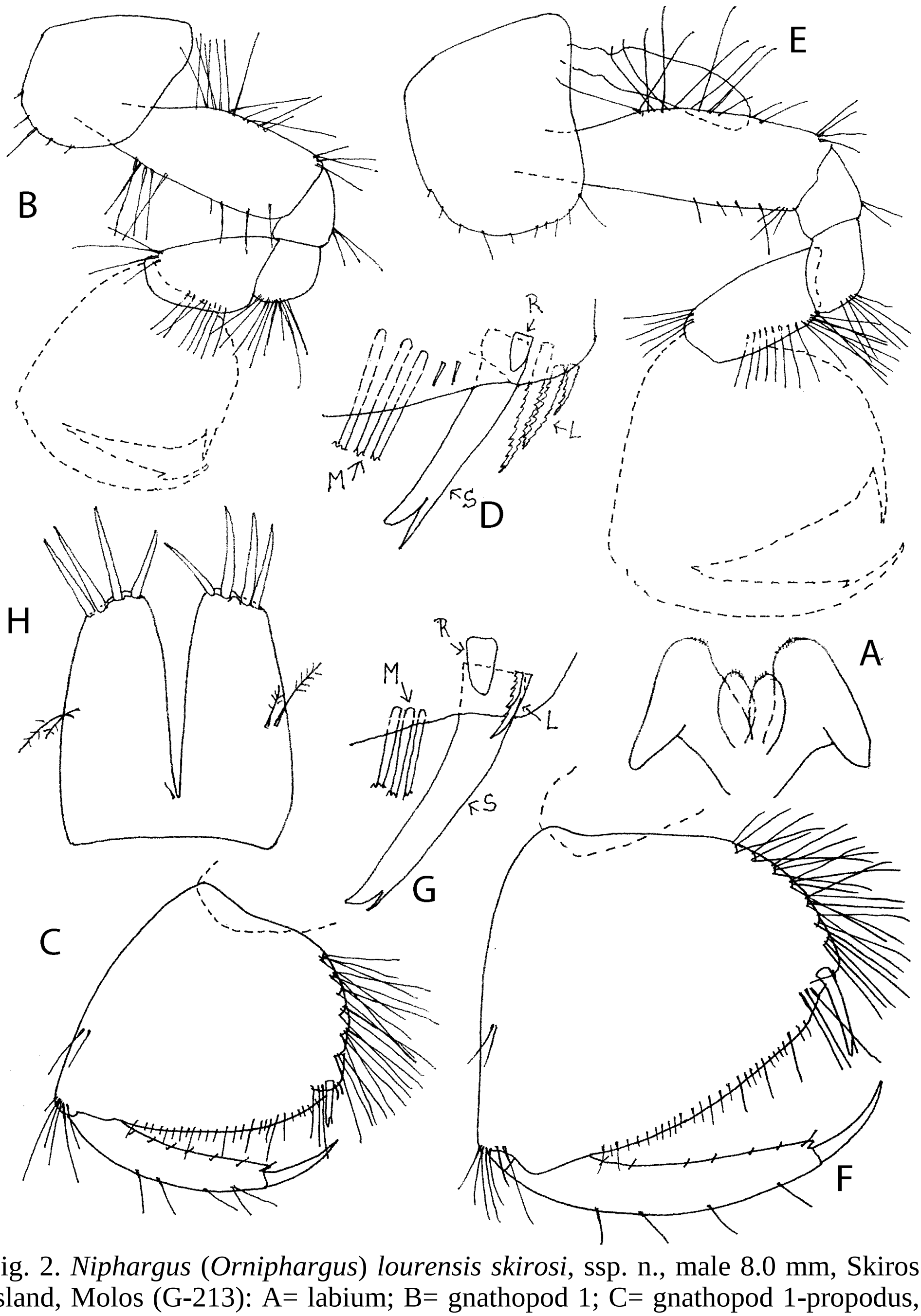

Fig. 2. *Niphargus* (*Orniphargus*) *lourensis skirosi*, ssp. n., male 8.0 mm, Skiros Island, Molos (G-213): A= labium; B= gnathopod 1; C= gnathopod 1-propodus, outer face; D= distal corner of gnathopod 1-propodus, inner face [S= corner S-spine; L= lateral serrate L-spines; R= subcorner spine; M= facial M-setae]; E= gnathopod 2, outer face; F= gnathopod 2-propodus, outer face; G= distal corner of gnathopod 2-propodus, inner face [S= corner S-spine; L= lateral serrate L-spines; R= subcorner spine; M= facial M-setae]; H= telson.

Gnathopods 1-2 are moderately large, with propodus larger than corresponding coxa (fig. 2B, E). Gnathopod 1: article 2 with long setae along anterior and posterior proximal margin, and shorter setae at distal anterior and posterior margin; article 3 at posterior margin with one bunch of setae (fig. 2B). Article 5 is shorter than propodus (ratio: 37:53), along anterior margin with one distal bunch of setae. Propodus is trapezoid, poorly longer than broad (ratio: 74:69), along posterior convex margin with 6 transverse rows of setae (fig. 2C). Palm is slightly convex, inclined hardly over half of propodus-length, defined on outer face by one corner S-spine accompanied laterally by 3 serrate L-spines and 3 facial M-setae, on inner face by one subcorner R-spine (fig. 2D). Dactylus reaching posterior margin of propodus, along outer margin with 5 median setae, along inner margin with row of several short setae (fig. 2C).

Gnathopod 2 is remarkably larger than gnathopod 1 (fig. 2B, E). Article 2 along anterior margin with several short setae in distal part, and no setae in proximal part; along posterior margin are attached numerous long setae in proximal part, and several short setae in distal part. Article 3 at posterior margin with one bunch of setae. Article 5 is shorter than propodus (ratio: 43:68), at anterior margin with distal bunch of setae (fig. 2E). Propodus is trapezoid, hardly broader than long (ratio: 94:91), along posterior margin with 7 transverse rows of setae (fig. 2F). Palm is convex, remarkably inclined over 2/3 of propodus-length, defined on outer face by one S-spine and 3 facial M-setae, on inner face by 2 slender L-spines sitting partially behind S-spine, and by one subcorner R-spine (fig. 2G). Dactylus reaching posterior margin of propodus, at outer margin with 4 median setae, along inner margin with several short setae (fig. 2F).

Pereopods 3 and 4 are moderately slender. Pereopod 3: article 2 at anterior margin with 2 proximal long setae and 3 short distal setae, along posterior margin with numerous long setae (fig. 3A). Articles 4-6 are of unequal length (ratio: 55:40:47); article 4 at anterior and posterior margin with several setae not exceeding diameter of article itself; article 5 along posterior margin with 4 spines and single short setae; article 6 along posterior margin with 6 groups of single or pairs of short spines. Dactylus is moderately slender, much shorter than article 6 (ratio: 16:47), at outer margin with one median plumose seta, at inner margin with one very slender spine near basis of the nail (fig. 3B); nail is hardly shorter than pedestal (ratio: 21:23).

Pereopod 4: article 2 at anterior margin with 2 proximal long setae and 3 mediodistal short setae; along posterior margin with numerous longer proximal setae and several mediodistal short setae. Articles 4-6 are of unequal length (ratio: 51:38:47): article 4 at anterior margin with 3-4 setae, at posterior margin with 4-5 bunches of 1-2 short setae; article 5 at anterior margin with 2 groups of short setae, at posterior margin with 3 single short spines and seta; article 6 along posterior margin with 5 bunches of short spines (fig. 3C). Dactylus is much shorter than article 6 (ratio: 20:47), at inner margin with one very slender spine near basis of the nail, at outer margin with one median plumose seta (fig. 3D), nail is hardly shorter than pedestal (ratio: 23:25).

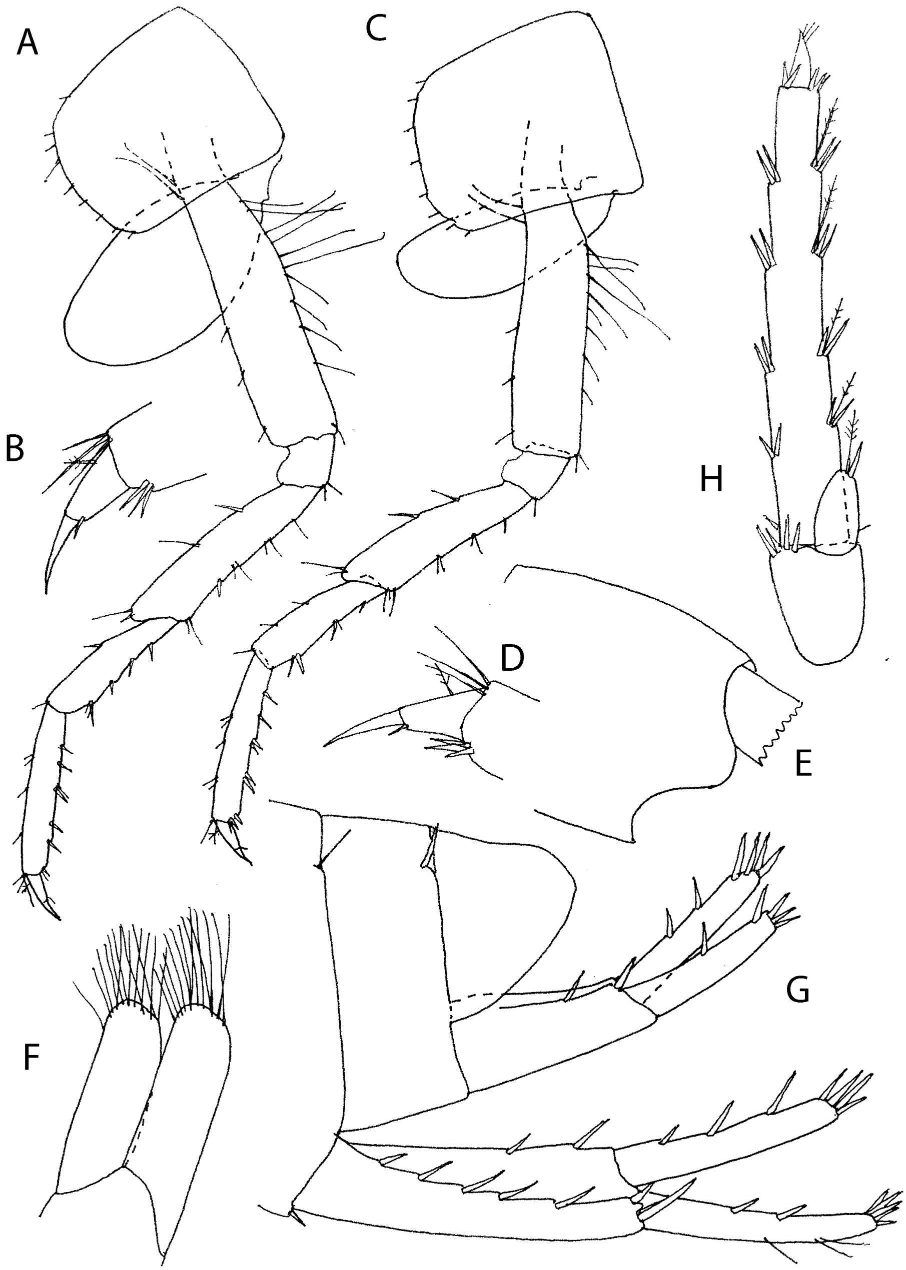

Fig. 3. *Niphargus* (*Orniphargus*) *lourensis skirosi*, ssp. n., male 8.0 mm, Skiros Island, Molos (G-213): A-B= pereopod 3; C-D= pereopod 4; E= head; F= maxilla 2; G= urosome with uropods 1-2; H= uropod 3.

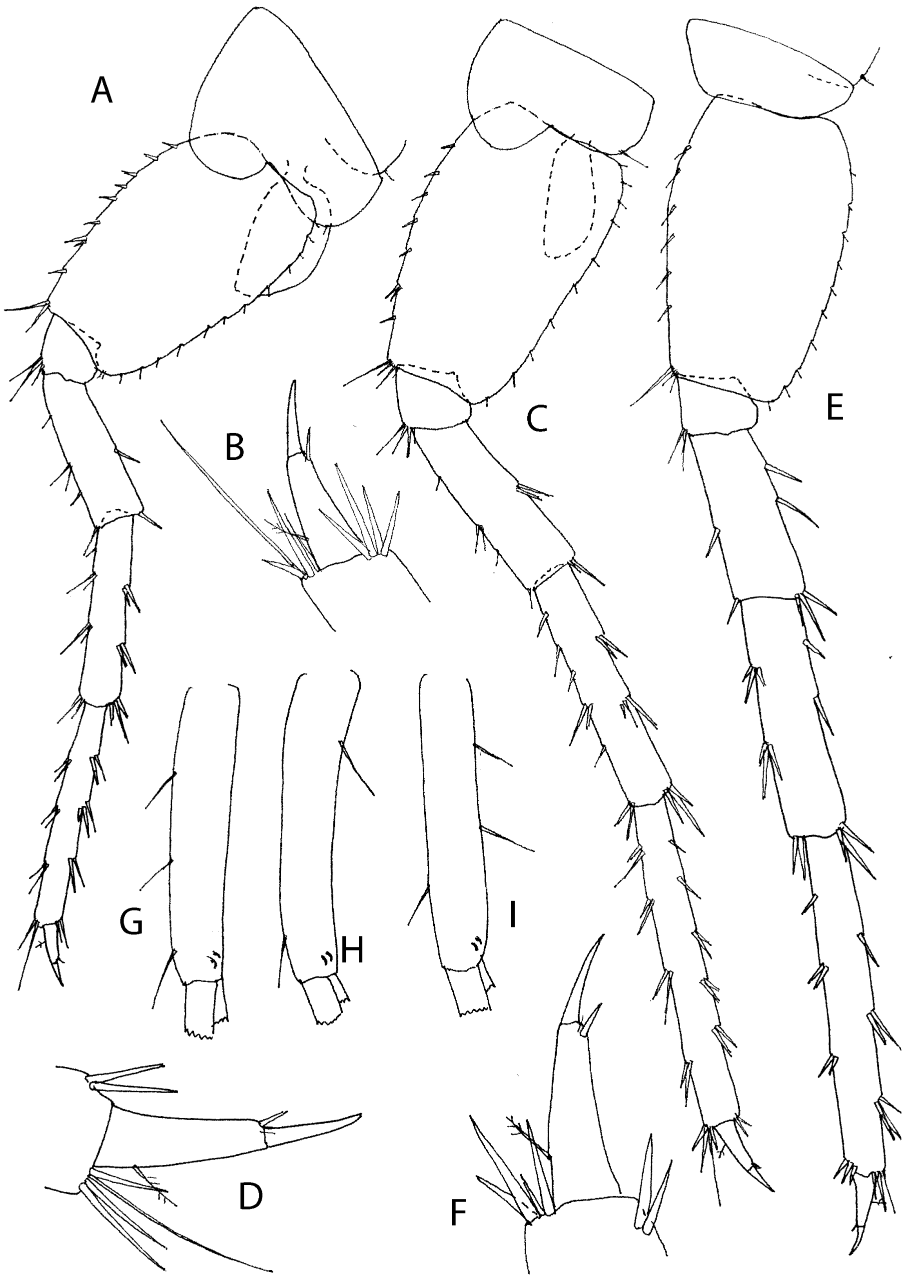

Fig. 4. *Niphargus (Orniphargus) lourensis skirosi*, ssp. n., male 8.0 mm, Skiros Island, Molos (G-213): A-B= pereopod 5; C-D= pereopod 6; E-F= pereopod 7; G= peduncle of pleopod 1; H= peduncle of pleopod 2; I= peduncle of pleopod 3.

Pereopods 5-7 are moderately long. Pereopod 5 is remarkably shorter than pereopods 6 and 7, with article 2 dilated, longer than broad (ratio: 73:46), along anterior convex margin are attached nearly 9-10 short spines, along posterior poorly convex margin are attached nearly 14 short setae, ventroposterior lobe is not developed (fig. 4A). Articles 4-6 are of unequal length (ratio: 44:52:61): article 4 at anterior margin with a row of short strong setae, at posterior margin with 2 single short spines; article 5 at anterior margin with 3 groups of slender spines and setae, at posterior margin with 3 bunches of spines; article 6 along posterior margin with 4 bunches of spines, along anterior margin with 4 groups of strong setae and one long distal seta. Article 2 is longer than article 6 (ratio: 73:61). Dactylus is much shorter than article 6 (ratio: 19:61), moderately slender, at inner margin with one slender spine, at outer margin with one median plumose seta (fig. 4B); nail is shorter than pedestal (ratio: 21:33).

Pereopod 6: article 2 is remarkably longer than broad (ratio: 79:50), along anterior margin with 8 groups of spines, along posterior margin with 10 short setae, ventroposterior lobe absent (fig. 4C). Articles 4-6 of unequal length (ratio: 55:69:88), article 4 along posterior margin with 2 groups of short spines; article 5 along anterior margin with 6 groups of short spines, along posterior margin with 3 groups of spines; article 6 along anterior and posterior margin with several bunches of spines; at tip are attached several spines and one long distal seta. Article 2 is shorter than article 6 (ratio: 79:88). Dactylus is much shorter than article 6 (ratio: 23:88), at inner margin with one slender spine near basis of the nail, at outer margin with one median plumose seta (fig. 4D); nail is shorter than pedestal (ratio: 27:48).

Pereopod 7: article 2 is longer than broad (ratio: 78:48), along anterior margin with 7 spines, along posterior slightly convex margin appear nearly 10 short setae, ventroposterior lobe absent (fig. 4E). Articles 4-6 are of unequal length (ratio: 48:65:93), along anterior and posterior margin with single or bunches of spines. Article 2 is shorter than article 6 (ratio: 78:93). Dactylus is moderately slender, along inner margin with one spine near basis of the nail, along outer margin with one median plumose seta (fig. 4F); nail is shorter than pedestal (ratio: 25:50).

Pleopods 1-3 are with 2 retinacula each. Peduncle of pleopod 1 along anterior margin with 3 long setae (fig. 4G); peduncle of pleopod 2 with one distal seta at anterior margin (fig. 4H); peduncle of pleopod 3 at anterior margin with one distal seta, along posterior margin with 2-3 long simple setae (fig. 4 I).

Uropod 1: peduncle longer than rami, with dorsoexternal row of strong spines; dorsointernal row is consisting on one median and one distal spine (fig. 3G). Outer ramus is as long as inner one or hardly longer, provided with 2 lateral and 5 distal short spines, as well as with 3 simple setae along outer margin; inner ramus is provided with 3 strong lateral and 4 distal spines.

Uropod 2: inner ramus is hardly longer than outer one, both rami with 1-2 single lateral and 4 distal short spines (fig. 3G).

Uropod 3 is relatively short; peduncle nearly as long as broad, with several distal spines; inner ramus is short, with one distal spine and plumose seta; outer ramus is consisting of 2 articles: first article is relatively slender, along outer margin with 5 bunches of short spines, along inner margin with 5 bunches of short spines mixed with single short plumose setae (fig. 3H); distal article is short, not exceeding diameter of first article and provided with 3 short distal simple setae.

Telson is relatively short, slightly longer than broad (ratio: 70:64), incised over ¾ of telson-length; each lobe with 4 distal spines shorter than half of telson-length; a pair of short plumose setae is attached in the middle of outer margin of telson (fig. 2H).

Coxal gills are relatively short, not reaching the ventral margin of corresponding article 2 of the legs (figs. 2E, 3A, C; 5A, C). Coxal gills on pereopod 3 are larger than these of pereopod 4.

FEMALE 7.0 mm with setose oostegites (from G-218) (slides G-218/1, 218/2, 218/3)

Head and body are very similar to the males, metasomal segments 1-3 with 4 dorsoposterior marginal setae each (fig. 6B). Urosomal segment 1 on each dorsolateral side with one seta, urosomal segment 2 on each dorsolateral side with one spine; urosomal segment 3 naked. Urosomal segment 1 on each ventroposterior corner with one short spine near basis of uropod 1-peduncle (fig. 6 I).

Epimeral plates are angular: epimeral plate 1 with slightly concave ventral margin, ventroposterior corner is distinctly marked, defined with corner seta, along posterior slightly convex margin appear several setae only (fig. 6B). Epimeral plate 2 is with hardly convex posterior margin provided with several setae only, ventral margin is slightly convex, with 3 subventral spines and well marked ventroposterior corner. Epimeral plate 3 is distinctly angular or slightly acute, with nearly straight and inclined posterior margin provided with 4 short setae, along ventral convex margin are attached 4 submarginal spines (fig. 6B).

Antenna 1 reaching nearly half of body, main flagellum is consisting of 19 articles. Accessory flagellum is 2-articulate, not reaching half of peduncular article 3-length. Flagellum of antenna 2 is longer than last peduncular article and consisting of 9 articles.

Mouthparts mainly like these in male. Mandibular palpus is similar to that in male. Maxilla 1 inner plate with 2-3 setae, outer plate with 7 spines [6 spines with one lateral tooth, one (mesial) spine with 5 lateral teeth (fig. 6A); palpus 2-articulated, provided with 5-7 setae.

Maxilliped inner plate is provided with 4 distal pointed spines and several setae, outer plate hardly exceeding half of palpus article 2 and provided with nearly 13 distomesial spines; palpus article 3 at outer margin with one median and one distal group of setae; palpus article 4 at inner margin with 1-2 setae near basis of the nail.

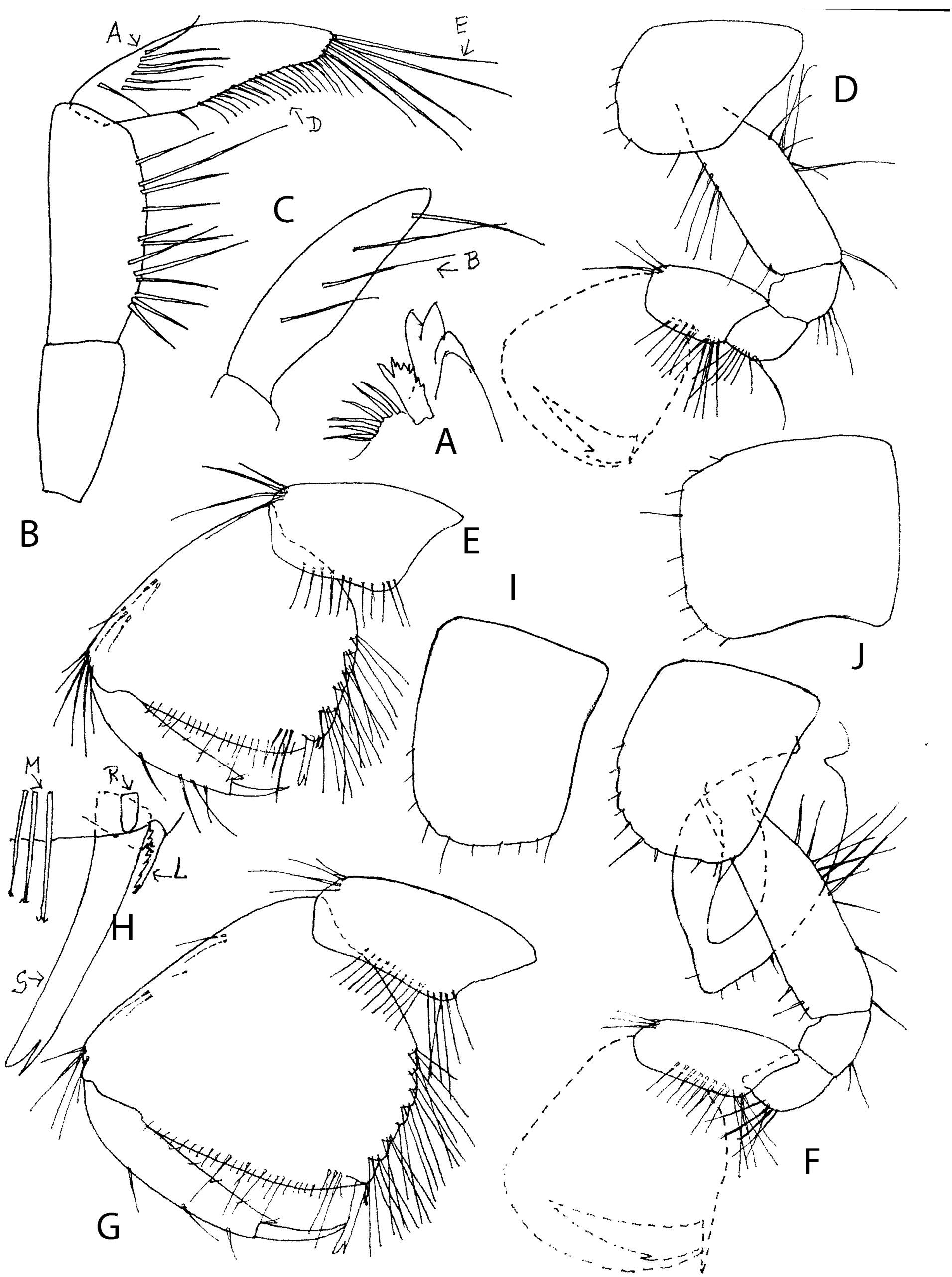

Fig. 5. *Niphargus* (*Orniphargus*) *lourensis skirosi*, ssp. n., male 8.0 mm, Skiros Island, Molos (G-213): A= incisor, lacinia mobilis and rakers of right mandible; B= mandibular palpus, outer face [A= outer facial A-setae; D= marginal D-setae; E= distal E-setae]; C= mandibular palpus article 3, inner face [facial B-setae, other setae omitted];
Female, 7.0 mm, Skiros Island, Aspous (G-218): D-E= gnathopod 1, outer face; F-G= gnathopod 2-propodus, outer face; H= distal corner of gnathopod 2-propodus, inner face [S= corner S-spine; L= lateral serrate L-spines; R= subcorner R-spine; M= facial M-setae]; I= coxa 3; J= coxa 4.

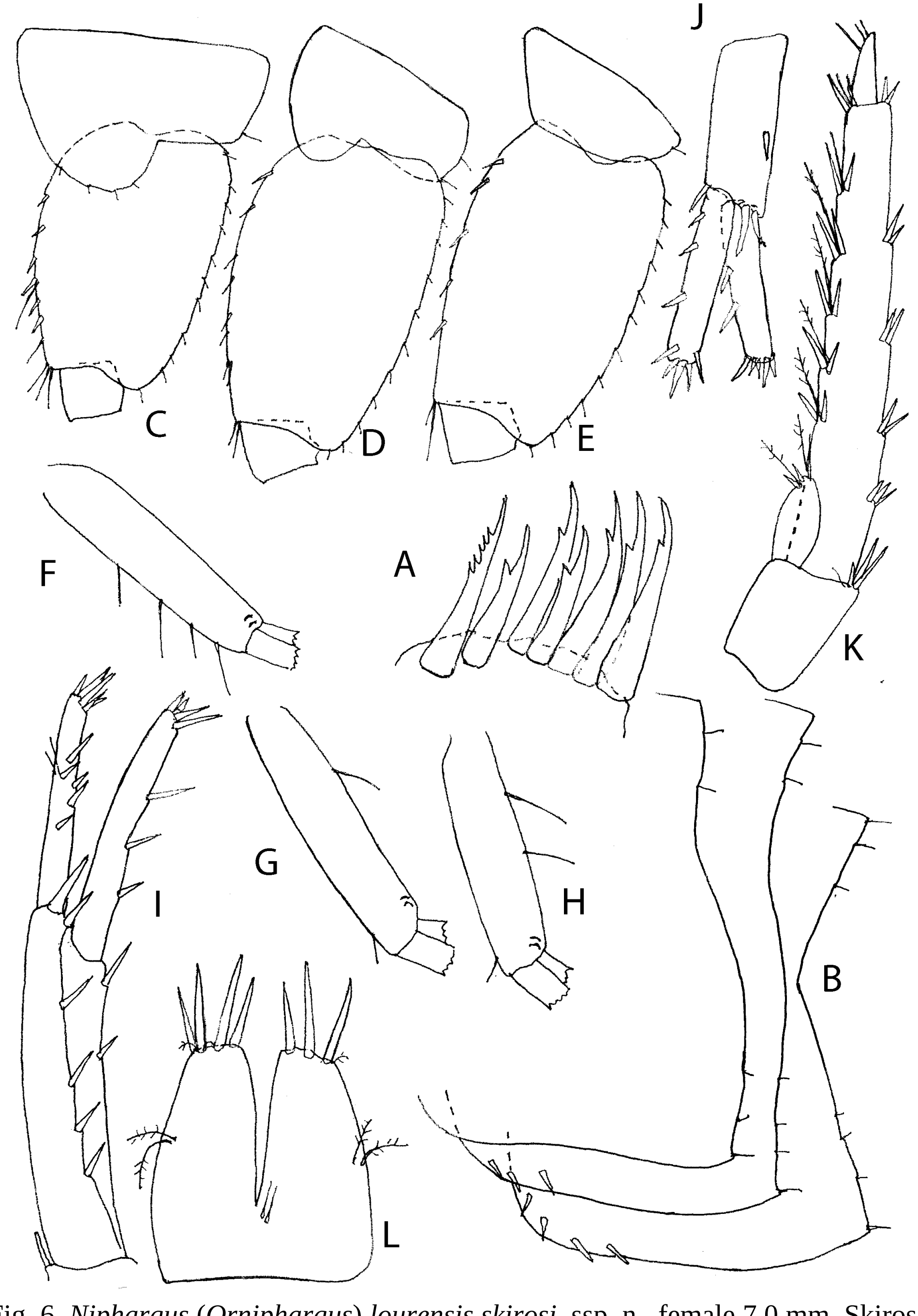

Fig. 6. *Niphargus* (*Orniphargus*) *lourensis skirosi*, ssp. n., female 7.0 mm, Skiros Island, Espouse (G-218): A= maxilla 1, outer plate; B= epimeral plates 1-3; C= pereopod 5 basipodit; D= pereopod 6 basipodit; E= pereopod 7 basipodit; F= pleopod 1 peduncle; G= pleopod 2 peduncle; H= pleopod 3 peduncle; I- uropod 1; J= uropod 2; K= uropod 3; L= telson.

Coxae are rather similar to these in male. Coxa 1 is broader than long (ratio: 40:36), with subrounded non produced ventroanterior corner provided with nearly 6 marginal setae (fig. 5D). Coxa 2 is longer than broad (ratio: 55:45), with subrounded ventroanterior corner provided with nearly 10 unequal setae (fig. 5F). Coxa 3 is longer than broad (ratio: 60:48) along ventral convex margin appear nearly 10 setae (fig. 5 I). Coxa 4 is only slightly longer than broad (ratio: 60:52), with convex ventral margin bearing nearly 9 setae, posterior margin is slightly concave, ventroposterior lobe absent (fig. 5J).

Coxa 5 is only slightly shorter than coxa 4, bilobed, broader than long (ratio: 70:44), anterior lobe is subrounded (fig. 6C). Coxa 6 is distinctly smaller than coxa 5, bilobed, broader than long (ratio: 52:35) (fig. 6D). Coxa 7 is entire, smaller than coxa 6, broader than long (ratio: 52:23) (fig. 6E).

Gnathopods 1 and 2 are rather smaller than these in male, with propodus nearly as large and corresponding coxa (fig. 5D, F).

Gnathopod 1 is remarkably smaller than gnathopod 2, article 2 along anterior margin with a row of long setae in proximal part and 2 short setae in distal part; along posterior margin appears one group of long setae in proximal part (fig. 5D); article 3 with one bunch of setae at posterior margin. Article 5 is shorter than propodus (ratio: 53:69), at anterior margin with one distal bunch of setae (fig. 5E). Propodus is trapezoid, hardly longer than broad (ratio: 69:65), along posterior margin with 5 transverse rows of setae (fig. 5E). Palm is convex, inclined nearly half of propodus-length, defined on outer face by one corner S-spine accompanied laterally by 3 slender L-spines and 3 facial M-setae, on inner face by one subcorner R-spine. Dactylus reaching posterior margin of propodus, provided with 4 median setae along outer margin and several short setae at inner margin (fig. 5E).

Gnathopod 2: article 2 along anterior margin with 3 short setae in distal part only (fig. 5F), along posterior margin with numerous long setae in proximal part and single short setae in distal part; article 3 at posterior margin with one bunch of setae (fig. 5F. Article 5 is shorter than propodus (ratio: 63:82), along anterior margin with distal bunch of setae (fig. 5G). Propodus is trapezoid, nearly as long as broad, along posterior margin with 7 transverse rows of setae (fig. 5G). Palm is convex, inclined nearly half of propodus-length, defined on outer face by one corner S-spine, 1-2 L-spines are sitting very close to the S-spine, not behind S-spine (fig. 5H), on outer face are attached 2-3 facial M setae; one subcorner short R-spine appear at inner face. Dactylus is reaching posterior margin of propodus, along outer margin with 3 median setae, along inner margin with several short setae (fig. 5G).

Pereopods 3-4 are similar to males, with short dactylus bearing one slender spine-like seta at inner margin near basis of the nail and one median plumose seta at outer margin; nail is shorter than pedestal.

Pereopods 5-7 are rather similar to males in shape and armature, and we figured here the basipodites (article 2) only. Pereopod 5: article 2 is rather longer than broad (ratio: 73:50), along anterior convex margin appear a row of short spines accompanied with several short setae or these setae are missing (fig. 6C), along posterior poorly convex margin are attached nearly 11 setae, ventroposterior lobe is not fully developed, ventroanterior corner is not produced (fig. 6C). Along inner margin of dactylus appears one slender spine near basis of the nail.

Pereopod 6: article 2 is remarkably larger than that of pereopod 5, longer than broad (ratio: 84:52), along anterior margin with row of short spine-like setae, along posterior convex margin with nearly 12 setae, ventroposterior lobe is not fully developed (fig. 6D); at inner margin of dactylus appear one slender spine near basis of the nail.

Pereopod 7: article 2 is longer than broad (ratio: 83:52), along anterior slightly convex margin with 5-6 slender spines, along posterior margin with nearly 12 setae, ventroposterior lobe is not distinctly developed (fig. 6E).

Pleopods 1-3 are provided with 2 retinacula each. Peduncle of pleopod 1 at anterior margin is with 4 setae (fig. 6F); peduncle of pleopod 2 along anterior margin with one distal seta, at posterior margin with one proximal seta (fig. 6G). Peduncle of pleopod 3 at anterior margin is with one short distal seta, at posterior margin with 2 medial long setae (fig. 6H).

Uropod 1: peduncle is rather longer than rami, with dorsoexternal row of strong spines, at dorsointernal side with one distal and one median strong spine (fig. 6 I); rami are of equal length, with several lateral spines and 4-5 distal short spines; 2 short simple setae are attached in the middle of outer ramus.

Uropod 2: peduncle is with one median and several distal spines; rami are almost of the same length, provided with several lateral and 5 distal short spines (fig. 6J).

Uropod 3 like that in male, relatively short: peduncle is short, with several distal spines; inner ramus is short, scale-like, with distal 2 spines and one plumose seta (fig. 6K). Outer ramus is 2-articulated: along outer margin appear 5 bunches of short spines, along inner (mesial) margin are attached 7 bunches of short spines accompanied by single plumose setae; second article is short, not exceeding diameter of first article and provided with 3 distolateral small simple setae.

Telson is relatively short, hardly longer than broad (ratio: 67:60), lobes are with 3 and 4 distal spines shorter than half of telson-length; a pair of short plumose setae is attached near the middle of outer margin of each lobe (fig. 6L).

Coxal gills are relatively small, not reaching ventral tip of corresponding article 2 (fig. 5F). Oostegites are broad, with short marginal setae (fig. 5F).

VARIABILITY

Urosomal segment 1 is always with one seta at each dorsolateral side; urosomal segment 2 on each dorsolateral side always with one strong spine.

Maxilla 1: inner plate is with 2-3 setae, outer plate with 7 spines [6 spines with one lateral tooth, mesial spine with 3-5 lateral teeth], only exceptionally one additional lateral tooth appear on one spine only [5-1-2-1-1-1-1]; palpus hardly exceeding basis of outer plate-spines, provided with 5-7 setae.

Maxilliped inner plate with 3-4 distal pointed spines accompanied by single setae. Additional A-seta on outer face of mandibular palpus article 3 is sometimes absent. Accessory flagellum is shorter than half of peduncular article 3 of antenna 1.

On palm of gnathopod 2-propodus appear 1-2 serrate L-spines sitting only partially behind S-spine or almost laterally of S-spine.

Three L-spines on gnathopod 1- propodus are sitting always laterally of corner S-spine.

Lobes of telson are provided with 3-4 distal spines distinctly shorter than half of telson-length; usually both lobes of telson are provided with 4 spines, sometimes one lobe with 3 and other with 4 spines, rarely both lobes were with 3 spines only.

G-222 (Skiros, Linaria), male 6.0 mm: This specimen agrees mainly with holotype. Urosomal segment 1 with one seta on each dorsolateral side; urosomal segment 2 with one strong spine on each dorsolateral side, urosomal segment 3 naked.

Maxilla 1: inner plate with 2 setae, outer plate with 7 spines [6 spines with one lateral tooth, one spine with 4-5 teeth), palpus with 5 distal setae. Maxilliped inner plate with 4 distal pointed spines, palpus article 4 at ventral margin with one seta near basis of the nail.

Palm of gnathopod 1- propodus with 3 serrate L-spines sitting laterally of corner S-spine. Gnathopod 2- propodus: palm with serrate L-spines sitting laterally or only partially behind corner S-spine, like that in female (fig. 5H). Lobes of telson are with 4 relatively short distal spines not reaching half of telson-length.

HOLOTYPE: male 8.0 mm (from G-213) (slides: 213/1, 213/2, 213/3). Holotype is deposited in KARAMAN`s Collection in Podgorica, Montenegro.

LOCUS TYPICUS: Molos, radar station, Skiros Island (Aegean Sea, Greece).

DISTRIBUTION: Skiros Island, Aegean Sea, Greece.

DERIVATIO NOMINIS: The name "*skirosi*" is made according to the name of locality, Skiros Island, where the taxon was collected.

REMARKS AND AFFINITIES.

Fišer et al. (2006) described a new species *Niphargus lourensis* from the subterranean waters of spring of Louros River, Vouliasta, Ioannina [Epirus, Greece] based on one adult female of 15 mm belonging to the subgenus *Orniphargus* S. Kar. 1950 (Karaman, S. 1950a, 1950b).

The holotype of *Niphargus lourensis* from type-locality [female 15 mm in final stage] differs from our populations of Skiros island in Aegean Sea by large body-size, very long distal spines on telson, by elevated number of spines on urosomal segments [2 spines on each side of urosomal segment 1, 4 spines on each side of urosomal segment 2], by very spinose outer ramus of uropod 3, by rather narrowed article 2 of pereopods 5-7 in female, by presence of one serrate

L-spine sitting completely behind corner S-spine on palm of gnathopod 2-propodus, by outer plate of maxilla 1 "with 7 uni-, bi- or pluri-toothed spines".

The variability of taxonomic characters and male of *N. lourensis* from type-locality remains unknown, because neither other localities nor redescription of this species were published later. By this way it is very difficult to understand the real value of established taxonomical differences between type-locality specimen and specimens from Skiros Island. Based on above mentioned taxonomic characters and zoogeographical data, we consider useful to describe the specimens from Skiros Island as not identical with these from Vouliasta, but as a distinct new subspecies, *Niphargus (Orniphargus) lourensis skirosi*, ssp. n., with above mentioned differences.

The further discovery of this species in type locality and other localities will probably put more light on the taxonomical relations between populations of these two distant localities, including the possibility that the populations from Skiros can be within extreme limits of the variability of *N. lourensis*.

The other known member of the subgenus *Orniphargus* known from Greece is *Niphargus lindbergi* S. Karaman 1956, described and known from Spilia Draconera Cave, Attica, Greece (Karaman, S. 1956), but this species differs remarkably from ssp. *skirosi* by presence of lateral spines on lobes of telson, by elevated number of retinacula on pleopods, presence of 2 spines at ventroposterior corner of urosomal segment 1 near basis of uropod 1-peduncle, etc. (see below).

In the adjacent region of Macedonia two species of *Niphargus (Orniphargus*) subgenus are present: *Niphargus macedonicus* S. Karaman 1929 (loc. typ.: Rašće spring near Skoplje) and *Niphargus pellagonicus* S. Karaman 1943 (loc. typ.: spring on road from Bitola towards the village Magarevo), but both these species differ from ssp. *skirosi* by elevated number of retinacula on pleopods 1-3 and various other morphological characters.

NIPHARGUS (*ORNIPHARGUS*) *LINDBERGI* S. Karaman 1956

Figures 7-9

Niphargus (*Orniphargus*) *lindbergi*, S. Karaman 1956: 1, figs. 1-6;
Niphargus lindbergi G. Karaman 1972: 7; Barnard, J. & Barnard, C. 1983: 692; G. Karaman & Ruffo 1986: 527: Fišer et al. 2006: 2315;
?*Niphargus lindbergi*, Pesce & Maggi, 1983: 58.

MATERIAL EXAMINED: GREECE

Sp. 424 (G-231)= Spilia Draconera Cave, Attica, 29.4.1954 (leg. K. Lindberg); one male 12 mm partially dissected, and slides 231/1, 231/2 (HOLOTYPE);

S-6361 (G-88)= Thessaly (=Tessalia), spring on main road Lamia-Larissa (=Laritza), water temp. 14*C, pH 6.7; NO_2 1 mg/l, 11.5.1977 (leg. Pesce, Maggi & Miranda); male 6.0 mm.

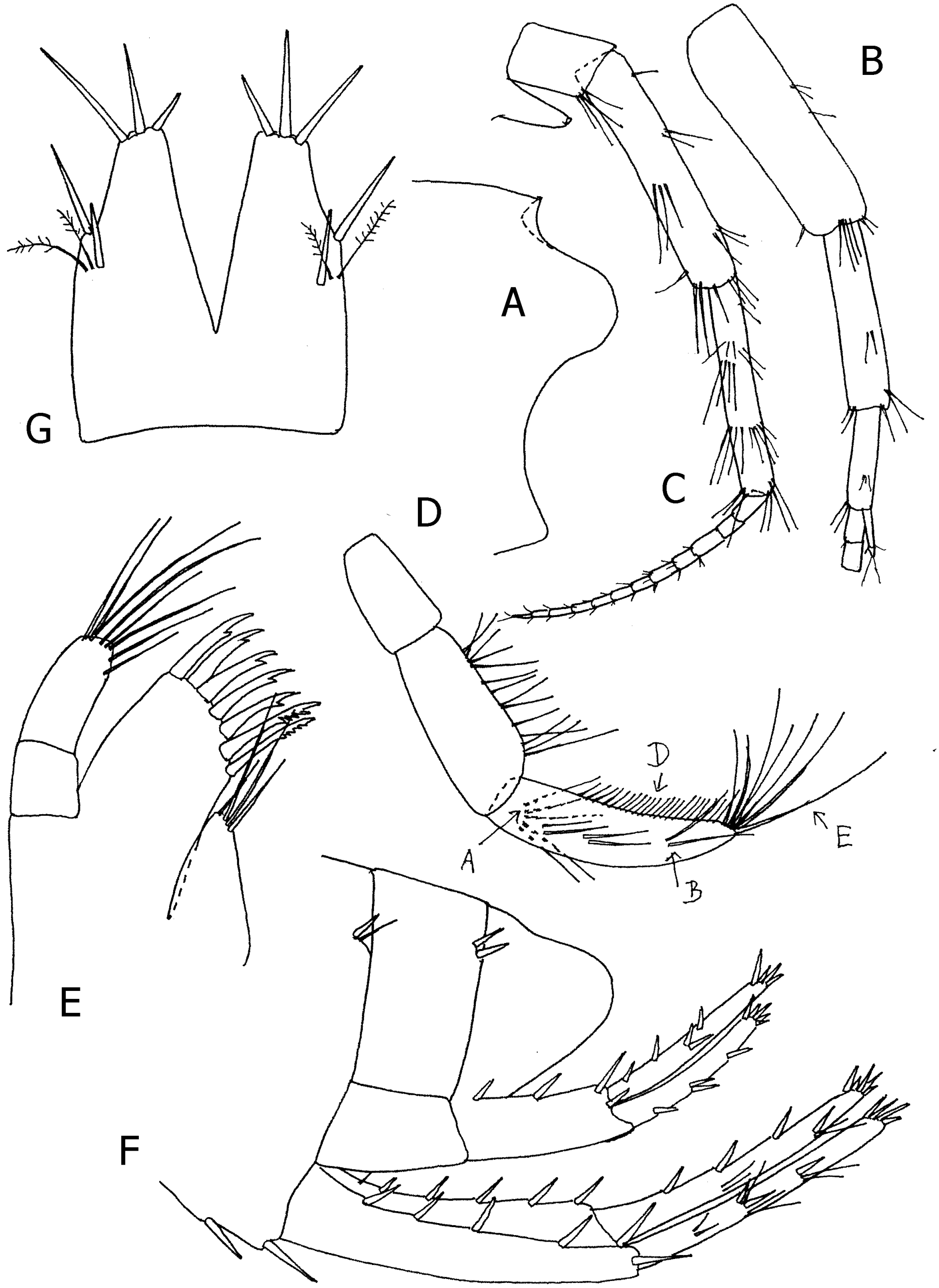

Fig. 7. *Niphargus (Orniphargus) lindbergi* S. Karaman 1956, male 12.0 mm, Draconera Cave (holotype): A= lateral cephalic lobe; B= peduncle of antenna 1; C= antenna 2; D= mandibular palpus, inner face [A= facial A-setae; B= facial B-setae; D= marginal D-setae; E= distal E-setae]; E= maxilla 1F= urosome with uropods 1-2; G= telson.

