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THE ESSENTIAL OIL EXTRACTED FROM *Thymus kotschyanus* BOISS. & HOHEN AS A NATURAL SUBSTANCE FOR MANAGEMENT OF THE LESSER GRAIN BORER, *Rhyzopertha dominica* F.

SUMMARY

Plant essential oils as promising bio-rational agents have been considered for management of damaging insect pests because they are naturally biodegradable and safe to the non-target organisms. In the present study, the fumigant toxicity of essential oil from *Thymus kotschyanus* aerial parts was assessed against a major Coleopteran insect pest *Rhyzopertha dominica* and this bio-effect was optimised and modelled using Response Surface Methodology (RSM).

Optimization of the fumigant toxicity displayed a concentration of 51.720 µl L⁻¹ was adequate to kill 72.752% of insect population after 60.0 h exposure time. The best model for predicting of insecticidal effect was a third-grade model. Results of the present study recommended a high potential of *T. kotschyanus* essential oil for management of a major stored-product insect pest *R. dominica* and prediction of this bio-effect using response surface methodology.

Keywords: Essential oil, fumigant toxicity, response surface, *Thymus kotschyanus*.

INTRODUCTION

Although effective synthetic chemicals for management of different detrimental pests are available, there adverse side-effects such as environmental pollution, toxicity to the non-target organisms and resistance of treated species encouraged researchers to search safe and efficient alternatives (Damalas and Eleftherohorinos, 2011; Lorini *et al.*, 2007; Pimentel *et al.*, 2008).

Plant-derived essential oils are complex blends of volatile components including terpenic, aromatic and aliphatic groups (Bakkali *et al.*, 2008). These materials produce by almost all living organisms of plants such as leaf, stem, root, and flowers against phytophagous pests and/or phytopathogenic fungi (Batish *et al.*, 2008). The volatile components have diverse biological effects and bio-efficiency of essential oils affected by them (Bakkali *et al.*, 2008). Essential oil biosynthesis is dependent on many different factors such as genetic make-up, climatic conditions, geographical position, cultivation measures, soil quality, water stress, harvesting time, and isolation method (Bakkali *et al.*, 2008; Tripathi

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et al., 2009). Essential oils have generally considered as safe and biodegradable materials (Isman, 2005). They have broad biological effects such as insecticidal, acaricidal, antifungal, antibacterial, and antioxidant activities (Isman *et al.*, 2011; Isman and Grieneisen, 2014; Asbahani *et al.*, 2015; Ebadollahi *et al.*, 2017). Further, it was found that the essential oils have multiple modes of actions and for this reason development of pest resistance to them is very low (Tangtrakulwanich and Reddy, 2014).

Thymus kotschyanus Boiss & Hohen with the name of “Avishane-Kohi” is one of the most popular medicinal plants in Iran (Mozaffarian, 1998). Aerial parts of this species are used in traditional medicine and as a herbal tea (Bahmani *et al.*, 2014). Further, some biological effects of the essential oil of *T. kotschyanus* comprising antibacterial, antifungal, antigenotoxic, and antioxidant activities were documented (Afshari *et al.*, 2016; Rasooli and Mirmostafa, 2003; Sevindik *et al.*, 2016).

According to our knowledge, there are not any documents on the application of a mathematical model to predict the insecticidal effect. Accordingly, the primary goals of present study were 1) evaluation of fumigant toxicity of *T. kotschyanus* essential oil against the lesser grain borer (*Rhyzopertha dominica* F.) as a destructive stored-product insect pest (Edde, 2012) 2) development of mathematical model to predict the insecticidal activity and 3) finding of the optimized conditions for this bio-effect.

MATERIAL AND METHODS

Extraction of the essential oil: Aerial parts of and *Thymus kotschyanus* were collected, respectfully, from Hassan Baroog and Sardabeh regions (Ardabil province, Iran). After drying at room temperature, the parts were chopped with an electric grinder. Extraction of essential oil was done using a Clevenger apparatus with 100 g of each plant sample, and 1500 ml distilled water within 3 hours.

Rearing of the insect: Adults of *Rhyzopertha dominica* were collected from infected wheat grains at the Moghan college of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Iran. One-litre cylindrical flasks filled with wheat kernels hosted the rearing of insect pest. In order to vent the kernels, a fine mesh cloth stopped each flasks mouth. Fifty pairs of adult insects were transferred in each flask and removed 48 hours later. Infested grains were kept at 27 ± 2 °C and $65 \pm 5\%$ relative humidity and 1-3 days old adult insects were selected for fumigation bioassay.