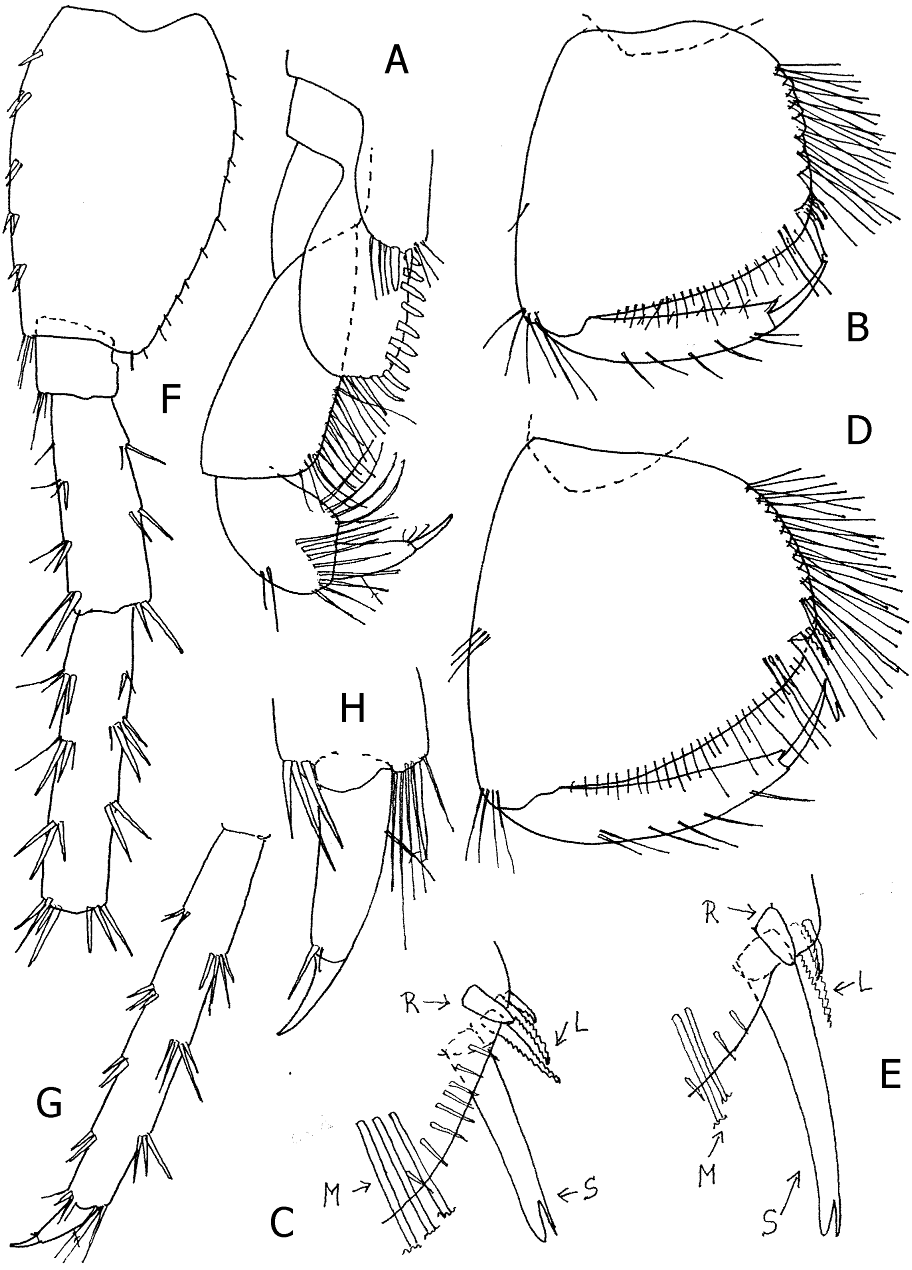

Fig. 8. *Niphargus* (*Orniphargus*) *lindbergi* S. Karaman 1956, male 12.0 mm, Draconera Cave (holotype): A= maxilliped; B= gnathopod 1 propodus, outer face; C= distal corner of gnathopod 1-propodus, inner face [S= corner S-spine; L= lateral serrate L-spines; R= subcorner R-spine; M= facial M-setae]; D= gnathopod 2-propodus, outer face; E= distal corner of gnathopod 2-propodus, inner face [S= corner S-spine; L= lateral serrate L-spines; R= subcorner R-spine; M= facial M-setae]; F-G= pereopod 7; H= dactylus of pereopod 7

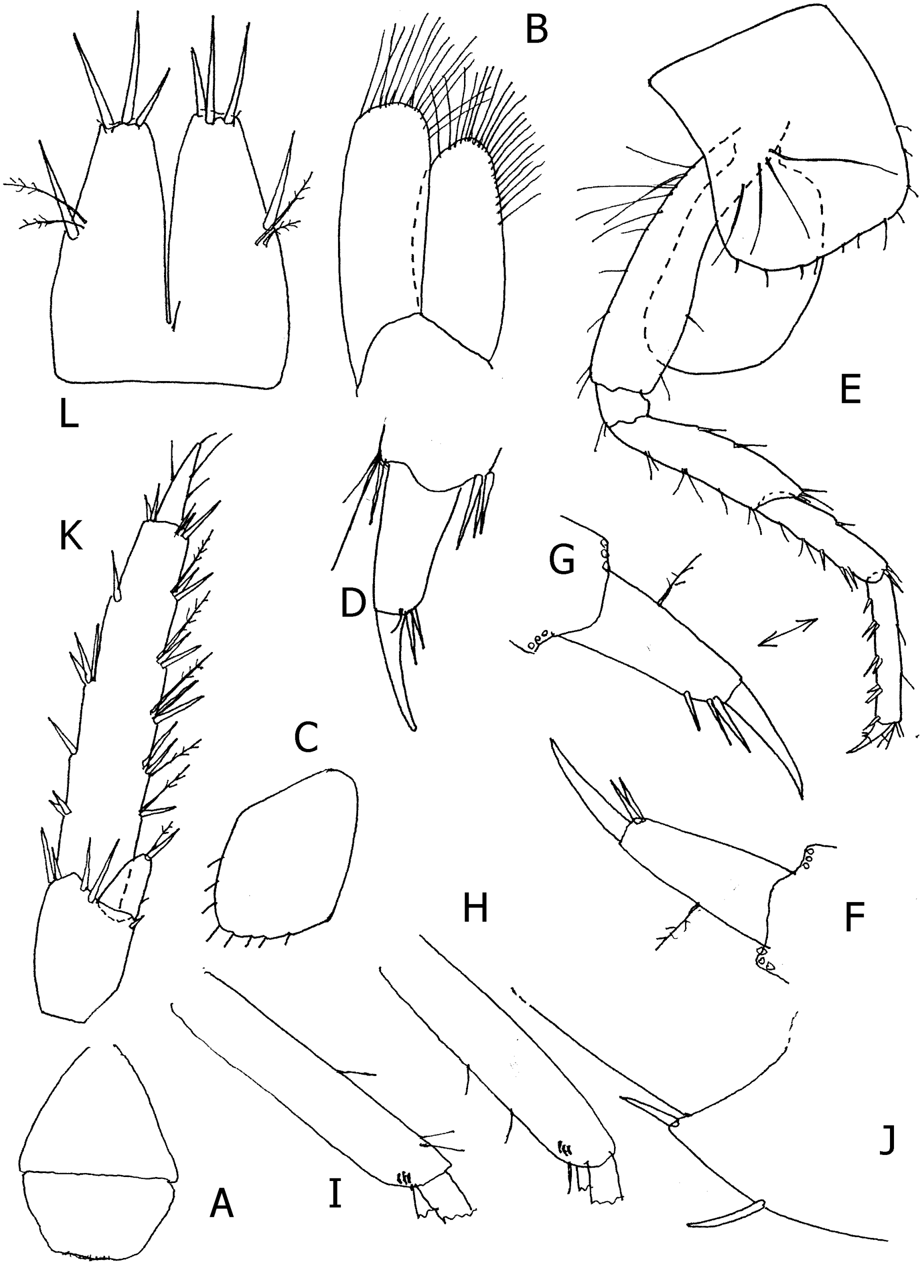

Fig. 9. *Niphargus* (*Orniphargus*) *lindbergi* S. Karaman 1956, male 12.0 mm, Draconera Cave (holotype): A= labrum; B= maxilla 2; C= coxa 1; D= dactylus of pereopod 3; E= pereopod 4; F-G= left and right dactylus of pereopod 4; H= pleopod 1 peduncle; I= pleopod 3-peduncle;
Male juv. 6.0 mm, Thessaly: J= urosomal segment 1, ventroposterior corner; K= uropod 3; L= telson.

DIAGNOSIS (males only): Moderately strong body, mesosomal segments smooth; metasomal segments 1-3 with dorsoposterior single setae; urosomal segments 1-2 with spines; telson with distal, marginal and 0-1 facial spines. Urosomal segment 1 on each ventroposterior corner with 2 strong single spines. Epimeral plates slightly acute. Maxilla 1 is provided with 4 setae, outer plate with 7 spines (most of them with one lateral tooth), palpus short, with numerous distal setae. Maxilliped inner plate short, with 3 distal spines.

Coxae 2-4 are slightly longer than broad, coxa 1 shallow, rhomboid, coxa 4 without distinct ventroposterior lobe, coxa 5 slightly shorter than coxa 4. Gnathopods 1-2 moderately strong, with trapezoid propodus having inclined palm bearing L-spines sitting laterally of corner S-spine, and dactylus with row of setae along outer margin. Pereopods 5-7 with unlobed article 2; dactylus of pereopods 3-7 strong, with one spine at inner margin (exceptionally with additional spine on pereopods 3 or 4). Pleopods with elevated number of retinacula, peduncles scarcely setose. Uropod 1 with equal rami; uropod 2 with inner ramus poorly longer than outer one. Uropod 3 short, with first article of outer ramus spinose, second article short. Lobes of telson are with distal; outer marginal and 0-1 facial spine.

DESCRIPTION of holotype (male 12.0 mm):

Stanko Karaman (1956) described and partially figured this species based on one adult male of 12 mm. We added some unknown important taxonomical character of this holotype.

Head is with short rostrum and subrounded lateral cephalic lobes (fig. 7A). Body is moderately strong, mesosomal segments 1-3 smooth; metasomal segments 1-3 with 4-5 short dorsoposterior marginal setae each. Urosomal segment 1 on each dorsolateral side with one spine and one seta, on urosomal segment 2 appear 2 strong spines on each dorsolateral side); urosomal segment 3 is naked (fig. 7F). Urosomal segment 1 at each ventroposterior margin with 2 single strong spines (fig. 7F) (overlooked by previous researches).

Epimeral plates 1-3 are rather acute, with well defined ventroposterior acute corner defined by one strong spine-like seta, posterior margin is slightly inclined, with setae or single spine-like setae; at ventral margin of epimeral plate 2 appear 2 subventral spines, that of epimeral plate 3 appear 3 strong spines (see Karaman, S. 1956, fig. 5).

Antenna 1 and antenna 2 are described well by S. Karaman (1956). Antenna 1: peduncular articles 1-3 progressively shorter (ratio: 67:50:28), very scarcely setose (fig. 7B); main flagellum with 28 articles (most of them with 2 aesthetascs). Accessory flagellum 2-articulate, not exceeding half of last peduncular article (fig. 7B).

Antenna 2 is moderately slender; peduncular article 3 is short, with distal bunch of setae. Peduncular article 4 is rather longer than article 5 (ratio: 68:60), both articles with several bunches of lateral and distal setae (the longest setae exceeding the diameter of articles themselves); flagellum longer than last peduncular article and consisting of 12 relatively slender articles; antennal gland cone reaching distal tip of peduncular article 3 (fig. 7C).

Mouthparts are well developed. Labrum broader than long, entire distally (fig. 9A). Labium with small inner lobes and subrounded outer lobes.

Left mandible: incisor with 5 teeth, lacinia mobilis with 4 teeth accompanied by 6 rakers. right mandible ?. Mandibular palpus article 2 with 14 setae (fig. 7D); article 3 is falciform, longer than article 2 (ratio: 66:48), with nearly 30 D-setae and 7 distal E-setae, on outer face appear 5 A-setae, on inner face are attached 6 B-setae (2+2+2).

Maxilla 1: inner plate is with 4 setae of unequal length (fig. 7E); outer plate with 7 spines bearing different number of lateral teeth [inner spine with 5 very short lateral teeth, one spine with 3 stronger lateral teeth, 5 spines with one strong lateral tooth]; palpus 2-articulated, short, not reaching distal tip of outer plate-spines and provided with 7-8 long setae.

Maxilla 2: inner plate is smaller than outer one, with numerous distal and distolateral marginal setae (fig. 9B), outer plate with numerous distal setae only, facial setae are absent.

Maxilliped: Inner plate is short, not reaching outer tip of palpus article 1 and provided with 3 distal spines mixed with several setae (fig. 8A); outer plate reaching nearly half of palpus article 2, and provided with nearly 9 distolateral smooth pointed spines. Palpus article 3 at outer margin with one median and one distal bunch of setae; palpus article 4 at inner margin with 4 setae near basis of the nail (fig. 8A).

Coxae 1-4 are not elongated, provided with several distal setae each. Coxa 1 is short, rhomboid, broader than long (ratio: 49:32), with subrounded ventroanterior margin (fig. 9C); coxae 2-3 are poorly longer than broad, with several longer distal setae; Coxa 4 is slightly longer than broad (ratio: 61:54), ventroposterior lobe is not fully developed and concave (fig. 9E), coxa 5 is only slightly shorter than coxa 4, bilobed. Coxa 6 is shorter than coxa 5, bilobed; coxa 7 is shallow, entire.

Gnathopods 1-2 are moderately large, with propodus slightly larger than corresponding coxa. Gnathopod 1 is smaller than 2, article 3 at posterior margin with one bunch of setae; article 5 is shorter than propodus, at anterior margin with one distal bunch of setae. Propodus is trapezoid, as long as broad, along posterior margin with 8 transverse rows of setae (fig. 8B); palm inclined distinctly half of propodus length, defined at outer face with one S-spine accompanied laterally by 3 slender L-spines and 3 facial M-setae, at inner margin by one subcorner R-spine (fig. 8C); dactylus along outer margin with row of 5-6 setae, along inner (mesial) margin with row of short setae (fig. 8B).

Gnathopod 2: article 3 is with one bunch of setae at posterior margin; article 5 is shorter than 6; with one bunch of setae at anterior distal margin. Propodus is trapezoid, hardly longer than broad (ratio: 95:90), along posterior margin with 10 transverse rows of setae (fig. 8D). Palm is inclined nearly half of propodus, defined on outer face by one corner S-spine accompanied laterally by

3 L-spines and facial 2-3 M-setae, on inner face by one subcorner R-spine (fig. 8E). Dactylus is with 6 setae at outer margin and several short setae at inner margin (fig. 8D).

Pereopods 3-4 are moderately slender, poorly setose. Pereopod 3 is rather similar to pereopod 4 but hardly longer; dactylus is short, at inner margin with bunch of 2 unequal slender spines near basis of the nail (fig. 9D), at outer margin with one median plumose seta; nail is shorter than pedestal (ratio: 31:42).

Pereopod 4: article 2 at anterior and posterior margin with several proximal long setae (fig. 9E). Articles 4-6 of unequal length (ratio: 45:38:41). Article 4 at anterior margin with 3 bunches of slender spines, at posterior margin with 4 bunches of setae; article 5 at posterior margin with several spines and short setae; article 6 along posterior margin with 5 groups of short spines. Left dactylus is much shorter than article 6 (ratio: 15:41), provided at inner margin with bunch of one stronger and one weak spine near basis of the nail, accompanied by one median slender spine (fig. 9G); right dactylus like left one but without median slender spine at inner margin (fig. 9F), outer margin of both dactyls with one median plumose seta, nail is shorter than pedestal.

Pereopods 5-7 are rather elongated, pereopod 5 is rather shorter than margin with several spines, pereopods 6 and 7. Article 2 of pereopod 5 and pereopod 6 is longer than broad, along anterior along posterior slightly convex margin with row of short setae, ventroposterior lobe is not developed.

Pereopod 7: article 2 is longer than broad (ratio: 91:60), along anterior slightly convex margin are attached 8 groups of short spines, along posterior slightly convex margin appear 13 short setae, ventroposterior lobe is not developed (fig. 8F). Articles 4-6 are of unequal length (ratio: 60:82:110), along both margins with strong spines mixed with single setae. Article 2 is rather shorter than 6 (ratio: 91:110) (fig. 8G). Dactylus is much shorter than article 6 (ratio: 27:110), strong, at inner margin with one spine and seta near basis of the nail, at outer margin with one median plumose seta (fig. 8H); nail is shorter than pedestal (ratio: 25:53).

Pleopods are with 3-5 retinacula; peduncle of pleopod 1 with 3 setae at anterior margin (fig. 9H); pleopod 2 is missing (figured by S. Karaman 1956, fig. 6); peduncle of pleopod 3 at posterior margin with 3 setae (fig. 9 I).

Uropod 1 peduncle strong, with dorsoexternal and dorsointernal row of strong spines (fig. 7F); rami of equal length, shorter than peduncle, outer ramus with several lateral and distal short spines and several short simple setae (fig. 7F); inner ramus with several lateral and distal spines and one bunch of 2 simple median setae.

Uropod 2: inner ramus is slightly longer than outer one, both rami with several lateral and distal short spines (fig. 7F).

Uropod 3 is well figured by S. Karaman (1956, fig. 4), relatively short: peduncle is short, with bunch of distal spines; inner ramus is scale-like, as long as broad, with one distal spine and one plumose seta; outer ramus with 2 articles: first article along outer margin with 5 bunches of spines; along inner (mesial) margin are attached 7 groups of spines accompanied by single plumose setae. Second article of outer ramus is much shorter than first article (ratio: 27:75), narrowed, but distinctly longer than diameter of first article-tip, provided with several short lateral and distal setae.

Telson is relatively short, slightly longer than broad (ratio: 38:32) [80:73 on slide] incised nearly 2/3 of telson-length, each lobe is tapering distally and provided with 3 distal spines, one spine at outer margin and one facial spine [the longest spines are remarkably shorter than half of telson-length]. A pair of plumose setae is attaches near the external middle of each lobe (fig. 7G).

The female is unknown, but probably similar to males, like other *Orniphargus* taxa.

The small male 6.0 mm (juv.) from Thessaly:

Body like that of holotype. Metasomal segments 1-3 with 4 short dorsoposterior setae.

Urosomal segment 1 on each dorsolateral side with 3 strong spines, urosomal segment 2 with 2 strong dorsolateral spines on each side, urosomal segment 3 naked. Urosomal segment 1 at ventroposterior margin with 2 single spines, like that in holotype (fig. 9J). Epimeral plates 1-3 are slightly acute, like these in holotype, epimeral plate 2 with 2 submarginal spines, epimeral plate 3 with 3 submarginal spines.

Antenna 1 reaching half of body-length, main flagellum consisting of 20 articles. Flagellum of antenna 2 is with 7 articles, antennal gland cone reaching distal tip of peduncular article 3.

Mouthparts mainly like that in holotype. Mandibular palpus article 2 with 9 setae, article 3 with 2 A-setae, 4 B-setae, 18 D-setae and 5 E-setae.

Maxilla 1: inner plate with 2 setae, outer plate with 7 spines bearing different number of lateral teeth [3-1-2-1-1-1-1]; palpus short, hardly exceeding basis of outer plate-spines, bearing 4 distal setae.

Maxilliped: inner plate with 3 distal spines, outer plate with nearly 8 distolateral spines; palpus article 4 at inner margin with 2 setae near basis of the nail.

Coxae and gnathopods 1-2 like these in holotype, with palm inclined up to half of propodus-length, 2 L spines are sitting laterally of S-spine, one subcorner R-spine is sitting on inner face; dactylus is with 3 setae at outer margin.

Dactylus of P3-P7 with one spine at inner margin near basis of the nail. Article 2 of pereopods 5-7 is without distinct ventroposterior lobe.

Pleopods 1-3 with 3 retinacula each. Peduncle of pleopod 1 along anterior margin with 2 median and one distal seta; peduncle of pleopod 2 naked; peduncle of pleopod 3 at posterior margin with 2 medial and one distal seta.

Uropod 1 with equal rami; uropod 2 with inner ramus slightly longer than outer one.

Uropod 3 is rather more stout than that of holotype, along outer margin with 5 bunches of spines, along inner (mesial) margin with 6 groups of spines mixed with single plumose setae; inner ramus is short, scale-like, with one distal spine and plumose seta (fig. 9K); second article of outer ramus is rather short, with 4 lateral short simple setae.

Telson is slightly longer than broad (ratio: 70:63), incised over ¾ of telson-length; each lobe with 3 distal long spines rather shorter than half of telson-length; one spine and one pair of plumose setae are attached near the middle of outer margin; facial spines absent (fig. 9L).

LOCUS TYPICUS: Cave Draconera, Attica (= Attique), Greece.

DISTRIBUTION: Draconera Cave in Attica (S. Karaman 1956); spring along road Lamia--Larissa in Thessaly (present work).

REMARKS.

As some body-parts of holotype on two slides were preserved not in Faure liquid, but in some other, not soluble liquid, we couldn't move the body parts for better drawing, and we figured them directly from the slides (gnathopods, pereopod 7, telson, maxilla 1, maxilla 2 and maxilliped); but some other parts we figured from holotype preserved in ethanol. Some body parts are very well figured and described by S. Karaman (1956) [telson, uropod 3, propodus of gnathopod 2, epimeral plates, retinacula] and we have not figured all of them again. Propodus of gnathopod 2 is figured here to show the relative size and shape of gnathopods 1 and 2.

The presence of pair of spines in some of dactylus in pereopods 3-4 seems to be not of taxonomical value but probably only occasional case. These additional spines are not present in the male from Thessaly.

The most visible taxonomic character of this species is presence of 2 ventroposterior single spines on urosomal segment 1 near basis of uropod 1-peduncle, overlooked by previous scientists (fig. 7F, 9J). This rare character is not present in other *Niphargus* taxa known from Greece. We found this character also in *Niphargus bodoni* G. Karaman, 1983 [loc. typ.: springs of Cassana, La Spezia, Italy] (G. Karaman, 1993) and only several other species of genus *Niphargus*, but all of them differ remarkably from *N. lindbergi* by combination of numerous other taxonomical characters.

Based on its taxonomical characters, *N. lindbergi* belongs to the subgenus *Orniphargus* S. Karaman 1950 [typus subgeneris: *Niphargus orcinus* Joseph 1862], presented in Greece by 2 taxa only: *Niphargus lindbergi* and *N. lourensis* Fišer et al. 2006 [loc. typ.: Spring of Louros River, Vouliasta, Ioannina (=Ionannina), Epirus, Greece]. *N. lourensis* differs remarkably from *N. lindbergi* by presence of 2 retinacula on pleopods 1-3, by absence of lateral and facial spines on telson, etc.

The subgenus *Orniphargus* is relatively well defined based on morphological characters mentioned by S. Karaman (1950a, 1950b, G. Karaman 1984) within the genus *Niphargus.*

Based on the new investigation on limited molecular-genetic level of members of genus *Niphargus,* numerous different taxonomical categories with or without established distinct morphological differences are created (Delić et al. 2017, etc.) what made difficult recognition of taxa, and category subspecies is ignored by this way. Only the combined data of all kinds of investigations (not only limited molecular-genetic) will show the real and useful taxonomical categories within present genus *Niphargus*. Various authors recently argued about these taxonomical problems (Padial et al. 2010, Timm, 2012, etc.), and the further studies will probably put more light on the validation of taxonomical categories.

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APPLYING NENDO DANGO TECHNIQUE FOR GERMINATION AND PRE-ESTABLISHMENT OF NATIVE SPECIES ON DEFORESTED AREAS

SUMMARY

Nendo Dango is a planting technique created by the agriculturist and microbiologist Masanobu Fukuoka, around the 1940's decade, to reforest some zones of the Asiatic continent with tendency to desertification, while developing the Fukuoka method with no-tillage, also called wild agriculture. This paper's goal was to evaluate this technique's efficiency on germination and pre-establishment of native species seeds in a deforested area in the Brejo Comprido's stream riverbank, in Palmas, state of Tocantins, Brazil. Eight types of native species seeds were selected, arranging them into lake-originated-clay balls around 2 to 3 cm large, disposed in 0.5 meters interspacing, forming an experiment field of 8 m², designed with 5 sample lines and 3 repetitions for statistical analysis, totalizing a 24 m² area. Nine data acquisitions were made to input into calculation the parameters germination percentage (GP), germination velocity rate (GVR) and required germination time (RGT). Results had shown a GP varying from 26.7% to 100%, where 5 of the 8 species seeds well-thrived the environment conditions applied, as well as a GVR varying from 0.36 to 1.35 individuals per day and an RGT varying from 0.17 to 0.26 seeds per day. In a general picture, the technique proved to be efficient for the proposed experiment and chosen native species, supplying the seeds with a good pre-environment for successful germination and pre-establishment.

Keywords: No-tillage, Seeds germination, Clay balls, Cerrado biome, Reforestation, Wild agriculture

INTRODUCTION

Human activities have become more frequently the reason of degraded environments appearance nowadays, in terms of deforestation, pollution, agriculture and a serie of other many economic-related actions required to sustain modern life. The recovery of these areas, direct or indirectly, demand priority on reestablish the biota's natural conditions, throughout initiatives and projects whose are less expensive and based on sustainability, primarily (Bastos &

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Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

Ferreira, 2010). Speaking of recovery, since the 1940's decade Masanobu Fukuoka, an agriculturist and microbiologist known by his minimally invasive planting methods, has developed a technique called "Nendo Dango", which consists of placing seeds into clay balls and release these into the environment for replant once-loss threes in deforested or with desertification tendency areas (Fukuoka, 1985). The main purpose of this technique is to protect the seeds from predators and inclement weather, without using any pesticides or herbicides, while involving the seed onto a clay ball, which provides a secure and stable environment for it to germinate and pre-establish into the soil (Fukuoka, 1991). This technique has been used all around the world for the most variated motives, as recovering green urban areas or filling green protected areas in underdeveloped countries in South America, Africa and Asia (Torres, 2010).

The area chosen for the experiment procedures was previously base for a sewage treatment plant, in the city of Palmas, which the degradation became from deforestation and soil pollution, altering the local ecosystem and affecting punctually the Brejo Comprido's riverbank, as well as the water. Once the plant was disabled, the area was abandoned and turned into a place for irregular domestic residues disposal. For the technique to work, the area was cleaned up from all the residues disposed and they were sent to the Palmas' landfill. Thus, this paper's main goal was to evaluate "Nendo Dango" efficiency on germination and pre-establishment of native species' seeds on a deforested area in the Brejo Comprido's stream riverbank, at the city of Palmas, state of Tocantins, Brazil.

MATERIAL AND METHODS

Species Selection and Management

Considering factors as species' genetic variability, most occurrence in remaining riparian wood and easy collection, eight native species, which occur in Cerrado biome, were selected. The seeds were cleaned, let exposed 2 days to dry and stored under temperature between 16ºC and 18º at the Laboratory of Environmental Impacts Characterization.

Table 1. Chosen species for the experiment

SCIENTIFIC NAME	SUCCESSIONAL CLASSIFICATION
Tabebuia roseoalba (Ridl.) Sandwith	Secondary
Sterculia chicha A. St.-Hil. ex Turpin	Pioneer
Tabebuia heptaphylla (Vell.) Toledo	Secondary
Anadenanthera falcata (Benth.) Speg	Pioneer
Calophyllum brasiliense Cambess	Secondary/Intermediary
Guazuma ulmifolia Lam.	Pioneer
Hymenaea stigonocarpa Mart. ex Hayne	Late Secondary and Climax
Bowdichia virgilioides Kunth	Secondary

For the Nendo Dango technique it is not necessary to scarify or break dormancy of the seeds. Table 1 shows the scientific name and successional classification of the chosen species.

Clay Origins and Management

For the experiment was used clay originated from the drain work of a lake within the Cesamar Park (Figure 1), also localized at the city of Palmas, under license and supervision of the park's authorized staff. To obtain the material was used pottery shovel and three tow bags.

Figure 1: Source of the clay for the experiment

Procedures for Clay Balls Production

For the method execution, the soil where the clay balls with seeds were released was not plowed, pruned or weeded, as well as applied any type of pesticide, fertilizer or herbicides, as essentially demanded by Fukuoka (1985). The clay removed from the drain work was previously arranged into blocks, which were broken down into smaller pieces with an octave hammer and sifted with a 0.005 mm sieve. After sifted, four kilos of the clay powder were set aside into a plastic tray, measuring 9.7 x 30 x 49 cm. The humidification process was executed by adding half a liter of water onto the powder and hand-mixed until form a consistent and homogeneous mass, enabling it to produce disks to insert the seed and then mold it into firm balls (Figure 2).

Figure 2: Clay mass and some of the clay balls already seeded

Each ball weighted an average of 70 g and had 2 to 3 cm diameter. This variance of size was dictated by the size of the seed, in order to supply enough clay mass for it to not crack and/or get external exposure for predators or inclement weather. Once ready, the seeded-clay-balls rested in room conditions to get some drying and prevent sudden cracking under the overheated external temperature. The release period occurred 30 days before rainy season in the city of Palmas, state of Tocantins, Brazil, as recommended by Fukuoka (1991).

Experiment Disposal onto the Soil

The Nendo Dango method essentially says the it must be applied through no-tillage and randomly sowing the seeded-clay-balls onto the soil, which are subject for recovery/reforestation, but, in order to get research control and data acquisition, the experiment had to be designed with clear guidelines and field markings. For that, the chosen disposition was the zig-zag rule (Figure 3) and the field was isolated with zebra ribbon.

Each ball was interspaced only by 0.5 m, whereas the experiment did not expect them grow into full form, i.e., just germination and pre-establishment period. At the end, the area was constituted by experiments of 8 m², with 5 sample lines, 8 treatments (species) and 3 repetitions, totalizing 24 m². The data acquisition occurred during 37 days, 3 times per week, using caliper rule, scaler and notepad. In the last day of counting/collecting data, the germination average rate was calculated.

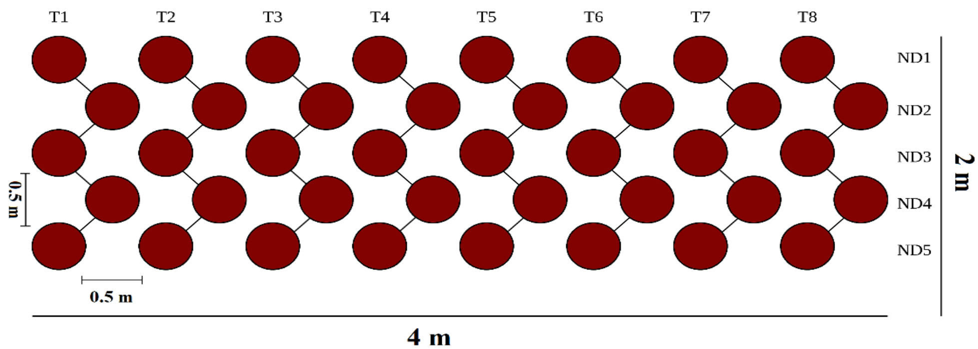

Figure 3: Design of the experimental field

Statistical Analysis

The treatments were outlined through completely randomized design with three repetitions/blocks, each repetition/block had eight treatments/species (Table 2) and each treatment had five samples, totalizing 40 clay balls for each repetition and 120 for the experiment.

For purposes of germination, the analyzed parameter was the number of germinated samples of each specie during the data acquisition period. Seeds which emerged seedlings elevating the cotyledons 5 cm above the soil were considered germinated (Brown, 1992).

Table 2: Indication for treatment into statistical terms

TREATMENT (T)	SCIENTIFIC NAME
T1	*Tabebuia roseoalba* (Ridl.) Sandwith
T2	*Sterculia chicha* A. St.-Hil. ex Turpin
T3	*Tabebuia heptaphylla* (Vell.) Toledo
T4	*Anadenanthera falcata* (Benth.) Speg
T5	*Calophyllum brasiliense* Cambess
T6	*Guazuma ulmifolia* Lam.
T7	*Hymenaea stigonocarpa* Mart. ex Hayne
T8	*Bowdichia virgilioides* Kunth

The data of germination percentage (GP) (Eq. 1), germination velocity rate (GVR) (Eq. 2) and required germination time (RGT) (Eq. 3) were analyzed under Tukey test with 5% of probability, using the software Assistat 7.7 (Maguire, 1976; Labouriau & Valadares, 1976; Silva & Azevedo, 2006). The GVR is a rate calculated by means of counting the germinated seeds and has the goal of establish the differences in the germination velocity on accesses, groups and batches of seed, i.e., the greater the GVR value is, greater will be the daily germination, indication good treatment choice (Brasileiro *et al*., 2008).

$$\mathbf{GP} = \frac{\mathrm{N}}{\mathrm{A}} \times 100 \qquad \text{(Eq. 1)}$$

Where GP means percentage of germination, N means total of germinated seeds and A means total of seeds sown to germinate.

$$\mathbf{GVR} = \frac{\mathrm{G1}}{\mathrm{N1}} + \frac{\mathrm{G2}}{\mathrm{N2}} + \frac{\mathrm{G3}}{\mathrm{N3}} + \cdots + \frac{\mathrm{Gn}}{\mathrm{Nn}} \qquad \text{(Eq. 2)}$$

Where GVR means germination velocity rate, G means number of seedlings in each counting, and N means number of days after clay balls release.

$$\mathbf{RGT} = \frac{\sum \frac{Ni}{Ti}}{\sum Ni} \qquad \text{(Eq. 3)}$$

Where RGT means required germination time, Ni means number of seeds germinated in the nth day, and Ti means time (in days).

RESULTS AND DISCUSSION

Germination Percentage (GP), Germination Velocity Rate (GVR) and Required Germination Time (RGT)

Table 3 shows results obtained from the experiment after 37 days. The data indicated a slight variation among the species, whereas they presented, in general, a different behavior because of the conditions induced by the clay ball and/or the external environment. These differences may also be explained by the necessities that each specie has in order to germinate, as said before, because of scarification and/or dormancy break, which any of those were made/required by the technique.

Table 3: Amount (N), Germination Percentage (GP), Germination Velocity Rate (GVR) and Required Germination Time (RGT) for the species in the experiment

SPECIE	N	GP	GVR	RGT
Tabebuia roseoalba (Ridl.) Sandwith	10/15	66.67%	0.90	0.22
Sterculia chicha A. St.-Hil. ex Turpin	12/15	80.00%	1.08	0.26
Tabebuia heptaphylla (Vell.) Toledo	8/15	53.33%	0.72	0.17
Anadenanthera falcata (Benth.) Speg	15/15	100.00%	1.35	0.26
Calophyllum brasiliense Cambess.	12/15	80.00%	1.08	0.26
Guazuma ulmifolia Lam.	4/15	26.67%	0.36	0.18
Hymenaea stigonocarpa Mart. ex Hayne	15/15	100.00%	1.35	0.26
Bowdichia virgilioides Kunth	11/15	73.33%	0.99	0.24
TOTAL	**87/120**	**72.5%**	**-**	**-**

Among the germinated species, only *Calophyllum brasiliense* Cambess and *Hymenaea stigonocarpa* Mart. ex Hayne are classified as secondary, which can indicate that even needing shading to grow within the ecological succession, they distinctly presented satisfactory outcomes when put under the “Nendo Dango” technique. This may have occurred because of the microclimate generated within the clay ball, protecting the seeds on their early stages of development under the local high temperatures, turning to be a positive factor for their fixation in reforesting projects using this method.

The table data also shows that the species presented very similar RGT, i.e., even all the seeds did not have turn into seedlings, their timing to germinated was almost the same. It is noticeable when looking at *Guazuma ulmifolia* Lam and *Tabebuia heptaphylla* (Vell.) Toledo, which had the lowest GP, but, even so, presented a high RGT.

Experiment Yields and Statistical Analysis

As mentioned before, the experiment counted with 3 repetitions. In the first (R1) there was a total of 40 seeds which 33 had germinated, yielding 82.5%. R2 had 40 seeds as well, germinating 30 and yielding 75%. Repetition R3 also had 40 seeds and had germinated 24, yielding 60%. The results from the Nendo Dango technique indicated differences when analyzing the yields of each repetition and of each specie. An example for that is the R3 yield of 60%, when compared to the 100% germination of *Hymenaea stigonocarpa* Mart. ex Hayne, within this same repetition. Table 4 shows the statistical analysis of the data obtained from GP, GVR and RGT. The species *Anadenanthera falcata* (Benth.) Speg. and *Calophyllum brasiliense* Cambess did not presented significant differences on the GP and GVR, and the main hypothesis may be because the results’ similarity and the way that the seeds were disposed next to each other, i.e., under the same external environment conditions.

Table 4: Statistical analysis for GP, GVR and RGT at the end of 37 days*

TREATMENT (T)	GP	GVR	RGT
T1	66.67^{b}	0.90^{b}	0.22^{a}
T2	80.00^{a}	1.08^{a}	0.26^{a}
T3	53.33^{b}	0.72^{b}	0.17^{a}
T4	100.00^{a}	1.35^{a}	0.26^{a}
T5	80.00^{a}	1.08^{a}	0.26^{a}
T6	26.67^{b}	0.36^{b}	0.18^{a}
T7	100.00^{a}	1.35^{a}	0.26^{a}
T8	73.33^{b}	0.99^{b}	0.24^{a}

*Tukey test at 5% probability. Data followed by the same letter did not differ statically among each other.

The other species from the experiment had shown significant differences when analyzed under the parameters of GP and GVR. An explanation for it is the results' difference related to the GP, influenced by the same variables. Overall, the experiment did not show significant differences related to RGT when compared among the species, possibly explained by the similar beginning time for germination, early in the 37 days of experimentation.

According to Santos *et al.* (2004), in a study carried out for *Sterculia chicha* A. St.-Hil. ex Turpin germination analysis, when adopted scarification, the GP results were between 20% and 60%, and, when not scarified, 50%. Silva *et al.* (2015), in a similar study, obtained GP between 20% and 40%. The same authors reported that the seed takes within 20 to 30 days to germinate. With the Nendo Dango technique it took 17 days.

Studies carried out by Oliveira *et al.* (2012) and Sena *et al.* (2017), using the *Anadenanthera falcata* (Benth.) Speg., registered GP varying from 68% to 100%, when treated with vermiculite, sand and humus, without any light, applying temperature around 25º to 30ºC. NCPI - Northern Center of Plants Information (2003) found out the germination time to be within 2 to 33 days. Using Nendo Dango, this specie took 2 days to germinate.

Grabias *et al.* (2013) performed an experiment using *Calophyllum brasiliense* Cambess, sown with vermiculite, obtaining GP between 60.83% and 63.33%. Nery *et al.* (2007) tested seeds of the same specie, scarifying it and putting under thermic conditions of 25 to 30ºC, also using as substratum a Germitest® paper roll wet with distillated water, which has resulted GP between 70% and 88%. Carvalho (1994) says that this specie's germination normally occurs within 6 months, and, for the experiment developed, the Nendo Dango technique presented germination in 17 days.

Figliolia *et al.* (2009) tested *Guazuma ulmifolia* Lam putting the seed under treatment with vermiculite in laboratory and registered GP of 55 to 66%. Barboza *et al.* (2014) says that the GP for this specie, when put under treatment with thermal shocks around 80ºC and 100ºC, can vary from 73% to 76%. Scalon *et al.* (2004) observed in seeds of *Guazuma ulmifolia* Lam GP from 71.7% to 73.3%, when treating it with infusions on boiling water during 5 and 10 minutes.

Nunes *et al*. (2006) verified that when exposing the seed to 70ºC heat and staying still until reaches 50ºC (taking approximately 30 minutes), it presented GP of 66.8%. Barroso *et al.* (1993) and Ramos *et al.* (1998) indicate that this specie takes within 6 to 14 days to germinate, and, when using Nendo Dango, it took 19 days.

In early studies, *Hymenaea stigonocarpa* Mart. ex Hayne presented GP between 7% to 78.3% (Moraes *et al*., 2001), taking between 9 to 60 days to germinate after sown, under conditions of pre-germination treatment, with manual scarification, using emery paper on the opposite side of the germinal axis (Carvalho, 2007). Using Nendo Dango this specie took 13 days to germinate. Other studies using different types of scarification obtained GP results from 37% to 52% in vegetation houses and 52% to 86% in laboratory (Carvalho *et al.*, 2005).

In the experiment of Duarte *et al*. (2010), the germination of *Tabebuia roseoalba* (Ridl.) Sandwith occurred between 8 to 18 days, considering that they were scarified, and presented GP of 40%. Lorenzi (1992) argues differently saying that its dormancy only breaks within 3 months, and, in his study, his experiment's goal was to confront some germination data and verify if, in natural conditions, the GP would occur using the same Duarte *et al.* (2010) applied methodology. In this experiment, using Nendo Dango, the specie took 15 days to germinate.

Lorenzi (1992) also tested the germination of *Tabebuia heptaphylla* (Vell.) Toledo and said that, if scarified, the specie germinates within 10 to 12 days. In his experiment the GP was 60%. Applying Nendo Dango enabled this specie to germinate in 20 days.

For *Bowdichia virgilioides* Kunth, according to Marcos Filho (2005), the main cause of its seed dormancy is the tegument impermeability and hardness. Studies made for this specie's germination presented GP from 2.1% to 11%, when manually or chemically scarified (Schatral & Fox, 1994; Allen & Meyer, 1998). The same authors point out that it takes up 5 to 20 days to germinate, and, in this study, Nendo Dango made it happen within 16 days.

CONCLUSIONS

The Nendo Dango technique, in this study, considering all the taken conditions, well proved its efficiency on germination and pre-establishment of the seeds and seedlings into the deforested area used as experimental field. Comparing to the literature, most of the chosen species for this experiment had similar germination time and percentage. Not specific studies for GVR (germination velocity rate) and RGT (required germination time) were found to compare the results to, which opens up possibilities to expand the literature data about this subject. Speaking of further studies, it's fundamental that improvements must be done to enhance the technique in terms of inserting scarification, breaking of dormancy and/or pre-treatment of the seed before sown at the field, helping it to be even more successful.

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INFLUENCE OF NITROGEN FERTILIZATION LEVELS ON GRAIN YIELD AND ITS COMPONENTS IN BARLEY (*Hordeum vulgare* L.)

SUMMARY

Grain yield and its components are very important and complicated in barley and highly have been influenced by agronomic applications and environmental factors. On the other hand; this is depend on combination varied plant traits (agronomic and quality). For this season, the study was designed to evaluate the effects of different nitrogen fertilization levels (0, 10, 20, 30 and 40 kg ha^{-1} of N) on the agronomic performance of five barley cultivars in two growing seasons. Split plot layout within randomized complete block design with 3 replications was used in both years. The response to fertilization levels were evaluated through GGE (Genotype main effects and Genotype x Environment interaction) biplot graphic methodologies and regression. Combined analysis of variance of nitrogen applications of five cultivars showed highly significant ($p<0.01$) difference between the cultivars, nitrogen applications and interaction. There were genetic variability among cultivars on grain yield and yield components in response to nitrogen fertilization. The results showed that biggest increases on yield and yield components were observed under 40 kg/ha^{-1} nitrogen fertilization level, while thousand grain weight was the biggest under without nitrogen application. The higher performance of yield and yield components was associated with higher nitrogen fertilization in regression analysis. The results of the this study has been recommended that it should be use and study higher nitrogen application levels than 40 kg ha^{-1} of N in the next barley studied.

Keywords: Nitrogen application, yield components, Barley, GGE biplot; regression

INTRODUCTION

Barley (*Hordeum vulgare* L.) is the major cereal in many dry areas of the world and is vital for the livelihoods of many farmers (Alazmani, 2015). It is the second important cereal crop of Turkey and accounts for about 25% of the total cereal production. In East Anatolia, barley has been cultivated for many years and has a significant role for livelihoods of majority farms. It is also grown mainly on rainfall conditions, but some application restricts the progress of yield improvement under rain fed and unpredictable climatic conditions during growing seasons (Kilic 2014). Therefore, experimental research needs to be

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carried out over multiple environment trials with different applications in order to identify and analyses the major factors that are responsible for genotype yields (Kendal et al., 2016). Nitrogen is needed for early tiller development of barley to set up the crop for a high yield potential. On the other hand; nitrogen fertilization has an important effect on the final harvest, thus if this element is not take from plant, yield is decreases (Mareno et al., 2003). The amount of nitrogen, barley crop needs to reach maximize yield and quality, will depend on the seasonal conditions, soil type, and rotational history of the soil as well as the potential yield of the cultivars (Alazmani, 2015).

Nitrogen is the key nutrient input for achieving higher yield of barley. Barley is very sensitive to insufficient nitrogen and very responsive to nitrogen fertilization. On the other hand, excessive use of nitrogen in barley causes lush succulent growth, more lodging, low thousand grain weight, low spike, delayed maturity and greater susceptibility to diseases and pests (Alam, 2007). Nitrogen application at proper dose is the most important for increasing crop production. The farmers have not enough information about use nitrogen fertilizers and adequate information concerning actual soil requirements. Therefore, the study of use N dozes in barley cultivars is necessary to recommend optimum nitrogen doses for high yield and quality in different environment conditions.

The yield of each variety in any environment is a sum of environment (E) main effect, genotype (G) main effect and genotype by environment interaction (GE or GEI) (Yan et al., 2000; Farshadfar et al 2013; Sayar et al., 2013). On the other hand; farmers need varieties that show high performance in terms of yield and other essential agronomic Traits by use nitrogen fertilizer. Modern barley breeding is largely directed towards the development of genotypes characterized with increased yield potential, wide adaptation and high responses to agronomic inputs (Przuli et al 2014). Some agronomic and technological traits such as lodging (LOG), plant height (PH), thousand-kernel weight (TKW), hectoliter mass (HM) and grain protein content (GPC) have significant influence on barley grain yield and quality.

Different statistical analysis, such as correlation, path coefficient and principal component analysis (PCA) can be used to reveal associations between yield and other agronomic traits. The impact of GGE Biplot methods and regression analysis has been clearly showed by different researchers using relationship among factors. This methods; provide the correlative size and interaction (Asfaw et al 2009; Sayar and Han, 2015; Kendal and Sayar 2016). So it is very important to identify the use of nitrogen fertilization doses to cultivars for high yield and best quality. The major objective of study reveal effect of nitrogen fertilization doses using GGE Biplot and regression analysis to recommend doses for application in farm areas.

MATERIAL AND METHODS

The experiment was conducted in the research field of the Department Faculty of Agricultural, University of 100. Van, Turkey. The experiment was conducted on the basis of split plot layout with completely randomized block design with 3 replications. Main plot was different level of nitrogen fertilizer (0, 10, 20, 30 and 40 kg ha^{-1} of N and sub plot was different five barley cultivars (Table 1).

Table 1. The information's about cultivars, used in experiment.

Number	Cultivar name	Spike type
1	Tokak 157/37	2 rows
2	Tarm-92	6 rows
3	Çetin-2000	6 rows
4	H-47	2 rows
5	Bülbül-89	2 rows

This research was conducted in 2001-2002 and 2002-2003 growing seasons. The seeding rates were 500 seeds m^{-2}. Plot size was 7.2 m^{-2} (1.2 × 6 m) consisting of 6 rows spaced 20 cm apart. Sowings were made by using an experimental drill. The fertilization rates for all plots were different N ha^{-1} doses and 60 kg P ha^{-1} with sowing time and different N ha-1 doses was applied to plots in double ridge stage. Harvests were made using Hege 140 harvester in 6 m^2. Other normal agronomic practices for barley production were followed. During both of growing seasons, heading time (date), spike per square(m^2), plant height(cm), length of spike (cm) , number of grains per spike, thousand grain weight(g), harvest index (%), biological yield(kg/ha^{-1}) and grain yield (kg/ha^{-1}) were examined(Kendal, 2016). The Soil analysis results was shown in Table 2 and the climate data of growing seasons were shown in Fig. 1 and Fig. 2 (Van Regional Directorate of Meteorology).

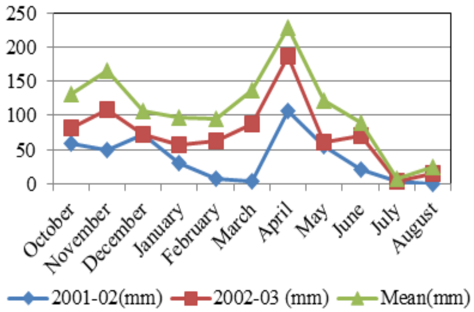

Fig. 1. The precipitation of 2001-02, 2002-03and mean of years(mm).

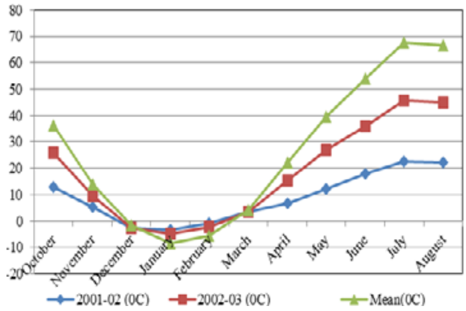

Fig. 2.The temperature of 2001-02, 2002-03 and mean of years(^{o}C).

Table 2. The main soil analysis results were

Depth (cm)	Texturing	pH	Lime (%)	Phosphorus (ppm)	Total N (me/100g)	Organic Subs. (%)	Total Salt (%)
0-20	Sandy clayey	7.75	18.7	336.2	0.076	1.41	0.091
20-40	Sandy clayey	7.60	19.2	375.4	0.072	1.21	0.080

The data obtained from the study related the investigated grain yield and yield components were analyzed respectively for each year and combined with nitrogen doses by using the JMP 5.0.1 statistical software package (SAS Institute, 2002), and the differences between means were compared using a least significant difference (LSD) test at the 0.05 probability level (Steel and Torrie, 1980). Also regression analysis was done by this program. On the other hand; GT biplot analyses were used to determine the differences among application nitrogen doses and crop characteristics and cultivar crop characteristics in two growing seasons (Dogan et al. 2016; Kilic, 2016). GGE biplot analysis also allows comparison amongst nitrogen doses in terms of their discriminating ability and representativeness. These values can be assessed using the discriminating power of the doses' biplot screen of the GGE biplot (Yan and Thinker, 2006). In a multi-application trial (MAT) for barley, biplot figures were constructed by plotting the first two principal components (PC1 and PC2) derived from subjecting nitrogen and cultivar-centered yield, and yield components data (yield variation due to GGE) to singular value separation (Yan et al., 2000). Also, with the GT biplot analysis graphs in the study: It was aimed at revealing relation among nitrogen doses and examined yield components for growing seasons means (Figs. 3A, 3B, 3C, 3D), and separately.

RESULTS

The combined ANOVA revealed highly significant differences among the years, cultivars and interaction of them for all components ($P < 0.01, 0.05$), the differences among nitrogen doses was highly significant ($P < 0.01, 0.05$) for all components without HI(harvest index), as shown in Table 3.

Table 3 The variance of analysis on grain yield and yield components of barley

Sources	DF	HT (date)	SS (m^2)	PH (cm)	SL (cm)	NGS	TGW (g)	HI (%)	BY (kg/ha^{-1})	GY (kg/ha^{-1})
Year	1	0.01**	129042**	1459.13**	34.92**	414.41**	66.84**	39.14**	2412**	25777.5**
Error 1	20	0.16	154.39	8.68667	0.009	0.867	1.04	0.64352	325.51	60.73
Cultivar	4	658.82**	389663**	492.55**	17.20**	1234.47**	194.54**	51.89**	109335**	206732**
Year* Cult.	4	34.28**	25460.4**	42.58*	0.80**	22.80**	4.29*	72.00**	20778.4**	2176.43**
Nitr. Doses	4	1.89**	10826**	392.88**	0.40**	18.61**	16.36**	1.29ns	93102.6**	15708.8**
Year*N.Dos.	4	0.35ns	92.57ns	8.6314ns	0.05**	1.49ns	0.43ns	0.92ns	973.26ns	40.88ns
Cul.*N.Dos	16	0.25ns	261.48ns	9.13ns	0.09**	1.40*	4.81**	6.70**	2474.81**	248.53**
Y*C*ND	16	0.18*	373.34*	8.70ns	0.01ns	0.36ns	2.75*	2.78**	1776.61**	69.09ns
Error 2	79	0.08	190.3	7.87	0.008	0.7678	1.06	0.52	491.2	76.6
C. Total	148									
CV(%)		20.8	3.32	4.12	1.32	3.33	2.56	1.82	2.93	2.92

HT:Heading Time, SS:Spike of per Square, PH:Plant Height, LS:Length of Spike, NGS:Number of grains per Spike, TGW:Thousand Grain Weight, HI:Harvest Index, BY:Biological Yield, GY: Grain Yield.

Moreover, the nitrogen doses × year's interaction (NYI) was only found to be highly significant ($P < 0.01$) for LS(length of spike), the nitrogen doses × cultivar's interaction were found to be highly significant($P < 0.01$) for LS, TGW, HI, BY and GY, while it was found significant ($P < 0.05$) for NGS. On the other hand, the nitrogen doses, years and cultivar's interaction were found to be highly significant ($P < 0.01$) for HI, BY and GY, while it was found significant ($P < 0.05$) for HT and SS. Generally, breeders interested in the genotypes with high genotypic main effect (average over years and nitrogen doses) and with low fluctuation in yield or yield components (stable). The results combined analysis of regression showed that the values of grain yield and yield component were high (positive) depend on application nitrogen doses, while it was low in TGW. The best results were obtained from the highest nitrogen dosing (N4-40 kg/ha-1) for all components, except TGW (Figs.-3A-3L).

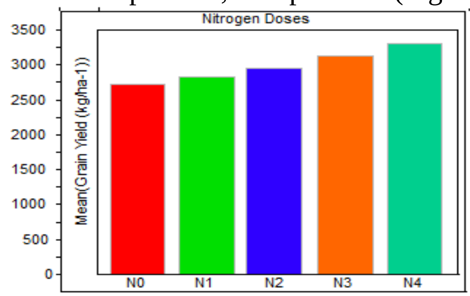

Fig. 3A. Regression analysis nitrogen doses of grain yield

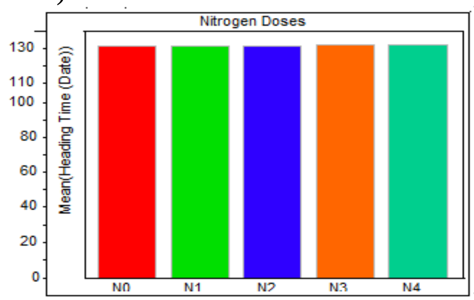

Fig. 3B. Regression analysis nitrogen doses of heading time

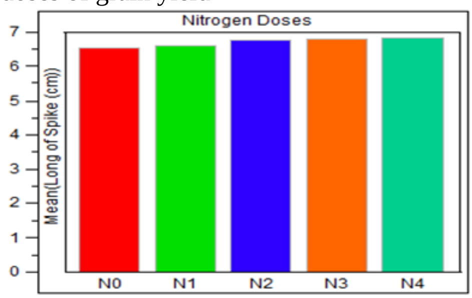

Fig. 3C. Regression analysis nitrogen doses of spike lenght

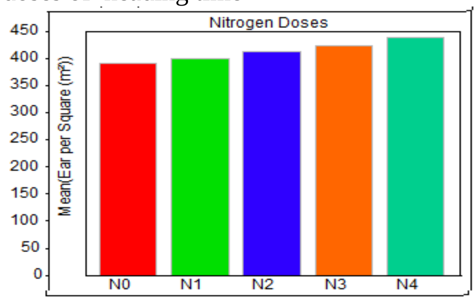

Fig. 3D. Regression analysis nitrogen doses of spike per squar

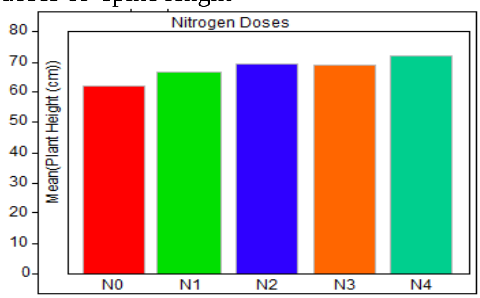

Fig. 3E. Regression analysis nitrogen doses of plant height

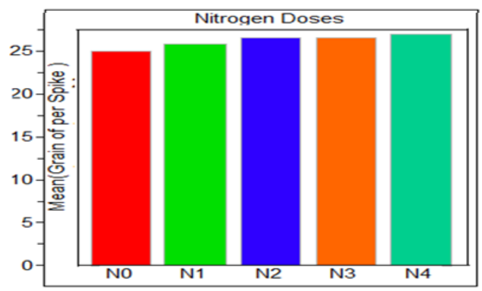

Fig. 3F. Regression analysis nitrogen doses of grain of per spike

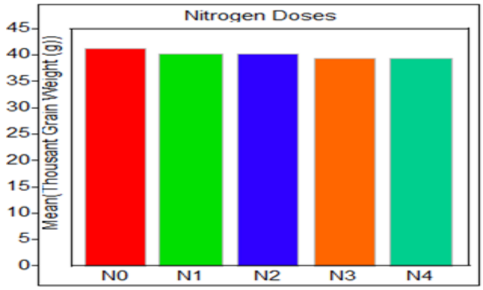

Fig. 3G. Regression analysis nitrogen doses of thausand grain weight

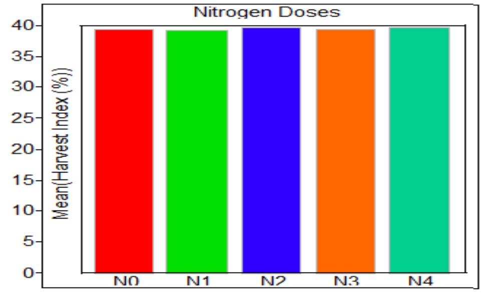

Fig. 3K. Regression analysis nitrogen doses of harvest index

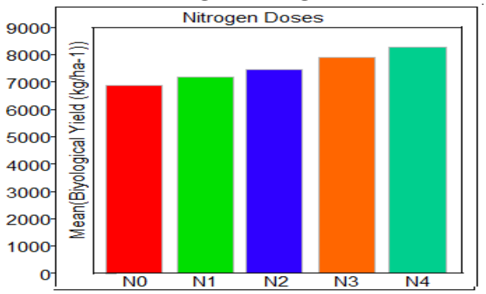

Fig. 3L. Regression analysis nitrogen doses of biological yield.

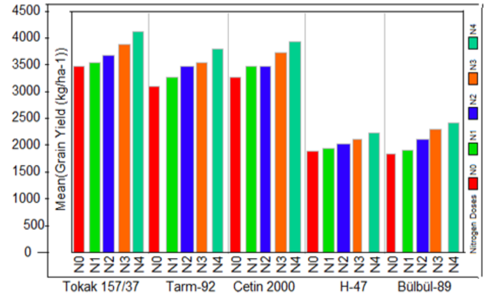

Fig.3M. Regression analysis of cultivar and nitrogen doses

The results of the data reviewed

The mean yield of 2001/02 growing season (3127 kg ha^{-1}) was high than 2002/03(2864 kg ha^{-1}), the mean yield of growing seasons was changed from 1953 kg ha^{-1} to 3952 kg ha^{-1}, and the best yield was obtained from Tokak 157/37 (3557 kg ha^{-1}(Table 4). The mean grain yield of both years was ranged from 2050 kg ha^{-1} to 3754 kg ha^{-1}, and the best yield was obtained from Tokak 157/37 (3557 kg ha^{-}1), while the low yield from H-47(2050 kg ha^{-1}). The yield of application nitrogen doses were ranged from 2726 kg ha^{-1}(N0) - 3310 kg ha^{-1}(N4)(Table 5). The yield of cultivar and nitrogen interaction were changed from 1855 kg ha^{-1} to 4120 kg ha^{-1} and the best yield was obtained by N4 nitrogen doses Tokak 157/37, while the low yield obtained from N0 (without nitrogen) doses and Bülbül-89 variety. The results of grain yield showed that first growing season had high yield than the second and the Tokak-157/37 variety was the best yielding during two grooving seasons. On the other hand; the grain yield was high in N4 nitrogen doses for all varieties and the yield increased in parallel with the dose increase for all varieties (Table 4 and Table 5).

The mean heading time of both growing season was the same (132 date), the mean heading time cultivars of both years was changed from 125 to 138 date, and the long duration was obtained from Tokak 157/37 (138 date) in Table 4. The mean heading time cultivar and year interaction of both years was ranged from 125 to 138 date, and the long duration of heading time was obtained from Tokak 157/37 (138 date) in 2001/02 season, while the low duration of heading time obtained from H-47 (125 date) variety. The heading time of application nitrogen doses were ranged from 132 date (N0) - 133 date (N4) in Table 5. The heading time of cultivar and nitrogen interaction of both year means were changed from 126 to 138 dates and the long duration was obtained by N4 nitrogen doses from Tokak 157/37, while the low duration obtained from N1 and N2 doses by Bülbül-89 variety. The results of heading time indicated that first growing season had long duration than the second as statistical analysis and the Tokak-157/37 variety had long duration during two grooving seasons. On the other hand; the duration was long in N4 nitrogen doses for all varieties and the heading time increased in parallel with the dose increase for all varieties (Table 4 and Table 5).

The mean ear per square of 2001/02 growing season (444 m^2) was high than 2002/03(383 m^2), the mean ear per square of cultivars of both years was changed from 250 to 591 m^2, and the high ears were obtained from Tokak 157/37(591 m^2) and 2001/02 season in Table 4. The application nitrogen doses of ear per square were ranged from 391 date (N0) - 440 date (N4) in Table 5. The ear per square of cultivar and nitrogen interaction of both year means were changed from 286 to 518 m^2.The study results revealed that first growing season had high values of ear per square than the second years as statistical analysis and the Tokak-157/37 variety had haigh ear per square during two grooving seasons. On the other hand; ear per square was high in N4 nitrogen doses for all varieties and the ear per square increased in parallel with the dose increase for all varieties (Table 4 and Table 5).

The mean plant height of 2001/02 growing season (71.2 cm) was high than 2002/03(64.9 cm), the mean plant height of cultivars of both years was changed from 61.1 to 76.7 cm, and the high plant height were obtained from Tokak 157/37 (76.7 cm) and 2001/02 season in Table 4. The application nitrogen doses of plant height were ranged from 62.4 cm (N0) to72.1 cm (N4) in Table 5. The plant height of cultivar of both year means were changed from 62.7 to 74.2 cm.The study results revealed that first growing season had high plant height than the second years as statistical analysis and the Tokak-157/37 variety had haigh plant height during two grooving seasons. On the other hand; plant height was high in N4 nitrogen doses for all varieties and the plant height increased in parallel with the dose increase for all varieties (Table 4 and Table 5).

The mean long ear of 2001/02 growing season (8.9 cm) was high than 2002/03(7.2 cm), the mean long ear of cultivars of both years was changed from 5.5 to 8.3 cm, and the high long ear was obtained from Tokak 157/37 (8.3 cm) and 2001/02 season in Table 4. The long of ears of application nitrogen doses and years interaction were ranged from 6.2 to 7.4 cm, and the best long of ear obtained from N4(7.4 cm) doses in 2001/02 season. The application nitrogen doses of long of ears were ranged from 6.6 cm (N0) to 6.9 cm (N4) in Table 5. The long ear of nitrogen doses and cultivar interaction of both year means were changed from (7.2 to 8.4 cm. The best long ear was obtained from Tokak 157/37 cultivar with (N4) doses. The study results revealed that first growing season had high long ear than the second years as statistical analysis and the Tokak-157/37 variety had haigh long ear during two grooving seasons. On the other hand; long ear was high in N4 nitrogen doses for all varieties and the long of ear increased in parallel with the dose increase for all varieties (Table 4 and Table 5).

The mean number of grain per spike of 2001/02 growing season (27.9) was high than 2002/03(24.6), the mean number of grain per spike of cultivars of both years was changed from 21.3 to 40.4, and the high number of grain per spike was obtained from Çetin-2000 (40.4) and 2001/02 season in Table 4. The application nitrogen doses of number of grain per spike were ranged from 25.0 (N0) to 27.0 (N4) in Table 5. The number of grain per spike of nitrogen doses and cultivar interaction of both year means were changed from 21.3 to 38.7. The number of grain per spike was obtained from Çetin-2000 cultivar with (N4) doses. Also, the number of grain per spike of among cultivars was ranged from 21.9 to 37.4 and the best grain of spike was obtained from Çetin-2000 cultivar. The study results revealed that first growing season had high number of grain per spike than the second years as statistical analysis and the Çetin-2000 variety had high number of grain per spike during two grooving seasons. On the other hand; number of grain per spike was high in N4 nitrogen doses for all varieties and the number of grain per spike increased in parallel with the dose increase for all varieties (Table 4 and Table 5).

The mean thousand grain weight (TGW) of 2001/02 growing season (40.8 g) was high than 2002/03(39.5 g), the mean thousand grain weight of cultivars of both years was changed from 36.7 g to 45.5 g, and the high thousand grain weight was obtained from Tokak 157/37 (45.5 g) during 2001/02 season in Table 4. The application nitrogen doses of thousand grain weight were ranged from 39.3 g (N4) to 41.2 g (N0) in Table 5. The number of thousand grain weight of nitrogen doses and cultivar interaction of both year means were changed from 36.8 g to 45.3 g, and the thousand grain weight was obtained from Tokak 157737 cultivar with N0 application doses. Also, the thousand grain weight of among cultivars was ranged from 38.8 g to 44.4 g, and the best thousand grain weights were obtained from Tokak 157/37 cultivar. The study results revealed that first growing season had high number of thousand grain weight than the second years as statistical analysis, and the Tokak 157/37 variety had haigh thousand grain weights during two grooving seasons. On the other hand; thousand grain weights was high in N0 nitrogen doses for all varieties, and the thousand grain weight decreased in parallel with the dose increase for all varieties (Table 4 and Table 5). The mean harvest index (HI) of 2001/02 growing season (40.0 %) was high than 2002/03(39.0 %), the mean harvest index of cultivars of both years was changed from 38.9 g to 43.1 g, and the high harvest index was obtained from Çetin-2000 (43.1 g) during 2001/02 season in Table 4. The application nitrogen doses, years and cultivar interaction was changed 38.1%-44.6%, and the best high harvest index was obtained from 2001/02 season in Çetin 2000 variety with N4 nitrogen application doses, while low harvest index from 2002/03 in same variety with N2 application nitrogen doses.

Table 4. Influence of different nitrogen levels on yield and yield components of barley cultivars.

Cultivars	Years											
	2001-2002						2002-2003					
	N0	N1	N2	N3	N4	Mean	N0	N1	N2	N3	N4	Mean
Heading Time (date)												
Tokak 157/37	138^{a}	138^{a}	138^{a}	138^{a}	138^{a}	138^{A}	136^{c}	136^{c}	136^{c}	136^{c}	137^{b}	136^{B}
Tarm-92	133^{g}	133^{g}	133^{g}	134^{f}	134^{f}	134^{E}	135de	136^{c}	136^{c}	136^{c}	13^{6c}	136^{C}
Çetin 2000	135^{d}	135^{d}	135^{d}	135^{d}	135^{d}	135^{D}	134^{ef}	135^{d}	135^{d}	135^{d}	136^{c}	135^{D}
H-47	127^{i}	127^{kl}	128^{jk}	128^{j}	128^{jk}	128^{G}	125^{n}	125^{n}	125^{n}	126^{m}	125^{mn}	125^{I}
Bülbül-89	127^{l}	127^{l}	127^{l}	127^{l}	127^{l}	127^{H}	129^{i}	12^{9i}	129^{i}	129^{i}	130^{h}	129^{F}
Mean of Doses	132^{EF}	132DE	132^{CE}	132^{B}	132^{BC}	132^{A}	132^{F}	132DE	132^{CE}	132^{BD}	133^{A}	132^{B}
Spike per Square (m^2)												
Tokak 157/37	551^{bd}	406o	572^{b}	419no	604^{a}	591^{A}	427mo	604^{a}	447^{lm}	625^{a}	470^{ik}	434^{E}
Tarm-92	510^{fg}	484^{hi}	530^{df}	504g^{h}	542de	540^{B}	529^{df}	552^{bd}	525^{eg}	565^{bc}	545^{ce}	518^{C}
Çetin 2000	437^{ln}	419no	454^{jl}	420no	45^{1kl}	459^{D}	428mo	477^{i}	433^{ln}	475^{ij}	448^{lm}	430^{C}
H-47	291^{tu}	247^{wx}	308^{it}	243^{vw}	330^{pr}	321^{F}	239^{x}	332^{pg}	259^{yx}	346^{p}	262^{vw}	250^{I}
Bülbül-89	305st	263^{vw}	296^{t}	271^{uv}	311^{qt}	310^{G}	294^{t}	307st	319^{qs}	333^{pq}	333^{pq}	296^{H}
Mean of Doses	419	432.2	448	454.1	469	444^{A}	364	371	384	396	412	385^{B}
Plant Height (cm)												
Tokak 157/37	69.3	71.7	80.7	79.7	82.0	76.7^{A}	62.1	74.0	73.3	73.0	76.0	71.7^{BC}
Tarm-92	63.7	68.0	72.3	71.7	73.7	69.9^{C}	59.7	62.0	63.3	65.0	70.0	64.0^{D}
Çetin 2000	68.3	74.3	74.0	73.7	76.3	73.3^{B}	60.0	62.3	65.3	64.7	68.0	64.1^{D}
H-47	59.7	64.7	65.7	64.3	67.3	64.3^{D}	56.0	61.0	62.0	62.7	63.7	61.1^{E}
Bülbül-89	66.3	68.0	75.3	73.7	75.3	71.7^{BC}	59.3	60.7	63.7	66.0	69.0	63.7^{D}
Mean of Doses	65.5	69.3	73.6	72.6	74.9	71.2^{A}	59.4	64.0	65.5	66.3	69.3	64.9^{B}
Spike Lenght (cm)												
Tokak 157/37	8.3	8.4	8.2	8.4	8.4	8.3^{A}	7.2	7.2	7.2	7.2	7.2	7.2^{C}
Tarm-92	6.6	6.7	6.9	6.9	7.1	6.8^{D}	6.0	6.1	6.2	6.2	6.2	6.2^{F}
Çetin 2000	7.7	7.7	8.0	8.0	8.1	7.9^{B}	6.5	6.5	6.5	6.6	6.6	6.5^{E}
H-47	6.0	6.1	6.2	6.1	6.1	6.1^{F}	5.5	5.5	5.6	5.5	5.5	5.5^{H}
Bülbül-89	6.5	6.6	7.0	7.1	7.1	6.9^{D}	5.5	5.5	5.7	6.0	6.2	5.8^{G}
Mean of Doses	7.0^{C}	7.1^{C}	7.3^{B}	7.3^{AB}	7.4^{A}	7.3^{A}	7.2^{F}	6.2^{EF}	6.2^{E}	6.3^{D}	6.3^{D}	6.3^{B}
Number of Grains per Spike												
Tokak 157/37	25.7	26.3	27.0	27.3	27.0	26.7^{C}	23.7	24.3	25.0	25.3	24.7	24.6DE
Tarm-92	24.3	24.7	24.7	26.0	26.0	25.1^{D}	21.3	22.7	23.0	22.7	24.0	22.7^{G}
Çetin 2000	38.0	40.0	41.3	41.3	41.3	40.4^{A}	32.0	34.0	35.3	34.7	36.0	34.4^{B}
H-47	22.0	23.3	23.3	24.0	24.0	23.3^{FG}	20.0	21.3	22.0	20.7	22.7	21.3^{H}
Bülbül-89	24.0	23.7	24.0	24.3	24.0	24.0^{EF}	18.7	19.3	20.0	20.0	20.7	19.7^{I}
Mean of Doses	26.8	27.6	28.1	28.6	28.5	27.9^{A}	23.1	24.3	25.1	24.7	25.6	24.6^{B}
Thousand Grain Weight (g)												
Tokak 157/37	45.7	46.3	46.7	45.0	44.0	45.5^{A}	44.8	44.3	42.7	42.7	42.3	43.4^{B}
Tarm-92	44.0	42.0	41.3	39.7	38.3	41.1^{C}	42.3	37.7	40.7	38.0	39.0	39.5^{D}
Çetin 2000	40.0	40.0	38.7	38.0	39.3	39.2^{DE}	38.3	40.0	39.0	37.7	37.0	38.4^{F}
H-47	38.7	39.3	38.0	38.0	38.7	38.5^{EF}	37.0	36.7	37.0	35.7	37.0	36.7^{G}
Bülbül-89	41.3	38.0	40.3	40.7	38.7	39.8^{D}	40.0	39.3	39.0	40.0	39.0	39.5^{D}
Mean of Doses	41.9	41.1	41.0	40.3	39.8	40.8^{A}	40.5	39.6	39.7	38.8	38.9	39.5^{B}
Harvest Index (%)												
Tokak 157/37	39.9^{gj}	39.03^{io}	38.9^{jp}	39.27^{io}	39^{io}	39.2^{DE}	43.9^{ab}	43.4^{ac}	43^{bc}	39.6^{hn}	39.3^{io}	41.8^{B}
Tarm-92	37.4^{gr}	39.03^{io}	39^{io}	39.53^{in}	39.6^{hn}	38.9^{DE}	39.3^{in}	39.2^{io}	39.7^{gl}	39.8^{gk}	41.1^{ef}	39.9^{C}
Çetin 2000	42.9^{bc}	42.4^{de}	42.7^{bd}	43.07^{bc}	44.6^{a}	43.1^{A}	39.7^{gm}	38.7^{kp}	38.1^{oq}	38.6^{lp}	39.5^{in}	38.9^{E}
H-47	37.8^{pg}	35.87^{s}	35.5st	34.53^{t}	35.3st	35.8^{G}	39.0^{jo}	39.9^{gj}	39.4^{in}	39.1^{io}	40.2^{fi}	39.5^{CD}
Bülbül-89	36.2^{rs}	36.37^{rs}	39.7^{gm}	39.2^{io}	38.5^{nq}	38.0^{F}	38.5^{mq}	38.8^{jp}	40.8^{eg}	41.5^{de}	40.8^{eh}	40.1^{C}
Mean of Doses	38.8	38.54	39.2	39.12	39.4	40.0^{A}	40.1	40	40.2	39.7	40.2	39.0^{B}

Biyological Yield (kg/ha^{-1})												
Tokak 157/37	9305^{ef}	9715^{cd}	10029^{c}	10406^{b}	10096^{a}	10088^{A}	7480^{m}	7722^{m}	8116^{kl}	9365^{df}	10060^{bc}	8549^{D}
Tarm-92	8788^{hj}	8651^{ij}	9248^{ef}	9372^{f}	9808^{c}	9173^{B}	7439^{m}	8180^{k}	8474^{ik}	8555^{ij}	9030^{fh}	8336^{E}
Çetin 2000	8174^{k}	$8809g^{i}$	8697^{ij}	9275^{ef}	9398^{de}	8871^{C}	7760^{lm}	$8377^{j}k$	8613^{ij}	9086^{fg}	9415^{de}	8650^{D}
H-47	5222^{st}	5739^{pr}	6197^{no}	6403^{p}	6506^{n}	6013^{F}	4647^{y}	4622^{v}	4768^{uv}	5257^{st}	5435^{rs}	4946^{I}
Bülbül-89	5246^{st}	5205^{st}	5358^{st}	5904^{op}	6434^{n}	5629^{G}	4673^{uy}	5022^{tu}	5210^{st}	5541^{qs}	5850^{oq}	5259^{H}
Mean of Doses	7347^{E}	7624^{D}	7906^{C}	8272^{B}	8626^{A}	7955^{A}	6400^{H}	6785^{G}	7036^{F}	7561^{D}	7958^{C}	7148^{B}
Grain Yield(kg/ha^{-1})												
Tokak 157/37	3710	3773	3903	4083	4290	3952^{A}	3278	3350	3493	3713	3950	3557^{C}
Tarm-92	3283	3377	3607	3723	3880	3574^{C}	2923	3210	3367	3410	3733	3329^{D}
Çetin 2000	3500	3733	3707	3990	4190	3824^{B}	3077	3237	3283	3510	3717	3365^{D}
H-47	1970	2057	2193	2210	2300	2146^{E}	1810	1843	1877	2053	2183	1953^{F}
Bülbül-89	1900	1893	2123	2313	2473	2141^{E}	1810	1953	2127	2297	2383	2114^{E}
Mean of Doses	2873	2967	3107	3264	3427	3127^{A}	2580	2719	2829	2997	3193	2864^{B}

The harvest index of nitrogen doses and cultivar interaction of both year means were changed from 36.8 % to 42.0 g, and the best harvest index were obtained from Çetin-2000 cultivar with N4 application doses, while the low harvest index obtained from H-47 variety with N2 application doses (Table 5). Also, the harvest index of among cultivars was ranged from 37.7% to 44.4 %, and the best harvest index was obtained from Tokak 157/37 cultivar. The study results revealed that first growing season had high harvest index than the second years as statistical analysis and the Tokak 157/37 variety had haigh harvest index of mean two grooving seasons. On the other hand; the high harvest index was changed depend on nitrogen doses and cultivar for and the harvest index showed fluctuate depend on dose increase for all varieties (Table 4 and Table 5).

The mean biological yield (BY) of 2001/02 growing season (7955 kg ha^{-1}) was high than 2002/03(7148 kg ha^{-1}), the mean biological yield of cultivars of both years was changed from 4946 to 10088 kg ha^{-1}, and the high biological yield was obtained from Tokak 157/37 (10088kg ha^{-1}g) during 2001/02 season in Table 4. The application nitrogen doses, years and cultivar interaction was changed 4647-10406 kg ha^{-1}, and the best biological yield was obtained from 2001/02 season in Tokak 157/37 variety with N3 nitrogen application doses, while low biological yield from 2002/03 in Çetin-2000 variety with N0 application nitrogen doses. The biological yield of nitrogen doses of both year means were changed from 6870 to 8292 kg ha^{-1}, and the best biological yield were obtained from N4 application doses, while the low biological yield obtained from N0 application doses (Table 5). Also, the biological yield of among cultivars was ranged from 5444 to 9318 kg ha^{-1}, and the best biological yield was obtained from Tokak 157/37 cultivar. The study results revealed that first growing season had high biological yield than the second years as statistical analysis, and the Tokak 157/37 variety had high biological yield of mean two grooving seasons. On the other hand; the high biological yield was high in N4 application doses for all cultivars, and the biological yield increased in parallel with dose increase for all varieties (Table 4 and Table 5).

Table 5. The data effect interaction of nitrogen levels and barley cultivars.

Cultivars	Heading Time(date)						Number of Grains per Spike					
	N0	N1	N2	N3	N4	Mean	N0	N1	N2	N3	N4	Mean
Tokak 157/37	137^{b}	137^{b}	137^{b}	137^{b}	138^{a}	137^{A}	26.3^{d}	26.0^{de}	24.7^{fg}	25.3^{df}	25.8^{de}	25.6^{B}
Tarm-92	134^{f}	135^{e}	135^{e}	135^{d}	135^{d}	135^{B}	24.3^{fh}	23.8^{gi}	22.8^{il}	23.7^{gj}	25.0^{ef}	23.9^{C}
Çetin 2000	135^{e}	135^{d}	135^{d}	135^{d}	136^{c}	135^{C}	35.0^{c}	37.0^{b}	38.3^{a}	38.0^{ab}	38.7^{a}	37.4^{A}
H-47	127^{l}	126^{jk}	126^{kl}	127^{i}	127^{j}	128^{D}	22.3^{km}	22.7^{jl}	21.0^{n}	22.3^{km}	23.3^{hk}	22.3^{D}
Bülbül-89	128^{h}	128^{h}	128^{h}	128^{h}	128^{g}	126^{E}	21.3^{mn}	21.5^{mn}	22.0^{ln}	22.2^{lm}	22.3^{km}	21.9^{D}
Mean of Doses	132^{D}	132^{C}	132^{C}	132^{B}	133^{A}		25.0	26.0^{C}	26.6^{B}	26.6^{AB}	27.0^{A}	
	Spike per Square(m^2)						Thousand Grain Weight(g)					
Tokak 157/37	497	517	536	538	555	513^{B}	45.2^{a}	45.3^{a}	44.7^{ab}	43.8^{bc}	43.2^{c}	44.4^{A}
Tarm-92	479	496	516	525	548	529^{A}	43.2^{c}	39.8^{dh}	41.0^{d}	38.8^{gj}	38.7^{hk}	40.3^{B}
Çetin 2000	428	437	440	455	462	444^{C}	39.2^{fi}	40.0^{dg}	38.8^{gj}	37.8^{jl}	38.2^{ik}	38.8^{D}
H-47	269	276	285	295	304	286^{E}	37.8^{jl}	38.0^{il}	37.5^{kl}	36.8^{l}	37.8^{jl}	37.6^{E}
Bülbül-89	284	283	303	313	333	303^{D}	40.7^{de}	38.7^{hk}	39.7^{eh}	40.3^{df}	38.8^{gj}	39.6^{C}
Mean of Doses	391^{E}	402^{D}	416^{C}	425^{B}	440^{A}		41.2^{A}	40.4^{B}	40.3^{B}	39.5^{C}	39.3^{C}	
	Plant Haigh (cm)						Harvest Index(%)					
Tokak 157/37	62.8	64.3	69.5	69.8	72.2	74.2^{A}	41.9^{a}	41.2ab	41bc	39.4ef	39.2fg	41.0^{A}
Tarm-92	64.2	68.3	69.7	69.2	72.2	66.9^{C}	38.3^{gh}	39.1fg	39.4f	39.7df	40.4cd	40.5^{B}
Çetin 2000	57.8	62.8	63.8	63.5	65.5	68.7^{B}	41.3ab	40.5^{bc}	40.4^{cd}	40.8^{bc}	42^{a}	39.4^{C}
H-47	61.7	65.0	67.8	68.3	71.8	62.7^{D}	38.4^{gh}	37.9^{hi}	37.5^{ij}	36.8^{j}	37.8^{hi}	37.7^{D}
Bülbül-89	65.7	72.8	77.0	76.3	79.0	67.7^{BC}	37.4^{ij}	37.6^{hj}	40.3^{ce}	40.4^{cd}	39.6^{df}	39.0^{C}
Mean of Doses	62.4^{D}	66.7^{C}	69.6^{B}	69.4^{B}	72.1^{A}		39.5	39.3	39.7	39.4	39.8	
	Spike Lenght (cm)						Biyological Yield(kg/ha^{-1})					
Tokak 157/37	7.7^{ab}	7.8^{ab}	7.7^{b}	7.8^{ab}	7.8^{a}	7.8^{A}	8393	8719	9073	9886	10523	9318^{A}
Tarm-92	6.3^{g}	6.4^{g}	6.6^{ef}	6.5^{f}	6.7^{e}	6.5^{C}	8114	8416	8861	8964	9419	8755^{B}
Çetin 2000	7.1^{d}	7.1^{d}	7.3^{c}	7.3^{c}	7.3^{c}	7.2^{B}	7967	8593	8655	9181	9407	8761^{B}
H-47	5.8^{k}	5.8^{jk}	5.9^{ij}	5.8^{jk}	5.8^{jk}	5.8^{E}	4935	5181	5482	5830	5970	5480^{C}
Bülbül-89	6.0^{hi}	6.1^{h}	6.4^{g}	6.6^{ef}	6.7^{e}	6.^{3D}	4959	5114	5284	5723	6142	5444^{C}
Mean of Doses	6.6^{D}	6.6^{D}	6.8^{B}	6.8^{C}	6.9^{A}		6870^{E}	7204^{D}	7471^{C}	7916^{B}	8292^{A}	
							Grain Yield(kg/ha^{-1})					
Tokak 157/37							3494^{f}	3562^{f}	3698^{e}	3898^{bc}	4120^{a}	3754^{A}
Tarm-92							3103^{h}	3293^{g}	3487^{f}	3567^{f}	3807^{cd}	3451^{C}
Çetin 2000							3288^{g}	3485^{f}	3495^{f}	3750de	3953^{b}	3594^{B}
H-47							1890^{m}	1950^{lm}	2035^{kl}	2132^{k}	2242^{j}	2050^{E}
Bülbül-89							1855^{m}	1923^{m}	2125^{k}	2305^{j}	2428^{i}	2127^{D}
Mean of Doses							2726^{E}	2843^{D}	2968^{C}	3130^{B}	3310^{A}	

GGE Biplot Analysis

Analysis of variance for nitrogen doses (ND) x component (C), and the cultivar(C) × component (C) interaction showed significant ($P < 0.01$) effect, and the total sum of squares explained for 97.56%, with PC1 and PC2 accounting 90.03% and 7.53% for nitrogen doses (ND) component (C) (Figs. 4A–4D), it was explained for 91.87%, with PC1 and PC2 accounting 77.48% and 14.39% for cultivar(C) × component (C) interaction (Figs. 5A–5D), respectively.

GGE biplot analysis of the means over years for nitrogen doses relationships among yield components (Figs. 4), the relationship between doses-components and components groups (Figs. 4A-4B), ranking of doses on components means (Fig. 4C), and comparison of components means of doses

(Fig. 4D) accounted for 97.56% (90.03% and 7.53%, for principal components [PCs] 1 and 2, respectively) of the total variation.

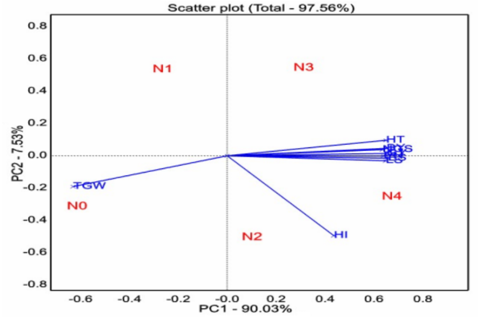

Fig. 4A. Relation among N doses and mean of components

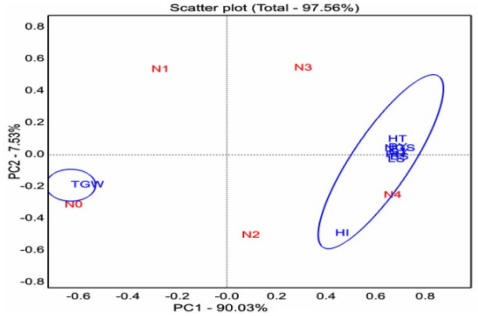

Fig. 4B. The grouping of components over doses

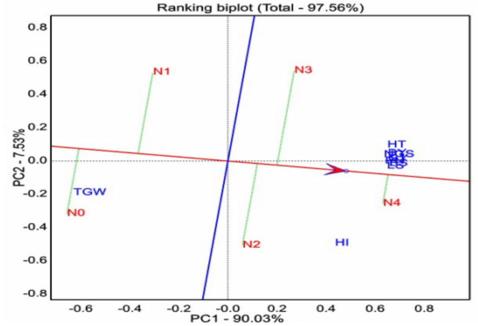

Fig.4C. Ranking of N doses on means of components

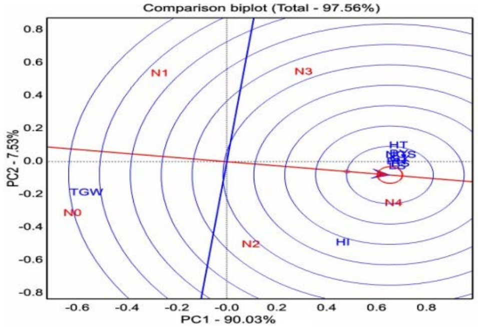

Fig. 4D. Comparison of N doses on means of components

The biplot analysis of the means over years for genotypes relationships among yield components (Figs. 5), the relationship between genotypes-components and components groups (Figs. 5A-5B), ranking of genotypes on components means (Fig. 5C), and comparison of components means of genotypes (Fig. 5D) accounted for 91.87% (77.48% and 14.39%, for principal components [PCs] 1 and 2, respectively) of the total variation.

The relationship between application N doses-components and grouping of components with doses: The nitrogen doses-components vectors and groups illustrate the specific interactions of each dose with each component (Figs. 4A, 4B, 4C, 4D). The biplot showed two groups that were highly correlated in terms of components means (Figs. 4A, 4B). Positive correlations were found among components without TGW, all components took places in(Group 1) and indicated by the acute angles (vector angles < 90°) of their respective vectors, while TGW in (Group 2) by the acute angles (vector angles > 90°). The relationships among doses, with regard to components, were observed. The biplot showed that majority of components means related with N4 application doses, while just N0 application doses related with doses (Figs. 4A, 4B) Thus, the biplot showed excellent discriminating ability in selecting specific component with particular doses and in recommending best application dose for each component.

Ranking and comparison of application nitrogen doses–components: The application dose with both high mean for components over years is called an ideal dose, and should have both high mean performances for all components (Figs. 4C, 4D). The application doses that are closer to the average axis (AEA) and are considered ideal application dose are more desirable than other dose. The ranking and comparison of application doses, based on t means of components over 2 years (Figs. 4C, 4D), showed that N4 was an ideal application dose. Some doses (N2, N3) performed favorably, as they were above the x-axis. On the other hand, some doses (N0, N1) were not desirable, as they were below the x-axis. The ranking and comparison of application doses based on component means of seasons (Figs. 4C, 4D) showed that N4 was ideal application for all components without TGW. The figure of biplot showed the best wives of to see the best application nitrogen doses for all components or each component.

The relationship between genotypes-components and grouping of components with genotypes: The genotypes-components vectors and groups illustrate the specific interactions of each genotype with each component over doses (Figs. 5A, 5B). The biplot showed two groups that were highly correlated in terms of components means (Figs. 5A, 5B). Positive correlations were found among components without NGS, all components took places in (Group 1) and indicated by the acute angles (vector angles < 90°) of their respective vectors, while NGS in (Group 2) by the acute angles (vector angles > 90°). The relationships among genotypes, with regard to components, were observed. The biplot showed that majority of components means related with Tokak 157/37 variety, while just Çetin-2000 related with NGS (Figs. 5A, 5B) Thus, the biplot showed excellent discriminating ability in selecting specific genotype with particular component and in recommending best genotype for all components or each component.

Ranking and comparison of genotypes–components over all doses: The genotype with both high mean for components over years is called an ideal genotype, and should have both high mean performances for all components (Figs. 5C, 5D). The genotypes that are closer to the average axis (AEA) and are considered ideal genotypes are more desirable than other genotype. The ranking and comparison of genotypes, based on means of components over 2 years (Figs. 5C, 5D), showed that Tokak 157/37 was an ideal genotype.

Two genotypes (Tarm 92, Çetin -2000) performed favorably, as they were above the x-axis. On the other hand, two genotypes (H-47 and Bülbül 89) were not desirable, as they were below the x-axis. The ranking and comparison of genotypes based on component means of seasons (Figs. 5C, 4D) showed that Tokak 157/37 was ideal application for all components without NGS. The figure of biplot showed the best wives of to see the best genotype for all components or each component.

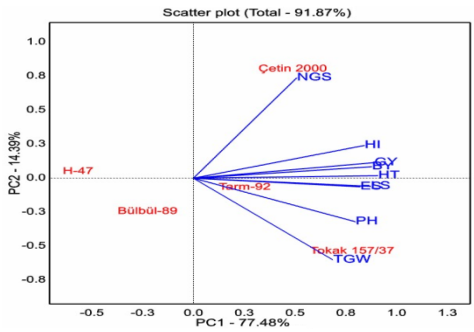

Fig. 5A. Relation among cultivars and mean of components

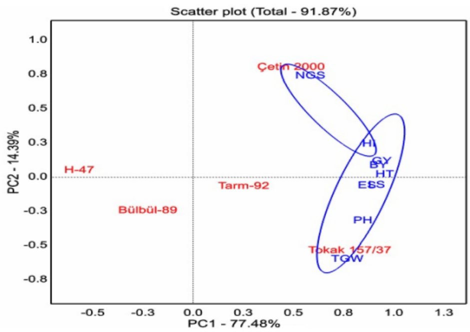

Fig. 5B. The grouping of components over cultivars

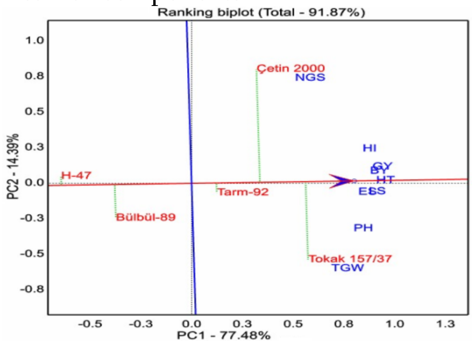

Fig. 5C. The ranking of cultivars over components

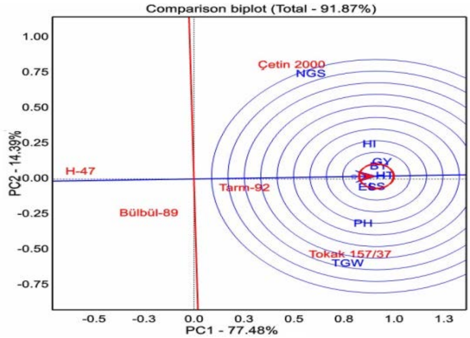

Fig. 5D. The comparison of cultivars over components

The regression analysis of component-application doses over years

The regression analysis showed positive or negative effect of nitrogen doses to genotypes or components in Figs. 3. For this purpose, the regression figures were obtained by analysis. In Fig. 3A-3M, the regression analysis nitrogen doses of grain yield and yield components showed that there was increasing linear performance for grain yield and all yield components in all cultivar as a function of the increase in the N doses. However, thousand grain weights were influenced negatively by application nitrogen doses as a function of decrease in the N doses (Fig. 3G).

DISCUSSION

The significant differences (P<0.001, P<0.005) found of the yield and yield components of the different years indicated the high influence of the year factor(Table 3), therefore the climatic conditions give a lead to high variable outputs in yield and it's all components every year. This results are accepted by Moreno at al. (2003), who proceed that the response of the barley to N fertilizer highly depend on growing seasons variations conditioned by environmental factors (Figs. 1 and 2). Climatic data of both growing season were indicated that the season of 2001/02 had more favorable climate conditions for barley growth, without more cold in winter and good rainfall in planting time (October, November) for early germination and for grain filling time occurs (April, May). On the other hand, the high temperature of grain filling time of 2002/03 season had negative affect to grain yield and it is components. The bad environmental conditions of grain filling occurs time is cause to reduce the grain yield and it's components. This is supported by Wallvork et al. (1998), who point that high temperatures have a strong effect on the structure of the mature barley grain and reducing its final weight.

According the results of the study, the application of nitrogen doses had positive effect on yield and yield components. The results increased in parallel with dose increase in all cultivars for all characters without TGW. The optimum N fertilizer doses to maximize barley yield and yield components are agreement with different studies (Moreno at al. 2003, Fallahi at al., 2008). There have been different studies described the positive effect of nitrogen doses on yield and yield components (Moselhy and Zahran, 2002; Alazmani, 2015). Nitrogen application had positive influence on all the yield components (Fallahi et al. 2008).

Heading time (HT) was affected by different N levels, years, cultivars and interactions. In the present study, the HT increased by increasing nitrogen doses and different climatic data of both years and all cultivars (Table 3, 4 and 5). The high rainfal and low temperature of HT of 2001/02 season, attributed to increasing HT, and so it was attributed positive effect on yield indirect, so the yield of N4 application doses was high than N0 doses. It was stated by Hadi et al. (2012), increase in number of time to spike by increasing N rate and it might be attributed to the increase in long time filling grain (Gürsoy, 2011;Shafi et al., 2011). Among the cultivars, Tokak 157/37 produced the latest HT, followed by Tarm 92. It can say that the HT of cultivars is more depend on genetic of cultivars.

Spike per square m^{-2} (SS) revealed that this component is significant affected by different N levels, years, cultivars and interactions. The study results showed that SS increased with parallel by increasing nitrogen doses and different climatic data of both years and genetic of cultivars (Table 3, 4 and 5). The high rainfal and low temperature of spike occurs period time in 2001/02 season, attributed to increasing ES, so the SS of N4 application doses was high than N0 doses. On the other hand, SS of 2001/02 was higher than 2002/03 depend on different climatic conditions. It was stated by Hadi et al. (2012), increase SS m^{-2} by increasing N rate and it might be attributed to increase the time of number spike. Among the cultivars, Tarm-92 produced the best SS followed by Tokak 157/37. It can say that the SS of cultivars is more depend on genetic of cultivars. So the six rows cultivars had high ES that of two rows (Table1)

Plant height (PH) was an important morphological character directly linked with the productive potential of plant in terms of grain yield. In the present investigation, PH increased by increasing nitrogen doses, different climatic conditions of both year and cultivars (Table 3, 4 and 5). The high rainfall of growing season of 2001/02 contributed positive effect PH, so the plant high of 2001/02 growing season was high than 2002/03. Similar results were reported in barley by Alazmani (2015) and Podsiadlo et al. (1999), in wheat. As stated by Hadi et al. (2012), increase in PH by increasing N rate might be attributed to the increase in vegetative of plant. Among the cultivars, Tokak 157/37 produced the tallest plant followed by Çetin 2000. The results of a study showed that increase in PH by increasing N rate might be attributed to the increase in internodes length and vegetative of plant since the number of internodes is greatly influenced by the genetic makeup of the plant (Subhan et al. 2004).

Spike length (SL) was affected by different N levels, years, cultivars and N x C interactions. In the present study, the LE increased by increasing nitrogen doses and different climatic data of both years and all cultivars (Table 3, 4 and 5). The high rainfall and low temperature of 2001/02 season, attributed to increasing SL, and so it was attributed positive effect on yield direct, so the yield of N4 application doses was high than N0 doses. The results of SL showed that increase in SL by increasing N rate and available environmental condition of growing season. Similar results were reported in barley by Gürsoy (2011) and Shafi et al. (2011), in wheat On the other hand; among the cultivars, Tokak 157/37 produced the longest SL, followed by Çetin-2000. It can say that the SL of cultivars is more depend on genetic of cultivars.

Number of grains per spike (NGS) indicated that this component is significant affected by different N levels, years, cultivars and N x C interactions. The study results showed that NGS increased with parallel by increasing nitrogen doses and different climatic data of both years and genetic of cultivars (Table 3, 4 and 5). The high rainfal and low temperature of grains occurs period time of 2001/02 season, contributed to increasing NGS, so the NGS of N4 application doses was high than control and other application doses. On the other hand, NGS of 2001/02 was haigh than 2002/03 depend on different climatic conditions (Figs.1 and 2). It was stated by Subhan et al. (2004) and Shafi et al. (2011), increase NGS by increasing N rate and it might be attributed to increase the time grain occurs. The results showed that among the cultivars, Çetin-2000 produced the best NGS followed by Tokak 157/37. It can say that the NGS of cultivars is more depend on genetic of cultivars. So, the six rows cultivars had high NGS that of two rows (Table1).