Toxicity of the essential oil against R. dominica: To investigate the fumigant toxicity of *T. kotschyanus*, 232 millilitres containers were used as a fumigant chamber. Twenty 1-3 days old adults of insects were located in plastic canisters (3.5×5 cm), which were cut on one side and covered with a fine mesh cloth, and were hanged from the centre of the fumigant chamber. Essential oil concentrations from 8.60 to 50.72 $\mu\text{l L}^{-1}$ was calculated and prepared during preliminary experiences. Each concentration was poured on 2×3 cm filter

papers rectangles, which were placed in the fumigant chambers. The lids of the containers were air-tightly closed, and mortality of the insect was counted at 12 hours intervals after treatment. All experiments were conducted for control groups without essential oil concentrations, and each experiment was repeated four times.

Modelling of the insecticidal effect of the essential oil and statistical analysis: The data were analysed by Design Expert versions 7.0.0 (2007, Stat-Ease company, USA). The coded independent variables for fumigant toxicity of *T. kotschyanus* essential oil against *R. dominica* are essential oil concentrations (X_1) and exposure time (X_2) in 5 levels and four replications. Mathematical model between the independent variables [Concentration ($\mu\text{l L}^{-1}$) and time (h)] and dependent variable [Mortality (%)] evaluated employing multiple linear regression analysis in the following form (Khuri and Cornell 1987):

$$Y = \beta_0 + \sum_{i=1}^n \beta_i X_i + \sum_{i=1}^n \beta_{ii} X_i^2 + \sum_{i=1}^n \sum_{j=i+1}^{n-1} \beta_{ij} X_i X_j + e$$

where β_0 , β_i , β_{ii} , β_{ij} are constant coefficients of regression, X_i and X_j are the independent variables, Y is the dependent variable, n is some independent variables, and e is the random error term. The relationships between the responses were checked by correlation coefficients of determination (R^2), adjusted R^2 , and predicted R^2 . A good model will have a large predicted R^2 and a low PRESS. ANOVA found the significant terms in the model. The significance was analysed with a confidence level of 95% ($P < 0.05$).

RESULTS AND DISCUSSION

The results of bioassays confirmed that the essential oil of *T. kotschyanus* has significant toxicity in the adults of *R. dominica*. Figure 1 shows the effect of different concentrations of essential oil and various exposure times on the mortality of insect pest: with the time and concentration increase, the mean mortality percentage increases.

The results of analysis of the variance for fumigant toxicity data, in Table 1, show that the effects of exposure time (A) and essential oil concentration (B) and their interactions (AB) on pest mortality are statistically significant. Also, the effects of factors AB^2 , B^2 , and B^3 on the mean mortality were significant ($P < 0.05$). The regression model is significant at 95% confidence level. The most significant effect based on the sum of squares was established in the essential oil concentration factor (76%), which indicates that this factor is more important than others (Table 1).

To model for the high estimating data potential, it is necessary to push the predicted and adjusted R^2 values for the highest count. The Coefficient of determination (R^2) is expressed as the ratio of described variations by the model to total variations. As R^2 value is closer to 1.0 (at least 0.8), the fitted model will have more ability to describe the response variations according to independent variables.

Table 1. Results of the analysis of variance of data on the toxicity of *T. kotschyanus* essential oil against the adults of *R. dominica*

Source	Sum of Squares	df	Mean Square	F value	p-value
Model	18506.62	6	3084.44	219.91	< 0.0001
A-Time	202.74	1	202.74	14.45	0.0003
B-Concentration	1782.44	1	1782.44	127.08	< 0.0001
AB	76.58	1	76.58	5.46	0.0216
B ²	69.59	1	69.59	4.96	0.0283
AB ²	115.18	1	115.18	8.21	0.0051
B ³	90.34	1	90.34	6.44	0.0128
Residual	1304.38	93	14.03		
Lack of Fit	141.88	18	7.88	0.51	0.9462
Pure Error	1162.50	75	15.50		

A and B, respectively, are the exposure time (h) and essential oil concentrations ($\mu\text{l L}^{-1}$).