Thousand grain weight (TGW) showed that this quality parameter is significant affected by different N levels, years, cultivars and N x C interactions. The results of study showed that TGW decreased with parallel by increasing nitrogen doses and different climatic data of both years and genetic of cultivars (Table 3, 4 and 5). The high rainfall and low temperature of grain filling period of 2001/02 season, contributed to decreasing TGW, so the TGW of N0 control doses was high than N application doses. On the other hand, TGW of 2002/03 was higher than 2001/02 depend on different climatic conditions (Figs.1 and 2). It was stated by Hadi et al. (2012) and Yesmin at al. (2014), increasing N rate and it might be attributed to increase the grains per spike, this is decreasing TGW. The results showed that among the cultivars, Tokak 157/37 produced the best TGW followed by Tarm 92. It can say that the TGW of cultivars is more depend on genetic of cultivars, and similar comments stated by different studies (Gürsoy, 2011;Kizilgeci at al., 2016).

Harvest index (HI) demonstrated that this parameter is significant affected by years, cultivars and N x C interaction and Y x N x C triple interactions. The results of study showed that HI decreased with different climatic data of both years and genetic of cultivars (Table 3, 4 and 5). The high rainfal and low temperature of period of 2001/02 season, contributed to increasing HI, so the HI of 2001/02 season was high than 2002/03(Figs.1 and 2). HI of 2002/03 was haigh than 2001/02 depends on different climatic conditions. It was stated by Alam et al. (2007) and Yesmin et al. (2014), increasing N rate and it might be attributed to increase the biological and grain yield, and this is increasing or decreasing HI in different growing season. On the other hand, The results showed that Çetin-2000 produced the best HI followed by Tokak 157/37. It can say that the HI of cultivars is more depend on genetic of cultivars and different of climatic condition. So, the six rows cultivar had high HI in 2001/02, while two rows barley variety had high HI in 2002/03 (Table1, 4, 5).

Biological yield (BY) indicated that this component is significant affected by N application doses, years, cultivars and Y x N x C triple interactions. The results of study showed that BY increased with N application doses in parallel and different climatic data of both years and genetic of cultivars (Table 3, 4 and 5). The high rainfal and low temperature of period of 2001/02 season, contributed to increasing BY, so the BY of 2001/02 season was high than 2002/03(Figs.1 and 2). Moreover, BY of N4 application doses was haigh than control (N0) and other application doses. It was stated by Gürsoy (2011) and Hadi et al. (2012), increasing N rate and it might be attributed to increase the grains per spike and plant height, and this is increasing the BY. On the other hand, The results showed that Tokak 157/37 produced the best BY followed by Çetin-2000 and Tarm-92 cultivars. It can say that the BY of cultivars is more depend on genetic of cultivars and different of climatic condition. So, the two rows cultivar had high BY in 2001/02, while six rows barley variety had high BY in 2002/03 (Table1, 4, 5).

Grain yield (GY) demonstrated that it is significant affected by N application doses, years, cultivars and N x C interaction. The results of study showed that GY increased with N application doses in parallel and different climatic data of both years and genetic of cultivars (Table 3, 4 and 5). The high rainfall and low temperature of period of grain occurs time in 2001/02 season, contributed to increasing GY, so the GY of 2001/02 season was high than 2002/03(Figs.1 and 2). Moreover, GY of N4 application doses was haigh than control (N0) and other application doses. It was stated by Subhan et al. (2004), Fallahi et al. (2008), Yesmin et al. (2014) and Alazmani (2015), Yildirim et al. (2016), increasing N rate and it might be attributed to increase the grains per spike, and this is increasing GY. On the other hand, The results showed that Tokak 157/37 produced the best GY followed by Çetin-2000 and Tarm-92 cultivars in 2001/02. It can say that the GY of cultivars is more depend on genetic of cultivars. So, the two rows cultivar had high GY both of season (Table1, 4, 5).

GGE Biplot Analysis

The GGE biplot method has been widely used to analyze the stability and performance of the genotypes for yield and other components (Yan and Rajcan, 2002; Goyal et al., 2011; Sabaghnia and Janmohammadi, 2014). The GGE biplot mainly allows the visualization of any crossover GE interaction, which is very important for the breeding program (Güngör and Akgöl, 2015; Sayar and Han, 2015; Kendal et al., 2016;). The GGE biplot method provides considerable flexibility, allowing plant breeders to simultaneously select for yield and stability (Rubio et al., 2004; Kilic et al., 2016). Moreover, GEI and yield stability analyses are important for their consideration of both varietal stability and suitability for cultivation across seasons and ecological circumstances (Adjabi et al., 2014). The GT (genotype-trait) biplot provides an excellent tool for visualizing genotype × trait data (Adjabi et al., 2014).

The GGE biplot could be used to interpret the relationships among nitrogen doses, components, and groups of component (Figs. 4). An understanding of the relationship between doses and components can aid in better understanding doses objectives and in identifying components that are positively or negatively correlated with nitrogen doses. This understanding can also aid in identifying components that can be indirectly selected by selecting for correlated components. It also helps to visualize the strengths and weaknesses of nitrogen doses, which is important for application in barley. If the angle of the vector was less than 90°, there was a positive correlation two observation factors. If the angle was equal to 90°, they were not correlated. There was a negative correlation if the angle was less than 90° (Yan and Thinker, 2006; Sabaghnia, 2015). The results of study showed that there is high positive correlation among large components and N application doses; while negative correlation with TGW in barley (Fig. 4A and Fig. 4B).The results of component was increase depend on application N nitrogen doses, while TGW was decrease (Table 5).Therefore, all components took place in a first group, except TGW in second. There is high correlation which is took places in same group (Kendal et al., 2016).On the other hand; the GGE biplot was accurate in interpreting the ranking and comparing genotypes and traits (Fig. 4C and Fig. 4D). The doses with both high mean performance and high stability for all of the components were called an ideal dose. The center of the concentric circles (i.e., ideal dose) was the AEA in the positive direction. Doses located closer to the ideal dose were more desirable than others (Yan &Tinker, 2006; Dogan et al., 2016). The result demonstrated that N4 was ideal application dose in the both season, as it was in the center circle for the ideal doses and on the AEA (Fig. 4C). N4 dose was the ideal dose for means over the two seasons, as it was closer to the ideal dose center on the AEA (Fig. 4D).

The GGE biplot could be used to interpret the relationships among cultivars, components, and groups of component (Figs. 4). An understanding of the relationship between cultivar and components can aid in better understanding cultivar objectives and in identifying components that are positively or negatively correlated with cultivars and each other. This understanding can also aid in identifying components that can be indirectly selected by selecting for correlated cultivars. It also helps to visualize the strengths and weaknesses of cultivars, which is important for selection in different environmental seasons. If the angle of the vector was less than 90°, there was a positive correlation two observation factors. If the angle was equal to 90°, they were not correlated. There was a negative correlation if the angle was less than 90° (Yan & Thinker, 2006). The results of study showed that there is high positive correlation among large components and large variation among cultivars (Fig. 5A and Fig. 5B).The results of cultivars was varied depend on genetic factor (Table 5). Therefore, all components took place in a first group with Tokak 157/37, Çetin-2000 with NGS in second. There is high correlation among components and cultivars which are took places in same group (Kendal et al., 2016).On the other hand; the GGE biplot was accurate in interpreting the ranking and comparing genotypes and traits (Fig. 5C and Fig. 5D). The doses with both high mean performance and high stability for all of the components were called an ideal cultivar. The center of the concentric circles (i.e., ideal cultivar) was the AEA in the positive direction. Cultivars located closer to the ideal cultivar were more desirable than others (Yan et al., 2000; Dogan et al., 2016). The result demonstrated that Tokak 157/37 was ideal cultivar in the both season for more components, as it was in the center circle for the ideal cultivar and on the AEA (Fig. 5C). Tokak 157737 was the ideal cultivar for means over the two seasons, as it was closer to the ideal cultivar center on the AEA (Fig. 5D).

CONCLUSIONS

The nitrogen application doses had positive effect on grain yield and yield components except thousand grain weight. Therefore, the values of component and grain yield increased in parallel with application nitrogen doses, while decreased in thousand grain weight. The nitrogen dose of N4 (40 kg ha^{-1}) are responsible for the maximum productivity of barley crop in Van environmental conditions. Tokak157/37 showed that it is best cultivar for more components and grain yield except TGW. On the other hand; GGE biplot analysis revealed that this analysis provided useful results and high image quality to show the correlation among doses, cultivars and components. The results of study recommended that the N application of doses in barley should be increased in next dose application studies to see the quadratic results of doses.

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EXAMINATION AND ANALYSIS OF YIELD, QUALITY AND ECONOMIC EFFECT WITHIN VARIETES OF BURLEY TOBACCO

SUMMARY

The purpose of these researches and analysis is to compare of some quantitative, qualitative and economic indicators between some standard foreign varieties with newly created varieties tobacco in Scientific Tobacco Institute – Prilep, R. Macedonia. During 2016 and 2017 investigations with 6 Burley tobacco varieties were made to study their influence on yield and quality of the obtained raw material, and to evaluate their economic effects. The results of investigations showed absolute dominance of hybrids B-204/15 and B-206A/16 over the other varieties, which was statistically confirmed. From a practical point of view, these results can be a good guideline to tobacco growers in selection of tobacco variety.

Key words: tobacco, variety, Burley, yields, economic effects

INTRODUCTION

Raw material of Burley and Virginia tobaccos participate in the composition of blend cigarettes with about 80%. The first steps towards introducing the type Burley in the Republic of Macedonia were made by Rudolf Gornik, who reported that this tobacco can be successfully cultivated only in rich soils and humid climate with frequent rainfalls (Gornik, 1953). During this timeframe, in former Yugoslavia examination of possibility for production of this type of tobacco, was conducted on the areas of Monte Negro as well, particularly in Bar area (Jovovič, 1957). In early 70ies efforts were made towards creating a variety which will prove to be the best in most of the properties, especially in yield and quality. In that period, the main representative of this type of tobacco in the Republic of Macedonia was the Croatian variety Chulinec. Later hybrids varieties: Burley B-96/85, Burley 1, B-2/93 and Pelagonec were created in Tobacco Institute - Prilep. These varieties were a satisfactory substitute for the variety Chulinec, and some of them found their way beyond the borders of Macedonia. The fact that there is no ideal variety created once and for all, but that some variety at a given moment is better than the others, motivated the breeders of Tobacco Institute - Prilep to create new varieties (genotypes) with

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improved characters, i.e. with higher yields and quality. Since these characters are governed by the genetic structure, parents in which these characters are predominant are used in the process of hybridization. This process is exclusively intervarietal and is conducted with the aim to obtain male sterile hybrid varieties. The best of them are tested in field, in comparative trials with other standard varieties (domestic and foreign) for a period of at least two years. If they show better results than the standard, they are submitted to the State Variety Commission for recognition. This paper will present the results of investigations on yield and quality of the raw material obtained from the varieties represented in the research.

MATERIAL AND METHODS

The investigations were carried out in the Experimental field of Tobacco Institute - Prilep during 2016 and 2017, on colluvial - alluvial soil. It included three introduced fertile varieties of Burley tobacco (Kentucky 12 from USA, L-8 from Zimbabwe and B-963 and B-1246 from Bulgaria), the hybrids varieties B-204/15 and B-206A/16, (Flowers of these hybrids are male sterile.) The variety L-8 was used as a check. Autumn ploughing was carried out at about 40 cm depth and prior to spring ploughing, the soil was fertilized with 300 kg/ha NPK 8:22:20. Before transplanting, the soil was treated with herbicide and, immediately after, it was incorporated into the soil by harrowing.

The trial was set up in randomized blocks with 4 replications, at 90×50 cm spacing. Two hoeing's of tobacco were applied, followed by addition of 5g of 26% CAN. A few additional irrigations during the growing period were applied when necessary. After harvest and stringing, tobacco was yellowed and air-cured in special curing barns for Burley tobacco. Qualitative estimation of dried tobacco was made according to the Rules for standard measurements of quality of leaf tobacco of the type Burley. Corrected yield per stalk and per hectare was estimated by the method of Rimker and gross income (US$/ha) was assessed when the yield per hectare was multiplied with the average price per 1 kg of raw tobacco. Conversion in US$ was calculated by middle exchange rate of National Bank of Republic of Macedonia on 01.02.2018 (49,3979 ден. for 1 US$, rounded on 49,40ден. for 1 US$). Statistical processing of data was performed using the analysis of variance.

RESULTS AND DISCUSSION

The yield of tobacco, as in many other crops, is affected by the genotype, as well as environment interaction. Tobacco yield as quantitative character is in close correlation with leaf number, size and thickness. There are differences between the varieties of the same type, but it still must be typical for that type (Budin, 1988) reports that the average yield of Burley tobacco in Zimbabwe in the period 1980-1985 ranged from 1 202 to 1 760 kg/ha. In the 1950ties, yield in Bar was around 1 276 kg/ha (Jovović, 1957). There, the production of type Burley in Monte Negro, to be on more richer soils, in the region of Skadar, where climate is suitable for good grow and development of Burley, as well (Klikovac, 1994). The development of selection of this tobacco in the world resulted in creation of new genotypes that produce significantly higher yields, without negative effects on quality. Researchers from Bulgaria reported that the yields of B-1317 variety in some parts of Bulgaria can reach up to 3 380 kg/ha (Stoyanov and Apostolova, 1999). Another researcher argues that the yield of Burley tobacco should not be lower than 3 500 kg/ha (Dyulgorski 2009). Researchers from Scientific Tobacco Institute from Prilep, reported that Burley varieties created in Tobacco Institute - Prilep gave a yield of 3 500-4 500 kg/ha. (Risteski and Kočoska 2012). The yield of this tobacco type is strongly affected by agrotechnical measures applied. Pelivanoska, (2001) reported that by different variants of fertilization and irrigation, the yields of B-2/93 in the regions of Ohrid and Struga can reach up to 6 000 kg/ha.

Yield per stalk (g/stalk)

Data on variations of yields per stalk in varieties investigated in the trial are presented on Table 1.

Table 1. Corrected yield, g/stalk

Varieties	Years	Average yield g/stalk	Average 2016/2017	Differences from the average		
				Absolute	Relative	Range
L-8 ø	2016	107,8	116,3	\	100	6
	2017	124,7				
Kentucky 12	2016	118,2^{+}	132,2	+16,9	114,53	5
	2017	148,2				
B-963	2016	132,4^{++}	146,3	+30,0	125,79	4
	2017	160,2^{++}				
B-1246	2016	158,0^{++}	148,7	+32,4	127,86	3
	2017	139,4				
B-204/15	2016	185,1^{++}	200,5	+84,2	172,39	1
	2017	215,9^{++}				
B-206A/16	2016	182,2^{++}	191,8	+78,5	164,91	2
	2017	201,5^{++}				

	2016		2017
LSD 5%$^{+}$	9,62 g/stalk	**LSD 5%$^{+}$**	25,09 g/stalk
LSD 1%$^{++}$	13,32 g/stalk	**LSD 1%$^{++}$**	34,75 g/stalk

According to the above data, the highest average yield per stalk of 200,5 g was recorded in the F_1 hybrid B-204/15. It is 84,2 g or 72,39% higher compared to the check variety L-8, which average yield was 116,3 g/stalk. In the other varieties, the average yield ranges from 132,2 g/stalk in variety Kentucky 12 to 191,8 g/stalk in F_1 hybrid B-206A/16. Sjeranj examining two Burley varieties (DKH-28 and DKH-33) in the region of Podgorica came to conclusion that 23 650 stalks in ha, yield per stalk was from 101 gr to 152 gr, and yield per hectare from 2 389 kg to 3 594 kg, or on average 2 829 kg/ha (Seranj et. al, 2008).

Yield per hectare (kg/ha)

The yield per hectare is closely related with quality per stalk. The combination of these two characters is a more expressive indicator in assessing the economic value of the genotype. Beside the impact of the variety, this character is also affected by some agro-technical measures. Topping of the inflorescence, combined with sucker control in Burley tobacco can result in 28% yield increase per hectare (Berenji and Nikolič, 1996)

Table 2. Corrected yield per hectare (kg/ha)

Varieties	Years	Average yield kg/ha	Average 2016/2017	Differences from the average		
				Absolute	Relative	Range
L-8 ø	2016	2 395	2 583	\	100	6
	2017	2 772				
Kentucky 12	2016	2 626^{+}	2 959	+376	114,55	5
	2017	3 293				
B-963	2016	2 943^{++}	3 251	+668	125,86	4
	2017	3 560^{++}				
B-1246	2016	3 512^{++}	3 305	+722	127,95	3
	2017	3 098				
B-204/15	2016	4 114^{++}	4 457	+1874	172,55	1
	2017	4 800^{++}				
B-206A/16	2016	4 050^{++}	4 263	+1680	165,04	2
	2017	4 477^{++}				

	2016		2017
LSD 5%$^{+}$	213,6 kg/ha	**LSD 5%$^{+}$**	557,8 kg/ha
LSD 1%$^{++}$	295,9 kg/ha	**LSD 1%$^{++}$**	772,5 kg/ha

According to the data presented in Table 2, the highest average yield per hectare of 4 477 kg was recorded in the variety B-204/15, which is 1 874 kg (72,55%) higher compared to the check variety L-8, which average yield was 2 583 kg/ha. In other varieties, the average yield per hectare ranged from 2 959 kg in the variety Kentucky 12 to 4236 kg in hybrid B-206A/16. In both years of investigations of yields, statistically significant differences at a level of 1%, compared to the check were registered with variety B-963 and B-204/15. In the period from 2012 till 2014 in experimental tobacco station in Markovo in Bulgaria, examining seven burley varieties, was found that line 1546 in three years of examination, showed highest yields with average of 3 578 kg/ha, which is 37% higher than control variety Pliska (Dylgorski and Dosheva, 2017). Hristoski from the reseach in region of Prilep, in period from 2009 to 2010 with three burley varieties, came to concusion that Pelagonec had average yield per stalk of 180,4 gr and average yield per hectare of 4 009 kg/ha. Control variety, B-21 achieved 144 gr per stalk and 3 221 kg per hectare, which was for 24% less compared with Pelagonec (Hristoski, 2014).

Average price, US$/kg

The average price is, in fact, an indicator of quality of the obtained tobacco raw expressed in monetary value. However, the quality of tobacco is a very complex concept, affected by many mutually dependent factors and influences.

Table 3. Average price, US$/kg

Varieties	Years	Average price US$/kg	Average 2016/2017	Differences from the average		
				Absolute	Relative	Range
L-8 ø	2016	0,71	0,79	\	100	6
	2017	0,88				
Kentucky 12	2016	$0,86^{++}$	+0,88	+0,09	111,39	3/4
	2017	0,91				
B-963	2016	$0,87^{++}$	+0,88	+0,09	111,39	3/4
	2017	0,90				
B-1246	2016	$0,86^{++}$	+0,85	+0,06	107,59	5
	2017	0,85				
B-204/15	2016	$0,98^{++}$	+1,01	+0,22	127,84	1
	2017	$1,04^{++}$				
B-206A/16	2016	$0,93^{++}$	+0,96	+0,17	121,52	2
	2017	$1,00^{++}$				

	2016		2017
LSD 5%$^{+}$	0,076 US$/kg	**LSD 5%$^{+}$**	0,088 US$/kg^{+}
LSD 1%$^{++}$	0,106 US$/kg^{++}	**LSD 1%$^{++}$**	0,122 US$/kg^{++}

So, this indicator is only the beginning of a series of procedures for estimation of tobacco quality (physical and chemical properties, degustation, etc.). The quality of tobacco raw and the average price depend on a number of adequately performed cultural practices in field, in the time of harvest, yellowing, curing, etc. Data on the average price per 1 kg of dry tobacco in investigated varieties are presented in Table 3.

Gross income, US$/ha

The most important factors in the formation of this character are the average yield per hectare and the average price of 1 kg raw tobacco, i.e. it represents the yield and quality achieved by the varieties investigated in the trial.

Table 4. Gross income, US$/ha

Varieties	Years	Gross income, US$/ha	Average 2016/2017	Differences from the average		
				Absolute	Relative	Range
L-8 ø	2016	1 675	2 057	\	100	6
	2017	2 439				
Kentucky 12	2016	2 258++	2 627	+570	127,71	5
	2017	2 996+				
B-963	2016	2 560++	2 882	+825	140,10	3
	2017	3 204++				
B-1246	2016	3 021++	2 826	+762	137,38	4
	2017	2 631				
B-204/15	2016	4 032++	4 512	+2455	219,34	1
	2017	4 992++				
B-206A/16	2016	3 767++	4 122	+2065	200,38	2
	2017	4 477++				

	2016		2017
LSD 5%+	363,68 US$/ha	**LSD 5%**	477,34 US$/ha
LSD 1%++	503,70 US$/ha	**LSD 1%**	661,11 US$/ha

According to the above data, the highest average gross income of 4 512 US$/ha was recorded in the hybrid B-204/15, which is 2 455 US$/ha, i.e. 119% higher than the check variety L-8, which achieved 2 057 US$/ha. In other varieties, the gross income ranges from 2 627 US$/ha in variety Kentucky 12 to 4 122 US$/ha in B-206A/16. Statistically significant differences at 1% level compared to the check variety were found in variety B-963, in hybrids B-204/15, B-206/16 in both years (2016, 2017) and only in 2016 variety Kentucky 12 and B-1246. In the area of Prilep, in 2009 and 2010, Hristoski examined three burley varieties and found that hybrid variety Pelagonec opposed to control (B-21) achieved, on average, higher economic effect by 25,8% (Hristoski, 2014).

CONCLUSION

Based on the data obtained during the investigation, the following conclusions can be drawn:

- All varieties and hybrids varieties included in the field trial developed under the same conditions of growing, but in the end, they showed different results, as a product of various reactions of the varieties dictated by their genetic structure.
- The yields per stalk and per hectare were the highest in the variety B-204/15 (200,5 g/stalk and 4 457 kg/ha), and the lowest in the variety L-8 (116,3 g/stalk and 2 583 kg/ha).
- The average price for 1 kg of raw tobacco was the highest in the variety B-204/15 (1,01 US$/kg) and the lowest in L-8 (0,79 US$/kg).
- The gross income was the highest in the variety B-204/15 (4 512 US$/ha), and the lowest in the variety L-8 (2 057 US$/ha).
- Data obtained from the investigations show absolute dominance of the hybrids B-204/15 and B-206A/16 over the other varieties, which has been confirmedstatistically.
- The obtained results lead to a conclusion that the varieties have a very big influence on some productional characters. For this reason, in selection of varieties it is very important to have a deep knowledge of their properties.

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EFFECTS OF SALINITY ON PHENOLIC COMPOUNDS IN TOLERANT AND SENSITIVE GRAPES

SUMMARY

Salinity is a major environmental stress and grape is classified as a moderately sensitive plant to salinity. Plants have anti-oxidative systems involving antioxidant enzymes and secondary metabolites like phenolic compounds. The aim of this study was to examine the salinity induced alterations, PAL enzyme activity and total phenolics contents in four grape genotypes. In this study two tolerant (Gharashani and H6) and two sensitive (Shirazi and GhezelUzum) grapes - according to screening experiments under salinity- were selected. 50 mM NaCl was used for salinity treatment.

Total phenolics content and phenylalanine ammonia-lyase (PAL) activity increased in all the genotypes under salinity. A positive significant correlation ($P<0.01$, $r>0.8$) was observed between total phenolics and PAL activity in leaves and roots of grapes. Ten phenolic acids including hydroxybenzoic acid and hydroxycinnamic acid derivatives were analyzed. Our tolerant and sensitive genotypes showed main differences in phenolic compounds accumulation under salinity. Some compounds like gallic, syringic and p-coumaric acids accumulated higher in tolerant genotypes and others like vanillic, caffeic and sinapic acids accumulated in sensitive genotypes. Considering our results it seems that syringic and p-coumaric acids -that was higher in leaves and roots of tolerant genotypes- had a key role in salinity tolerance.

Keywords: Abiotic stress, phenylalanine ammonia-lyase activity, caffeic acid, p-coumaric acid

INTRODUCTION

Abiotic stresses like salinity decreases the plants growth and causes the osmotic stress (Xiong et al., 2002). Salinity induced some of the biochemical and physiological processes in plants. High level of salt produce reactive O_2 species (ROS). To reduce damage, plants have evolved complex anti-oxidative systems, involving antioxidant enzymes and secondary metabolites like phenolic compounds (Posmyk et al., 2009). Antioxidant activity of phenolic compounds have been reported before (Wang and Nii, 2000). Flavonoids and other phenolics are scavengers of free radicals (Rice-Evans et al., 1997).

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Grape (*Vitis vinifera*) is the plant that cultivated extensively in the world and is economically important. Salt stress have decreased grape yield. *Vitis vinifera* family is moderately sensitive to salt (Maas and Hoffman, 1977). Grapes contain valuable phenolic compounds (Fuleki and Ricardo-Da-Silva, 2003). Phenolics are considered for their important role in grape development and their beneficial health effects (Bub et al., 2003).

Phenols are important secondary metabolites that show antioxidant properties and play a key role in grape quality (Solecka and Kacperska, 2003) and defense under abiotic stresses (Amarowicz and Weidner, 2009). Polyphenols accumulated in *Mentha pulegium* plant under salt stress (Oueslati et al., 2010).

Phenolics mainly synthesize from cinnamic acid, which is produced by phenylalanine ammonia-lyase (PAL; EC 4.3.1.5) activity, the key enzyme between shikimate pathway and phenylpropanoid metabolism. Abiotic stresses increased PAL enzyme activity, which cause the accumulation of phenolic acids (Solecka and Kacperska, 2003). So phenylalanine ammonia-lyase enzyme may induce stress tolerance via regulating the synthesis of phenolic compounds.

Some phenols like phenolic acids exists in most plants (Jwa et al., 2006). Phenolic acids including hydroxycinnamic acid and hydroxybenzoic acid derivatives are chemical compounds with one or more hydroxyl groups (Hounsome et al., 2008). Phenolics are good ROS scavengers that helping to plants in salt tolerance. The radical scavenging antioxidants inhibit oxidation of lipids, proteins and DNA. Phenolics act as antioxidants by inhibiting ROS production (Castellano et al., 2012).

The composition and quantity of phenolics is different in various species, varieties and the regions that grapes grown (Bautista-Ortin et al., 2007). Various treatments affect the final composition of phenolics content in natural fruits.

In this study four grape genotypes were compared from the view point of phenolic compounds under salinity. In previous experiments 18 grape genotypes was screened based on salinity tolerance (Mohammadkhani et al. 2013, 2014). The genotypes with higher (Gharashani and H6) and lower (Shirazi and GhezelUzum) salinity tolerance selected for determining phenolic compounds. In order to investigate salinity induced alterations in four grape genotypes, PAL enzyme activity and total phenolics contents were studied under salt stress.

MATERIAL AND METHODS

Plant growth conditions

Hardwood cuttings of grape genotypes [Gharashani, H_6 (*V. vinifera* cv. GharaUzum × *V. riparia* cv. Kober 5BB), Shirazi, and GhezelUzum] were obtained from Kahriz vineyard (agricultural research center, grape genotypes collection). The cuttings were soaked in IBA (indol-3-butyric acid) 0.1 % (w/v) for 5-10 s and put in a mist house (relative humidity of 80%) with a heat-bed temperature of 20-30 ºC. The rooted cuttings were transferred to aerated pots containing modified Hoagland nutrient solution (Mohammadkhani et al., 2014). Solutions were replaced every 2 days, also nutrient concentrations and the pH of the solutions were monitored daily (maintained within a range of 6.0-6.5). Our experimental design was Complete Randomized Design (CRD).

Salinity treatments

Two months old plants were treated with 50 mM NaCl (threshold salinity that induced osmotic stress in the genotypes according to our screening). Plants were collected at different time points (0, 24 hours, 7 days and 14 days) after salinity and stored at -80 °C until phenolic compounds extraction.

Total phenolic content assay

Total phenolics were determined using Folin-Ciocalteu's reagent by Bonilla et al. (2003) method. Total phenolic content was reported as gallic acid in $mg.g^{-1}$ of fresh weight.

Determination of PAL activity

Phenylalanine ammonia-lyase enzyme activity was measured by Solecka and Kacperska (2003) method. One unit of PAL activity was the enzyme amount that produce 1 µmol of cinnamic acid in 1 h.

Extraction and measurement of phenolic acids by HPLC

Phenolic acids were extracted by Hakkinen et al. (1998) method. Leaves and roots were grounded and rinsed with extraction solution (methanol, HCl and ascorbic acid) and sonicated for 2 min. After 16 h shake in 35 ºC water bath in the dark, the extract was centrifuged at 12000g for 20 min and the samples filtered through a 0.45 µm filter. Quantitative and qualitative analysis was done by reverse phase HPLC (Guillen et al., 1996). The HPLC apparatus consisted of a KNAUER (Germany), UV–Vis detector, and reverse-phase C_{18} column at 25 ºC. The volume injected was 20 µl. A flow rate of 0.8 ml/min was used with two solvents: solvent A, 10% methanol–2% acetic acid in water; solvent B, 90% methanol–2% acetic acid in water. The following proportions of solvent B were used: 0–15 min, 0–15% B; 15–25 min, 15–50% B; 25–30 min, 50–0% B. The chromatograms were obtained at 280 nm. Phenolic acids were identified by authentic standards. Standard acids (ascorbic acid, rutin, ferulic acid, caffeic acid, p-coumaric acid, p-hydroxybenzoic acid, vanillic acid, syringic acid, sinapic acid and gallic acid) were purchased from Sigma-Aldrich. Stock solutions of the standard acids were prepared in a concentration of 0.1 g/10 ml in pure methanol (Vekiari et al., 2008). ChromGate software was used for data processing.

Statistical analysis

Statistical analyses were done using SPSS (Version 19.0). Tukey’s multiple range tests ($P<0.05$) and GLM (General Linear Model) were performed to determine the significant difference among salinity treatments and genotypes. Correlations between different factors were calculated for all genotypes.

RESULTS

Effects of salinity on total phenolics contents and PAL activity

Salinity accumulated the phenolics in roots and leaves of all genotypes (Figures 1A and 1B).

After 14 days salinity, significant increase (*P<0.05*) in phenolics contents was observed in roots of all genotypes. Total phenolic contents increased under salinity; that increase in leaves and roots of H6 genotype was higher than others (8.25 and 4.24 fold more than control, respectively).

GLM (General Linear Model) analysis showed that the difference in increase of total phenolics contents among genotypes was significant (*P<0.05*) in leaves. In leaves and roots the difference among all treatments was significant.

Salinity induced PAL activity in all treatments and all genotypes (Figures 1C and 1D). Increase in PAL activity of leaves was higher compare to roots. A maximum activity was observed in roots of Gharashani and leaves of H6 genotypes (46% and 71% more than control, respectively).

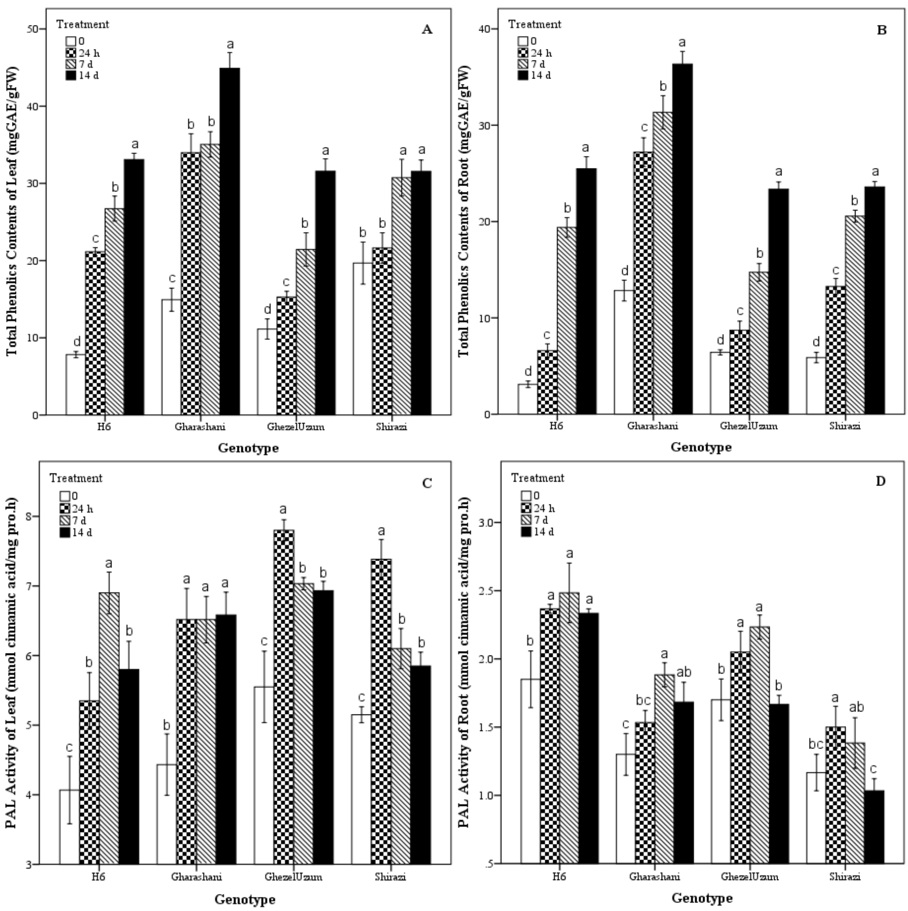

Figure 1. Total Phenolics contents in leaves (A) and roots (B) and PAL Activity in leaves (C) and roots (D) of four grape genotypes after 0, 24 hours, 7 days and 14 days treated by 50 mM NaCl. Different letters above the columns indicate significant difference (*P<0.05*) between the treatments according to Tukey's test.

The difference in accumulation of PAL activity among genotypes was significant ($P<0.05$) in roots. Also in roots the difference among treatments was significant ($P<0.05$), but in leaves the difference between 24 hours and 7 days salinity was not significant.

Effects of salinity on phenylpropanoids

Ten phenolic acids, including 4 hydroxybenzoic acid derivatives (gallic acid, p-hydroxybenzoic acid, vanillic acid, and syringic acid), and 4 hydroxycinnamic acid derivatives (caffeic acid, p-coumaric acid, ferulic acid, and sinapic acid) as well as ascorbic acid and rutin were analyzed by HPLC. As shown in figures 2 to 5 and table 1, four hydroxybenzoic acid derivatives and five hydroxycinnamic acid derivatives accumulated significantly under salinity in our grape genotypes.

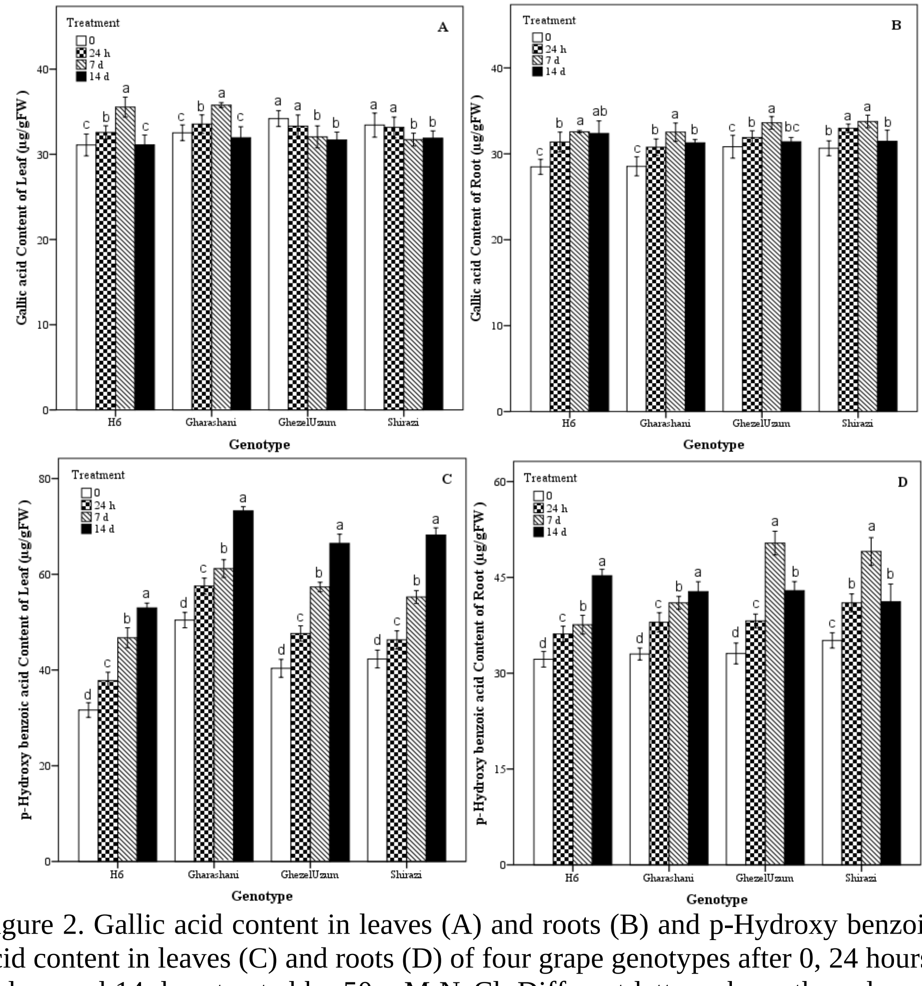

Figure 2. Gallic acid content in leaves (A) and roots (B) and p-Hydroxy benzoic acid content in leaves (C) and roots (D) of four grape genotypes after 0, 24 hours, 7 days and 14 days treated by 50 mM NaCl. Different letters above the columns indicate significant difference ($P<0.05$) between the treatments according to Tukey’s test.

Effects of salinity on hydroxybenzoic acid derivatives

Gallic acid

Gallic acid content increased in leaves of tolerant genotypes (Gharashani and H6), but after long time (14 days) there was no difference ($P<0.05$) compare to control. Whereas in leaves of sensitive genotypes (GhezelUzum and Shirazi) it was decreased. In roots gallic acid accumulated under salinity, that accumulation in tolerant genotypes was higher compare to sensitive ones (Figures 2A and 2B).

The difference in gallic acid content of leaves between Gharashani and other genotypes was significant ($P<0.05$), but H6, GhezelUzum and Shirazi genotypes showed no significant difference.

P-hydroxy Benzoic Acid

Leaves of our genotypes showed significant increase ($P<0.05$) in p-hydroxy benzoic acid content, that increase in sensitive genotypes (GhezelUzum and Shirazi) was higher compare to tolerant ones (Gharashani and H6). We observed a significant increase in roots of tolerant genotypes. However all salinity treatments (time points) showed higher p-hydroxy benzoic acid content compare to control (Figures 2C and 2D).

The difference between sensitive genotypes in leaves and roots was not significant ($P<0.05$), but tolerant genotypes showed significant difference. Also the difference between treatments was significant.

Vanillic acid

Under salinity vanillic acid content increased in leaves and roots compare to control, that increase in sensitive genotypes was higher compare to tolerant (Figures 3A and 3B). In leaves of tolerant genotypes we observed first increase and then decrease, but all time points showed higher content compare to control.

GLM (General Linear Model) analysis showed that the difference among genotypes and also among treatments in leaves and roots was significant ($P<0.05$).

Syringic acid

Syringic acid increased in leaves and roots with time passing, that increase in tolerant genotypes was higher compare to sensitive ones (Figures 3C and 3D). In leaves the difference among genotypes was significant. The difference among all treatments in leaves and roots was significant ($P<0.05$).

Effects of salinity on hydroxycinnamic acid derivatives

Caffeic acid

Caffeic acid content increased in leaves and roots with time passing, that increase in sensitive genotypes (GhezelUzum and Shirazi) was higher compare to tolerant ones (Figures 4A and 4B). Caffeic acid content increased in roots but we observed decrease under long time salinity.

The difference among genotypes in leaves was significant ($P<0.05$), but roots of H6 and GhezelUzum showed no significant difference. The difference among treatments in leaves and roots was significant.

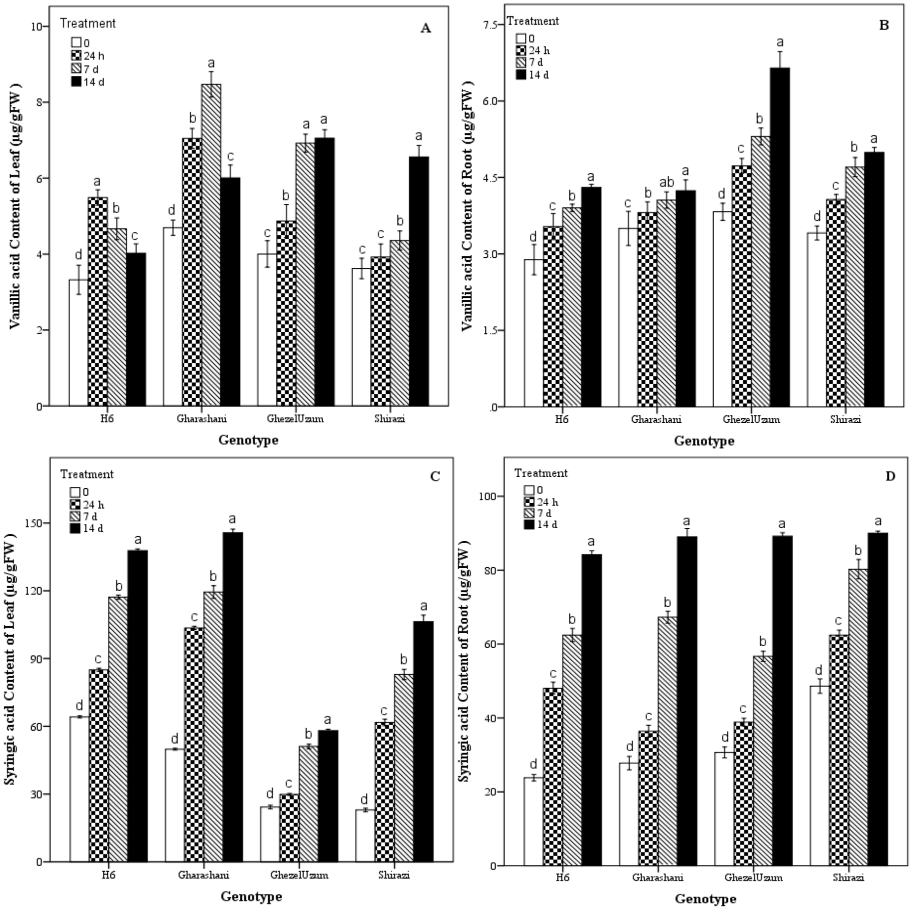

Figure 3. Vanillic acid content in leaves (A) and roots (B) and Syringic acid content in leaves (C) and roots (D) of four grape genotypes after 0, 24 hours, 7 days and 14 days treated by 50 mM NaCl. Different letters above the columns indicate significant difference (*P<0.05*) between the treatments according to Tukey's test.

P-coumaric acid

Higher content of p-coumaric was observed in leaves of tolerant genotypes after 7 days salinity and in sensitive ones after 24 hours (Figure 4C). In roots p-coumaric acid accumulated in tolerant genotypes (H6 and Gharashani) with time passing (Figure 4D). However p-coumaric acid content increased compare to control.

The difference among genotypes and also among treatments (time points) in leaves and roots was significant (*P<0.05*).

Ferulic acid

Ferulic acid content increased under salinity, that increase in leaves of sensitive genotypes and roots of tolerant genotypes was higher than others

(Figures 5A and 5B). Leaves of GhezelUzum and roots of H6 showed higher ferulic acid accumulation compare to control.

The difference among all genotypes in leaves was significant (*P<0.05*), but in roots Gharashani and GhezelUzum showed no significant difference. Also the difference among all treatments was significant.

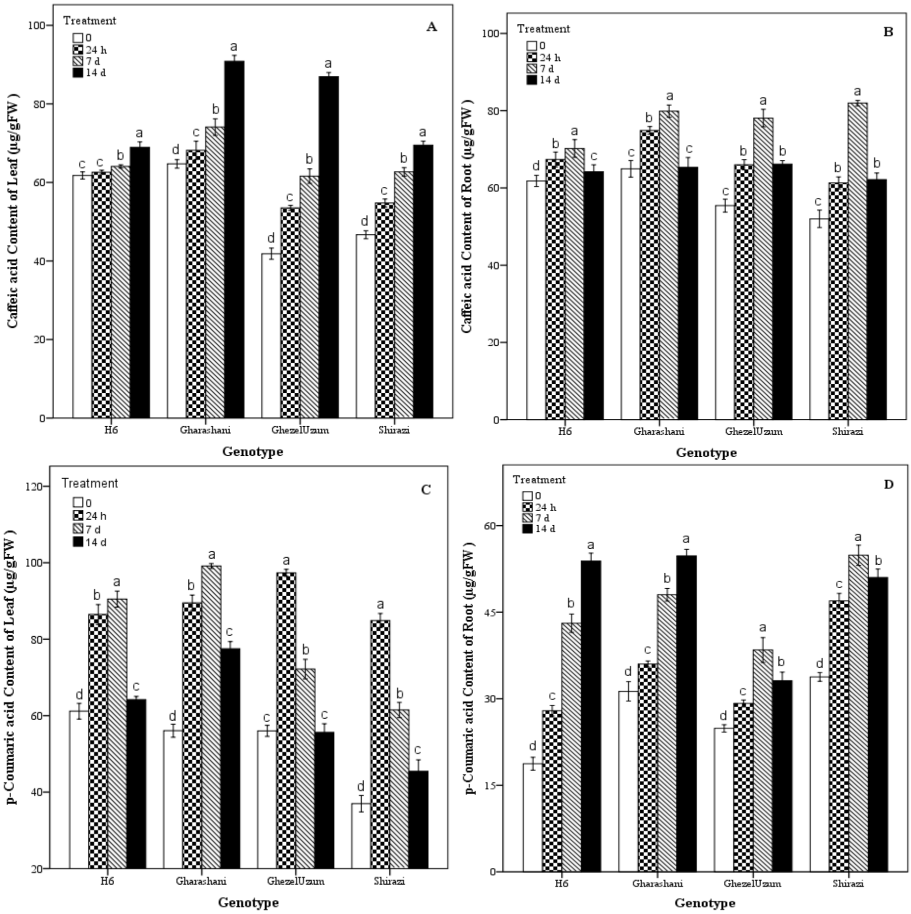

Figure 4. Caffeic acid content in leaves (A) and roots (B) and p-Coumaric acid content in leaves (C) and roots (D) of four grape genotypes after 0, 24 hours, 7 days and 14 days treated by 50 mM NaCl. Different letters above the columns indicate significant difference (*P<0.05*) between the treatments according to Tukey’s test.

Sinapic acid

Sinapic acid accumulated significantly (*P<0.05*) in leaves and roots with time passing under salinity (50 mM NaCl). Leaves of Gharashani and roots of GhezelUzum showed higher content of sinapic acid compare to control (Figures

5C and 5D). The difference among genotypes and also among treatments (time points) in leaves and roots was significant.

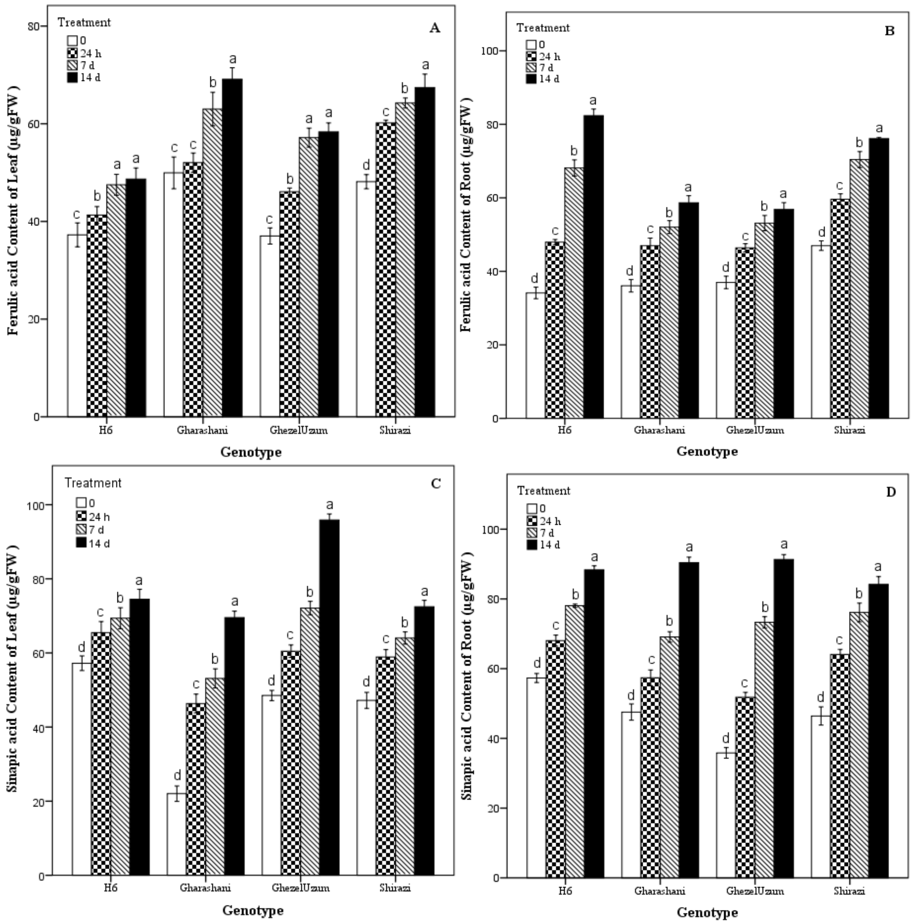

Figure 5. Ferulic acid content in leaves (A) and roots (B) and Sinapic acid content in leaves (C) and roots (D) of four grape genotypes after 0, 24 hours, 7 days and 14 days treated by 50 mM NaCl. Different letters above the columns indicate significant difference (*P<0.05*) between the treatments according to Tukey's test.

Effects of salinity on other contents

We assayed ascorbic acid and rutin contents, too.

Ascorbic acid increased in leaves and roots (Table 1). Leaves of Shirazi and roots of H6 showed higher accumulation of ascorbic acid compare to control. The difference among genotypes and also among treatments (time points) in leaves and roots was significant.

Rutin content first increased and then decreased in our genotypes, leaves of tolerant genotypes and roots of sensitive genotypes showed higher increase compare to control (Table 1). There was no significant difference between tolerant genotypes (Gharashani and H6) and also between sensitive genotypes (Shirazi and GhezelUzum) in leaves. The differences among all time points in leaves and roots was significant.

Table 1. Ascorbic acid and rutin content (µg/g FW) in leaves and roots of four grape genotypes after 0, 24 hours, 7 days and 14 days treated by 50 mM NaCl.

Genotype & 50 mM NaCl	Ascorbic acid Content of Leaf (µg/g FW)	Ascorbic acid Content of Root (µg/g FW)	Rutin Content of Leaf (µg/g FW)	Rutin Content of Root (µg/g FW)
H6				
0	4.81±0.15 d[a]	8.73±0.35 d	15.56±0.18 b	15.61±0.26 d
24 h	52.65±0.82 b	19.50±0.33 c	19.35±0.16 a	16.71±0.15 c
7 d	60.30±0.45 a	35.30±0.66 b	16.35±0.23 b	19.14±0.06 a
14 d	31.22±0.28 c	54.46±0.53 a	16.41±0.24 b	17.78±0.17 b
Gharashani				
0	11.17±0.31 d	25.28±0.37 d	14.73±0.24 c	15.75±0.12 c
24 h	41.39±0.66 c	30.18±0.36 c	20.64±0.19 a	16.30±0.03 b
7 d	100.54±0.36 a	48.33±0.48 b	17.48±0.20 b	18.99±0.12 a
14 d	87.73±0.55 b	62.01±0.36 a	15.50±0.25 c	16.60±0.13 b
GhezelUzum				
0	9.71±0.28 d	4.72±0.06 d	16.53±0.25 c	15.61±0.12 d
24 h	27.59±0.52 c	7.69±0.18 c	19.55±0.32 a	19.38±0.26 b
7 d	33.90±0.43 b	14.97±0.38 b	18.40±0.26 ab	21.06±0.15 a
14 d	61.29±0.49 a	19.59±0.32 a	17.47±0.26 bc	17.77±0.07 c
Shirazi				
0	5.68±0.20 d	5.43±0.18 d	16.21±0.18 d	15.27±0.10 d
24 h	13.75±0.15 c	8.28±0.20 c	19.59±0.09 a	18.17±0.12 a
7 d	51.62±0.24 b	13.79±0.21 b	18.74±0.25 b	19.21±0.09 a
14 d	109.04±0.25 a	25.33±0.26 a	17.63±0.16 c	16.74±0.07 c

[a] Different letters indicate significant differences (*P<0.05*) according Tukey's multiple range tests.

DISSCUSION

Grapevine plants are moderately sensitive to salinity (Walker et al., 1981). Increase in phenol content could be an adaptive mechanism for tolerate stress. Several studies have reported that salt and drought stresses induce total phenol production (Hanen et al., 2008). Our results verified them, so that a regular ascendant process from control to long term salinity (14 days under 50 mM NaCl) was observed in all genotypes. However, this process was higher in tolerant genotypes (75%) compare to sensitive ones (61%). It seems that production of total phenolic compounds in tolerant genotypes was a part of their defense system against salinity.

Phenylalanine ammonia-lyase is a key enzyme that regulates the biosynthesis of phenolic compounds from phenylalanine. Phenolic acids are accumulated under stress as a result of increase in PAL activity and protect plants against abiotic stresses (Dixon and Paiva, 1995). In present study PAL activity increased under salinity. A significant positive correlation ($P<0.05$, r≥0.8) was observed between total phenolics and PAL activity in the leaves and roots of tolerant genotypes. Secondary metabolites as antioxidants, help the plants to tolerate oxidative stress. The accumulation of caffeic acid and p-coumaric acid help to reduce oxidative pressure, also caffeic acid and p-coumaric acid are good ROS scavengers (Rezazadeh et al., 2012). Szwajgier et al. (2005) reported that p-coumaric and ferulic acids showed the highest reducing power of free radicals.

In present study caffeic acid, p-coumaric and ferulic acid contents increased in all genotypes, but increase in caffeic acid content in leaves and roots of sensitive genotypes (GhezelUzum and Shirazi) was higher compare to tolerant ones. Whereas increase in p-coumaric in leaves and roots of tolerant genotypes was higher compare to sensitive ones. Also increase in ferulic acid content in leaves of sensitive genotypes and roots of tolerant ones was higher. It means that caffeic acid help to free radicals scavenging in sensitive genotypes more than tolerant and vice versa about p-coumaric acid.

The hydroxycinnamic acid derivatives are more strong antioxidants than hydroxybenzoic acid derivatives (Steenkamp et al., 2013). Increase in both hydroxycinnamic acid and hydroxybenzoic acid derivatives was observed in present study, it seems that accumulation of hydroxycinnamic acid derivatives was higher than hydroxybenzoic acid derivatives. Among hydroxybenzoic acid derivatives, Syringic acid increased higher. There was a significant positive correlation ($P<0.01$, r>0.8) between p- coumaric acid and ferulic acid contents (hydroxycinnamic acid derivatives) and also between p- hydroxyl benzoic acid and gallic acid (hydroxybenzoic acid derivatives) in roots of all genotypes. Most of phenolic acids in leaves was higher compare to roots, therefore grape leaves contain more phenolic acids than roots.

The synthesis of phenolic compounds is affected positively or negatively under different stresses. Plants synthesize different phenolic acids in response to abiotic stresses (Caldwell et al., 2007). Many authors reported the accumulation of phenols in plants under abiotic stresses (Dixon and Paiva, 1995; Weidner et al., 2009a). Differences in results can be related to type of stress, development stage, intensity and duration of stress and plant parts, such as roots or leaves (Weidner et al., 2009a). Variation among rootstocks in accumulation of biochemical compounds in response to salinity stresses observed. Increase in phenolic compounds was highest at the end of the stress (Parida et al., 2004).

Our results was consistent with them. In present study phenolic compounds accumulated in leaves and roots of both tolerant and sensitive grape genotypes under salinity, also our genotypes showed differences in accumulation of each compound. Accumulation of phenolic compounds was higher after 14 days salinity, but about some phenolic compounds some fluctuations was observed.

Jogaiah et al. (2014) reported the increase in phenols of tolerant grape rootstocks under salinity. That result is similar to Latha et al. (1989) study, where tolerant genotypes showed a higher total phenolics and decrease was observed in sensitive genotypes. Therefore increase in total phenols content of tolerant genotypes could be an adaptive mechanism for preventing damage during stress.

Our results verified them about total phenolic compounds increase in tolerant genotypes. The sensitive genotypes showed increase in total phenolic compounds too, but that increase was higher in tolerant genotypes. Some phenolic derivatives increased higher in sensitive genotypes and others was higher in tolerant ones.

Phenolic acids were decreased in tissues during long-term stress (Kro´l et al., 2014). Similar results were reported by Weidner et al. (2011) in grape plants under osmotic stress, but caffeic acid increased. Phenolic synthesis in Shiraz variety during drought stress could also be limited (Ojeda et al., 2002).

Under chilling stress in leaves of *V. vinifera*, ferulic and caffeic acid contents increased, while p-coumaric acid decreased (Amarowicz et al., 2010). In addition, decrease in ferulic, caffeic and p-coumaric acids also have been reported in roots of grape plants at the same conditions. The highest phenolic in roots was p-coumaric acid and the lowest was caffeic (Weidner et al., 2009b).

Our results verified Kro´l et al. (2014), Ojeda et al. (2002) and Amarowicz et al. (2010) reports for some phenolic acids. Caffeic acid content in roots and p-coumaric acid content in leaves decreased at long term salinity, but the most of our phenolic compounds increased even under high salinity.

A significant positive correlation ($P<0.05$, r>0.7) was observed between total phenolics contents and many of phenolic compounds in both tolerant and sensitive genotypes, r value was higher in tolerant genotypes.

CONCLUSION

In conclusion PAL activity and total phenolics showed a significant positive correlations ($P<0.01$) in tolerant genotypes. Phenolic compounds increased under salinity, some compounds accumulated higher in tolerant genotypes and some accumulated in sensitive genotypes. Therefore our tolerant and sensitive genotypes showed different defense mechanisms against salinity. But our tolerant genotypes were more successful and showed higher accumulation in phenolics content and PAL activity. Considering above mentioned results obtained in this study, because tolerant genotypes (H6 and Gharashani) possessed higher efficiency in its anti-oxidative system and can tolerate salinity better than sensitive ones, it seems that syringic and p-coumaric acids that was higher in tolerant genotypes, had an important role in salinity tolerance.

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MORPHO-PHYSIOLOGICAL CHARACTERIZATION AND PHOTOSYNTHETIC PIGMENT CONTENTS OF ACACIA KARROO HAYNE SEEDLINGS UNDER SALINE CONDITIONS

SUMMARY

Acacia karroo is a leguminous tree listed in most of the Algerian territory. It is a salt-tolerant species and a multipurpose tree in agroforestry. However, the defence mechanisms underlying salt tolerance of this species are still unknown. In this study, the effects of salt stress on various morpho-physiological and biochemical traits of *A. karroo* were investigated. Three-months-old plants were submitted to increasing salt concentrations (0, 200, 400 and 600 mM NaCl), for a period of 21 days. Stem length was not significantly affected by salinity. Increasing salinity reduced the length of root. Number of leaves was maintained constant at 200 and 400 mM NaCl but was reduced slightly at 600 mM NaCl. Also, an increase in crown diameter by 30% under mild and high salt stress was observed. Furthermore, salt tolerance index was not affected at all salinity levels. The leaf mass area was not affected by saline conditions. Salt treatments did not produce a notable change in the relative water content of leaves, indicating a relatively high resistance as well to dehydration, which will certainly contribute to some degree of salt tolerance in *A. karroo*. Relative water loss from excised leaves was significantly higher at 200 mM and similar at high concentration of NaCl as compared to control. The result of variance analysis for the major effect of salinity showed that salt stress significantly decreased the content of photosynthetic pigment in leaves at higher concentrations of NaCl. However, at 200 mM of NaCl, an enhancement of chlorophyll b, total chlorophylls and carotenoids content was observed. At the same level, chlorophyll a presented a constant content compared with control. In conclusion, although plants suffered from salt stress, as shown by the degradation of photosynthetic, they continued their vegetative growth and maintained their internal water potential under salinity conditions. Therefore, *A. karroo* is a potential halophytic species to be cultivated in saline lands and make it favourable for agroforestry practices.

Keywords: *Acacia karroo*, agroforestry, halophyte, NaCl, water potential.

INTRODUCTION

Salinity is a widespread problem, affecting around 831 million hectares of lands that include 397 and 434 million hectares of saline and sodic soils,

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respectively (Teakle and Tyerman, 2010). Salinization of soil and groundwater has been considered as the most critical environmental issue, hindering sustainable agricultural productivity and presenting a challenging task for ecologists and physiologists (Lal, 2009; Mansouri and Kheloufi, 2017). Salinity can affect growth and yield of most plants (Munns, 2002; Nasrin *et al*., 2016), by inducing reduced cell division in roots and leaves (Munns and Tester, 2008), cell elongation, cell differentiation, along with genetic, biochemical, physiological, morphological, and ecological processes, as well as their complex interactions followed by significant tissue damage, leading to the plants' death in case of prolonged exposure to salinity (Ashraf and Harris, 2004).

Halophytes have been regarded as potential new crops for use as forage, vegetable, and oil seed crop (Glenn *et al.*, 2013). However, the potential utilization of halophytic species to grow in salt-affected soil and to facilitate saline soil phytoremediation depends on several factors such as salt accumulation, relative growth rate and biomass conversion, multipurpose utilization, and economic returns to the farmers (Panta *et al*., 2014). *Acacia karroo* Hayne, commonly known as the sweet thorn, is a species of acacia, native to southern Africa from southern Angola to east Mozambique and south Africa (Archibald and Bond, 2003). It belongs to the family of Fabacea (Leguminosae) with the main advantage to make symbiosis with soil microorganisms (rhizobium and mycorrhizae) conferring them the capacity to survive in very poor grounds in nutritional elements (Bashan *et al*., 2012; Boukhatem *et al*., 2016). *A. karroo* varies from a shrub up to 2 m tall to a tree more than 20 m in height, with distinctive white thorns and attractive yellow flowers. The leaves comprise about five pairs of leaflets, each divided into ten or more pairs of smaller leaflets of about 5 mm long (Maroyi, 2017). This species is used for chemical products, forage livestock, domestic uses and environmental management. *A. karroo* is the most widespread acacia in southern Africa and occupies a diverse range of environments from acacia savannahs and woodlands on hills and rocky soils to the banks of dry watercourses in Algeria (Kheloufi *et al*., 2018). *A. karroo* tree can produce seeds prolifically from an early age and is resistant to fire (Midgley and Bond, 2001). It has a lot of potential as a possible source of pharmaceutical products for the treatment of a wide range of both human and animal diseases and ailments. Indeed, *A. karroo* has been used as herbal medicine by the indigenous people of southern Africa for several centuries and several diseases (Maroyi, 2017).

In Algeria, it has been reported that *A. karroo* can germinate under 400 mM of NaCl with 66% of final germination (Kheloufi *et al*., 2017). Thus, introduction of *A. karroo*, as a salt-tolerant species, could be an important strategy in conserving ecology and wood production in the salt-affected regions of Algeria. Moreover, no study has been conducted at morpho-physiological and biochemical levels to understand the mechanisms associated with the adaptability of *A. karroo* under salt stress. Therefore, in the present study, we aimed to examine the effects of various levels of salinity on some morpho-physiological parameters and photosynthetic pigment contents (chlorophylls and carotenoids) of *A. karroo* seedlings.

MATERIALS AND METHODS

Plant material, growth condition and salt treatment

The seeds of *A. karroo* Hayne were collected from Aïn El Baïda salt farm area (Oran, Algeria) (latitude: 35°39'34.96" N; longitude: 0°40'4.68" W; elevation: 136 m). The pods were collected from 10 trees and the seeds were then mixed. The thousand-seed-weight was 39 g. Sieving and flotation were used to sort out seeds. The clean seeds were then spread on filter paper to dry. Once dried, the seeds undergo a chemical treatment which consisted of immersion in 96% sulphuric acid for 30 minutes followed by washing in distilled water. *A. karroo* seeds need this pre-treatment to break down the seed coat and induce a high germination rate in a short time (Kheloufi, 2017). Seeds were germinated in plastic pot (Top diameter: 10 cm; Bottom diameter: 7 cm; Height: 14 cm) containing 1 kg of mixed substrate (two volumes of sand mixed with one volume of compost) (EC = 49 $mS.m^{-1}$; pH = 6.2; N = 89 $g.m^{-3}$; P_2O_5 = 42 $g.m^{-3}$; K_2O = 27 $g.m^{3}$) and arranged according to the method of complete randomized blocks with four replicates under greenhouse conditions. Sand was sieved at 2 mm to eliminate wastes and coarser material then washed repeatedly with tap water to eliminate all carbonates and chlorides. The experiment was conducted in the green house of Ecology and Environment Department, University of Batna 2, Algeria (latitude: 35°38'10.32"N; longitude: 6°16'31.52"E; elevation: 926 m).

Table 1. Preparation of saline solution and corresponding hydric potential.

NaCl (mM)	NaCl (g/L)	Ψos Level (MPa) (Braccini *et al.*, 1996)
0	0	0
200	11.68	-0.83
400	23.37	-1.67
600	35.06	-2.50

Three months (90 days) old healthy seedlings of uniform size were selected as initial material and further grown in KNOP's nutrient medium. The plants were subjected to salt treatment by supplementing the nutrient medium with varied sodium chloride (NaCl) concentrations (200, 400 and 600 mM) (Table 1). The control plants were grown in the nutrient medium devoid of NaCl. The nutrient solutions were replaced with freshly prepared solutions at every 7 days intervals. After 21 days of salt treatment, leaf, stem, and root samples were harvested from control and NaCl-treated plants for estimation of various parameters. Leaves occupying the same position were sampled from control and NaCl-treated plants for estimation of photosynthetic pigment contents.

Measurement of morphological parameters

Total stem length (SL), total root length (RL), leaves number per plant (LP) and crown diameter (CD) of four plants (n=4) from each treatment were recorded after 21 days of treatment. For measurement of fresh and dry weights, leaves were excised from control and NaCl-treated plants and the fresh weight was noted immediately. Later, they were wrapped in pre-weighed aluminium foils and kept in an incubator at 80°C for 48h before the dry weight was recorded. Total green leaf area per plant was measured in both control and NaCl-treated plants, using Image analysis system Digimizer software (version 4.6.1, MedCalc Software, Belgium).

Measurement of physiological parameters

Salt Tolerance Index

Salt tolerance index (STI) was calculated by using the following formula developed by Seydi (2003):

$$\mathrm{STI} = \frac{\mathrm{TDW\ at\ Sx}}{\mathrm{TDW\ at\ SI}} \times 100$$

TDW: Total dry weight (oven at 80°C for 48h)
SI: Control treatment
Sx: Salt treatment

Leaf mass area

The leaf mass area (LMA) was calculated using (Hernández and Kubota, 2016) formula:

$$\mathrm{LMA\ (mg/cm2)} = \frac{\mathrm{LDW}}{\mathrm{LA}}$$

LDW: Leaf dry weight (mg)
LA: Leaf area (cm^2)

Leaf relative water content

Leaf fresh weight (LFW) was immediately noted after sampling and subsequently immersed into distilled water for 8 h at room temperature. Leaves were then blotted dry and leaf turgid weight (LTW) was taken prior to incubating at 80°C for 48h. After incubation period, leaf dry weight (LDW) was also noted. The leaf RWC was calculated using following formula (Barrs and Weatherley, 1962):

$$\mathrm{RWC\ (\%)} = \left[\frac{(\mathrm{LFW} - \mathrm{LDW})}{(\mathrm{LTW} - \mathrm{LDW})}\right] \times 100$$

Rate water loss

The rate water loss (RWL) was calculated using (Clarke *et al.*, 1989) formula:

$$\mathrm{RWL\ (mg/cm2.min)} = \frac{(\mathrm{FW} - \mathrm{FW2h})/\mathrm{DW}}{\mathrm{LA} \times 120}$$

FW: Leaf fresh weight determined immediately after leaf harvesting
FW2h: Leaf fresh weight measured after 120 minutes under laboratory conditions
DW: Leaves dry weight measured after drying in an oven at 50°C for 2 hours.
LA: Leaf area (cm^2).

Chlorophylls and Carotenoids

Chlorophylls (Chl a, Chl b and Total Chl) and carotenoids (mg.g^{-1} LFW) were extracted by 100% acetone from fresh leaves samples (LFW). After centrifugation (10 000 rpm for 5 minutes), supernatants were used for the analysis of pigments. Absorbances were determined at 645, 652, 662, and 470 nm, respectively, using UV/visible light spectrometer (4-16K, Sigma) and the following equations were used for calculations (Lichtenthaler and Wellburm, 1983):

$$\text{Total Chl} = \frac{A652 \times 27.8 \times 20}{\text{mg LFW}}$$

$$\text{Chl a} = \frac{[(11.75 \times A662) - (2.35 \times A645)] \times 20}{\text{mg LFW}}$$

$$\text{Chl b} = \frac{[(18.61 \times A645) - (3.96 \times A662)] \times 20}{\text{mg LFW}}$$

$$\text{Car} = \frac{[((1000 \times A470) - (2.27 \times \text{Chl a}) - (81.4 \times \text{Chl b}))/227] \times 20}{\text{mg LFW}}$$

Statistical analysis

All the experiments were conducted with four replicates (n=4) and the results were expressed as mean ± standard deviation (SD). All the data were subjected to one-way analysis of variance (ANOVA) and Duncan's multiple-range test (P<0.05) using SAS Version 9.0 (Statistical Analysis System) (2002) software.

RESULTS AND DISCUSSION

Morphological traits

The effect of sodium chloride was significant for root length (p = 0.0199), leaves number per plant (p = 0.0278) and crown diameter (p = 0.0010), except stem length, which was not significantly affected by salinity (p = 0.2178) (Figure 1, Figure 2, Table 2).

Figure 1. *Acacia karroo* seedlings of 111 days-old cultivated under different salinity levels (0, 200, 400 and 600 mM NaCl) after 21 days of treatment.

Table 2. Mean comparison and analysis of variance effects of salinity on stem length, root length, leaves per plant, crown diameter, crown diameter, leaf mass area, rate water loss, rate water loss, relative water content, salinity tolerance index, total chlorophylls content, chlorophyll a content, chlorophyll b content and carotenoids content.

Parameters	Sources of variation	Df	F	P
Stem length (SL)	NaCl treatment	3	1.73	0.2178
Root length (RL)	NaCl treatment	3	4.82	0.0199
Leaves per plant (LP)	NaCl treatment	3	4.32	0.0278
Crown diameter (CD)	NaCl treatment	3	10.81	0.0010
Salinity tolerance index (STI)	NaCl treatment	3	1.96	0.1738
Leaf Mass Area (LMA)	NaCl treatment	3	7.36	0.0047
Relative water content (RWC)	NaCl treatment	3	1163.37	<0.0001
Rate water loss (RWL)	NaCl treatment	3	8.04	0.0033
Chlorophyll a content (Chl a)	NaCl treatment	3	27.41	<0.0001
Chlorophyll b content (Chl b)	NaCl treatment	3	24.65	<0.0001
Total chlorophyll content (TChl)	NaCl treatment	3	25.17	<0.0001
Carotenoid content (Car)	NaCl treatment	3	20.81	<0.0001

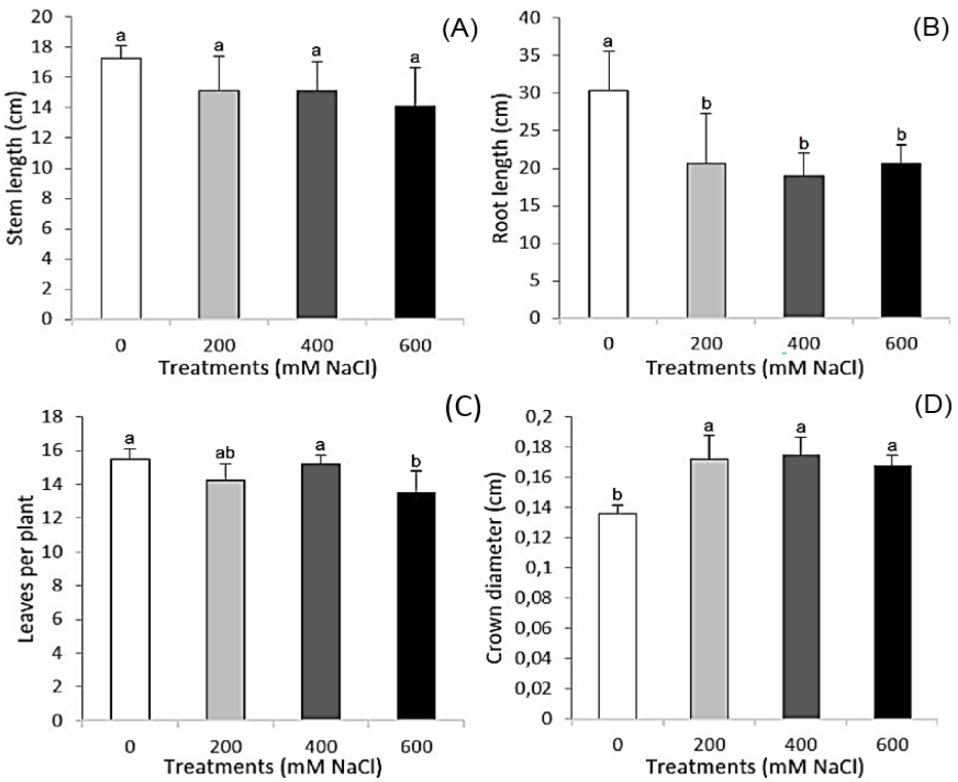

Figure 2. Effects of salt stress on (A) Stem length, (B) Root length, (C) Leaves per plant and (D) Crown diameter of *Acacia karroo* seedlings after 21 days of various levels of saline treatments. Means, in each box, with similar letters are not significantly different at the 5% probability level using Duncan's test.

Increasing salinity reduced the length of root by 10 cm at lower and higher concentrations compared with control. Reduction in plant height and other growth parameters are the most distinct and obvious effects of salt stress, since inhibition of growth is probably the most general response of plants to stress (Munns and Tester, 2008).

In this study, all results indicated that different growing characteristics were significantly affected by salinity stress, except stem length. Depressed growth due to high salinity is attributed to several factors such as osmotic stress, specific ion toxicity and ion imbalance, and induced nutritional deficiency (Giri *et al*., 2003; Morant-Manceau *et al*., 2004; Meloni *et al*., 2008).

The first plant part interacts with salt is the roots and it is almost inevitable that the crops are affected by salt concentration. Therefore, the results obtained in present study agree with previous studies on *A. karroo* seedlings and other species of the same genus, reporting the negative effect of salt concentration on plant height: Kheloufi *et al*., 2016a (*A. saligna* and *A. decurrens*); Kheloufi *et al*., 2016b (*A. tortilis*, *A. ehrenbergiana* and *A. dealbata*), Kheloufi *et al*., 2017 (*A. karroo*); Rahman *et al*., 2017 (*A. auriculiformis*) and Theerawitaya *et al*., 2015 (*A. ampliceps*). The delay of the radicle growth under salt stress may be due to the reduction in the turgor of the radicle cells (Bradford, 1995; Saroj and Soumana, 2014). The reason that the root and shoot length are affected negatively by salt stress is due to toxic effect of salts as well as inhibition of cytokinesis and cell expansion (Kurum *et al*., 2013). The increase in osmotic pressure around the roots because of saline environment can also prevent water uptake by root and results with short root (Aroca *et al*., 2011).

Number of leaves was maintained constant at 200 and 400 mM but was reduced by two leaves at 600 mM of NaCl treatment. The crown diameter was the most affected of the morphological parameters and showed an overall increase as salinity increased (Figure 2). Salinity stress had also remarkable effects on other plant growth parameters such as leaf number and crown diameter. Salinity usually results in a biochemical loosening of the cell wall under turgor pressure, which initiates cell expansion followed by water and solute uptake, and an increased succulence (Chen *et al*., 2015). In this investigation, an increase in crown diameter (30%) under mild and high salt stress in this salt tolerant species may be vital under physiological drought for its better water storage, which is an adaptation for ion dilution to minimize the effect of Na^+ and Cl^- in plant tissues (El-Lamey, 2015). Reduction in cell size was also attributed to the plant ability to reduce its size to minimize salt uptake (Zapryanova and Atanassova, 2009). The reduction in biomass increased with the increase in salinity which is obvious because of disturbances in physiological and biochemical activities under saline conditions as shown by Vinocur and Altman (2005) that may be due to the reduction in leaf area and number of leaves.

Physiological traits

STI, a reliable criterion for salt tolerance (Ali *et al*., 2013), was not affected by salt stress at low and high levels (p = 0.1738) (Figure 3A, Table 2).

The higher STI at seedling stage indicate that the key mechanisms of salt tolerance in plants may be associated with (i) accumulation of compatible solutes like proline, total sugars, reducing sugars and total free amino acids; (ii) increase amount of K^+, Ca^{2+} and Mg^{2+} in phyllodes than roots; (iii) increase K^+ retention in photosynthetic tissues through hindering Na^+ uptake; (iv) anatomical adjustment by increasing the size of spongy parenchymal tissue of phyllodes, endodermal thickness of stems and roots, and pith area of roots; (v) efficient Na^+ sequestration in vacuoles that would be facilitated by a decrease in stomatal density and (vi) the enhanced Na^+ exclusion **(**Rahman *et al*., 2017).

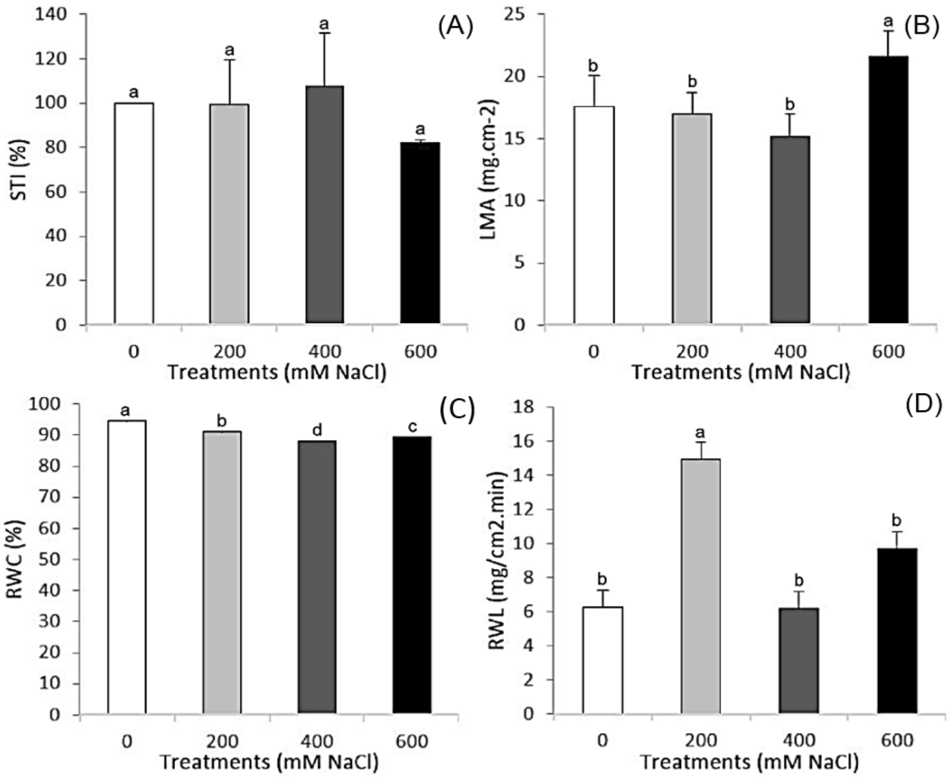

Figure 3. Effects of salt stress on (A) Salinity tolerance index, (B) Leaf mass area, (C) Relative water content (D) Rate water loss of *Acacia karroo* seedlings after 21 days of various levels of saline treatments. Means, in each box, with similar letters are not significantly different at the 5% probability level using Duncan's test.

A meta-analysis on Figure 3 showed that leaf-related parameters (leaf mass area, relative water content and rate water loss) were significantly affected by salinity (Table 2). The LMA was not affected by salinity at 200 and 400 mM compared with control but was improved by 22.2% at 600 mM of NaCl treatment. Our results are inconsistence with Munns and Termaat (1986) and Franco *et al*. (1997) who reported that NaCl highly reduced leaf mass area. Leaf mass per area is a composite structural parameter.

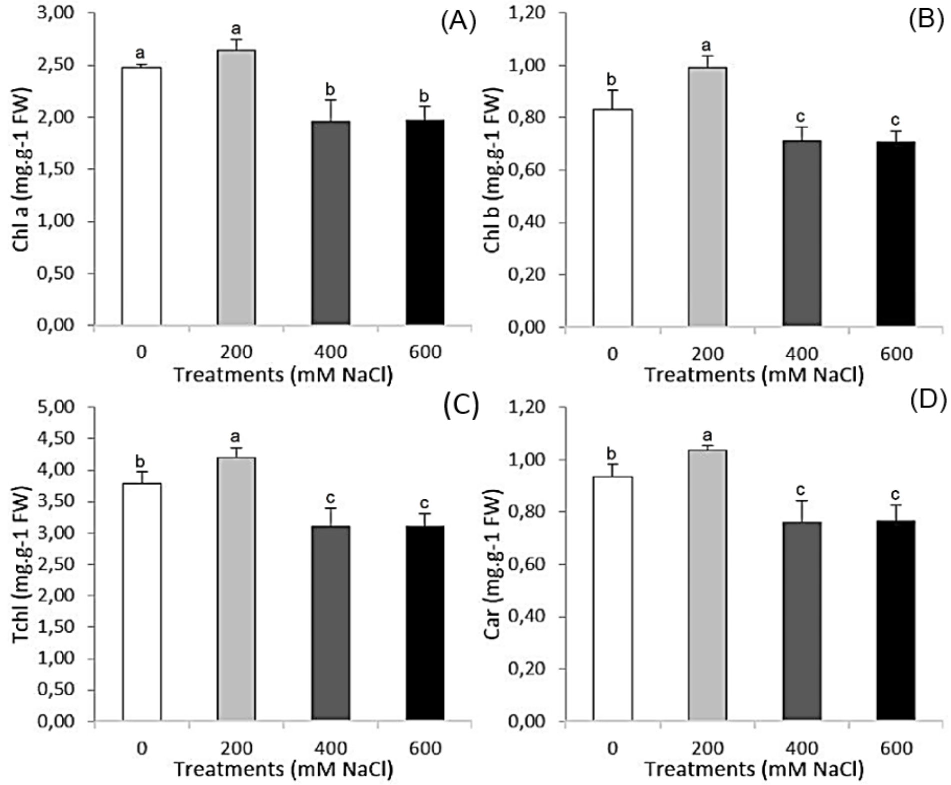

Figure 4. Effects of salt stress on (A) Total chlorophylls content, (B) Chlorophyll a content, (C) Chlorophyll b content and (D) Carotenoids content of *Acacia karroo* leaves after 21 days of various levels of saline treatments. Means, in each box, with similar letters are not significantly different at the 5% probability level using Duncan's test.

It is not only closely related to many physiological responses of plants, but also can measure the investment of dry mass per unit of light-intercepting leaf area (Poorter *et al.*, 2009). LMA is considered an important indicator of plant ecological strategies and has been studied widely in plant ecology, agronomy, forestry, and plant physiology (Liu and Liang, 2016).

The RWC decreased slightly with increase in salinity levels (Figure 3C). Indeed, salt treatments did not produce a notable change in the water content of the plants leaves, indicating a relatively high resistance as well to dehydration, which will certainly contribute to some degree of salt tolerance in *A. karroo*. Salt tolerance is also depending on the plant capacity to accumulate Na^+ and Cl^- in the vacuole, to avoid reaching toxic concentrations in the cytoplasm, a mechanism that is especially efficient in some succulent, highly tolerant dicotyledonous halophytes (Haque *et al.*, 2016).

RWL from excised leaves was significantly higher at 200 mM of NaCl by 5 mg/cm^2.min and similar at high level of NaCl compared with control (Figure 3D, Table 2). This improvement could be due to stomatal closure, it will typically induce the limitation of gas exchange and alter the rate of photosynthesis and metabolism (Wang and Nii, 2000). RWL has been suggested as a screening technique to identify genotypes under drought stress (Gunes *et al.*, 2008). Indeed, this trait is a direct measurement of plant water deficit and a good criterion for the selection of drought tolerant plants (Farshadfar *et al.*, 2001).

Chlorophylls and carotenoids

Chlorophyll a is the principal photosynthetic pigment while chlorophyll b is an accessory one. The result of ANOVA for the major effect of salinity showed that salt stress significantly decreased ($p < 0.0001$) the content of photosynthetic pigment in leaves (Table 2) at higher concentrations of NaCl (400 mM and 600 mM). Indeed, Chl a, Chl b, Tchl and Car were degraded by 20.5, 14.4, 17.7 and 18.2% of control, respectively, under extreme concentration of NaCl (400 and 600 mM NaCl). However, at 200 mM of NaCl, an enhancement of chlorophyll (b), total chlorophylls and carotenoids content was observed as compared to control (Figure 4). At the same level, chlorophyll (a) presented a constant content compared with control (Figure 4A).

The reduction of photosynthetic pigment content is likely due to chlorophyll degradation induced by toxic levels of NaCl (Hassanein *et al.*, 2009). These results are consistent with those reported by Theerawitaya *et al.* (2015), who indicated that chlorophyll content significantly decreased in the leaves of *A. ampliceps* with increasing NaCl concentration. Reduction of chlorophyll levels in salt-treated plants is due to the inhibition of chlorophyll synthesis, together with the activation of its degradation by the enzyme chlorophyllase. Yet, this is not the only reason for the inhibition of photosynthesis in the presence of salt, since NaCl also inhibits key enzymes involved in this process (Parihar *et al.*, 2015).

Under salt stress, leaf chlorophyll content could be altered due to impaired biosynthesis and accelerated degradation of the pigments (Mäkelä *et al.*, 2000). Therefore, the levels of photosynthetic pigments, such as Chl a and Chl b, are vital for steady photosynthesis in plants during salt stress (Richardson *et al.*, 2002). It has been reported that photosynthesis in some halophytes remains unaffected by salinity or even increases at low salinity (Flowers and Colmer, 2015). Increased chlorophyll and carotenoid content under saline stress may be related to a decrease in leaf area, it also can be a defensive response to reduce the harmful effects of drought stress (Farooq *et al.*, 2009).

CONCLUSION

In conclusion, although plants suffered from salt stress, as shown by the degradation of photosynthetic, they continued their vegetative growth and maintained their internal water potential under salinity conditions. Therefore, *A. karroo* is a potential halophytic species to be cultivated in saline lands and make it favourable for agroforestry practices. However, this screening is not sufficient for a complete characterization of *A. karroo* Hayne as a halophyte. It will be necessary to go further at the biochemical (e.g., proline, soluble sugar, ion accumulation) and molecular levels, and to explore other stages of development such as flowering and fruiting in response to salt conditions in situ.

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STUDY OF THE EVOLUTION OF CLAY MINERALS IN PADDY (Pa) AND NON-PADDY (NPa) SOILS ON A TOPOSEQUENCE IN GHAEMSHAHR CITY, MAZANDARAN PROVINCE

SUMMARY

This research was carried out to investigate the effect of rice cultivation and topography on the development of soil clay minerals in Ghaemshahr City in Mazandaran province. A total of 10 profiles were selected on a toposequence– five profiles with rice fields and five with rangeland land uses. The soils studied were classified as Inceptisols and Mollisols. The results of clay mineralogy show that the amount of smectite is higher in paddy (Pa) soils, while the amount of vermiculite is higher in non-paddy (NPa) soils. The higher presence of smectite in rice fields is probably due to the neoformation process. Also, smectite was found to be higher in lower regions with shallow groundwater. The cation exchange capacity, clay content, and bulk density are higher in the Pa soils. Rice cultivation increases the amount of iron extracted in oxalate (Fe_o) form and decreases the amount of iron extracted in dithionate (Fe_d) form. Also, during the toposequence from lower areas to areas with higher altitudes, the amount of Fe dithionate increased while Fe oxalate decreased respectively. The amount of pedogenic iron (Fe_d) has a direct correlation with the degree of evolution of the soil; this amount increased during the toposequence from low to higher regions.

Keywords: Clay mineralogy, evolution, toposequence, paddy soil, Ghaemshahr City

INTRODUCTION

Rice is an annual plant that is often cultivated under hydromorphic conditions. It is the most important source of food for more than 50% of the world's population and is cultivated across 155 million hectares in 114 countries. Pa soils are affected by intense human activities (Gong, 1983) because rice cultivation requires specific management (flooding, puddling, and drainage). The formation of Pa soil under the influence of flooding, puddling, and drainage generates redoximorphic properties. The formation of redoximorphic

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complications occurs with the accumulation and depletion of iron and manganese with the fluctuation of groundwater level in the soil (Zeng-Yei and Zeng-Sang 2001). In the management of rice cultivation, the soil is under flooding condition for a long time, resulting in different morphological, physical, chemical, and mineralogical characteristics.

The quantitative and qualitative identification of soil clay minerals is important in terms of not only their usage and management but also their evolution process. In addition to determining the soil genesis, evolution, and development manner, it can throw a broad scientific light on their formation and evolutionary process. These studies also provide valuable information on the status of the absorption, stabilization, and release of cations (Torabi Golsefidi, 2001).

Clay mineralogy studies in Pa soils have been extensively performed by Chang (1961), Egashira et al. (1997), Hassannezhad et al. (2008), Chen et al. (2011), and Han and Zhang (2013). By studying the Pa soils in China, Chang (1961) report that the combination of clay minerals in Pa soils is based on the combination of their parent materials. Hassannezhad et al. (2008) investigated the effect of rice cultivation on the clay mineralogy characteristics of soils in the Amol region of Mazandaran province, in which the hydromorphic conditions cause smectite neoformation. The study by Han and Zhang (2013) on the chronosequence of Pa soils in southern China shows that the evolution of clay minerals can be determined based on their compounds, which are strongly influenced by their parent materials. They also found that due to complex oxidoreduction processes, long-term rice cultivation could create major differences in the soil's morphology and properties; in contrast, in younger rice field soils, the combination of clay minerals is similar to the primary soils without cultivation. Comparing the physicochemical and mineralogical characteristics of Pa soils in the region of Noorabad and the adjacent virgin lands, Owliaie et al. (2014) found that rice cultivation has led to a sharp reduction in palygorskite and an increase in smectite in the Pa soil. The increase in smectite content in Pa soil, in addition to the deformation of palygorskite to smectite, is also likely to be related to the alteration of the illite and neoformation of smectite in the soil solution.

Absolute amounts of iron compounds, in addition to the pedogenic processes, are dependent on the parent material. Therefore, the values of Fe_d-Fe_o (iron oxide crystallization degree), Fe_o/Fe (amorphous iron to free iron ratio), and Fe_d-Fe_o/Fe_t (crystalline iron ratio) are used to compare the evolution of soils. The Fe_d-Fe_o index represents the amount of crystalline iron oxides. The largeness of this value indicates that most of the iron compounds are crystalline (Hassannezhad et al. 2008).

So far, no study has been done regarding the effect of rice cultivation and topography on the evolution and clay mineralogy of soil in the region of Ghaemshahr in Mazandaran province, this study was performed to investigate the aforementioned cases.

MATERIAL AND METHODS

Study area and sampling

The study took place in Qaemshahr, Mazandaran province, Northern Iran (Figure 1). The average annual temperature and precipitation in this region are 16 °C and 734 mm respectively. The soil moisture and temperature regimes in the study area are xeric and thermic respectively (Banaei, 1998). The study area is related to the fourth period of geology. The parent materials are alluvial. They are transported to the region due to the flow of rivers and floods; in proportion to the slope of the lands and the speed of the river water, coarse and fine sediments are left. For the purpose of this study, 10 profiles were selected during a toposequence—five profiles in Pa soils and five in NPa soils. Excavated profiles are described based on the American classification (soil taxonomy, 2014) (Table 1). From its different horizons, an intact and a non-intact sample have been prepared to measure the physical and chemical parameters of the soil.

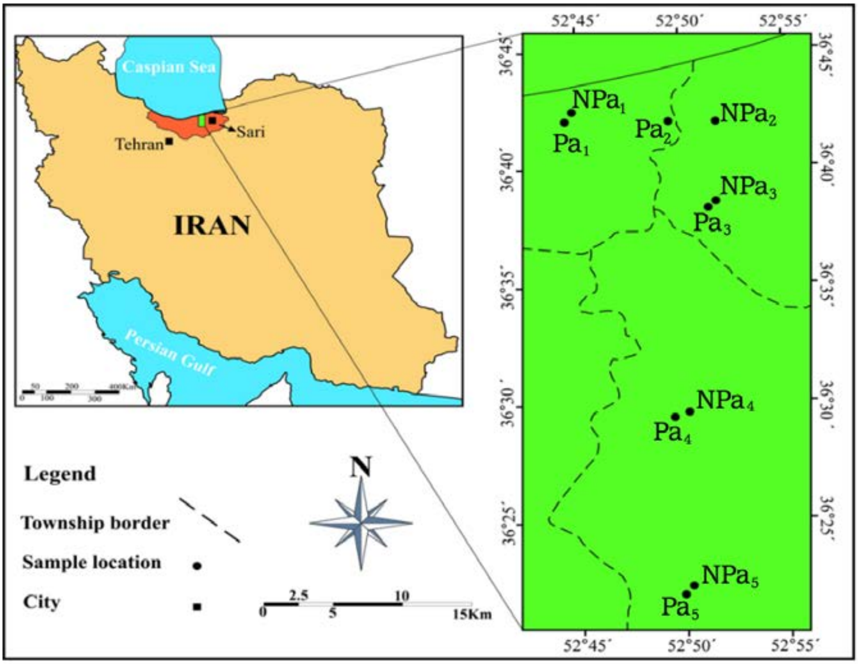

Figure 1. Location of the study area and sampling sites

Physicochemical analysis

The distribution of soil particles size was determined using the hydrometer method (Bouyoucos, 1962). The organic carbon content of the soil samples was evaluated by wet oxidation using chromic acid and by return titration with ferrous ammonium sulfate, based on Nelson's method (1982). The pH of the soil samples was measured with a pH electrod in a suspension of 1:1 (soil: H_2O). The sodium acetate method was used at pH 8.2 to measure soil CEC (Chapman, 1965). The titration method was used to determine the amount of carbonate (US Salinity Laboratory Staff, 1954). The amount of iron extractable by dithionate (Fed) in soil samples was determined using the Mehra and Jackson method. Soil samples were dried and weighed in oven for 24 hours at 105 °C and the paraffin method was used to determine the bulk density (Blake and Hartge 1986). The iron extractable by ammonium oxalate was measured using 0.3M ammonium oxalate at pH 3 in a dark place (McKeague et al. 1971).

Mineralogy analysis

The methods given by Mehra and Jackson (1960) and Kittrick and Hope (1963) were used to eliminate the cementations chemical agents and separate the clay particles from each other. To separate clay particles (<2 μm), iron-free samples were centrifuged for 4.5 minutes at 750 rpm (Kittrick and Hope 1963). For X-ray analysis on the clay section, a Phillips diffractometer was used (Jackson, 1975). To obtain an acceptable comparison between the relative intensity of the peaks in different samples, equal concentrations of clay suspensions were used. After saturation with potassium, magnesium, and ethylene glycol, the 001 reflection was considered. Potassium-saturated samples were heated at 550 °C for four hours. The semi-quantitative evaluation of clay minerals was performed based on the levels of sub-peaks resulting from X-ray diffraction in the glycerol treatment of samples (Johns and Grim, 1954).