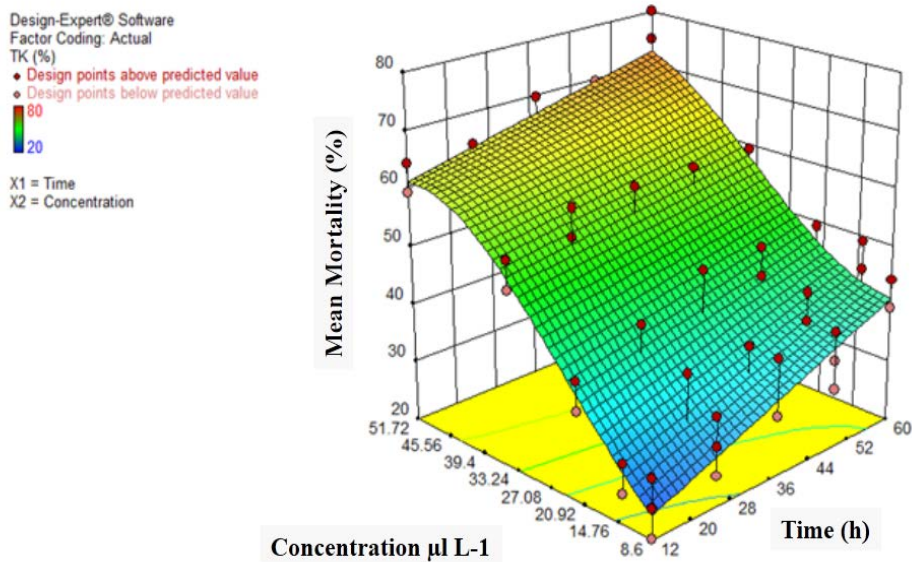


Figure 1. Three-dimensional diagrams of the fumigant toxicity of *T. kotschyanus* essential oil against *R. dominica*.

According to the model presented in Table 2 and the coefficient of variations, it can be concluded that this is the best model for estimating of the insecticidal effect of *T. kotschyanus* essential oil. The coefficients of the independent variables (concentration and time) are positive: an increase in each of the variables is an incremental effect on the response variable. The negative sign of the variables in the model indicates the decreasing effect of the variable on the amount of insect mortality.

Table 2. Estimated regression models of fumigant toxicity of *T. kotschyanus* essential oil against *R. dominica*.

Equation	R ² value	Adj R ²	Pred R ²	C.V. (%)
$52.08 + 3.49A + 22.84B - 1.72AB - 2.06B^2 + 3.73AB^2 - 5.60B^3$	0.93	0.93	0.93	8.02

*A and B, respectively, are exposure time (h) and essential oil concentrations ($\mu\text{l L}^{-1}$).

The optimum conditions for achieving 50% and maximum mortality of insect pest are shown in Table 3. Essential oil concentrations and the time were selected from 60.68 to 51.72 $\mu\text{l/l}$ and from 12 to 60 hours. The concentration of 51.72 $\mu\text{l/l}$ and 60 hours-time was estimated as the optimum conditions for 75.72% mortality with the desirability of 88%. Based on the results, the amount of mortality increased with increasing concentrations and time. For 50% mortality of the pest population, a concentration of 24.62 $\mu\text{l/l}$ and a time of 98.57 hours are adequate (Table 3).

Table 3. Optimization of the fumigant toxicity of *T. kotschyanus* essential oil against *R. dominica*.

Response variable	Time (h)	Concentration ($\mu\text{l L}^{-1}$)	Desirability
Mortality (%) = 50	57.98	24.62	100
Mortality (%) = 72.75	60.00	51.72	0.88

Although susceptibility of the key stored-products insect pest *R. dominica* to the plant essential oils was documented in some recent researches (Ebadollahi *et al.*, 2010; Hanif *et al.*, 2016), fumigant toxicity of the essential oil of *T. kotschyanus* was initially recognized in the present study against this pest. However, the antifeedant, repellency and oviposition detergency of *T. kotschyanus* essential oil were documented against *Tribolium castaneum* Herbst and *Callosobruchus maculatus* Fabricus (Akrami *et al.*, 2011a and b). Further, the modelling and optimisation of this bio-effect were assessed in the present study for the first time.

Chemical profile of the *T. kotschyanus* essential oil has investigated in some of the recent studies. For example, carvacrol (22.75%), thymol (16.52%), myrcene (12.65%), thymoquinone (11.39%), and borneol (4.52%) were introduced as main components in the essential oil of *T. kotschyanus* (Rasooli and Mirmostafa, 2003). In another study, thymol (46.72%), benzene (6.88%), carvacrol (3.73%), caryophyllene (3.39%), and γ -terpinene (3.58%) were the main components in the *T. kotschyanus* essential oil (Mohammadi *et al.*, 2014). 1,8-cineole (4.6%), p-cymene (16.6%), γ -terpinene (6.6%), and thymol (52.2%) were recognised as the major components in the essential oil of *T. kotschyanus* in the other work (Sevindik *et al.*, 2016).

The differences can be due to various geographic location, weather conditions, plant organs, extraction methods, soil types of the harvested areas and

some other factors (Afshari *et al.*, 2016; Pluhár *et al.*, 2016; Rowshan *et al.*, 2013). Further, recent studies have shown that the biological effects of plant essential oils are directly related to their principal components and even their synergistic effects with other components (Isman *et al.*, 2011; Regnault-Roger *et al.*, 2011). Some terpenic compounds such as borneol, camphene, Linalool, linalyl acetate, and β -ocimene indicated potential insecticidal activities (Ogendo *et al.*, 2008; Rozman *et al.*, 2007). So, it can be said that the insecticidal effect of *T. kotschyanus* essential oil may be related to the mentioned components.

CONCLUSIONS

R. dominica is among the most detrimental agent in the many countries, which has economic damage to many agricultural stored-products. Control of this destructive agent is currently carried out using chemical pesticides, but utilisation of synthetic chemicals caused several adverse side-effects such as environmental pollution, resistance to pests, outbreaks of secondary pests and effects on non-target organisms. Therefore, the use of low-risk and natural compounds in the management of such detrimental agent is necessary.

In the present study, the fumigant toxicity of *T. kotschyanus* essential oil was proved against *R. dominica*. Further, results of the present study were also revealed that the response surface methodology (RSM) was efficaciously used for the modelling and optimisation of the insecticidal effect of *T. kotschyanus* essential oil.

REFERENCES

- Afshari V., Elahian F., Ayari Y., Yazdinezhad A., Mirzaei S.A. (2016). Diversity and ecotypic variation in the antioxidant and antigenotoxic effects of *Thymus kotschyanus* Boiss & Hohen. *Flavour Fragr. J.*, 31: 429-437.
- Akrami H., Moharrampour S., Imani S. (2011a). Efficacy of *Mentha longifolia* and *Thymus kotschyanus* essential oils on nutrition indices and oviposition deterrence of *Tribolium castaneum*. *IOBC/wprs Bulletin.*, 69: 263-269.
- Akrami H., Moharrampour S., Imani S. (2011b). Comparative effect of *Thymus kotschyanus* and *Mentha longifolia* essential oils on oviposition deterrence and repellency of *Callosobruchus maculatus* F. *Iranian J. Med. Arom. Plants*, 27: 2-10.
- Asbahani A.E., Miladi K., Badri W., Sala M., Addi E.H.A., Casabianca H. (2015). Essential oils: From extraction to encapsulation. *Int. J. Pharm.*, 483: 220-243.
- Bahmani M., Zargaran A., Rafieian-Kopaei M. (2014). Identification of medicinal plants of Urmia for treatment of gastrointestinal disorders. *Rev Bras Farmacogn* 24: 468-480.
- Bakkali F., Averbeck S., Averbeck D., Idaomar M. (2008). Biological effects of essential oils – A review. *Food Chem. Toxicol.*, 46: 446-475.
- Batish D.R., Singh H.P., Kohli R.K., Kaur S. (2008). Eucalyptus essential oil as a natural pesticide. *For. Ecol. Manag.*, 256: 2166-2174.
- Damalas C.A., Eleftherohorinos I.G. (2011). Pesticide exposure, safety issues, and risk assessment indicators. *Int. J. Env. Res. Public. Health.*, 8: 1402-1419.
- Ebadollahi A., Davari M., Razmjou J., Naseri B. (2017). Separate and combined effects of *Mentha piperata* and *Mentha pulegium* essential oils and a pathogenic fungus *Lecanicillium muscarium* against *Aphis gossypii* (Hemiptera: Aphididae). *J. Econ. Entomol.* 110: 1025-1030.