RESULTS AND DISCUTION

Physico-chemical properties

The major physicochemical and morphological properties of the studied soils are presented in Table 1. As shown in Table 1, the studied Pa soils and NPa soils are classified in two categories of Inceptisols and Mollisols respectively, according to the soil taxonomy (2014).

The color of the soils varies from 2.5Y to 10YR according to the Munsell soil chart. Pa soils have poor drainage and the presence of hydromorphic conditions over the years causes the 2.5Y hue. There is a great difference between the textures of Pa and NPa soils (from clay to silty clay). The highest amount of clay was observed in the lowland of the region (Table 1), which can be explained by the sedimentation of clay-sized particles leached from higher regions (Khormali and Ajami 2009). Wilding et al. (1982) report that the soil texture in the lowland is heavier than other areas in the same region. According to Table 1, the NPa_5 profile has the coarsest texture in comparison to other profiles (silty clay loam). The cation exchange capacity (CEC) in Pa soils is higher than that in rangeland fields due to the higher percentage of clay in rice-cultivated land.

This case can also be justified on the basis of the clay mineralogy, because NPa soils have a greater amount of smectite than others. The percentage of calcium carbonate varies from 2% on the horizon C of the NPa_4 profile to 27% on the horizon A of the Pa_4 profile. The bulk density (BD) has been shown to be very variable in both land uses; it is higher in the Pa soils than in rangeland soils.

Table 1. Morphological and physicochemical properties of the studied soils

horizon	Depth (cm)	Color (moist)	pH	CEC[a] (cmol(+) kg^{-1})	sand (%)	silt (%)	clay (%)	Textural class[b]	CCE[c] (%)	OC[d] (%)	BD[e]
					NPa$_1$, Haplaquolls						
A	0-20	10YR3/2	7.76	32	4	44	52	SiC	15	1.26	1.78
AB	20-40	2.5Y3/2	7.80	34.1	8	44	50	SiC	16	1.19	1.81
B_g	40-60	2.5Y5/3	8.02	34.1	6	48	46	SiC	14	0.86	1.84
BC_g	60-100	2.5Y4.5/3	8.22	34.5	6	44	50	SiC	18	2.15	1.80
					Pa$_1$, Haplaquepts						
A_p	0-15	2.5Y4.5/4	7.79	41.8	10	32	58	C	19	1.02	1.89
AB_g	15-40	2.5Y4.5/4	7.89	38	8	33	59	C	18	1.06	1.96
B_g	40-70	2.5Y4.5/3	7.82	37	7	37	56	C	23	0.16	1.95
BC_g	70-100	2.5Y4/2	7.93	32	8	42	50	SiC	20	0.62	1.94
					NPa$_2$, Calciaquepts						
A	0-10	10YR4/4	6.04	38.3	14	40	46	SiC	4	3.06	1.83
AB	10-35	2.5Y3/2	7.65	35.8	10	41	49	SiC	7	1.17	1.82
B_{kg1}	35-70	2.5Y4/2	7.57	30.0	14	42	44	SiC	18	0.66	1.80
B_{kg2}	70-100	2.5Y4/1.5	7.50	34.9	13	42	45	SiC	14	0.64	1.81
C_g	100-130	2.5Y4/1.5	7.47	21.6	12	40	48	SiC	12	0.46	1.78
					Pa$_2$, Haplaquepts						
A	0-20	10YR4/3	8.04	45	10	36	54	C	13	2.03	1.99
B_{wg1}	20-50	2.5Y3/4	7.83	35	12	38	58	C	5	0.47	1.99
B_{wg2}	50-70	2.5Y3/4	7.78	28.3	14	40	56	C	5	1.40	1.91
C_g	70-90	2.5Y3/5	7.70	31	8	42	50	SiC	10	1.63	1.89
					NPa$_3$, Calciaquolls						
A	0-25	10YR3/2	7.24	38	9	42	49	SiC	11	3.54	1.78
B_g	25-55	2.5Y3.5/2	7.80	33	11	41	48	SiC	13	0.78	1.72
C_{kg}	55-80	10YR4/2	7.99	32	14	34	52	C	18	0.51	1.70
					Pa$_3$, Cslciaquepts						
A_{pg}	0-20	2.5Y4/1	7.75	41	12	36	52	C	13	1.62	1.90
B_g	20-45	2.5Y4/1	7.70	42	12	34	54	C	12	1.24	1.92
B_{kg}	45-65	2.5Y4/2	7.81	44.1	10	34	56	C	13	0.62	1.91
C_g	65-90	2.5Y4/2	8.01	40	18	32	50	C	9	0.64	1.89
					NPa$_4$, Haploxerolls						
A	0-19	10YR3/2	7.67	30	17	41	42	SiC	9	1.48	1.70
B_{w1}	19-45	10YR3/3	7.78	28.7	17	43	40	SiC	10	0.78	1.68
B_{w2}	45-65	10YR3/4	7.84	27.9	19	42	39	SiCL	5	0.55	1.72
C	65-100	10YR3/3.5	7.69	31.2	23	35	42	C	2	0.4	1.71
					Pa$_4$, Endoaquepts						
A_g	0-18	2.5Y4/1	7.65	40	20	29	51	C	27	1.87	1.97
CB_g	18-45	2.5Y4/4	7.85	32	17	30	53	C	24	0.66	1.95
C_g	45-90	10YR4/6	7.77	30	26	34	40	C	25	0.35	1.92
					NPa$_5$, Calcixerolls						
A	0-27	10YR3/2	7.75	32.4	16	44	40	SiCL	9	1.37	1.67
AB	27-44	10YR3/3	7.81	29.9	18	43	39	SiCL	3	0.73	1.65
B_K	44-66	10YR3/4	7.77	32	20	41	39	SiCL	12	0.8	1.68
CB	66-90	10YR3/3.5	7.65	22.8	21	37	42	C	13	1.0	1.63
					Pa$_5$, Haplaquepts						
A_{pg}	0-21	2.5Y3.5/2	7.28	37	18	32	50	C	16	1.84	1.92
B_{g1}	21-47	2.5Y3/3	7.82	32	15	35	50	C	18	1.18	1.93
B_{g2}	47-65	2.5YR4/1	7.81	30	19	34	47	C	17	1.08	1.90
C_g	65-95	2.5YR3/1	7.92	30	20	35	45	C	10	0.62	1.91

[a]CEC: Cation Exchange Capacity
[b]C: clay, si: Silty, L: loam, S: sandy.
[c]CCE: Calcium Carbonate Equivalent
[d]OC: Organic Carbon
[e]BD: Bulk Density

Table 2. Concentration of various forms of iron and their ratio in studied soils

horizon	Fe_d	Fe_o	Fe_o/Fe_d
	(g/kg)		
	NPa_1, Haplaquolls		
A	20.55	5.62	0.27
AB	20.36	5.45	0.26
B_g	20.20	4.92	0.24
BC_g	19.78	4.86	0.24
	Pa_1, Haplaquepts		
A_p	15.15	5.82	0.38
AB_g	15.86	5.66	0.35
B_g	15.52	5.35	0.34
BC_g	15.01	5.01	0.33
	NPa_2, Calciaquepts		
A	20.48	4.2	0.20
AB	21.86	3.56	0.16
B_{kg1}	21.14	3.92	0.18
B_{kg2}	20.20	3.47	0.17
C_g	20.14	3.04	0.15
	Pa_2, Haplaquepts		
A	18.47	4.5	0.24
B_{wg1}	17.52	4.42	0.25
B_{wg2}	17.12	4.7	0.27
C_g	16.96	4.83	0.28
	NPa_3, Calciaquolls		
A	23.33	3.01	0.12
B_g	23.1	2.60	0.11
C_{kg}	22.61	2.45	0.10
	Pa_3, Calciaquepts		
A_{pg}	19.94	3.35	0.16
B_g	19.36	3.18	0.16
B_{kg}	19.62	2.95	0.15
C_g	19.20	2.80	0.15
	NPa_4, Haploxerolls		
A	25.40	2.62	0.10
B_{w1}	25.24	2.65	0.10
B_{w2}	24.87	2.44	0.09
C	24.48	2.33	0.09
	Pa_4, Endoaquepts		
A_g	22.8	2.73	0.11
CB_g	22.62	2.65	0.11
C_g	21.89	2.48	0.11
	NPa_5, Calcixerolls		
A	25.02	2.45	0.09
AB	24.56	1.90	0.07
B_K	26.95	1.75	0.06
CB	25.24	1.50	0.05
	Pa_5, Haplaquepts		
A	23.95	2.34	0.09
B_{g1}	23.78	2.71	0.08
B_{g2}	23.2	2.65	0.08
C_g	23.45	2.15	0.07

Table 2 shows the concentrations of different forms of iron (Fe_d and Fe_o) and their ratios (Fe_d/Fe_o) in all studied soils. In most studies, soil age is measured based on the morphological characteristics and the amount of pedogenic iron and clay (Costantini and Damiani 2004). Detier et al. (2012) report that the most important forms of iron in the soil are Fe_t (total iron), Fe_d, and Fe_o. Therefore, (Fe_d-Fe_o) shows the amount of crystalline form of Fe, Fe_o/Fe_d indicates the degree of iron crystallization, and Fe_d-Fe_O/Fe_t represents the ratio of crystallized iron to total iron (Schaetzl, 2005). Rezapoor et al. (2010) studied different forms of Fe oxides and their dispersion in the Northwest of Iran, and report that increased weathering leads to increased Fe_d and decreased Fe_o/Fe_d ratio. In this study, Pa soils were found to have a higher Fe_o content than NPa soils, while NPa soils had a higher content of Fe_d. Based on the results obtained, the Fe_o content in all profiles decreases with increasing depth. Decreasing organic matter in depth leads to microbial activity reduction, resulting in a decrease in the conversion rate of Fe^{3+} to Fe^{2+} (Bouma, 1990). In hydromorphic conditions, the possibility of converting amorphous iron oxides and hydroxides into crystals is minimal (Scarciglia et al. 2011). The Fe_d amount is reduced from surface to depth in all profiles, except NPa_5. Fe_o/Fe_d ratios for all profiles except Pa_2 decreases with increasing depth. The amount of Fe_d increases with the increase in the weathering of iron-containing minerals, but the Fe_o/Fe_d ratio—which indicates the ratio of amorphous iron to crystalline iron—decreases. This index, which is used to compare the evolution of soils, decreases with increasing evolution.

In hydromorphic conditions and lack of oxygen, iron is formed as Fe (II); in such conditions, formation of iron oxides is less, resulting in an increase in Fe_o/Fe_d ratio. According to McKeague and Day (1966), if the Fe_o/Fe_d ratio is greater than 0.25, it indicates hydromorphic conditions. In old Pa soils, the continuation of iron accumulation leads to the formation of a rich horizon of iron (Gong, 1986). In this study, the B_k horizon of NPa_5 profile has the highest amount of Fe_d and the lowest amount of Fe_o; in contrast, the C_g horizon of Pa_1 profile has the lowest amount of Fe_d and the highest amount of Fe_o. Compared to rangeland soils, Pa soils had more amounts of Fe_o, resulting in higher Fe_o/Fe_d values (as much as 1.36 times). During the topography row, this ratio is reduced from lower regions to regions at higher altitudes, indicating that the soils of regions at higher altitudes are more developed.

Mineralogy analysis

The results of clay mineralogy of the studied soils are shown in Table 3 and Figure 2. According to this table, illite, chlorite, smectite, kaolinite, vermiculite, and interlayer minerals such as illite-smectite and illite-vermiculite have been observed in clay fraction of soils.

Illite is dominant in most of profiles due to its heritable origin in these soils (Wilson, 1999; Khormali and Abtahi 2003). This mineral has the highest frequency in almost all profiles (Table 3 and Figure 2). In almost all profiles, the amount of illite increases with increasing depth, indicating that the mineral has hereditary origin. Due to the relatively poor condition of moisture in the xeric moisture regime compared to that in the udic regime for the conversion of illite to other minerals, the amount of illite is high. Khormali et al. (2003) argue that illite is converted to other minerals, which are often smectite.

Table 3. Mineralogical composition of clay fractions of studied samples

Profile	Horizon	Depth(cm)	Sme	Ill	Ver	Kao	Chl	Ill-ver Ill-Sme
NPa_1	A	0-20	x	xx	xx	x	tr	-
	Bg_1	40-60	xx	xx	xx	tr	-	x
	BCg	60-100	x	xxx	tr	x	xx	-
Pa_1	Ap	0-15	-	xxx	x	tr	x	-
	Bg	40-70	xxx	xx	-	tr	tr	-
	BCg	70-100	x	xxx	-	tr	xx	-
NPa_2	A	0-10	xx	xx	x	tr	x	-
	Bkg_1	35-70	xx	xx	xx	tr	tr	x
	Cg	100-130	xx	xxx	x	x	-	-
Pa_2	A	0-20	-	xx	xx	x	x	-
	Bw_1	20-50	xxx	x	x	tr	tr	-
	Cg	70-90	xxx	xx	x	x	tr	-
NPa_3	A	0-25	x	xxx	-	tr	xx	-
	Bg	25-55	x	xx	xxx	tr	tr	-
	Ckg	55-80	x	xxx	xx	x	tr	-
Pa_3	Apg	0-20	xxx	xx	-	tr	x	-
	Bkg	45-65	xxx	xx	tr	x	x	-
	Cg	65-90	xx	xxx	x	tr	tr	-
NPa_4	A	0-19	x	xx	xxx	x	tr	-
	Bw_2	45-65	x	xx	xx	x	tr	-
	C	65-100	x	xxx	xx	x	-	-
Pa_4	A	0-18	xx	xx	x	x	tr	-
	CBg	18-45	xx	xx	x	x	tr	-
	Cg	45-90	xx	xxx	-	x	tr	-
NPa_5	A	0-27	x	xx	xx	x	x	-
	Bk	44-66	x	x	xxx	x	tr	-
	CB	66-90	-	xxx	xxx	x	tr	-
Pa_5	A	0-21	xx	xx	x	x	x	-
	Bg_2	47-65	xx	xx	x	x	tr	-
	Cg	65-95	-	xxx	xxx	x	tr	-

xxx= large(25-50%) xx=moderate (10-25%) x=small (<10%)
tr= Trace (<5%) -= Not detected Sme= Smectite
Ill= Illite Ver= Vermiculite Kao= Kaolinite
Chl= Chlorite

Chlorite is detectable in a small amount in X-ray fluorescents in clay fraction of all profiles. No change in the peak of 14 Angstrom in magnesium glycerol and potassium with a temperature of 550 °C treatment indicates this mineral (Fig 2). The origin of this mineral in these soils is hereditary (Wilson, 1999; Khormali and Abtahi 2003). This mineral often originates from metamorphic rocks with low to moderate degrees (Grim, 1968). Barnhisel and Bertsch (1989) report that in acidic conditions (expandable minerals 2:1), first the brucite layer (hydroxy interlayer) and then pedogenic chlorite are formed. Illite and chlorite are abundant in areas where soil formation is limited (Wilson, 1999). The abundance of these two minerals in the soil is generally due to the presence of parent materials.

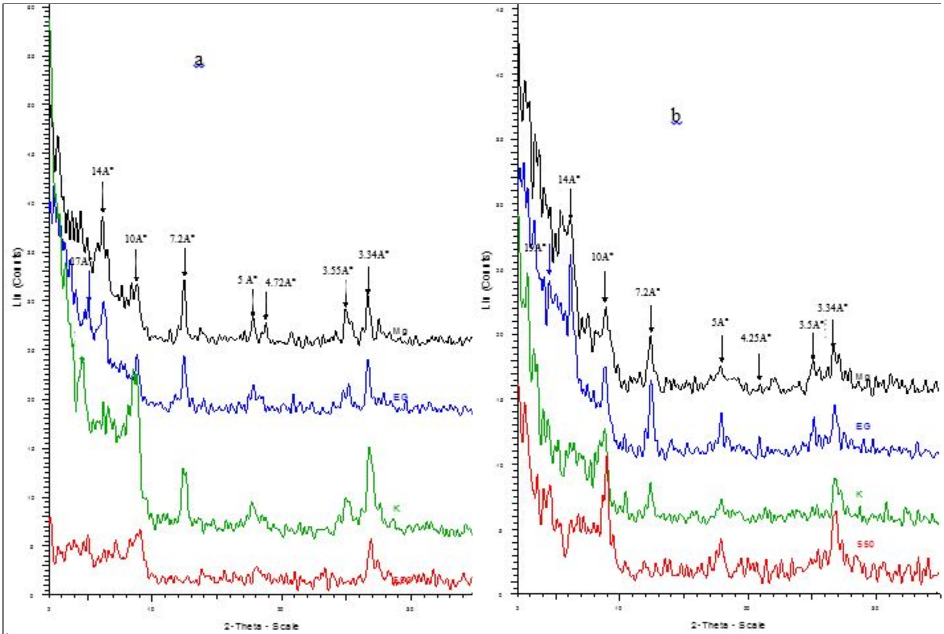

Figure 2. X-Ray diffraction patterns of clay fraction for the representative soil profiles. (a) B_g horizon of NPa_1, (b) B_g horizon of Pa_1

The presence of vermiculite has been reported in most soils of Iran (Khormali and Abtahi 2001 and 2003). Khormali and Abtahi (2003) state that vermiculite is unstable under high pH conditions with the presence of Si and Mg^{2+} and low Al solubility. An increase of Angstrom 10 peak in potassium and heat treatment is probably due to the presence of vermiculite (Figure 2a). There is a lot of vermiculite in NPa soils, mainly due to leaching conditions and the removal of potassium from mica. The probability of formation of vermiculite as an intermediate product of mica and its subsequent conversion to smectite has been confirmed (Douglas, 1989).

In this study, smectite is the dominant mineral of Pa soils (Figure 2b) because these soils are under poor drainage and submerging conditions for several months of year. Under these conditions, iron is reduced; the Si, Al, and Mg ions are dissolved from the higher regions and provide conditions for the neoformation of the smectite (Borchart, 1989). Khormali and Abtahi (2003) describe there are three main sources of smectite in soils: 1. neoformation in soil solution, 2. detrital origin or inheritance, and 3. transformation of other clay minerals. Hassannezhad et al. (2008) report that natural or human-induced flooding conditions lead to increase in smectite. Ghergherechi and Khormali

(2008) studied the effect of groundwater level and the type of land use on the origin and distribution of clay minerals. They found that in soils with poor drainage, the amount of smectite was maximum and was mostly from the neoformation process.

Kaolinite is present on all horizons in a low amount. Considering that Khormali and Abtahi (2003) expressed the acidic conditions with the average activity of Si and the low alkaline cations suitable for the neoformation of kaolinite from the soil solution, the studied soils are not suitable for the formation of kaolinite. It can be said that the reason for the presence of this mineral is its hereditary origin in these soils. Angstrom 7.2 peak in the magnesium saturation treatment is related to this mineral (Figure 2).

Illite-smectite and illite-vermiculite have been observed only in the profiles of NPa_1 and NPa_2. These types of mixed layer minerals are very common in soils where illite is formed from smectite (Moore and Reynolds 1997).

A semi-quantitative study of minerals during the toposequence shows that smectite content in lowland with higher groundwater was higher; in contrast, the vermiculite content was higher in highlands with better drainage. The changes in other clay minerals were not significant.

CONCLUSIONS

The results show that Pa soils have less evolution due to flooding and are classified in Inceptisols, whereas in the rangelands, the soils have more evolution, and except in one case, the soils were classified in Mollisols. Clay mineralogy studies show that illite is the dominated clay mineral in most of the soils. Rice cultivation and management result in flooding and hydromorphic conditions; the presence of these conditions and poor drainage can lead to an increase in the amount of smectite. The smectite is probably formed due to the neoformation process in these soils. In contrast, in NPa soils, the amount of vermiculite is higher than that of smectite. Also, the results show that in the toposequence, from the lowland to the highland, the amount of smectite is reduced while the amount of vermiculite is increased.

Rice cultivation has caused changes in the physical, chemical, and mineralogical characteristics of the studied soils. The soil BD in Pa soils has increased due to the compaction caused by soil tillage operations. The CEC is higher in this usage, which can be explained by the amount and type of clay mineral (presence of more smectite). The amount of pedogenic iron (Fe_d) has a direct correlation with the degree of soil evolution; this amount increases during toposequence from lowland areas to higher elevations and is also higher in rangeland soils than in Pa soils. However, the amount of iron extracted with oxalate is higher in the lower areas and in Pa soils. The average ratio of Fe_d/Fe_o in Pa soils is 1.36 times higher than that in NPa soils.

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PHENOTYPE CORRELATION BETWEEN PRODUCTION TRAITS AND BODY WEIGHT OF HEAVY BROILER BREEDER HENS

SUMMARY

Research was conducted on two broiler parent flocks of Ross 308 and Cobb 500 hybrids. At the beginning of productive cycle (24 weeks of age) for Ross 308 hybrid average body weight of laying hens was 2680.40g, while for Cobb 500 hybrid it was 2697.80g. In the middle of the productive cycle (42nd week) body weight was 3565.10 g (Ross 308) and 3599.05 g (Cobb 500), while at the end of productive cycle (61st week) body weight for Ross 308 hybrid was 3841.50 g, and for Cobb 500 3850.00 g. Determined differences in body weights of laying hens in specific periods of productive cycle (17.40 g, 33.95 g and 8.50 g), as well as difference in weight for entire productive period (23.26 g) were not statistically significant (P>0.05). More thorough research of broiler laying hen body weight influence on productive performances was done by determining phenotype correlation coefficient between researched parameters. Therefore between hen body weight and most of productive parameters statistically significant (P<0.001; P<0.01; P<0.05) phenotype correlation coefficients were determined, while between body weight of laying hens and laying intensity of breeding and fertilized eggs determined correlation coefficients were not statistically significant (P>0.05).

Keywords: broiler breeder, laying hens, body weight, production, correlation.

INTRODUCTION

Productive abilities of broiler parents are influenced by many non-genetic factors. One of important non-genetic factors that influences productive performances is change inbody weight of laying hens during productive cycle (Savić et al., 2004; Ciacciariaello et al., 2005; Vieira et al., 2005; Almeida et al., 2006; Đermanović et al., 2005; 2008; 2010; Djermanovic, 2010; Mitrović et al., 2005; 2009; 2010; 2011; Pandurevic et al., 2013; Djermanovic et al., 2009; 2016; 2017). Proper hormonal function of endocrine system of laying hens is significantly influenced, next to age and photo stimulation, by body development of breeding animals (Lewis et al., 2005; Lewis and Gous, 2006; 2007; Usturoi et al., 2007). At optimal body weight at specific age ovaries are stimulated and maturing speed of egg cells is increased (egg production).

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Only with proper diet and adequate utilization technique of the flock pre condition for necessary laying hen vitality and incubation egg quality can be created (Barnett et al., 2004; Luquetti et al., 2004; Maiorka et al., 2004). In order for fertilized egg production to last for longer time period it is needed to keep laying hens in reproductive condition, where special attention should be given to their development during productive cycle.

For the most of heavy line hybrids egg production starts in 24th week when laying intensity is about 5% and more. From that period egg production is increased till it reaches maximum and then productivity of broiler parents in smaller or higher rate deteriorates. As parameter for more through research of parent flock body weight influence on productive parameters determined phenotype correlation coefficients between laying hen body weight and productive parameters during productive cycle can have significant contribution.

MATERIALS AND METHODS

In this research two parent flocks of heavy hybrids Ross 308 and Cobb 500 were taken. During productive cycle breeding technology suggested by selection scientists for this type of hybrids was used. Flocks were reared till 61st week of age, both flocks started laying at the beginning of 22nd week, and for incubation eggs laid from 24th week and until the end of production cycle were used, because in that period eggs had minimal weight needed for incubation (>50.00 g). Therefore egg production period lasted 38 weeks.

As starting experimental material total number of 5200 birds of both sexes Ross 308 and Cobb 500 were used, they were reared in two separate facilities. First facility was inhabited by 4750 ♀ and 450 ♂ Ross 308 hybrid birds, and in second 4960 ♀ and 470 ♂ Cobb 500 hybrid birds, so that gender ratio was 1:10.56 (Ross 308) and 1:10.55 (Cobb 500). In preparation period from 21st until 24th week mortality and exclusion for Ross 308 was 13 birds (0.279%), and for Cobb 500 12 birds (0.24%). Which further means that at the beginning of utilization of eggs for incubation there were 4737 Ross 308 parents in the flock, and 4948 for Cobb 500 flock.

In order to control body weight, 200 Ross 308 and Cobb 500 laying hens individual body weights were taken, by method of random sample. Using this readings uniformity of laying hens of researched flocks during production cycle was monitored, then, influence of laying hen body weight on broiler parent production parameters was researched (brooding egg laying intensity, fertilized egg laying intensity, egg weight, daily food consumption per bird, food consumption per brooding egg and food consumption per fertilized egg).

Basic data analysis was done by applying variation - statistical methods, testing of the differences between hybrids was done with T-test. Moreover, obtained results were used to calculate correlation of researched parameters by applying correlation analysis. Statistical data rendering was done using statistical program SAS/STAT (2000).

RESULTS AND DISCUSSION

Average values, variability and significance of laying hen body weight in specific periods of production cycle, and for entire period of egg production is shown in Table 1.

Table 1. Average values, variability and significance of laying hen body weight (g) weight in specific periods of production cycle (Djermanovic et al., 2017).

Production cycle period	Weeks of age (production)	Hybrid	$\bar{x}$ ±SEM	S	$\bar{d}$
Beginning	24 (1)	Ross 308	2680.40±14.63	206.93	17.40^{ns}
		Cobb 500	2697.80±17.09	241.66	
Middle	42 (19)	Ross 308	3565.10±19.86	280.92	33.95^{ns}
		Cobb 500	3599.05±20.12	275.28	
End	61 (38)	Ross 308	3841.50±21.39	302.56	8.50^{ns}
		Cobb 500	3850.00±21.68	306.59	
Entire production cycle	61 (38)	Ross 308	3411.15±61.58	394.33	23.26^{ns}
		Cobb 500	3434.41±61.03	390.76	

$^{ns}P>0.05$.

Data from table 1 shows that average body weight of laying hens of both hybrids gradually increased during the production cycle. Body weight of laying hens in 24th week was 2680.40 g (Ross 308) and 2697.80 g (Cobb 500), and at the end of the cycle 3841.50 g for Ross 308 and 3850.00 g for Cobb 500. During the production cycle Cobb 500 laying hens compared to Ross 308 hens had higher average body weight which was not statistically significant (P>0.05). Average body weight for Ross 308 hybrid for entire cycle was 3411.15 g, and for Cobb 500 3434.41 g, where difference of (23.26 g) in laying hen body weight between researched hybrids was not statistically significant (P>0.05), and that shows that genotype haven't had significant influence on laying hen body weight.

Body weight of researched hybrids was slightly higher than the weight proposed by their genetic potential. However, in their researches (Djermanovic, 2010; Djermanovic et al., 2009; 2016; 2017; Mitrovic et al., 2010; 2011; Pandurevic et al., 2013) other authors also obtained similar results regarding the body weight of laying hens of above mentioned hybrids. Depending on breeding conditions and technologies used, some researchers have achieved different results, where for broiler parents of Ross 308 hybrid they detected slightly lower body weight in 60th week of age which was between 3988.95 g and 3990.44 g (Usturoi et al., 2007). Moreover, on the contrary to above mentioned in 60th week for Cobb 500 significantly higher body weight of laying hens was determined, it was between 4.21 and 4.25 kg (Lewis et al., 2005; Lewis and Gous, 2006), and in 59th week of age (Lewis and Gous, 2007), significantly higher body weight for Ross 308 was determined Ross 308 (4.43 kg) and Cobb 500 (4.56 kg).

Production abilities of parent flocks depend on large number of para genetic factors and mostly from laying hen body weights. Therefore, special attention should be given to larger number of parameters (Table 2) on which success of specific type of production depends.

Table 2. Average values, variability and difference significance of broiler parents production parameters

Parameters	Hybrid	$\bar{x}$ ±SEM	S	$\bar{d}$
Brooding eggs laying intensity %	Ross 308	59.29±2.62	16.16	1.39^{ns}
	Cobb 500	60.68±2.67	16.45	
Fertilized eggs laying intensity %	Ross 308	56.92±2.54	15.66	1.15^{ns}
	Cobb 500	58.07±2.56	15.77	
Egg weight, g	Ross 308	62.03±0.80	4.96	0.22^{ns}
	Cobb 500	62.25±0.90	5.57	
Daily feed consumption per bird g/day	Ross 308	173.59±1.89	11.68	4.05^{ns}
	Cobb 500	177.64±1.95	12.01	
Food consumption per brooding egg g/egg	Ross 308	325.25±22.45	138.38	2.03^{ns}
	Cobb 500	327.28±23.39	144.16	
Food consumption per fertilized egg g/egg	Ross 308	339.38±23.71	146.18	2.54^{ns}
	Cobb 500	341.92±24.28	149.66	

$^{ns}P>0.05$

Similar to the values of average body weights of laying hens from table 1, between researched parameters for broiler parents, no statistically significant (P>0.05) differences were determined (Table 2). Regardless to genotype other authors who researched this subject concluded that with age of the flock, increase of the body weight of broiler parents laying hens of different genotype egg weight increases. Same as for laying hen body weight, for egg weight some authors obtained similar values (Lewis and Gous, 2007; Djermanovic et al., 2016; 2017), higher (Luquetti et al., 2004; Vieira et al., 2005; Almeida et al., 2006) and lower values (Barnett et al., 2004; Maiorka et al., 2004; Ciacciariaello et al., 2005). However, in contrast to laying hen body weight and egg weight, laying intensity depending on exploitation period of the flock is variable. Therefore, results of some research point out at similar (Savić et al., 2004; Đermanović et al., 2005; 2008; 2010; Djermanovic, 2010), and totally different values (Mitrović et al., 2005; 2009) when it comes to laying intensity of brooding and fertilized eggs in approximately similar period of flock exploitation.

Next to determined variation results for body weight of laying hens and productive parameters of analyzed parent flocks, with aim to better understand influence of body weight of laying hens on productive performance, phenotype correlation coefficients between researched parameters were calculated (Table 3).

Table 3. Phenotype correlation between laying hen body weight and productive parameters of broiler parents.

Paramenter	Hybrid	Correlation coefficients
Body weight hens (g) : Brooding egg laying intensity (%)	Ross 308	0.046^{ns}
	Cobb 500	0.122^{ns}
Body weight hens (g) : Fertilized egg laying intensity (%)	Ross 308	0.045^{ns}
	Cobb 500	0.122^{ns}
Body weight hens (g) : Egg weight (g)	Ross 308	0.986^{***}
	Cobb 500	0.981^{***}
Body weight hens (g) : Daily feed consumption per bird (g/day)	Ross 308	0.376^{**}
	Cobb 500	0.669^{***}
Body weight hens (g) : Food consumption per brooding egg, g/egg	Ross 308	-0.305^{*}
	Cobb 500	-0.272^{*}
Body weight hens (g) : Food consumption per fertilized egg, g/egg	Ross 308	-0.307^{*}
	Cobb 500	-0.270^{*}

* P<0.05; ** P<0.01; *** P<0.001; ^{ns} P>0.05.

For both researched hybrids between hen body weight and brooding, fertilized egg laying intensity for entire productive cycle phenotype correlation coefficients were determined and they were not statistically significant (P>0.05). However, between body weights of laying hens and egg weight complete correlation was determined for both parent flocks (P<0.001). Moreover, between body weight of laying hens and feed consumption per birds and produced eggs statistically significant (P<0.05; P<0.01; P<0.001) phenotype correlation coefficients were determined. Determined correlation coefficients between laying hen body weight and food consumption for brooding and fertilized eggs were negative, and between hen weight and daily feed consumption per bird results were positive (Table 3).

Between hen body weight and egg weight total correlation was determined for both parental flocks, and between hen body weight and food consumption per bird and per produced eggs statistically significant (P<0.05; P<0.01; P<0.001) correlation coefficients were determined. Depending on breeding conditions and technology used some researchers determined similar (Djermanovic, 2010; Đermanović et al., 2005; 2008; 2010; 2016; 2017; Mitrovic et al., 2010), and totally opposite values (Mitrović et al., 2009) of correlation coefficients connected to link between hen body weight and productive performances for different broiler parent genotypes.

CONCLUSION

Compared to technological normative for researched hybrids, average laying hen body weight was lower, at the start and at the end of production cycle. However, differences between body weights of laying hens for both hybrids were not statistically significant (P>0.05), genotype had no significant influence on hen body weight.

Based on calculated phenotype correlation coefficient and their significance it can be concluded that body weight of laying hens had significant influence to production performances because for both parent flocks, between laying hen body weight and most of monitored parameters statistically significant (P<0.001; P<0.01; P<0.05) correlation coefficients were determined, while between body weight of laying hen and laying intensity for brooding and fertilized eggs determined correlation coefficients were not statistically significant (P>0.05). From above said it can be noted that with increase of laying hen body weight production ability of laying hens decreases which leads to shorter productive cycle.

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IMPROVEMENT THE EVAPOTRANSPIRATION ESTIMATES USING REMOTE SENSING TECHNIQUES AND FUZZY REGRESSION

SUMMARY

Through the use of remote sensing techniques, multiple regression analysis is related to evapotranspiration computed from two components: thermal infrared and spectral reflectance bands. Improvement the regression analysis with emphasis on the used components is the aim of this research which the vegetation indices is the desired one. In this regard, the performance of Soil Adjusted Vegetation Index (SAVI) in some synoptic stations of East Azarbaijan Province (Ahar, Tabriz and Mianeh stations) was compared with Normalized Difference Vegetation Index (NDVI) performance. Increasing of L in the SAVI calculation caused the increasing of estimated evapotranspiration. The change of vegetation index led to error decreasing for example the value of RMSE decreasing was 14.29% and 9.9% in case of Mianeh and Ahar stations, respectively. The vegetation cover was the main factor in improvement of evapotranspiration estimates using SAVI. The precise estimation of fuzzy regression parameters is more important which the variation of confidence level had no effect on the center of fuzzy number but the increasing of confidence level parameter led to increasing the spread of fuzzy number.

Keywords: Remote Sensing, Evapotranspiration, SAVI, NDVI, Fuzzy Regression

INTRODUCTION

Evapotranspiration with representation the water loss by evaporation and plants transpiration is the main factor in the climate change, land use, water budget and irrigation studies (Soskic et al., 2001; Dragovic et al., 2009). Over the past years, several evapotranspiration estimation methods are divided into four groups such as: the hydrological method (using the principle of water balance), micro-meteorological method (using the equations of energy balance and aerodynamic), combinations approach (Thornthwaite) and measurement into directly (lysimeter) (Matinfar, 2012).

In some ways, the application of methods is associated with a problem for example difficulty in making public the micro-meteorological method due to great cost of instrument manufacturing and small scale of evapotranspiration estimation using lysimeter (Lingling et al., 2013).

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Remote sensing methods have already reached a significant level of accuracy and reliability over the last forty years, thus becoming attractive for evapotranspiration estimation, since they have a very high resolution and cover large areas (Blanta et al., 2011; Djurovic and Nikolic, 2016; Chivulescu and Schiteanu, 2017). Empirical models with discovering the relationship between evapotranspiration and vegetation indices derived from satellite images and physical models with solving the energy balance equations using the surface temperature of satellites images are the evapotranspiration models with the information of satellite images. The empirical approach needs lesser additional information and rather to the physically models is simpler (Helman et al., 2015).

The relationship between evapotranspiration flux and Normalized Difference Vegetation Index (NDVI), surface temperature derived from satellite images have been analyzed in the variety studies. Surface temperature data modify the evapotranspiration flux with incorporating the effects of topography, surface water and wind (Di Bella et al., 2000).

Anbazhagan and Paramasivam (2016) investigated the correlation between NDVI and land surface temperature from thermal bands of Landsat TM. The results indicated the negative anomaly of vegetation index with increasing of emissivity. The standardized regression coefficient value derived from the statistical regression analysis of NDVI and land surface temperature was negative. The results of research emphasized the importance of land surface temperature (Anbazhagan and Paramasivam, 2016). Di Bella et al. (2000) using NDVI and surface temperature data of the Advanced Very High Resolution Radiometer (AVHRR) sensor have been applied the predictive power of models for evapotranspiration estimation in the Argentine Pampas. Based on the approach, the reliable evapotranspiration estimation was obtained using remotely sensed data. In order to generate the models, the high sensitivity of relation between spectral data and evapotranspiration was related to the dates (Di Bella et al., 2000).

Han et al. (2006) explained the spatial relationships of NDVI and land surface temperature in the form of triangular or trapezoid. For explication the existent meaning of a triangular space, the Temperature Vegetation Dryness Index (TVDI) has been used. Occurrence of that can be done after reaching NDVI to the saturated state with applying the relationships of NDVI, leaf area index (LAI) and evapotranspiration. The validation and updating of land surface models can be done accurately using the relations of NDVI and land surface temperature (Han et al., 2006).

Zhang et al. (2009) obtained the correlation of the groundwater use efficiency estimation derived from eddy covariance tower with vegetation index and ground micro-meteorological data. The correlation coefficient of water use efficiency and the Enhanced Vegetation Index (EVI) of moderate resolution imaging spectrometer (MODIS) ($r = 0.82$) was more than NDVI ($r = 0.64$). The annual curves illustrated the better correspondence between the values of observed and predicted water use efficiency and evapotranspiration in 8 days temporal resolution (Zhang et al., 2009).

Chang and Sun (2013) have been applied the Adaptive Network based Fuzzy Inference System (ANFIS) for modeling the regional evaporation in Taiwan using EVI and land surface temperature of Landsat image products. The better results were related to the EVI and land surface temperature regard to evapotranspiration estimation, therefore improvement of estimation was linked to EVI (Chang and Sun, 2013).

Helman et al. (2015) estimated the actual evapotranspiration with a model basis on the relation of NDVI, EVI derived from MODIS and annual evapotranspiration at 16 FLUXNET sites with diversity of plant functional. The dominant variance was explained with vegetation indices also the regression (multiple variables) and the Temperature and Greenness model (modified version) with land surface temperature did not improve the correlations. The intra-annual relationships had high mean relative error rather than the interannual relationships (Helman et al., 2015).

Reyes-Gonzalez et al. (2018) estimated the crop evapotranspiration using vegetation index in northern Mexico during four growing seasons. The used index was NDVI. The results shows that Etc maps derived from multispectral vegetation indices are useful tool to find crop water consumption at regional and field scale (Reyes-Gonzalez et al., 2018).

The main objective of research is the evapotranspiration estimates improvement based on using land surface temperature and vegetation indices. Validation of evapotranspiration values related to the weather stations using the traditional method need to the large number of weather data which led to satellite data using in comparison to the other methods.

In this regard, we calibrated (16 data) and tested (8 data) the models which data of them derived from MODIS sensor to estimate evapotranspiration in some synoptic stations of East Azarbaijan Province (Ahar, Tabriz and Mianeh stations). In this regard, fuzzy regression models- symmetric and non symmetric- were used for modeling the relation between evapotranspiration and NDVI, land surface temperature. The improvement of evapotranspiration estimation was related to the vegetation index part which the Soil Adjusted Vegetation Index (SAVI) was applied instead of using NDVI.

MATERIAL AND METHODS

Vegetation index

Many studies indicated that NDVI and land surface temperature space can be representative of soil surface water content and vegetation coverage (Gilabert et al., 2002). Therefore, vegetation indices and land surface temperature are the most important component for evapotranspiration estimation.

The data derived satellite images in the form of vegetation indices can be monitored the variety of vegetation cover (Gilabert et al., 2002). NDVI is an important and applied vegetation index with combination of two spectral bands, the visible and near-infrared bands of the electromagnetic spectrum in the form of numerical indicator. NDVI can be defined by equation 1.

$$NDVI = \frac{\rho_{nir} - \rho_{red}}{\rho_{nir} + \rho_{red}} \quad (1)$$

Where ρ_{nir} and ρ_{red} are the near infrared and red reflectance band.

The range of NDVI is between -1 to 1 which higher value of NDVI is indicative of dense vegetation.

Despite the NDVI advantages for vegetation cover describing, the soil background brightness with separation the NDVI relationships with canopy biophysical properties limited the application of NDVI. In this regard, a soil-adjustment factor, L basis on the proposal of Huete (1988) was presented for description the first-order, non-linear, differential near infrared (Huete 1988) and red radiative transfer through a canopy, and obtained the SAVI with equation 2 (Jiang et al., 2008).

$$SAVI = (1 + L)\frac{\rho_{nir} - \rho_{red}}{\rho_{nir} + \rho_{red} + L} \quad (2)$$

Where ρ_{nir} and ρ_{red} are the near infrared and red reflectance band, L is a soil adjustment factor.

One source of error in NDVI calculation was related to the soil background, therefore SAVI have been used to get high accuracy calculation. Minimizing of brightness-related soil effects using SAVI can be conducted with considering the first-order soil vegetation interaction using L. Values for L range from 0 to 1. L varies with vegetation density which is zero for dense and 1 for low vegetation cover. The suggestion about the values of L is o.5 but prior knowledge about vegetation amounts is necessary, otherwise an iterative function would require (Qi et al., 1994). In this regard, the soil line has been used to describing the vegetation indices function. The soil line describes the variation of red and near infrared reflectance bands and for each soil types, the different soil line must be determined (Gilabert et al., 2002). L can be determined using the equation 3 and 4.

$$L = 1 - 2.a.NDVI.WDVI \quad (3)$$

$$WDVI = \rho_{nir} - \gamma\rho_{red} \quad (4)$$

Where ρ_{nir} and ρ_{red} are the near infrared and red reflectance band and L is a soil adjustment factor, a=1.6, γ is the soil line slope (Allen et al., 2010).

Land surface temperature

Two major factors that affected evpotranspiration is land surface temperature and vegetation index. Split window technique is used for land surface temperature estimation which based on differential absorption of two close infrared bands. In this case, the role of atmospheric gases in the absorption can be determined. In order to calculate land surface using brightness temperature derived from multiple thermal bands, several split window algorithms are accessible. The algorithm of Price (1984) was applied in the study. Accurate performance of Price algorithm rather than to the other algorithms was stated by Vazquez et al. 1997 (Hong et al., 2009).

Fuzzy Regression

The development of the classical regression analysis was related to the suggestion of Asai et al. (1982) as the fuzzy linear regression. Describing the vague relationship of dependent and independent variables was developed using the fuzzy regression (Asai, 1982). In the regression, representation the some elements of the regression models is possible with imprecise data. The linear programming (LP) and the least-squares (LS) are the methods which used for the fuzzy regression coefficients estimation. The total spread of the estimated dependent variables was minimized using the LP methods. The constraint of optimization is related to the estimated dependent variables within a certain confidence level parameter (h). The LP computational complexity is low which led to method usefulness. Their sensitivity to outliers and growing the estimated intervals with more data collection are the thinking points of LP methods. To overcome the shortcoming of the LP methods, multi-objective fuzzy regression techniques were developed. Minimizing the total difference of estimated and observed dependent variables has conducted using the LS methods. The error comparison of LP and LS methods indicated that the LS methods have small error values but the computational complexity of the LS methods is high (Lu and Wang, 2009). The structure of describing the dependency between output and input variables in the fuzzy linear regression are brought in equation 5.

$$\tilde{Y} = \tilde{A}_0 + \tilde{A}_1 x_1 + \ldots + \tilde{A}_n x_n \qquad (5)$$

$$\tilde{A} = (\tilde{A}_0, \tilde{A}_1, \ldots, \tilde{A}_n) \qquad (6)$$

Where $\tilde{Y}$ is the fuzzy output, $x = [x_1, x_2, \ldots, x_{n1}]^T$ is the real valued input vector, $\tilde{A}$ is a set of fuzzy numbers.

Each number coefficient $\tilde{A}_i$ can be expressed as equation 7 in case of triangular membership functions of $\tilde{A}_i s$.

$$\tilde{A}_i = \{a_i^L, a_i^C, a_i^U\} \qquad (7)$$

Where a_i^U is the upper limit, a_i^L is the lower limit and a_i^C is the point with the property $\mu_{\tilde{A}_i}(a_i^C) = 1$. The relations of equations 8 and 9 are derived based on the symmetry property of the fuzzy coefficient $\tilde{A}_i$.

$$a_i^C = \frac{a_i^L + a_i^U}{2} \qquad (8)$$

$$a_i^S = a_i^U - a_i^C = a_i^C - a_i^L \qquad (9)$$

Where a_i^C is the center, a_i^S is the spread of $\tilde{A}_i$. Therefore, the parameters for describing the symmetric fuzzy number coefficient $\tilde{A}_i$ can be defined such as a_i^C, a_i^S or a_i^L, a_i^U, as $\tilde{A}_i = \{a_i^C, a_i^S\}$ or $\tilde{A}_i = \{a_i^L, a_i^U\}$. The vector form of the fuzzy coefficient can be explained in terms of a_i^C and a_i^S as $\tilde{A} = \{a^C, a^S\}$ where $a^C = [a_1^C, a_2^C, ..., a_n^C]^T$ and $a^S = [a_1^S, a_2^S, ..., a_n^S]^T$.

Determination the parameter $\tilde{A}_i$ with the fuzzy output set, $\{y_j\}$ with a membership value greater than h, is the objective of the fuzzy regression method

$$\mu_{\tilde{Y}}(y_j) \geq h, \quad j = 1, ..., m \qquad (10)$$

The best-fitting model generation is the criteria in h selection.

The selection of confidence level parameter is based on the best-fitting model generation.

Therefore, minimization the spread of fuzzy output of all data was considered for finding the fuzzy coefficients, at the end the cost function was described such as equation 11.

$$\begin{aligned}
&\textit{Objective function} \quad a_0^S + \sum_{i=1}^{n} a_i^S \sum_{j=1}^{m} |x_{ij}| \\
&\textit{Subject to} \\
&a_0^C + \sum_{i=1}^{n} a_i^C x_{ij} - (1-h)\left[a_0^S + \sum_{i=1}^{n} a_i^S x_{ij}\right] \leq y_j \\
&a_0^C + \sum_{i=1}^{n} a_i^C x_{ij} + (1-h)\left[a_0^S + \sum_{i=1}^{n} a_i^S x_{ij}\right] \geq y_j
\end{aligned} \qquad (11)$$

Where *y* is a dependent parameter, *x* is an independent parameter, a_i^C is the center and a_i^S the spread of $\tilde{A}_i$ and h is the confidence level parameter.

If the triangular, is not symmetric, minimally three parameters are need. For example, $\tilde{A}_i$ can be described by the triplets $\{a_i^L, a_i^P, a_i^U\}$ or by $\{s_i^L, a_i^P, s_i^R\}$ where a_i^p is the point in which $\mu_{\tilde{A}_i}(a_i^P) = 1$, peak point, s_i^L is the left-side spread from the peak point a_i^p and s_i^R represents the right-side spread.

Another representation is also possible, if the spreads are normalized. Since $s_i^L = a_i^P - a_i^L$ and $s_i^R = a_i^U - a_i^P$. If s_i^L is chosen as the base, therefore s_i^R expressed as $s_i^R = k_i\, s_i^L$ where k_i are the skew factors (positive real number). The cost function in non-symmetric case can be expressed by following equations.

$$\textit{Objective function} \quad (1+k_0)S_0^L + \sum_{i=1}^{n}\left[(1+k_i)S_i^L \sum_{j=1}^{m}\left|x_{ji}\right|\right]$$

$$\textit{Subject to}$$

$$(1-h)S_0^L + (1-h)\sum_{i=1} S_i^L\left|x_i\right| + \sum_i a_i^P x_i + a_0^P \le y_j \tag{12}$$

$$(1-h)k_0 S_0^L + (1-h)\sum_{i=1} k_i S_i^L\left|x_i\right| - \sum_i a_i^P x_i - a_0^P \le -y_i$$

Where y is a dependent parameter, x is an independent parameter, h is the confidence level parameter, a_i^P is the point in which $\mu_{\tilde{A}_i}(a_i^P) = 1$, k is the skew factor, s_i^L is the left-side spread from the peak point a_i^P (Yen et al. 1999).