- Ebadollahi A., Safaralizadeh M.H., Pourmirza A.A. (2010). Fumigant toxicity of essential oils of *Eucalyptus globulus* Labill and *Lavandula stoechas* L., grown in Iran, against the two coleopteran insect pests; *Lasioderma serricorne* F. and *Rhyzopertha dominica* F. *Egypt J. Biol. Pest Control.*, 20: 1-5.
- Edde P.A. (2012). A review of the biology and control of *Rhyzopertha dominica* (F.) the lesser grain borer. *J. Stored Prod. Res.*, 48: 1-18.
- Hanif C.M.S., -Ul-Hasan M., Sagheer M., Saleem S., Akhtar S., Ijaz M. (2016). Insecticidal and repellent activities of essential oils of three medicinal plants towards insect pests of stored wheat. *Bulg. J. Agri. Sci.*, 22: 470-476.
- Isman M.B. (2005). Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annu. Rev. Entomol.* 51: 45-66.
- Isman M.B., Grieneisen M.L. (2014). Botanical insecticide research: many publications, limited useful data. *Trends Plant Sci.*, 19: 140-145.
- Isman M.B., Miresmailli S., Machial C. (2011). Commercial opportunities for pesticides based on plant essential oils in agriculture, industry and consumer products. *Phytochem. Rev.*, 10: 197-204.
- Khuri A.I., Cornell J.A. (1987). *Response Surfaces*. Marcel Dekker, New York, 105p.
- Lorini I., Collins P.J., Daghli G.J., Nayak M.K., Pavic H. (2007). Detection and characterisation of strong resistance to phosphine in Brazilian *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae). *Pest Manag. Sci.*, 63: 358-364.
- Mohammadi A., Nazari H., Imani S., Amrollahi H. (2014). Antifungal activities and chemical composition of some medicinal plants. *J. Mycol. Med.*, 24: 1-8.
- Mozaffarian V. (1998). *A Dictionary of Iranian Plant Names*, Farhang Moaser, Tehran, 739p.
- Ogendo J.O., Kostyukovsky M., Ravid U., Matasyoh J.C., Deng A.L., Omolo E.O. (2008). Bioactivity of *Ocimum gratissimum* L. oil and two of its constituents against five insect pests attacking stored food products. *J. Stored Prod. Res.*, 44: 328-334.
- Pimentel M.A.G., Faroni L.R.A., Batista M.D., da Silva F.H. (2008). Resistance of stored-product insects to phosphine. *Pesqu. Agropecu. Bras.*, 43: 1671-1676.
- Pluhár Z., Szabó D., Sárosi S. (2016). Effects of different factors influencing the essential oil properties of *Thymus vulgaris* L. *Plant Sci. Today.*, doi:10.14719/pst.2016.3.3.241.
- Rasooli I., Mirmostafa S.A., (2003). Bacterial susceptibility to and chemical composition of essential oils from *Thymus kotschyanus* and *Thymus persicus*. *J. Agric. Food Chem.*, 51: 2200-2205.
- Regnault-Roger C., Vincent C., Arnason J.T. (2011). Essential oils in insect control: low-risk products in a high-stakes world. *Annu. Rev. Entomol.* 57: 405-424.
- Rowshan V., Bahmanzadegan A., Saharkhiz M.J. (2013). Influence of storage conditions on the essential oil composition of *Thymus daenensis* Celak. *Ind. Crops Prod.*, 49: 97-101.
- Rozman V., Kalinovic I., Korunic Z. (2007). Toxicity of naturally occurring compounds of Lamiaceae and Lauraceae to three stored-product insects. *J. Stored Prod. Res.*, 43: 349-355.
- Sevindik H.G., Özek T., Yerdelen K.Ö., Önal M., Özbek H., Güvenalp Z., Demirezer L.Ö. (2016). Chemical composition, antioxidant capacity, acetyl- and butyrylcholinesterase inhibitory activities of the essential oil of *Thymus haussknechtii* Velen. *Rec. Nat. Prod.*, 10: 503-507.
- Tangtrakulwanich K., Reddy G.V.P. (2014). Development of insect resistance to plant biopesticides: An Overview. In: *Advances in plant biopesticides*, (ed.: D Singh). Springer, Delhi, pp 407-462.
- Tripathi A.K., Upadhyay S., Bhuiyan M., Bhattacharya P.R. (2009). A review on prospects of essential oils as biopesticide in insect-pest management. *J. Pharmacognosy Phytother.*, 1: 52-63.