The sensitivity analysis must be conducted on two parameters of symmetric and non-symmetric membership function of fuzzy regression: confidence level parameter and skew factor.

The main objective of the research is the comparison of fuzzy regression performance using different vegetation indices for evapotranspiration estimation. In this regard, some criteria were used which their mathematical forms are brought in the following equations. The minimum values of criteria are related to the best performance of model.

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(O_i - S_i)^2} \tag{13}$$

$$RRMSE = \frac{\sqrt{\frac{1}{n}\sum_{i=1}^{n}(O_i - S_i)^2}}{\overline{O}} \tag{14}$$

$$MRE = \frac{\sum_{i=1}^{N}\left|\frac{O_i - S_i}{O_i}\right|}{n} \tag{15}$$

$$MAE = \frac{\sum_{i=11}^{N}\left|O_i - S_i\right|}{n} \tag{16}$$

Where O_i are observed data, S_i are simulated data, *RMSE* is root mean square error, *RRMSE* is relative root mean square error, *MRE* is mean relative error, and *MAE* is the mean absolute error.

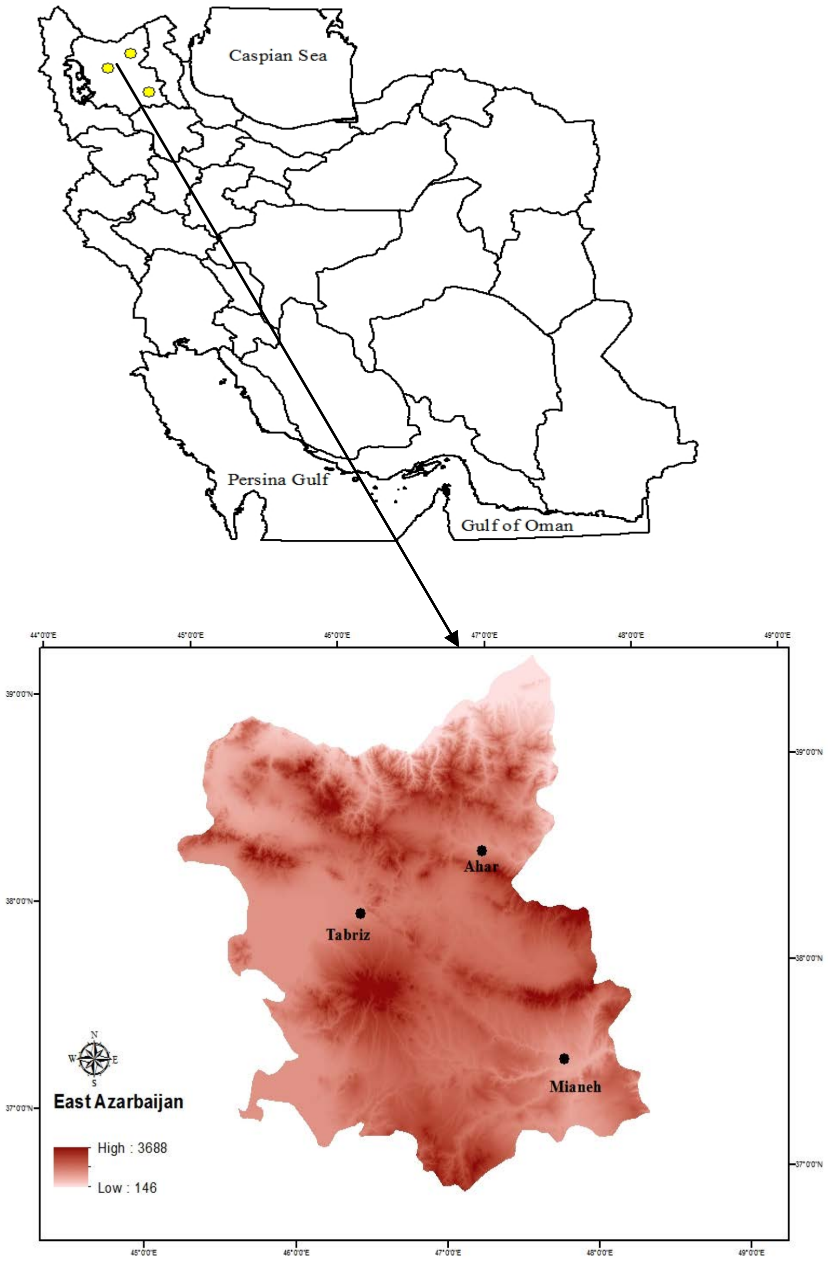

Figure 1: Location of used synoptic stations

Case study

East Azarbaijan Province was the region chosen as the study area. The province covers an area of approximately 47830 km^2 which was in the semi-arid climatic region based on De Martonne climate classification. According to the climate change and the effect of that on the agricultural activities, water requirement and etc., evapotranspiration estimation is the major issue in the Province. In this regard, synoptic stations must be selected for evapotranspiration using the FAO Penman-Monteith method which is proposed by The FAO as the standard method (Raki et al., 2013) . The synoptic stations are Tabriz, Ahar and Mianeh stations with different vegetation cover. NDVI and land surface temperature are derived from MODIS data because of the sensor's moderate spectral spatial resolution. Figure 1 shows the location of synoptic stations in the East Azarbaijan Province.

RESULTS AND DISCUSSION

Evapotranspiration is affected by vegetation indices and land surface temperature. In this study, improvement the evapotranspiration estimates was related to the vegetation indices. Due to the high efficiency of FAO Penman-Monteith method, it was used for evapotranspiration calculation. This method has been chosen as the standard method for estimating evapotranspiration (Serban et al., 2010). The images were sampled every 10 days in each month, June and July, 2007- 2014 which 16 and 8 data are presented the calibration and validation periods, respectively. The climatological parameters which are used for evapotranspiration estimates using FAO Penman-Monteith were: mean air temperature, maximum air temperature, minimum temperature, wind speed at 2 m above the ground, relative humidity.

Table 1. Variation of confidence level, center and spread.

Confidence level parameter	Tabriz-July		Ahar-July	
	spread	Center	spread	Center
0.1	1.51	3.94	0.036	4.49
0.2	1.7	3.94	0.04	4.49
0.3	1.94	3.94	0.046	4.49
0.4	2.26	3.94	0.054	4.49
0.5	2.72	3.94	0.065	4.49
0.6	3.4	3.94	0.081	4.49
0.7	4.53	3.94	0.1	4.49
0.8	6.8	3.94	0.16	4.49
0.9	13.6	3.94	0.32	4.49

The fuzzy linear regression is an alternative modeling tool which is employed instead of classical regression for evapotranspiration modeling using NDVI and land surface temperature. Independent variables of fuzzy regression which are NDVI and land surface temperature derived from satellite images.

Coefficient estimation of fuzzy regression model is the first step in the modeling process. The variation of confidence level, center and spread of fuzzy number are brought in Table 1. The variation of confidence level had no effect on the center of fuzzy number but the increasing the confidence level parameter led to increasing spread of fuzzy number. Yen et al. (1999) indicated the spread increasing of fuzzy number coefficient with increasing the confidence level parameter.

The results indicated the negligible variation of fuzzy regression performance against the confidence level parameter. Therefore in the non symmetric fuzzy regression, the sensitivity analysis must be taken on the skew factor. The results of skew factor sensitivity analysis are listed in Table 2.

Table 2. Skew factor sensitivity analysis.

K_0	K_1	K_2	RMSE
1.1	1.25	1.4	5.2
1.4	1.6	1.9	5.73
1.9	2.3	2.6	6.1
2.7	2.9	3.2	6.47
1	1	1	5.15
1.25	1	1	5.47
1.4	1	1	5.73
1.9	1	1	6.1
2.6	1	1	6.46

According to table 2, $K_0= K_1= K_2=1$ has the minimum error, therefore the selected skew factor are applied for the next modeling process. The variation of skew factor versus spread peak point variation in non symmetric fuzzy regression is listed in Table 3.

Table 3. Skew factor variation versus spread peak point variation-July.

K_0	Tabriz		Mianeh	
	spread	center	spread	center
1	2.39	3.6	2.97	7.51
1.1	2.28	3.66	2.82	7.58
1.25	2.13	3.73	2.64	7.68
1.4	1.99	3.73	2.47	7.76
1.9	1.65	3.8	2.04	7.97
2.6	1.33	3.97	1.65	8.17
2.7	1.29	4.15	1.6	8.19

The left-side spread decreases with the increase of skew factor and the peak point is increased. In the research of Yen et al. (1999), the increasing of skew factor in the case of non-symmetric membership functions led to decreasing of the spread S_0^L and increasing of the center, a_0^P. The variation of skew factor and coefficient of fuzzy regression are presented in Table 4.

Table 4. Variation of skew factor and coefficient of fuzzy regression.

Skew factor			Mianeh-June			Ahar-July		
K_0	K_1	K_2	C_0	C_1	C_2	C_0	C_1	C_2
1.1	1.25	1.4	5.69	1.37	0.007	3.94	4.79	0.015
1.4	1.6	1.9	5.8	1.37	0.007	4.1	4.79	0.015
1.9	2.3	2.6	5.92	1.37	0.007	4.3	4.79	0.015
2.7	2.9	3.2	6	1.37	0.007	4.5	4.79	0.015
1	1	1	5.65	1.37	0.007	3.88	4.79	0.015
1.25	1	1	5.75	1.37	0.007	4.03	4.79	0.015
1.4	1	1	5.8	1.37	0.007	4.1	4.79	0.015
1.9	1	1	5.92	1.37	0.007	4.3	4.79	0.015
2.6	1	1	6.04	1.37	0.007	4.48	4.79	0.015

The peak point of constant parameter changes with the skew factor variations but skew factor variations cannot change the peak point of other coefficients. The model performance for evaporation estimation using NDVI and land surface temperature is listed in Table 5.

Table 5. Performance of fuzzy regression in regard to evapotraspiration estimation.

Station	Month	RMSE		RRMSE		MAE		MRE	
		Symmetric	Non symmetric	Symmetric	Non symmetric	Symmetric	Non symmetric	Symmetric	Non symmetric
Tabriz	June	0.86	0.88	0.13	0.14	0.61	0.63	0.11	0.11
	July	0.68	0.65	0.093	0.089	0.52	0.52	0.074	0.074
Mianeh	June	0.82	0.87	0.12	0.12	0.63	0.65	0.09	0.092
	July	0.54	0.54	0.093	0.094	0.45	0.45	0.084	0.084
Ahar	June	1.21	1.11	0.23	0.21	1.05	0.87	0.23	0.2
	July	0.94	0.78	0.13	0.1	0.76	0.67	0.11	0.099

Based on Table 5, there is not major difference between symmetric and non symmetric fuzzy regression but in general, the decreasing of error in non symmetric case is more than symmetric (Parviz and Paymai, 2017), for example the error decreasing of Ahar from symmetric to non symmetric case are: RMSE of July=17%, RRMSE of June= 8.69%, MAE of June= 27.61%, and MRE of June=13%. In the research of Shayannejad et al. (2008) for daily potential evapotranspiration estimation, fuzzy regression had minimum error in comparison to the used methods such as Penman-Monteith, ANN.

The representation of green vegetation cover is basis on high and low reflectance of near infrared and red reflectance, respectively. The difference between values of reflectance in each band can be illustrated with the scatterplot of image cell values using near infer red and red brightness.

In our study, the improvement of evapotranspiration estimates is focused on the vegetation indices which SAVI were used in this case. Therefore, fuzzy regression performance was evaluated in June in all stations. L is one of the most important parameters to determine SAVI which soil line is used in this regard.

For L calculation process, the shape of soil line must be calculated which Figure 2 shows the scatter plot of near infrared and red reflectance.

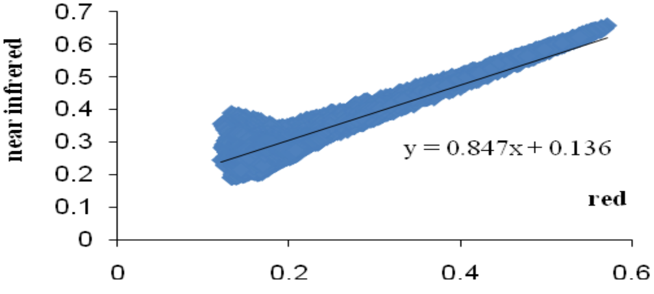

Figure 2: Scatterplot of near infrared versus red reflectance.

The range of L is zero to 1 for dense and low vegetation cover, respectively. The variation of NDVI and L in the studied stations and some days are shown in Fig. 3. For 2007-159, the maximum and minimum of NDVI happened in Ahar and Tabriz stations, respectively. In this regard, the minimum and maximum of L happened in Ahar and Tabriz stations. Therefore, the maximum case of NDVI is related to the minimum case of L. This trend is preserved in the other days such as 200-158, 2009-155 and 2014-158. At the end, Figure 3 satisfied the variation of L against the vegetation cover, because NDVI is related to the vegetation cover.

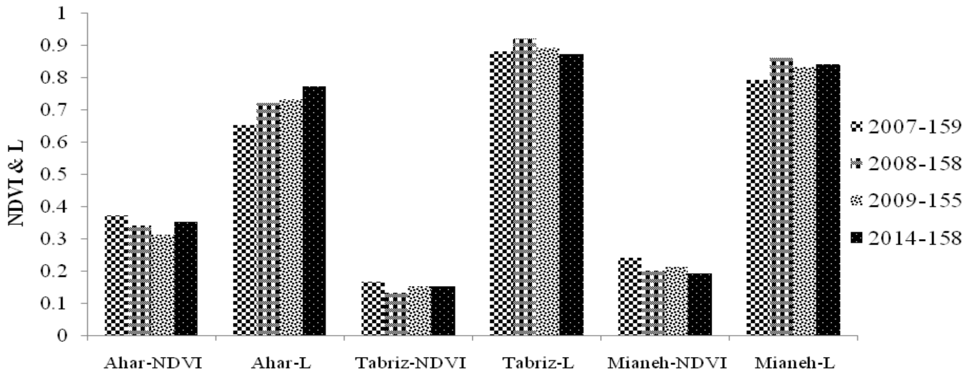

Figure 3: Diurnal variation of NDVI and L in some stations.

Comparison between observed and estimated evpotranspiration (using SAVI) in three stations showed the 7.85% and 5.51% underestimation in Mianeh and Ahar stations and 6.53% overestimation in Tabriz station.

The evpotranspiration estimates using SAVI and NDVI are compared and the results of comparison are shown in Figure 4.

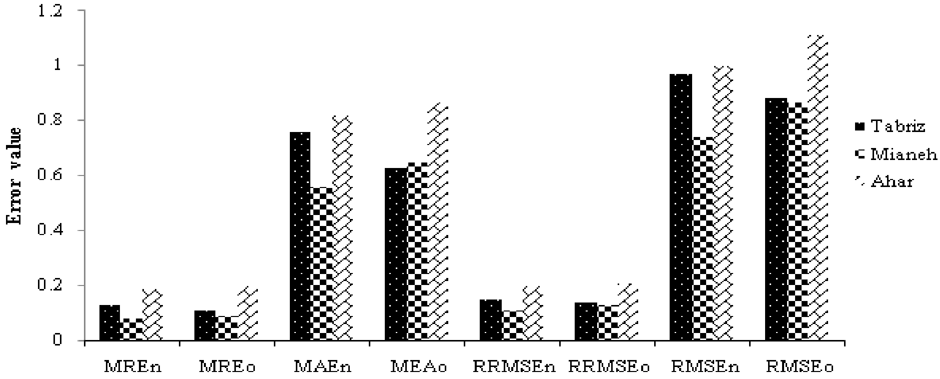

Figure 4: Comparison of fuzzy regression performance in two cases: NDVI(o), SAVI (n).

The values of error are decreased in case of using SAVI for evapotranspiration estimation in Ahar and Mianeh stations but any improvement were not observed in evapotarspiration estimation of Tabriz. The value of RMSE decreasing is 14.29% and 9.9% in case of Mianeh and Ahar, respectively. The value of MRE decreasing is 13.84% and 5.74% in case of Mianeh and Ahar, respectively. The evapotranspiration improvement was observed in case of using other indices instead of NDVI such Zhang et al. (2009), Chang and Sun (2013) and Helman et al. (2015) which their comparison was related to NDVI and EVI indices.

The amount of error decreasing in Mianeh is higher than Ahar station. The reason of that may be related to the vegetation cover. The average NDVI of Ahar and Mianeh stations are 0.32 and 0.18. Therefore, the region with minimum NDVI corrected better than maximum NDVI and this matter shows the impact of vegetation index improvement for evapotranspiration estimates. The variation of L and estimated evapotranspiration are compared in Figure 5.

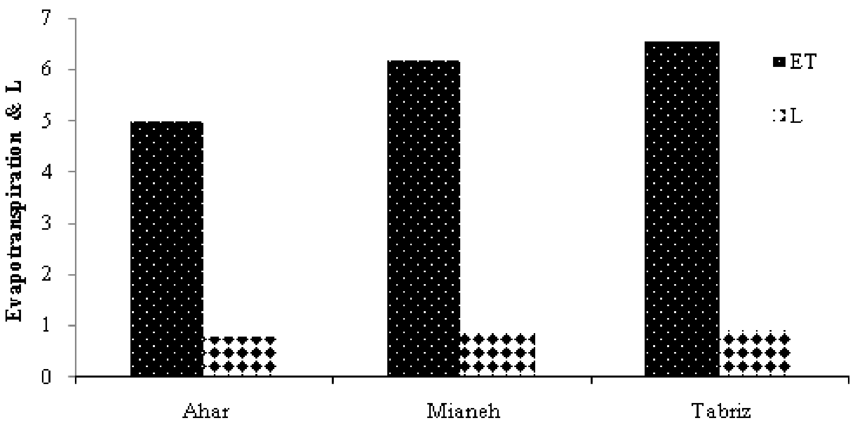

Figure 5: Variation of L and estimated evapotrasnpiration.

Results showed that increasing of L causes increasing of estimated evapotranspiration which are consisting of other results (Hassanpour et al., 2011). The spatial variation of indices showed the importance of spatial distribution studies (Moradi et al., 2015; Milos and Bensa, 2014).

The reason of error decreasing and increasing in the studied stations can be related to the vegetation cover. The main vegetation cover in Maineh and Ahar stations are rain fed areas and irrigated farming lands as agriculture and garden, whereas most parts of Tabriz station is related to urban areas. According to the vegetation cover of stations, it can be said that in the areas with low vegetation in Maineh and Ahar station, using SAVI led to correction the spectral bands, but in the Tabriz station, SAVI has not better results and several advanced vegetating indices which are from SAVI family such as Transformed Soil Adjusted Index (TSAVI) must be used.

CONCLUSIONS

Accurate evapotranspiration is one of the major issues in the many fields such as water resources and land management, land surface and vegetation processes, and agricultural activities. The aim of this study is the component of evapotranspiration improvement with emphasis on vegetation index. Using SAVI index in most stations had minimum error because of reducing the impact of background soil surface. The vegetation cover is important factor in the evapotranspiration improvement determination. This shows that if land use map is not available in areas with low vegetation, L parameter would be important. Therefore, for evapotranspiration improvement, the precise and accurate vegetation index must be developed with emphasis on the correction of spectral and thermal bands. The land surface temperature, vegetation index and evapotranspiration modeling conducted with linear fuzzy regression which the efficiency of fuzzy regression was proved in many studies for modeling. The decreasing of error in non symmetric case is more than symmetric. The coefficient determination of fuzzy regression and skew factor are affected the evapotranspiration estimation. The evapotranspiration improvement is affected by type of modeling and the determination the precise components of modeling. Therefore, the suggestion of the research can be divided in two parts: 1- using the other improved satellite indices in order to model the behavior of spectral data 2- improving the regression methods such as using the support vector regression.

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DEVELOPMENT AND ASSESSMENT OF TECHNOLOGIES OF MISCANTHUS AND SWITCHGRASS GROWING IN FOREST-STEPPE ZONE OF UKRAINE

SUMMARY

The relation between weather conditions and yield of energy crops in the conditions of forest-Steppe of Ukraine was established. Optimum condititions for *Miscanthus × giganteus* rhizome density and depth, method of sowing of switchgrass seeds, row spacing and terms of conducting maintenance during the first year of vegetation have been established. The yield of *M.×giganteus* increases due to rhizomes planting in the early stages and planting depth - 8-10 cm.

The article presents the results of studies on the effect of cultivation technology for *Miscanthus × giganteus* and switchgrass biomass purposed for the production of solid biofuel. Methods of planting, optimal row width and conditions of care during the first year of vegetation are substantiated. The highest switchgrass yield of dry biomass and the energy output was provided in options with marker crop sowing and the width between rows 30 and 45 cm. The factors under study, namely methods of planting and tillage are essential only in the first year of vegetation. The optimal row width, methods of planting and tending switchgrass sowing were established.

Keywords: energy crops, planting dates, row width, planting density, marker crop, productivity.

INTRODUCTION

The involvenment of alternative sources in energy balance of agricultural sector, reduce energy dependence of Ukraine (Geletukha et al., 2016). Production of fuel pellets and briquettes based on biomass energy crops is economically viable biofuel production (Mitchell et al., 2012, Hodgson et al., 2010; Bouriaud et al., 2015). The main advantage of solid biofuels is renewable, reducing the greenhouse effect, environmentally closed-loop energy system, the potential for growing cellulosic feedstock (Karp. and Sheid, 2008; Heaton et al., 2010). Biomass of Miscanthus (*Miscanthus Anders*) and switchgrass (*Panicum virgatum L.*) has a high content of cellulose and lignin and thus it is high quality raw material for the production of solid biofuels (Butkutė et all, 2013; Cassie et al., 2015). *Miscanthus × giganteus* and switchgrass are perennial grasses with C4-

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Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

type photosynthesis. At a certain point these plants had attracted the interest of researchers as a promising source of cellulose containing biomass for chemical industry and bioenergy production (Christian et al., 2008, Dohleman et al., 2009, Brosse et al., 2012; Mitchell et al., 2012). The aboveground plant biomass can be used as renewable raw materials for the production of solid (pellets, briquettes), liquid or gaseous (ethanol, butanol, ethylene) types of fuels (Hodgson et al., 2010, Han et al., 2011, Parrish et al., 2012; Cassie et al., 2015). Recent researches (Lewandowski and Schmidt, 2005; Milovanovic et al., 2012; Arnoult and Brancourt-Hulmel, 2015; Powlson et al., 2005) have shown that many different factors can influence on Miscanthus and switchgrass biomass production efficiency (geographical location of the cultivation area, climate conditions, water supply, crop management, mineral element availability, genotypic variability). In this connection, thorough studying of productivity parameters under introduction conditions will allow to determine of potential and prospects of this crop cultivation. Meantime, adaptation of the technology of second generation energy crops growing to soil and climatic conditions of Ukraine is still missing. The objective of this study was to develop and evaluate technologies of switchgrass sowing and planting of *Miscanthus* × *giganteus* rhizome in conditions of forest-steppe of Ukraine.

MATERIAL AND METHODS

Experimental plots located in the western part of forest-steppe zone of Ukraine the Borshchiv district, Ternopyl region. The soil of the experimental plots is turf podzol with acidity (pH) is 6.0. The climate is moderately continental with minor amplitude fluctuations in temperature, the sum of positive temperatures is within 2500 and.2600ºC. Period with average temperature above 10 ° C lasts 160-165 days. There are 370-420 mm of rainfall during this period, the value of hydrothermal factor is from 1.4 to 1.6. Weather conditions prevailing in the study region for 2009-2015 years are shown in tables 1 and 2. Moisture level was limiting factor, since precipitation is distributed unevenly. A small amount of precipitation was registered during the period 2009-2011, which resulted in drought conditions for the studies plants. Weather conditions in 2012 year could be caracterized by warm early growing season.

The average daily air temperature in April was 10.3°. The summer of 2012 year was characterised warm weather conditions. It was enough for energy crops growth and development. Meanwhile, during this time, precipitations were at sufficient level. The less rainy summer of 2012 year, when in May precipitation rate registered at level 21.5 mm, had negatively affected the germination of plants. However, a large amount of precipitation fell in June – 105.5 mm and 77.5 mm – in August. 2012-2014 years weather was hot and humid. Average temperature in 2012-2013 years was 16.4 °C, while in 2014 – only 14.9 °C. In May of 2014 precipitation twice higher than long-term observations average was registered.

Table 1.The average long-term values of air temperature in Ternopyl region

Month	2009	2010	2011	2012	2013	2014	2015
Apr	11.1	9.4	10.4	10.3	12	9.5	8
May	14.5	15.3	14.9	16.0	16.4	14.9	14
June	17.3	17.9	18.4	19.3	18	16	18
July	20.9	20.3	19.7	23.0	19.5	20	21.5
Aug	19.7	21.1	19.3	19.1	20	18.5	22.5
Sept	16.0	12.8	17.2	15.1	13	15	17.5

Table 2.The average long-term values of precipitation in Ternopyl region

Month	2009	2010	2011	2012	2013	2014	2015
Apr	3.4	26.3	37.5	83.0	93	75	47
May	41.0	108.6	21.2	21.5	100	133	113
June	81.3	143.9	92.4	105.5	143	38	64
July	27.3	122.9	66.0	82.2	44	120	38
Aug	49.9	42.1	63.0	77.5	52	67	11
Sept	3.8	134.8	13.3	19.0	115	30	47

The weather was hot during summer of 2015 year. Irregularity of precipitations could be observed during this period. Such weather conditions had adverse impact on growth and development of all types of studied plants and their yields.

All observations could be resulted in following: during 2009-2015 years dry weather was prevailing, temperatures higher of average were registered. As it generally known, optimal conditions for the crop can be supported by certain agronomic measures before and after sowing, if those are variable and timely. The sun radiation level can be optimized by adjusting planting density and direction of rows and the width between rows. Temperature regime has great impact on germinating quality of cereals and the transition from the tillering phase to the next phases of development.

Two factors field experiment were conducted on *Miscanthus × giganteus*. Plants were grown during 2009-2012 years. Factor A – planting dates (I – II decade of Apryl; II–III decade of Apryl; I decade of May). Factor B is planting depth (6 cm; 8cm; 10cm; 12cm).

Field experiments with switchgrass were conducted from 2009 to 2015 years. Two factors were considered as well: factor A - method of seeding (with and without marker plant). Mustard was used as marker crop. Factor B is the width of inter-row spacing. The total area of the experiments was 0.40 ha, repetition - four times.

RESULTS AND DISCUSSION

The impact the timing and depth of planting rhizomes on the yield of miscanthus are shown in the table 3.

Table 3**.** Yield of *Miscanthus × giganteus* biomass depending on the timing and depth of planting rhizomes

Planting time	Year				
	2009	2010	2011	2012	Average
	Planting depth - 6cm				
I	2.2	3.7	1.8	3.2	2.7
II	2.1	3.1	1.5	2.5	2.3
III	1.7	2.8	1.3	2.1	2.0
	Planting depth - 8cm				
I	2.8	3.7	1.9	3.3	2.9
II	2.1	3.2	1.7	2.6	2.4
III	1.7	2.8	1.4	2.3	2.0
	Planting depth - 10cm				
I	2.9	3.9	2.2	3.6	3.2
II	2.2	3.5	1.9	2.6	2.5
III	1.8	2.9	1.4	2.4	2.1
	Planting depth - 12cm				
I	2.9	3.9	2.0	3.1	3.0
II	2.1	3.5	1.8	2.3	2.4
III	1.7	2.8	1.3	2.1	2.0
LSD_{05}	0.4	0.4	0.3	0.3	

Weather conditions had a significant effect on the *Miscanthus × giganteus* biomass yield. The yield of *Miscanthus × giganteus* in the favourable weather conditions of 2010 and 2012 years was 2 times higher compared to 2009 and 2011 years. It is established that the yield of *Miscanthus × giganteus* biomass rises with the increase in depth of planting rhizomes. Best result is fixed when Rhizomes are planted 10 cm deep. Depending on the time of planting the rhizome during the four years of studies, yield of biomass ranged: the first period – from 1.8 to 3.9 t/ha, second term: from 1.5 to 3.5 t/ha; the third period –from 1.3 to 2.9 t/ha. These factors influence on the yield was observed in the first year of vegetation. Meanwhile, the trend continued in subsequent years. However, the effect in subsequent years was small. Plants of *Miscanthus × giganteus* were in the same conditions. The main shoot regeneration started at the same time regardless of the timing and depth of planting in the previous year. The yield of *Miscanthus × giganteus* at different planting dates during four years is shown in Fig.1. The yield of biomass in all options changed from 13.1 to 15.2 t/ha (plants after the second year of vegetation), 21.1-21.8 t/ha after the third year and 24.1-24.8 t/ha after the fourth year. Decreasing harvest of *Miscanthus × giganteus* in the second and third planting dates was associated with deficient rainfall in 2009 and 2011years.Seed germination in experiments with switchgrass starts at the temperature of +6 - 8

°C. Amicable germination occurs when soil was warming to +15-16 °C. Seedlings appeared only after 15-18 days.

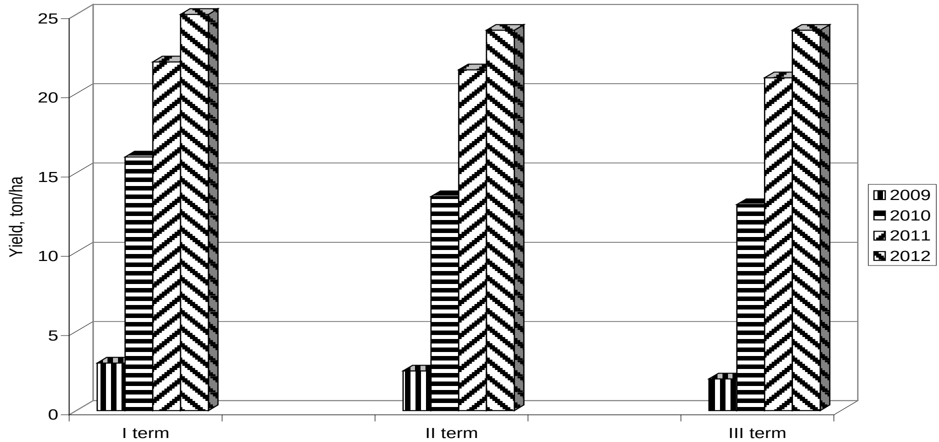

Figure 1. The miscanthus yield in different planting dates during four years

Seedlings can withstand weak frosts to - 2 °C. The sprouts were killed or significantly damaged at the temperature 3-5 °C. Long-term effect of low positive temperatures (+6 -10 °C) and cloudy weather was very harmful for switchgrass seedlings. In the first year of vegetation in the early phase of tillering roots develop poorly, deep into the soil slowly to a depth of 12-15 cm. The increase of the root mass occurs until late autumn. Regrowth of switchgrass started simultaneously and its density depended largely on the degree of tillering of the plants in previous years. The data of number of switchgrass stems before maturation of the seed depending on the width of inter-row spacing and mustard as marker crop are presented in the table 4.

Table 4.The number of stems of switchgrass depending on the width of inter-row spacing and marker crop.

Width of row spacing, marker crop	Years					Average
	2011	2012	2013	2014	2015	
Width 15 cm, marker crop sowing	242	250	225	233	240	238
Width 30 cm, marker crop sowing	233	276	280	285	288	272
Width 45 cm, marker crop sowing	260	284	288	292	290	283
Width 15 cm, without marker crop sowing	236	256	262	269	273	259
LSD_{05}	30.3	33.3	32.9	33.7	34.1	32.8

The number of stems was large compared with the row - spacing width of 15 cm in the trials when a width between rows - 30 and 45 cm. The increase in height of stems up to 40 cm was fixed in the same trials (table 5).

Table 5. Height of switchgrass stems depending on the width of inter-row spacing and marker crop, cm

Width of row spacing, marker crop	Years					Average
	2011	2012	2013	2014	2015	
Width 15 cm, marker crop sowing	167	174	180	183	185	178
Width 30 cm, marker crop sowing	191	212	214	220	224	212
Width 45 cm, marker crop sowing	196	218	221	223	219	215
Width 15 cm, without marker crop sowing	169	172	179	181	180	176
LSD_{05}	22.6	24.3	24.8	25.2	25.3	24.4

In the variants with a width 30 cm between rows switchgrass yield was 20.3 t/ha, 45 cm – 19,5 t/ha, whereas the width of the row spacing of 15 cm – from 16.5 to 15.8 t/ha (table. 6).

Table. 6.The switchgrass yield depending on the width of inter-row spacing and marker crop, t/ha

Width of row spacing, marker crop	Years					Average
	2011	2012	2013	2014	2015	
Width 15 cm, marker crop sowing	12.5	16.1	17.2	18.1	18.2	16.5
Width 30 cm, marker crop sowing	17.1	19.7	21.6	20.9	22.2	20.3
Width 45 cm, marker crop sowing	16.3	19.7	19.1	20.3	21.6	19.5
Width 15 cm, without marker crop sowing	11.8	14.0	17.3	17.9	18.0	15.8
LSD_{05}	1.8	2.2	2.4	2.4	2.5	2.3

Additional biomass in comparison with the fourth embodiment (inter-row spacing of 15 cm, seeding without the marker crop) was 2.9-4.8 t/ha. The lowest yield of dry biomass switchgrass was observed in the variant with the marker crop. An important indicator characterizing the value of switchgrass as bioenergy crop is possible energy output with yield. Calculations of this index are given in table 7. The highest energy output 367,2 GJ/ha was obtained at switchgrass sowing with the 45 cm width between rows with the marker crop, and the lowest

– 304,3 GJ/ha with a width of row spacing of 15 cm without marker crop. The calculations for the five years indicate that the greatest productivity in miscanthus – 25.3 t/ha of dry matter and 516.2 GJ/ha.

Table 7. Energy uptake with switchgrass depending on the method of preparation of soil for sowing, (mean for 2011-2015 years)

Width of row spacing, marker crop	Yield of raw mass, t/ha	Dry matter, %	Yield of dry biomass, t/ha	The yield of solid fuel, t/ha	Energy output, GJ/ha
Width 15 cm, marker crop sowing	21.2	80.2	16.5	18.6	316.2
Width 30 cm, marker crop sowing	25.0	79.5	18.3	20.7	351.0
Width 45 cm, marker crop sowing	24.7	79.2	19.5	21.6	367.2
Width 15 cm, without marker crop sowing	20.6	81.2	15.8	17.9	304.3
LSD_{05}	2.9	10.0	2.3	2.6	41.1

The switchgrass yield – 18.2 t/ha, energy uptake – 371.5 GJ/ha (Table. 8). The results indicate that the higher effect can be obtained by creating energy chain supply of raw materials to the consumer. Realization of raw biomass for biofuel production on domestic and foreign markets, as well as planting material of miscanthus (ryzoms) and switchgrass (seeds), can bring additional economic benefits and reduce costs during the laying of plantation energy.

Table 8. The energy uptake of perennial bioenergy crops in the third year of vegetation (average over 2012-2016 years)

Energy crop	Yield of raw mass, t/ha	Dry matter, %	Yield of dry biomass, t/ha	The yield of solid fuel, t/ha	Energy output, GJ/ha
Miscanthus	58.8	42.6	25.3	30.3	516.2
Switchgrass	23.2	78.5	18.2	21.8	371.5

Indexes of energy crops production economic efficiency were evaluated at actual cost realized in the field experiments. In the experiments provided the traditional system of primary tillage, plowing with a wrapping layer. The fertilizers and pesticides responded to the need of plants to produce a crop according to get the 25.3 t/ha of miscanthus dry biomass and 18.2 t/ha of switchgrass. Analysis of the funds structure distribution per 1 ha of sowing such energy crops as miscanthus and switchgrass (table. 9) indicates that the largest part of these items are mineral fertilizers, pesticides and seeds that take up to 50 % of the total cost. A significant portion of energy costs were for planting material, fuel, fertilizer and herbicides.

Table 9. Economic efficiency of energy crops cultivation, euro

Item of expenditure	Miscanthus		Switchgrass
	for the 2nd year of vegetation	for the 3rd year and subsequent years	From 2nd year of vegetation
Salary	40.3	9.1	40.9
Fuel material	102,06	13.7	65.6
Amortisation	26.1	9.2	19.8
Routine maintenance	18.3	12.8	13.9
Seeds, cuttings, rhizome	500	-	16.7
Mineral fertilizer	112.5	22.1	23.5
Pesticides	39,3	-	74.8
Other expenses	19,3	6.1	6.0
The rent of land	62.5	20.9	42.0
Total production costs	923	73.0	310
Transport cost	46.2	3.6	15.5
Market price raw materials, ton	31.7	31.7	30.0
Gross proceeds	1013	633	267
Tax income			274.5
Profitability, %	4,5	726,8	84.0
Yield, ton/ha	25,3	25	18.0
Cost, 1 ton	38,3	3.8	16,3
Income, 1 ton	1,38	27.8	13.7

Therefore it is necessary to implement such technologies of cultivation of bioenergy crops, which could contribute to a reduction in total energy costs. In the system of technology of growing take place a differentiated use of natural resources, anthropogenic factors and adaptive capacity of cultivated species, varieties, weed control etc. The second largest cost item is fuel. The amount of energy expenditure accounted for the total energy on the cultivation of energy crop. Costs the total energy for cultivation of miscanthus and switchgrass was not the same. A special feature of both crops is the low yield in the first three years of vegetation associated with significant costs for the construction of plantations. Considerable expenditure is incurred in laying plantations of miscanthus because of its vegetative propagation by rhizome planting. However, in subsequent years the exploitation of plantation expenses incurred are fully justified. This is with minimal care, mineral fertilizing and harvesting biomass. Switchgrass has a slightly lower economic performance than miscanthus. Switchgrass is indispensable in the arid southern regions of Ukraine, where miscanthus growing is much more difficult. That's why, for the rational use of energy plantations need to consider the advantages and disadvantages of each crop as a whole.

It is known that Giant Miscanthus is a very cold-tolerant warm-season grass.The optimal planting time for rhizomes is from March to April but planting can continue into May and even early June and still be successful(Christian et al., 2008). In our research the early stages (first and second) planting helped to increase yields from 19 to 45% compared to the third planting time. Early planting takes advantage of spring-time soil moisture and allows an extended first season of growth. This is important, because it enables larger rhizome systems to develop and allow the crop to tolerate drought and frost better We approved also that best depth for *Miscanthus x giganteus* rhizomes is 10 cm deep (Pyter et al., 2010). Results of this study suggest that rhizomes should be 60–75 g, planted to a depth of 10 cm and kept in cold storage for as little time as possible. It is known, that in the first year of growing switchgrass gives up to 30% of its potential, in the second year - up to 70%. 100% of its potential can be reached from the third year of cultivation (Burli et al., 2017). Depending on the cultivation conditions, the switchgrass yield of two-year plants can reach 9 to 18 t/ha (Mitchell et al., 2012). In our experiment, the productivity of three years old plants with inter-row spacing width 30 cm reached 21.6 t/ha.

CONCLUSIONS

The yield of miscanthus increases due to rhizomes planting in the early stages. Optimum planting depth is 8...10 cm. The effect of date and depth of planting on the yield of biomass was observed only in the first year of vegetation. The trend continues due to the difference of plant density.The greatest difficulty in switchgrass growing technology is the increased sensitivity of plants to the conditions of life support in the first year of vegetation. The highest switchgrass yield of dry biomass and the energy output was provided in options with marker crop sowing and the width between rows 30 and 45 cm. A high yield of solid biofuels (20.7 t/ha) and energy (351.0 GJ/ha) with switchgrass is achieved farming with row-spacing width of 45 cm with the sowing of marker crop. It is proved that miscanthus is the most profitable energy crop. The profitability of growing miscanthus for year 3 and subsequent years is 726 %.

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POSSIBILITY OF USING LAND COMPONENTS FOR ESTIMATION OF SOIL EROSION: A CASE STUDY OF A WATERSHED OF THE SECOND URBAN PHASE, MASHHAD, KHORASAN PROVINCE

SUMMARY

In most parts of Iran, due to population growth, deforestation, over-grazing of pastures and other factors, soil erosion is more than the world's average and increase as time passes. Therefore, its correct evaluation is very important. The Mashhad-Chenaran is the biggest and most important sub-basin of Khorasan, with an extension of about 223989 acres. Two models, the MPSIAC and the Erosion Potential Method – EPM (Gavrilovic, 1972), used for evaluation of sediment amounts and soil erosion stations showed 2.74 t ha^{-1} per year. However, the MPSIAC model showed 1.56 t ha^{-1}; whereas the EPM model showed larger amounts of 5.73 t ha^{-1} per year. In soil erosion studies in watersheds, researchers have often introduced hydrological units of work. In this research, physical-geographical factors such as geological factors, soil type, vegetation, slope were utilized in the erosion estimation models used in addition to the hydrological units in the land components. The present study attempted to measure the erosion and sediment in hydrological units (sub-basins) and land components. The accuracy of estimates of erosion was tested; in order to ensure that the accuracy of the results or possibly the superiority of the homogeneous units to the hydrological units is ensured, it can be used in the same areas in the future.

Key words: Erosion, Erosion Potential Method – EPM, Land use, MPSIAC, Sediment.

INTRODUCTION

Soil is one of the most important natural resources in every country. Soil erosion is a serious issue and can be considered as a big threat for civilized mankind (Kavian *et al.*, 2018; Ahamdi *et al.*, 2011, Hessel and Jetten, 2007, Fanetti, 2007; Hadley, 1984). In recent times, the increase in erosion and its effect on the economy and environment result in a serious problem (Lim *et al.*, 2005). Due to the lack of sufficient and reliable information, regarding the

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amounts and kinds of soil erosion in most watersheds in Iran and most other parts of the world, several models have been designed and accomplished to estimate soil erosion and sedimentation. However, the determination of erosion and sedimentation by using available models has some difficulties and problems due to inconsistency and inadaptability in the intended areas. The conflicts in this concern caused researchers to start finding a suitable solution to the amounts of soil erosion and subsequently prevent soil erosion (Ahmadi *et al.*, 2011).

Although soil erosion quality and quantity can be studied with different models, most of them were unsuitable for application. So, it is essential to validate the accuracy and performance of models in different watersheds (Merrit *et al.*, 2003). By comparing MPSIAC, Hydrophysical model with the EPM model, using the GIS tool in Nozhian watershed in Lorestan province, Davari *et al.* (2005) estimated the quality and quantity of erosion and sedimentation. The result showed that the hydrophysical method, using the EMPSIAC model is more precise compared with the EPM model. The result of proficiency estimation by using different experimental methods to determine erosion and sedimentation in Babol Rood watershed in Mazandaran province indicated that among the seven experimental methods namely MPSIAC, EPM, Fornier, Doglas, Kirkby, Geomorphology and Hydrophysics, the MPSIAC model is the most appropriate model for evaluating erosion and sedimentation (Khosravi *et al.*, 2011; Amiri, 2010; Arekhi and Nazari, 2008).

Big watersheds have been divided into sub-basins in several studies. The main reasons for their dissociation are: circumstance of hydrographic network in watershed, watershed area, damaged areas because of flooding or susceptible areas to flooding and study purposes (Esmaili, 2011). To divide watersheds into hydrologic units, some features were considered such as soil gravel volume, depth of porosity, soil texture, depth and type of limiting layer etc. The soil can be divided into four groups based on the ability of runoff potential namely: Hydrologic group A (very low runoff potential), Hydrologic group B (low runoff potential), Hydrologic group C (high runoff potential), Hydrologic group D (very high runoff potential) (Refahi, 1999).

In the first level, the land type is divided into nine main forms and one extra form, based on slope, phsiography etc. At the second level, each land form is divided into several land types according to topographic features. Again in the lower level, each land type is divided into several land components according to other characteristics like parent materials, vegetation type, soil type etc. (Ayoubi, 2006). From the theoretical viewpoint, it was assumed that land unit components have the highest homogeneity (Bagheri, 2008).

The working units which have been used by researchers in soil erosion studies of watersheds are mainly hydrologic units (Ahmadi, 2009). However, in the present study more variables and influencing factors were used namely: geology, soil type, vegetation, slope; to evaluate the amounts of soil erosion and sediment amounts, because we have accessibility to land unit maps and their land components in most regions in Iran, in order to test the effects of these factors.

Blinkov and Kostadinov (2010) evaluated the applicability of various erosion risk assessment methods for engineering purposes. The factors taken into consideration depended on scale, various erosion tasks, as well as various sector needs. According to them, the erosion potential method (EPM) was the most suitable on catchment level for the watershed management needs in this region. It was created, developed, and calibrated in Yugoslavia (Gavrilovic, 1972).

This methodology is in use in: Bosnia & Herzegovina, Bulgaria, Croatia, Czech Republic, Italy, Iran, Montenegro, Macedonia, Serbia and Slovenia (Spalevic *et al.*, 2017a, Spalevic *et al.*, 2017b; Vujacic *et al.*, 2017; Spalevic *et al.*, 2016a, Spalevic *et al.*, 2016b; Tazioli *et al.*, 2015; Barovic *et al.*, 2015; Spalevic *et al.*, 2015; Behzadfar *et al.*, 2014; Spalevic, 2014a; Kostadinov *et al.*, 2014; Spalevic *et al.*, 2014b; Tazioli, 2009; Milevski, 2008; Fustic and Spalevic, 2000; Curovic *et al.*, 1999. The use of this methodology in research on runoff and the intensity of soil erosion have been demonstrated in Montenegro, specifically in the Region of Polimlje (Vujacic *et al.*, 2016; Vujacic *et al.*, 2015; Spalevic *et al.*, 2013a, 2013b, 2013c, 2013d, 2013e, 2012a, 2012b, 2011, Spalevic *et al.*, 2004, 2000a, 2000b, 1999. The EPM is distinguished by its high degree of reliability in calculating sediment yields as well as reservoir sedimentation (Ristic *et al.*, 2011).

MATERIAL AND METHODS

The study area of Mashhad-Chenaran is the biggest and most important sub-basin of Kashaf Rood, extending 224009 acres. Mahshad-Chenaran is a relatively big steeped plain located between Hezar-Masjed and Binaloud mountains (Fig. 1). This plain is rectangular, with 120 km in length and 28 km width extending from the northeast to south east between Hezar-Masjed and Binaloud mountains.

The study area of Mashhad urban phase II was divided into 4 hydrologic groups (Table 2, Fig.2) and 37 sub-basins or hydrologic units (Fig.1, Table 4).

Based on the results of resource assessment and land capability classification, it contains 7 main land types including mountains, hills, plateaus, upper terraces, piedmont plains, flood plains, gravel debris, fan-shaped alluvial gravel, and a miscellaneous type as well as composite and non-arable lands (Fig. 3, Table 6). According to geology, Paleozoic formations such as Lalun and Mila can be found in this area and the middle section includes a quaternary deposit. The diversity of formations is one of the important factors which constitute different soil types and different geomorphological forms, hence the major effect is on soil erosion and sedimentation.

This area has a Mediterranean type of rainfall, with dry season in summers and rainy season in cold winters. Due to sparse vegetation this area is very susceptible to erosion. The average rainfall in Mashhad station is 250 mm. As a result of the type of geology and little vegetation, some sporadic snowing reduces the erosion in this area. In this study, the Gavrilovic (EPM) and MPSIAC models were considered in the estimation of erosion and rates. Based on the contemplation of 9 factors in erosion and sediment yield (Table 1) including surface geology, soil, climate, runoff, slope vegetation and land use, the current erosion status and fluvial erosion and sediment transfer were calculated by the MPSIAC model and compared with the models used in Iran (Refahi, 2001). In order to use this model, the watershed was divided into hydrologic units (sub-basins) or land unit components or geomorphological homogenous work units based on intended objectives. The used units and their values are presented in Table 1. The sum of these scores shows the amounts of sediment and erosion intensity in each area (Ahmadi, 2009).

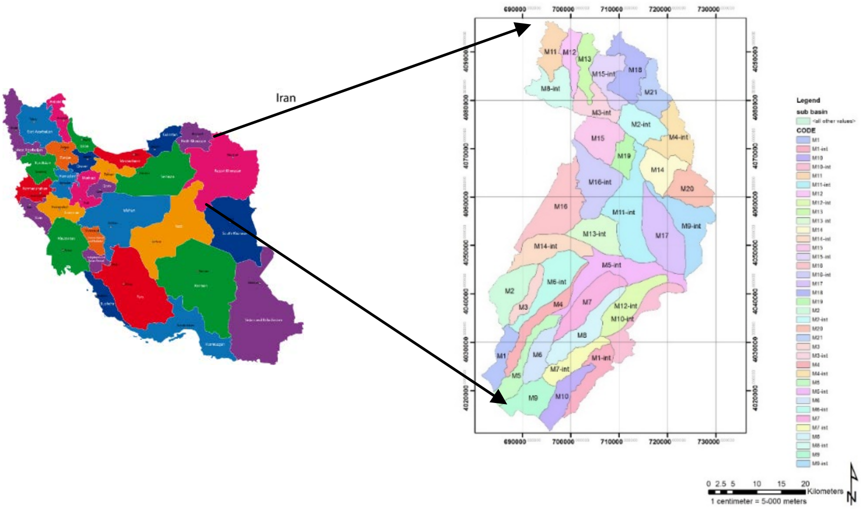

Fig 1: Study Area: Mashhad watershed phase II and intended area location

Table1: The factors and their values used in MPSIAC model

Factors	Calculated points	**Definitions**
Geology	$Y_1=X_1$	X_1: stone sensitive point
Soil	$Y_2=16.6K$	K: erodibility factor in USLE
Climate	$Y_3=0.2X_3$	X_3: precipitation intensity with 2 year interval return
Water runoff	$Y4=0.006R+10Q_p$	R: annual runoff depth (mm), Q_p: annual specific discharge (CmS/km^2)
Topography	$Y_5=0.33S$	S: average watershed slope (%)
Land cover	$Y_6=0.2X_6$	X_6: bare soil (%)
Land use	$Y_7=20-0.2X_7$	X_7: canopy cover (%)
Surface erosion	$Y_8=0.25X_8$	X_8: points summation in BLM model
Gully erosion	$Y_9=0.16X_9$	X_9: point of Gully erosion in BLM model
$R= Y_1+Y_2+Y_3+Y_4+Y_5+Y_6+Y_7+Y_7+Y_8+Y_9$		

The EPM method is used to determine 3 subjects namely: 1) Erosion intensity and specific erosion, 2) sediment coefficient and specific sediment discharge and 3) total sediment discharge in the determination of soil erosion intensity. Four factors including soil erodibility index (Y), land use coefficient (X_a), erosion coefficient (Ψ), and mean slope of the watershed (I) are used in this model.

Considering that there are several effective layers in EPM and MPSIAC, and these layers are used to achieve erosion intensity and sediment yield maps, the first step is to overlap these layers and then by merging these data, the erosion status of the study area can be determined. Map drawing steps are as follows:

1.Georeferencing information layers;

2.Matching watershed and sub-basin boundaries in different layers;

3.Polygoning of target units;

4.Converting polygons to raster layers;

5.Superpositioning and calculating raster layers (9 MPSIAC factors, Table 1 and the factors affecting EPM). Obtaining maps of erosion intensity based on the weight of each layer.

RESULTS AND DISCUSSION

Mashhad urban watershed phase II (Fig. 1) is divided into 4 hydrologic Groups (Fig. 2, Table 4), 21 main sub-basins and 16 subsidiary sub-basins or 37 Hydrologic Units (Fig.1). Further, this watershed has been divided into 10 land types, 23 land units and 35 land unit components (Fig. 3, Table 7). Sub-basins and land unit components were contemplated as study units to examine erosion rates and intensity.

Table 2: The area of hydrologic Groups in Mashhad watershed phase II

Hydrologic Groups	Area (ha)
A	35206
B	76778.448
C	49170.237
C+D	1661.268
D	61193.28
Sum	224009

The erosion types in the studied area were categorized as follows: Sheet erosion (S) was observed more in most parts of the area. This type of erosion is classified into three (3) namely: S_1 with lowest erosion, S_2 and severe erosion (S_3). Rill erosion (R) appears because of runoff on the soil surface with not very deep furrows and will disappear by farming operations (Refahi, 1999). This type of erosion is divided into 3 classes including R_1 with furrows less than 10 cm depth, R_2 with depth of furrows ranging between 10 to 30 cm, and R_3 with furrows more than 30 cm depth. Gully erosion (G) which is an advanced type of rill erosion and waterways or water streams is clear on the land surface (Refahi, 1999).

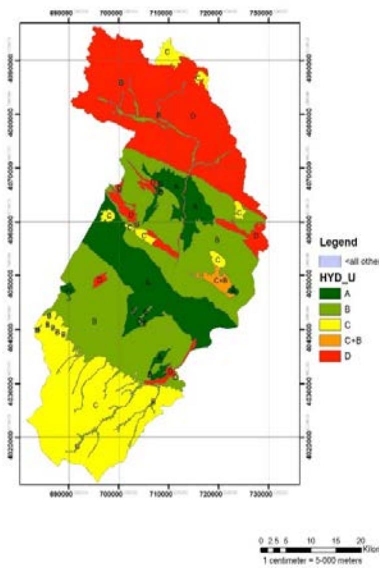

Fig. 2 Hydrological map Groups

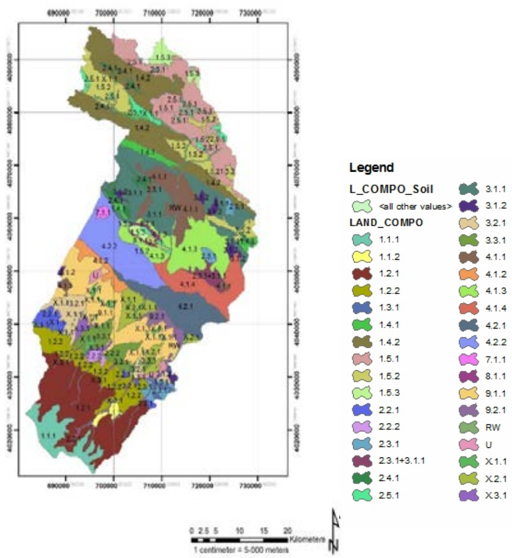

Fig. 3 Land components map

This type can also be divided into 3 classes: G_1, G_2 and G_3. By using EPM and MPSIAC, erosion was calculated in the studied units and dominant erosion types in sub-basins and land unit components are shown in Tables 4, 5 and 7, respectively. Estimated erosion and sedimentation in work units (Table 3) showed that changing the studied units (sub-basins and land components) has no effect on estimation accuracy.

Table 3: erosion and sediment Rates in Mashhad urban watershed phase II by studied units dissociation

Area (ha)	Studied unit	Sediment (ton.ha^{-1} per year)			Erosion (ton.ha^{-1} per year)	
		Hydrometric stations	MPSIAC	EPM	MPSIAC	EPM
223989.7	Sub basin	2.74	1.56	5.73	3.19	9.45
	Land Component		1.50	-	3.39	9.66

There is a difference between calculated sediment amount by using 2 used models in this study and reported sediment amount in sediment measurement stations (Table 3, Diagram 1). The sediment amount calculated using the MPSIAC model is 1.5 $ton.ha^{-1}$ per year which is about half of the reported studies in sediment measurement stations. It shows less estimation in this model, however this variable calculated by EPM was 5.73 $ton.ha^{-1}$ per year which is 2.9 times higher than reported studies and it shows overestimation in this model.

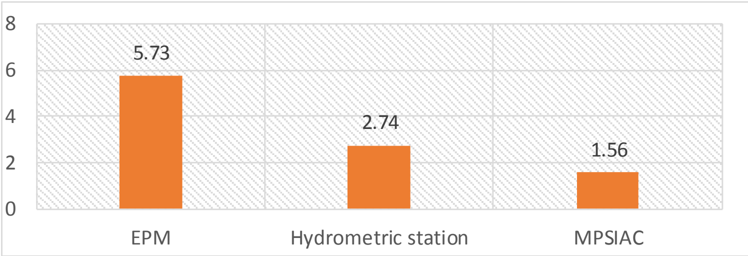

Diagram 1: Estimated sediment amount ($ton.ha^{-1}$ per year) Comparing the results of EPM, Hydrometric stations and MPSIC.

The findings in this study confirm previous studies conducted by Davari *et al.* (2005), Ahmadi *et al.* (2011), and Abedini *et al.* (2013). The erosion level of each sub-basin and the differences of estimation by using EPM and MPSIAC models and dominant erosion types in each sub-basin are shown in Table 4.

Studies conducted in Iran, in relation to this topic include the following research teams lead by: Bagherzade Karimi (1993), Faraji (1994), Tahmasbipour (1994), Shakeri and Balaeepour (1994), Nikjoo (1994), Asadi (1995), Rafahi and Nemati (1995), Sarkhosh (1996), Koupayi (1997), Azamirad (1998), Ghaderi Choukanlou (1998), Bayat (1999), Rafahi (1999); Nabipei Lashkarian (2000), Agharazi and Ghodousi (2001), Bayat *et al.* (2001), Ghodoosi (2002); Tangestani (2006), Khodabash (2010). In these studies, the MPSIAC model compared to the EPM model or one of the experimental methods was introduced as the more effective model (Davari *et al.*, 2005). Also, in studies which employed GIS, regardless of the result of erosion and sediment yield, the utilization of modern technologies like remote sensing (RS) is recommended because of accuracy and time saving compared to the traditional methods (Rastgou, 2006; Malekian *et al.*, 2012).

A difference between observational estimated sediment and these 2 models (Table 3, Diagram 1) might be found due to the in-acquisition and comprehensiveness of these models with different conditions of watershed. When the study unit defined land components, the length of the longest waterway or water stream will not be calculable by using conventional methods and this parameter cannot be defined in land unit components. This is the reason why the sediment amount is not calculable by the EPM model. The erosion types of the land components (Table 5) and estimated erosion by using the EPM and MPSIAC models, soil types, vegetation types and land usage are shown in Table 7. Based on the soil map of Iran (reference), this area is covered dominantly (48.32%) by shallow soils (Lithic Xerorthens), based on the slope (25%) they are categorized into class C and D hydrologic groups. The land slope was 25% and categorized into hydrologic groups C and D. Mountains and hill types with rock outcrop are dominant and usually there is no vegetation or canopy or low canopy can be observed (Table 7).

Table 4: Amount and types of erosion in each sub basin (ton.ha-1 per year)

Sub basin	Area (ha)	Erosion (t ha^{-1} year)		Erosion type
		MPSIAC	EPM	
M_{1-int}	5029.73	2.98	7.64	$S_1R_2G_1$
M_{2-int}	5312.76	7.36	18.80	$S_1R_2G_3$
M_{3-int}	4226.73	4.36	17.42	$S_2R_2G_1$
M_{4-in}t	6278.54	7.72	9.78	$S_1R_2G_3$
M_{5-int}	9570.36	1.70	2.16	S_1R_1
M_1	3935.53	4.27	12.05	$S_2R_1G_3$
M_2	6576.25	1.80	8.53	S_1R_1
M_3	2729.25	2.00	7.16	$S_1R_1G_1$
M_{6-int}	7820.80	1.51	4.29	S_1R_1
M_4	5119.68	2.78	9.28	$S_1R_1G_1$
M_5	5013.32	3.96	12.92	S_2G_1
M_6	4540.47	3.70	7.72	S_2G_1
M_7	6663.45	1.56	9.02	S_1R_2
M_8	6815.01	2.53	9.62	$S_1R_1G_1$
M_9	5450.10	4.62	12.32	$S_2R_1G_1$
M_{7-int}	4525.03	3.19	10.51	$S_1R_2G_1$
M_{10}	4994.99	4.10	7.16	$S_2R_1G_1$
M_{11}	4250.62	4.81	13.20	$S_2R_1G_1$
M_{8-int}	5440.12	5.55	18.90	$S_1R_2G_2$
M_{12}	3446.04	5.84	14.70	$S_1R_1G_2$
M_{13}	3750.15	5.72	16.44	$S_1R_1G_2$
M_{20}	5415.62	3.48	27.50	S_2R_2
M_{15}	6597.36	4.85	24.56	$S_2R_2G_2$
M_{9-int}	8588.61	2.98	8.03	$S_1R_2G_1$
M_{16}	11333.53	1.44	4.55	S_1R_1
M_{10-int}	5507.97	1.95	7.38	$S_1R_1G_1$
M_{11-int}	12059.95	2.73	5.55	$S_1R_2G_1$
M_{17}	10041.66	1.74	2.88	S_2R_2
M_{12-int}	6462.16	1.42	4.95	S_1R_1
M_{13-int}	5806.96	1.36	1.47	S_1R_1
M_{14-int}	6727.10	1.41	3.58	S_1R_1
M21	4928.14	3.64	4.92	$S_1R_1G_1$
M_{15-int}	5020.31	4.25	13.10	$S_2R_1G_1$
M_{18}	6834.11	3.97	9.29	$S_1R_1G_1$
M_{16-int}	9014.04	2.23	11.14	$S_1R_2G_1$
M_{14}	5301.95	6.38	10.43	$S_2R_2G_2$
M_{19}	2880.64	3.90	10.62	$S_2R_2G_2$

Table 5: Types of erosion in each land unit components

Land Component	Erosion Type	Land Component	Erosion Type	Land Component	Erosion Type
1.1.1	$S_2R_1G_1$-S_2G_1-$S_1R_1G_1$	3.1.1	$S_1R_2G_1$-S_2R_2-$S_2R_2G_2$-$S_1R_2G_3$-S_1R_1	7.1.1	S_1R_1
1.1.2	$S_1R_2G_1$- $S_2R_1G_1$	3.1.2	$S_1R_2G_1$-$S_2R_2G_2$-S_2R_2	8.1.1	$S_1R_2G_1$- S_1R_1
1.2.1	$S_1R_2G_1$-$S_2R_1G_1$-$S_1R_1G_1$- S_1R_1-S_2G_1- S_1R_2	3.2.1	S_1R_1-$S_1R_1G_1$	9.1.1	S_1R_1- $S_1R_1G_1$-S_1R_2
1.2.2	$S_1R_2G_1$-$S_2R_1G_1$- S_1R_1-$S_1R_1G_1$- S_2G_1-S_1R_2	3.3.1	S_1R_1-$S_1R_1G_1$-S_2G_1- S_1R_2	9.2.1	S_1R_1- $S_1R_1G_1$
1.3.1	$S_1R_1G_1$-$S_1R_2G_1$-	4.1.1	S_1R_1-S_2R2-$S_1R_2G_2$	X.1.1	$S_1R_1G_1$- S_1R_1-S_1R_2
1.4.1	$S_2R_2G_2$-S1R2G1- S_1R_1	4.1.2	S_1R_1	X.2.1	S_1R_1- $S_1R_1G_1$-S_1R_2
1.4.2	$S_1R_2G_1$-$S_2R_1G_1$-$S_1R_1G_1$-$S_1R_1G_2$- $S_2R_2G_1$	4.1.3	$S_1R_2G_1$-S_2R_2	X.3.1	$S_1R_1G_1$- S_1R_1-S_2G_1
1.5.1	$S_1R_2G_1$-$S_1R_1G_2$- S_2R_2-$S_1R_1G_1$- $S_2R_2G_2$	4.1.4	S_1R_1-S1R_2G_1-S_2R_2		
1.5.2	$S_1R_1G_1$-$S_1R_2G_3$-$S_2R_2G_1$-$S_1R_1G_2$- $S_2R_1G_1$	4.2.1	$S_1R_1G_1$-S_1R_1- S_1R_2		
1.5.3	$S_1R_1G_1$-$S_1R_2G_1$-$S_2R_1G_1$- S_1R_1	4.2.2	S_1R_1		
2.2.1	$S_1R_2G_1$- S_1R_1-$S_1R_1G_1$				
2.2.2	S_2G_1- S_1R_2				
2.3.1	$S_1R_2G_1$-$S_1R_1G_1$- S_2R_2				
2.4.1	$S_1R_2G_1$- S_1R_1-$S_1R_1G_2$-$S_1R_2G_2$-$S_2R_1G_1$- $S_2R_2G_1$				
2.5.1	$S_1R_2G_1$-$S_1R_2G_3$-$S_1R_1G_1$-$S_2R_1G_1$-$S_1R_1G_2$-$S_1R_2G_2$- $S_2R_2G_1$				

Based on the similarity of soil types, similar land types and being rocky, having many outcrops in these lands, high slope and low vegetation are the main factors of the high erosion rate. EPM and MPSIAC have been innovated in those countries with different climate and geology attributes. Hence, the coefficients and effective factors in erosion does not correspond to conditions in Iran completely.

For example, in the EPM model, the rock and soil sensitivity to erosion due to the lack of uniformity of weather conditions, weathering and natural erosion of rock and geological formations, as well as differences in land use and vegetation type, have very high values for erosion and are far from reality. In the MPSIAC model, to determine the second effective factor of erosion (soil factor), soil erodibility coefficient (K) was used in the global erosion equation. In this model, the rock outcrop level and its effect on estimation of erosion are not considered and this can affect high calculated erosion in land unit components (mountains and hills). Furthermore, the percentage of canopy can be considered as land use index. This index is suitable for pastures and is not suitable for arable and gardens which have specific canopy (Ahmadi, 2011).

The homogeneity of studied units is very important in determining the accuracy of the estimated erosion amounts in the Otan sub-basin (M_{16}), with 11333.53 hectares which contains 5.06% of the total studied area (Fig. 4), has been dissociated into 10 land unit components (Table 6). Based on information in Table 4, erosion type in this hydrologic Unit (M_{16}) S_1R_1 showed low sheet and rill erosions. Table -5 shows the different types of erosion in the Otan sub-basins.

There are different erosion types in each land component of the Otan sub-basin (M_{16}) (Fig. 4, Table 6). It is necessary to attend to erosion types numbers to reduce erosion in land usage. If our focus is on sub-basins, excess erosion in a small expanse of land will not be important.

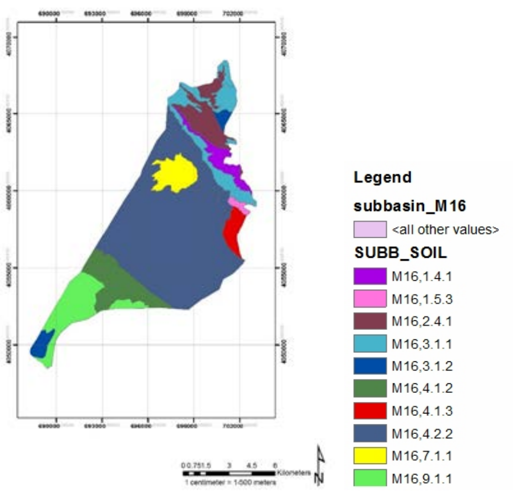

Fig. 4 Map of Otan sub basin (M_{16}), dissociation of land components

In the Otan sub-basin (M_{16}), erosion type is defined as S_1R_1 (Table 4), however rill erosion (R_2) with 10 to 30 cm depth and moderate gully erosion (G_2) with 1 to 3 m width were also observed. Furthermore, gullies in this group affected 25 to 50% of the total area (Refahi, 1999). Soil erosion can be prevented or limited by giving adequate attention to erosion types, even in a small expanse of land and designation of suitable usage.

Table 6: The amount and types of erosion in Otan sub basin (M16) by dissociating the land components

Sub basin	Land components	Erosion(ton/ha.year)	Erosion type	Soil type
		MPSIAC		
Otan (M_{16})	1.4.1	1.45	$S_2R_2G_2$- $S_1R_2G_1$- S_1R_1	Lithic Xerorthents
	1.5.3	1.35	$S_1R_1G_1$- $S_1R_2G_1$- $S_2R_1G_1$- S_1R_1	Lithic Xerorthents
	2.4.1	1.44	$S_1R_2G_1$-S_1R_1- $S_1R_1G_2$- $S_1R_2G_2$- $S_2R_1G_1$- $S_2R_2G_1$	Lithic Xerorthents
	3.1.1	1.41	$S_1R_2G_1$- S_2R_2- $S_2R_2G_2$- $S_1R_2G_3$- S_1R_1	Xeric Hoplogypsids
	3.1.2	1.41	$S_1R_2G_1$- $S_2R_2G_2$- S_2R_2	Xeric Hoplogypsids
	4.1.2	1.56	S_1R_1	Xeric Hoplogypsids
	4.1.3	1.51	$S_1R_2G_1$- S_2R_2	Xeric Hoplogypsids
	4.2.2	1.39	S_1R_1	Typic Haplocambids
	7.1.1	1.62	S_1R_1	Sodic Haplocambids
	9.1.1	1.53	S_1R_1- $S_1R_1G_1$- S_1R_2	Xeric Torrifluvent

Erosion is high as a result of the soil type (Lithic Xerorthents) and similarity of land types (mountain and hill) in the hydrologic group D, and because of high rock outcrop and low canopy or no canopy (Table 7). The mountains (1.4.1 ‹1.4.2 ‹1.5.1 ‹1.5.2) constituted a salty formation with Red bed and gypsum and in some parts contained conglomerate (1.4.1) and in some parts included valleys with Mozdouran parental material (1.4.2) (Table 7). Another part of the hydrologic group D contains the Mozdouran formation (2.5.1, 1.5.2, 1.5.1) (Table 7). One of the reasons for the high amount of erosion in these areas is probably the ineffectiveness and inappropriateness of the EPM and MPSIAC models for this study area.

CONCLUSIONS

In general, erosion factors in the researched area refer to high slope in folding, texture of parent material, geological formations, rainfall regime, the premature grazing, and the excess capacity, and untechnical exploitation such as: cultivation in high slopes, plowing in the direction of slope and no consideration to crop rotation. The highest amount of erosion occurs in gypsum formations, which naturally forms due to loosening, and also the high dissolution coefficient, erosion acts physically. Preventing and reducing erosion in these areas is difficult but necessary. Due to the aristocracy of these lands to agricultural lands and also the passage of water, it can lead to degradation and low land salinity.

Unfortunately, the minor slopes of these heights have been cultivated and plowed due to the provision of more soil and conditions for erosion. Dry farming in a part of the land, especially in high slope lands, is one of the most effective factors in land degradation, erosion and sediment yield. Considering the importance of dry farming and technical principles in these lands can be very effective in reducing erosion. Also, the determination of land suitability and land use change, inappropriate for utilization, can be an important step towards sustainable land use.

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COMPARATIVE EFFICACY OF SOME EMPIRICAL MODELS TO ESTIMATE SEDIMENT YIELD IN SMALL CATCHMENTS

SUMMARY

Empirical models have been developed to estimate erosion for a certain area and their calibration is essential for use in off-site conditions. Empirical modeling with high accuracy and efficiency for estimating sediment load can lead to better estimates of sediment load, resulting in a more appropriate design of soil and water conservation practices. The aim of this study was to compare the efficiency of FSM, MPSIAC and PSIAC models in estimating erosion and sedimentation. In this study, 10 small watersheds were selected with areas between 14.3 and 556 hectares as the field of research. The model parameters were determined using available maps, satellite images and field operations. The amount of watershed sediment was estimated using empirical models described above. Observed sediment was calculated by determining sediment volume in check dam by field method. The minimum, average and maximum observed sediment were respectively 0.41, 0.82 and 1.18 m^3ha^{-1} $year^{-1}$. The correlation coefficients between the observed sediment and the estimated sediment by the empirical models of FSM, MPSIAC and PSIAC, respectively, were 0.56, 0.47 and 0.54. The estimated sediment data of the empirical models were compared with the observed sediment data of the relative error test. Values of the relative error estimated for FSM, MPSIAC and PSIAC models were 38.67, 1.22 and 2.35. Efficiency index of Nash and Sutcliffe for FSM, PSIAC and MPSIAC models were, respectively, -17386.37, -45.46 and -11.48. These results indicated that the FSM model compared with the other two models had more errors, and this model with the baseline coefficients has no efficiency to estimate the sediment of small watersheds, and the two PSIAC and MPSIAC models also faced with sediment overestimation, but the MPSIAC model showed relatively acceptable results.

Keywords: Sediment, Empirical Models, FSM, MPSIAC, Small Watersheds

INTRODUCTION

Soil erosion and sediment yield is one of the main challenges in Iran, and quantity estimation of them is an important issue (Amini et al., 2014). In the

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current situation, statistics and information are collected by data acquisition from sediment measurement in stations of large-scale watersheds. The results obtained from the data are not easily generalizable to the small sub-basins and watersheds. On the other hand, enough information is not available on consistent rate of empirical methods to estimate erosion and sedimentation concerning condition of watersheds in the country, and this leads to distrust the statistics and information about erosion and the estimated sediment in the basins. Based on the evidences, the greatest difficulty caused by sedimentation is related to small-scale watersheds. Lack of appropriate data on sediment for small watersheds has created many problems for watershed management experts. One way is to use empirical models to estimate soil erosion and sediment yield.

All these models consist of empirical coefficients and constants, which may not be suitable for other regions (Hadley, 1985) so that Pourkarimi et al. (2017) recommended that both the EPM and MPSIAC models were created in countries with climates and geology attributes that differ from those of Iran. Hence, the coefficients and factors affecting erosion do not correspond precisely to the conditions in Iran. Mahmoudzadeh (2002) measured the specific sediment yield on 19 small watersheds with end dam in New South Wales, Australia, and considered the trapping coefficient due to sediment outflow; the age of structure was 4 to 31 years. Martin-Rosales et al (2003) measured sediments 107 of Gabion, concrete, stone and cement dams in a semi-arid region with Mediterranean climate in the south of Spain. Verstraeten et al. (2003) provided a quantitative model of FSM (Factorial Scoring Model) for the first time in Spain, and used five factors of topography, lithology, basin shape, ditch and vegetation to calculate sediment yield in catchments. De Vente et al. (2005) calibrated the FSM model for the sub watersheds and resolved existing problems. Haregeweyn et al. (2005) also evaluated the FSM and PSIAC models in Tigre region of Ethiopia, and concluded that the FSM model has less fit with the observed values than the PSIAC model. Khodami (2005) used for the first time this model in Iran on Lateshur watershed, Northeast of Pakdasht, and found that the FSM model has more suitable match than the PSIAC model. Gholami (2013) by applying the FSM and MPSIAC models in Nahand watershed of East Azerbaijan, Iran, and reported the sediment values respectively equal to 3.27 and 4.18 tons per hectare per year, representing the superiority of the FSM model compared to MPSIAC model in this research. Neil (1988) through farm dam in the outlet of fourteen small watersheds at River Valley at Yasi found that the sediment value varies from six to 54m^3/km^2/year. Management factors in sediment yield are more crucial than morphological parameters. De Vente et al. (2006) in another study in Italy, added landslide factor to the FSM, the results of this study compared to the previous method showed better agreement with measured sediment value. Nichols (2006) in southern Arizona, the United States, measured sediment in eight sub-basins, with an average of 30 to 47 year life of the dams. Hashemi (2010) assessed sediment volume deposited in the reservoir of nine soil dams in Semnan province, Iran, which based on the evidence, there was no overflow of sediment; the lifespan of the structures was 14 years. Mohamadiha (2011) used the FSM model in Eivaneki watershed in Iran and compared the results with data obtained from the MPSIAC models and simulators, and concluded that the data of FSM model are more consistent compared to the MPSIAC model. Atapourfard (2012) using the FSM model on Tehran watershed found that the efficiency was 0.62 and the PRMSE was equal to 0.27. Gusman 2012 estimated the FSM of the sediment load in upper Liobregat watershed greater than the actual value.

This study was carried out to evaluate the efficiency of the empirical models of FSM, MPSIAC and PSIAC to determine their accuracy in estimating sediment of small watersheds with an area up to 10 square kilometers.

MATERIAL AND METHODS

This study was carried out in West Azerbaijan province that is located at latitude 38°58' to 39°47' N and longitude 44°14 to 47°17''E in Iran. Based on data achieved from 10 small watersheds with an area between 14.35 and 556 hectares. The study basins had a variety of slope, land use, topography and geology.

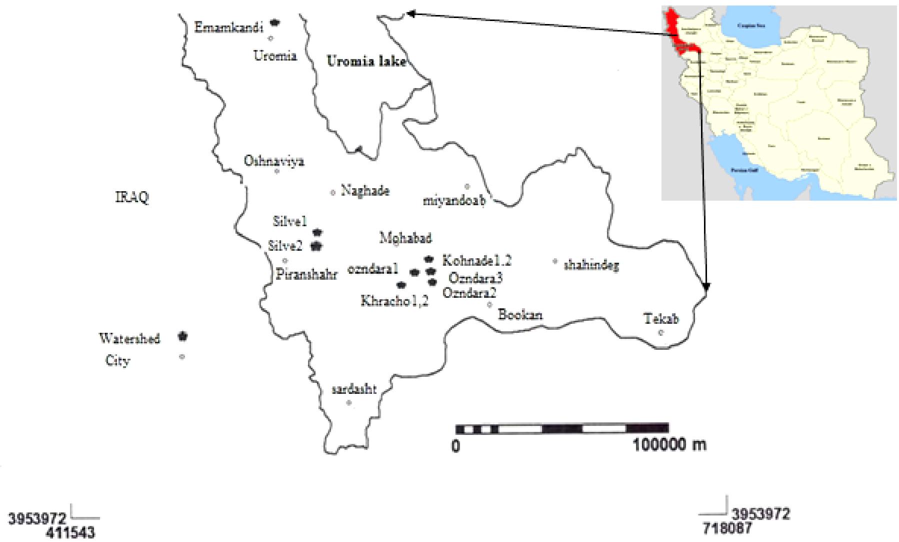

Fig. 1: Location of the study area

The sedimentation volumes were measured in reservoirs of 53 small dams, which had sufficient volume for trapping sediment. The sediment control structures were in kinds of concrete, stone or mortar and Gabion sometimes; there was a check dam in a number of watersheds and over 14 small dams in some of them, which had been constructed in continuous across the drainage path and had enough space for trapping sediment behind the dams.

The steps of the research were as follows:

- First, selection of 10 small watersheds having check dam with a minimum of 10 years construction history,

- Studies on physiographic features, lithology, soil science, meteorology, hydrology, topography, vegetation, land use and erosion for each of the watershed,

- Scoring the model factors using various tools, including previous studies, satellite images, aerial photos, topographic maps, field operations and table on how to score the factors in the FSM, MPSIAC and PSIAC models.

Table 1: How to scoring factors in the FSM model (Verstraeten et al., 2003)

Factor	Score	Description
Topography	1	Very gentle slopes near reservoir and main rivers; elevation difference !200 m within 5 km
	2	Moderate slopes near reservoir and main rivers; elevation difference 200–500 m within 5 km
	3	Steep slopes near reservoir and main rivers; elevation difference O500 m within 5 km
Vegetation cover	1	Good contact cover of the soil (O75% surface protected)
	2	Moderate contact cover (25–75% protected surface)
	3	Poor contact cover (25% protected)
Gullies	1	Bank and ephemeral gullies are very rare
	2	Few bank and/or ephemeral gullies can be observed
	3	Many bank and/or ephemeral gullies can be observed
Lithology	1	Dominant limestone, sandstone or conglomerate(low weathering degree)
	2	Dominant Neogene sedimentary deposits (gravels, etc.)
	3	Strongly weathered (loose) material loams and/or marls
Watershed shape	1	Elongated basin shape with one main river channel draining to the reservoir. No significant direct runoff
	2	Between elongated and (semi-) circular basin shape
	3	(Semi-) circular basin shape with many rivers draining into the reservoir and/or much direct runoff from hill slopes to the reservoir

- Sediment yield in the watersheds was determined using equation (1) in the FSM model.

$SSY = 4193A^{-0.44} + 7.77(FSMIndex) - 310.99$ Equation (1)

Which, SSY: specific suspended-sediment yield (t/km^2), A: area (km^2) and FSM Index: obtained by multiplying the five factors of the model.

- The range of scores for nine parameters of the PSIAC and MPSIAC models was determined as Table 2 using a pointed tools, field studies and expert work.

Table 2: The range of scores for the parameters of PSIAC and MPSIAC models (Johnson and Gebhardt, 1982)

Erosion factors	Psiac	Mpsiac	parameters
lithology	0-10	Y1=X1	X1=Geological erosion index
Soil	0-10	Y2=16.67X2	X2=Soil erodibility factor
climate	0-10	Y3=0.2X3	X3=6-hour rainfall with a 2-year return period
runoff	0-10	Y4=0.006R+10Qp	Qp= annual specific Debi (m^3/skm^2)R=annual of runoff Height (mm^3)
topography	0-20	Y5=0.33X5	X5=Percentage of the average basin slope
vegetation	-10-(+10)	Y6=0.2X6	X6=Percentage of land without vegetation
Land use	-10-(+10)	Y7=20-0.2X7	X7=Percentage of vegetation cover
Surface erosion	0-25	Y8=0.25X8	X8=total surface soil factor scoring in BLM*
Channel erosion	0-25	Y9=1.67X9	X9=Gully scoring in BLM*

*BLM: Breau of Land Management (Refahi 2006)

- In the PSIAC model, the rate of annual sediment yield was calculated after scoring for each factor and the sum of these scores using equation (2),

$Qs = 38.77\ e^{0.0353R}$ Equation (2)

Where: Qs: the rate of sediment yield (m^3/km^2/year), and R: the sediment yield degree.

-In the MPSIAC model, equation (3) was used to estimate the sediment.

$Qs = 0.253e^{0.036R}$ Equation (3)

-The observed sediment in each watershed was determine via the sediment volume by measuring the sediment of the reservoirs accumulated in behind the check dam.
-The estimated sediment of three FSM, MPSIAC and PSIAC models was investigated and analyzed with measured sediment from the sediment measurement in dams, by the statistical method of relative error in equation (4) (Moore et al., 2009).

$$\sigma x = \frac{\Delta x}{x} = \frac{xo - x}{x} = \frac{xo}{x} - 1 \quad \text{Equation (4)}$$

Where: X_o = estimated value, X = observed value and δ_X = relative error
- Nash and Sutcliffe method was used to evaluate the efficiency of model, equation (5) (Nash and Sutcliffe 1970).

$$ME = 1 - \frac{\sum_{i=1}^{n}(Q_i - Pi)^2}{\sum_{i=1}^{n}(Q_i - \overline{Q})^2} \quad \text{Equation (5)}$$

Where: ME: model efficiency, Qi: measured values, Q̄: average measured values, Pi: predicted values and n: number of samples used

RESULTS AND DISCUSSION

Some of the features of study areas including watershed size, check dam age, type of land use, weighted average slope, annual rainfall and basin lithology have been presented in Table 3.

Table 3: General characteristics of the study area

small catchments	Area (ha)	Age (year)	Land use	Slope (%)	Precipitation (mm)	Litology
Emamkandi	174.03	12	Rangeland	44.94	346/7	Limestone, dolomite, shale,
Silve1	79.00	13	Rangeland & dry farming	16.92	549	Limestone, old terraces
Silve2	78.00	12	Rangeland & dry farming	15.70	549	Limestone,
Ozondara1	14.35	11	Rangeland & dry farming	35.11	496	Phyllite, gneiss, volcanic rocks
Ozondara2	104.70	11	Rangeland & dry farming	38. 85	496	rhyolite
Ozondara3	129.04	11	Rangeland	40.47	496	Rhyolite, red sandstone, siltstone
Kohnede1	556.00	10	Rangeland & dry farming	32.62	496	rhyolite
Kohnede2	408.00	10	Rangeland & dry farming	30.74	496	Rhyolite, red sandstone, siltstone,
Khracho1	97.06	10	Rangeland & dry farming	37.24	496	gneiss, granite
Khracho2	118.75	10	Rangeland & dry farming	36.87	496	gneiss, granite

The scores of five factors of the FSM model were determined using various tools including satellite images, aerial photos, topographic maps, field operations, integrating mentioned data and information of Table 1. The FSM model applying equation (1) with the unit of ton/ha/year estimated the sediment and then was converted to m^3/ha/year based on the specific weight of sediment (Table 4).

Table 4: Final scores of five factor and sediment yield calculation results by FSM method

parameters	Emamkandi	Silve1	Silve2	Ozondara1	Ozondara2	Ozondara3	Kohnede1	Kohnede2	Khracho1	Khracho2
Topography	2.91	2.02	1.95	2.74	2.88	2.29	2.85	2.75	2.84	2.84
vegetation cover	2.00	2.00	2.00	2.00	2.00	2.00	2.52	2.57	2.60	2.67
Gulli	1.00	1.00	1.00	1.00	1.00	1.00	2.00	2.00	3.00	3.00
Litology	1.85	1.00	1.00	2.00	2.00	1.84	1.48	1.29	2.00	2.00
Shape	2.00	1.00	1.00	3.00	2.00	2.00	2.00	2.00	2.00	2.00
Area(ha)	1.82	0.79	0.78	0.14	1.04	1.29	5.56	4.08	0.97	1.12
FSM(Index)	21.53	4.04	3.90	32.88	23.04	21.49	41.03	36.60	88.60	90.99
Predicted SY (ton/ha/y)	30.92	43.83	44.07	100.25	40.07	36.23	19.18	22.07	40.70	37.06
Predicted SY (m^3/ha/y)	23.35	31.31	33.90	38.75	26.37	25.88	14.31	15.12	29.72	28.08

According to Table 4, the average estimated sediment from the FSM model in the study watersheds was 30.30±17.07m^3. The minimum observed sediment was 13.14m^3 and the maximum value was 38.75m^3. Nine parameters of the PSIAC model and the amount of sediment (m^3/ha/year) have been shown in Table 5.

Table 5: Final scores of nine factors and sediment yield calculation results by PSIAC method

parameters	Emamkandi	Silve 1	Silve 2	Ozondara 1	Ozondara 2	Ozondara 3	Kohnede 1	Kohnede 2	Khracho 1	Khracho 2
lithology	4.8	3.0	3.0	5.5	5.5	5.0	4.2	3.6	5.3	5.2
Soil	4.5	6.5	6.5	4.5	4.5	4.0	4.0	4.0	6.0	6.0
climate	3.0	2.0	2.0	2.5	2.4	2.5	2.0	2.0	2.5	2.4
runoff	8.0	5.5	5.0	7.0	8.5	8.0	6.5	7.0	6.0	6.0
topograghy	16.0	7.0	7.0	11.0	12.0	13.0	12.0	11.0	13.0	12.7
vegetation	1.0	2.0	2.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Land use	2.0	5.0	5.0	4.0	4.0	4. 0	4.0	4.0	6.0	6.0
Surface erosion	7.0	7.5	7.5	9.0	10.0	9.5	8.5	8.5	11.0	12.0
Channel erosion	3.0	3.0	3.0	6.0	4.0	4.0	6.0	5.0	7.0	7.0
Sediment Rate	49.3	41.6	41.0	52.5	53.9	53.0	50.2	48.1	59.8	60.4
Predicted SY $m^3/km^2/y$	226.6	171.8	168.2	253.9	267.0	259.0	233.7	216.9	329.3	366.9
Predicted SY m^3/ha/y	2.2	1.72	1.68	2.54	2.67	2.59	2.34	2.17	3.29	3.37

In the PSIAC model the minimum, average and maximum estimated sediment were, respectively, 1.67, 2.46 and 3.37m^3/ha/year (±0.57). The estimated values have three times overestimation compared with the observational data. Skewness of data for the observed values was -0.08, and for the estimated data was 0.29, and correlation coefficient among the sediment data estimated from model and the observational data was 0.54. Nine parameters of the MPSIAC model and the amount of sediment (m^3/ha/year) are found in Table 6.

Table 6: Final scores of nine factors and sediment yield calculation results by MPSIAC method

parameters	Emamkandi	Silve 1	Silve 2	Ozondara 1	Ozondara 2	Ozondara 3	Kohnede 1	Kohnede 2	Khracho 1	Khracho 2
lithology	4.82	3.08	3.00	5.50	5.50	5.05	4.17	3.60	5.26	5.29
Soil	2.33	2.17	2.17	3.00	3.00	3.00	3.17	3.17	1.67	1.67
climate	2.60	3.20	3.20	2.60	2.60	2.60	2.40	2.40	2.80	2.80
runoff	8.66	6.57	6.31	8.09	9.04	8.52	6.19	6.88	7.08	7.08
topograghy	14.80	5.60	5.20	11.60	12.80	13.40	10.80	10.13	12.28	12.16
vegetation	3.60	6.40	6.10	6.80	7.60	7.30	6.74	7.20	7.50	7.40
Land use	10.58	13.20	12.52	13.82	15.25	14.59	14.12	14.52	14.89	14.76
Surface erosion	5.20	6.20	6.40	7.60	7.40	7.00	8.60	8.40	9.00	9.60
Channel erosion	1.67	1.67	1.67	3.34	1.67	1.67	5.01	5.01	6.68	6.68
Sediment Rate	54.26	48.09	46.57	62.35	64.86	63.13	61.20	61.31	67.16	67.44
Predicted SY (t/ha/yr)	1.78	1.43	1.35	2.39	2.61	2.46	2.29	2.30	2.84	2.87
Predicted SY (m^3/ha/yr)	1.34	1.02	1.04	1.84	1.72	1.75	1.71	1.58	2.07	2.17

In the MPSIAC model the minimum, average and maximum estimated sediment were, respectively, 1.02, 1.62 and 2.17m^3/ha/year (±0.39). The skewness of data for the observed values was -0.08, and for the estimated data was 0.42, and correlation coefficient among the sediment data estimated from model and the observational data was equal to 0.47. The estimated values had twice overestimation compared with the observed sediment.

Results of the estimated data from the three models of FSM, MPSIAC and PSIAC were compared with the observed sediment by the statistical method of relative error in equation (4), and the results have been reported in Table 7.

According to Table 7, the average observed sediment in 10 study watersheds was 0.74±0.42 m^3. The minimum and maximum observed sediment were 0.41 and 1.18 m^3/ha/year, respectively.

As shown in Table 7, the relative error values in the FSM, PSIAC and MPSIAC models have been calculated 38.67, 2.35 and 1.22 with observational data, respectively.

The relative error value closer to one indicates acceptable estimation of the model and vice versa. In this study, the FSM model with maximum relative error of 38.67 is far from observed data. The PSIAC model with overestimation of almost 2.35 for observed data showed better results compared with the FSM model and the estimated data of the MPSIAC model with the relative error of 1.22 had the lowest relative error compared to the two previous models as well as had the acceptable overestimation. The results correspond to previous findings, including Mohamadiha (2011), Atapourfard (2012), heravi et al. (2012), which confirmed overestimation for the FSM model. De Vente et al. 2005 pointed to the limitation of watershed areas between 10 and 10000 km^2.

Table 7: Results of calculating relative error of FSM, MPSIAC and PSIAC models

small catchments	Measured sediment yield m³/ha/y)	FSM sediment yield (m³/ha/y)	PSIAC sediment yield (m³/ha/y)	MPSIAC sediment yield (m³/ha/y)	FSM relative error	PSIAC relative error	MPSIAC relative error
Emamkandi	0.93	23.25	2.27	1.34	24.00	1.44	0.44
Silve1	0.87	31.31	1.72	1.02	34.99	0.98	0.17
Silve2	0.41	33.90	1.68	1.04	81.68	3.07	1.54
Ozondara1	1.18	75.38	2.54	1.84	62.88	1.15	0.56
Ozondara2	0.57	26.37	2.67	1.72	45.26	3.67	2.02
Ozondara3	0.87	25.88	2.59	1.75	28.75	1.98	1.01
Kohnede1	0.49	14.31	2.34	1.71	28.20	3.78	2.49
Kohnede2	0.56	15.12	2.17	1.58	26.00	2.88	1.82
Khracho1	0.94	29.72	3.29	2.07	30.62	2.50	1.20
Khracho2	1.11	28.08	3.37	2.17	24.30	2.04	0.95
relative error		38.67	2.35	1.22			

Based on the research findings in Iran, the efficiency of MPSIAC model has been confirmed by Tahmasebipoor and Najafi Disfani (1994), Nikjoo et al. (1995), Sokouti et al. (2005) and Bayat and Mahmoodabadi (2005). Brooshkeh et al. (2004) examined 25 small watershed sediments in West Azerbaijan province in Iran, who concluded that the average specific sediment yield and the highest measured sediment were, respectively, 1.3 and 12 m^3/ha/year for one of the watersheds with lithology sensitive to erosion and with rainfed lands. According to investigations of Toy et al. (2002), differences in measured soil loss from the same plot with erosion rates under one and 20 tons per hectare per year, were up to 400 percent and 30 percent, respectively. Nichols (2006) reported a 47-year long-term average sediment yield of the watershed with an area of 43.8 ha equal to 3 tons per hectare per year and the 10-year long-term average equal to 1.2 tons per hectare per year, representing major changes in sediment yield over time.

CONCLUSIONS

Regarding the mentioned items, we can concluded that the amounts of sediment and soil loss rate are the functions of various factors; and during the course of the investigation in relation to the reservoir sediment control has important role in determining sediment. If the duration of sediment yield behind the check dam is greater, the erosion and sediment will be measured with higher accuracy. Watersheds studied in this research had no much difference in terms of climatic conditions. However, lithological characteristics, type of land use and prevailing occupation in the region had major role in the sediment yield; the minimum and maximum observed sediment showed a three-fold difference.

Efficiency index of Nash and Sutcliffe for FSM, PSIAC and MPSIAC empirical models were, respectively, -17386.37, -45.46 and -11.48. Base on the coefficient calculated, the FSM model showed high difference with the measured values and had no efficiency for small and several hundred hectares watersheds with primary coefficients. The efficiency index in the PSIAC model was better than the FSM model, but differed with measured sediment data; and its overestimation was 2.5 times. The index calculated in the MPSIAC model compared to the previous models had a better efficiency.

It is obvious that due to the intervention of various parameters in the sediment yield, certain values and ranges could not be declared with high confidence for sediment production in an area.

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In memoriam

Dr Bogdan Bulatović
(1950-2018)

Dr Bogdan Bulatović, naš kolega, preminuo je 08. Aprila 2018. godine. Na Biotehničkom fakultetu u Podgorici, u Centru za agroekonomska proučavanja i ruralni razvoj, dr Bogdan Bulatović završio je svoj radni vijek.

Bogdan Jeftov Bulatović rođen je 13.05.1950. godine u Cerovu, - opština Kolašin. Osnovnu školu završio je u rodnom mjestu, a srednju poljoprivrednu školu u Baru. Na Poljoprivrednom fakultetu u Zemunu (Agroekonomski odsjek) diplomirao je 1975.godine. Na istom fakultetu odbranio je magistarski rad 1992.godine, a doktorirao iz oblasti agroekonomike 1996.godine.

Nakon završenih studija radio je u odjeljenju za privredu opštine Kolašin do 1980.godine, kada dolazi u Poljoprivredni institut. Obavljao je brojne dužnosti, između kojih, rukovodioca Centra za agroekonomska proučavanja. Na Univerzitetu Crne Gore stekao je zvanja Naučnog saradnika, Višeg naučnog saradnika i Naučnog savjetnika.

Boravio je u inostranstvu, radi stručnog usavršavanja i na naučnim skupovima u Izraelu, Rusiji i drugim zemljama. Učestvovao je u reealizaciji više naučnoistraživačkih projekata, posebno iz oblasti međunarodne saradnje sa Ekonomskim fakultetom Rostovskog univerziteta (razvoj agrokompleksa u Crnoj Gori i u Rostovskoj oblasti), Institutom za istraživanja i razvoj, Otava- Kanada (Crnogorski poljoprivredni proizvodni potencijali) i Poljoprivrednim Fakultetom u Tirani. Kao autor ili koautor objavio je preko 80 naučnih i stručnih radova, od čega knjige, kao i znatan broj studija, programa, analiza i sl. Bio je član savjeta ili redakcija više naučnih i stručnih časopisa i strukovnih organizacija.

Osnivanjem studija poljoprivrede, bio je angažovan u nastavi kao predavač na akademskim i primijenjenim studijama Biotehničkog fakulteta, koje se realizuju u Podgorici, Baru i Bijelom Polju.

U ime kolega sa Biotehničkog fakulteta, dragi Bogdane, hvala ti na godinama zajedničkog rada i uspješne saradnje.

Dr Miomir Jovanović

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If received significant help in designing, or carrying out the work, or received materials from someone who did a favour by supplying them, their assistance must be acknowledged. Acknowledgments are always brief and never flowery.

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