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THE USE OF *ZINGIBER OFFICINALE* (GINGER) EXTRACT FOR THE CONTROL OF *ALTERNARIA ALTERNATA* FUNGAL PATHOGEN

SUMMARY

This study presents the treatment of pepper seeds with ginger extract for their germination and emergence, as well as the reduction of pesticides using alternative non-polluting methods to control pathogens.

The observations followed both the germination and emergence of the seeds, as well as the development of the pathogen *Alternaria alternata* and the appearance of necrosis and symptoms of alternariosis. Numerous data from the literature offer recommendations on the biological and chemical control of *A. alternata*, however more frequently there is the problem of maintaining their effectiveness and the detection of new products that prevent and combat the pathogen.

This research states that the ginger stimulates seed germination and has both preventive and curative action against the pathogen *A. alternata*.

Keywords: extract, ginger, pathogen, pepper

INTRODUCTION

Ginger (*Zingiber officinale*) is a herbaceous plant found in the tropics, which has an aromatic rhizome, rich in essential oils. It is highly valued as a spice and is used in all types of dishes, from sauces, soups and main courses, to desserts and drinks. Moreover, it is used since antiquity in medicine, to relieve nausea, relieve cramps and improve blood circulation.The rhizome of *Z. officinale* contains pungent phenolic substances, with a variety of biological activities (Wang *et al*., 2014).

It was shown that the consumption of ginger could influence the weight loss, by decreasing the body mass index and serum insulin (Ebrahimzadeh Attari *et al*., 2016.)

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Researchers have found that gingerol, the main bioactive compound, would inhibit the growth of several types of bacteria (Park et al., 2008; Karuppiah and Rajaram, 2012). In addition to gingerol, ginger contains large amounts of vitamin C and B6, along with magnesium, potassium, copper, manganese. Gingerols and their corresponding dehydration products shogaols were considered as the active compounds of ginger, the rhizome of the plant *Zingiber officinale*, for its antioxidant, anti-inflammatory, anti-diabetic and antitumor activities (Feng *et al*., 2011; Poltronieli *et al*., 2014; Khandouzi *et al.*, 2015). It is also used in the perfume and cosmetics industry, for hair toning and revitalization, ubiquitous in the revitalizing formulas of face creams.

There has been a growing global concern about the use of chemicals in the cultivation of vegetables, flowers, fruit trees and vines, due to their effects on human health and the environment. Fungicides from natural extracts have proven to be extremely useful in controlling plant pathogens, being a future alternative to synthetic fungicides.

Recent studies have shown that ginger oil has a very strong antimicrobial and therapeutic effect (Imamović *et al*., 2021).

This study presents the treatment of pepper seeds with ginger extract for their germination and emergence, as well as the reduction of pesticides using alternative non-polluting methods to control pathogens.

The observations followed both the germination and emergence of the seeds, as well as the development of the pathogen *Alternaria alternata* and the appearance of necrosis and symptoms of alternariosis.

Numerous data from the literature offer recommendations on the biological (Feng and Zheng, 2007; Tozlu *et al*., 2018) and chemical (Kapsa, 2009) control of the pathogen *Alternaria alternata*, however more frequently there is the problem of maintaining their effectiveness and the detection of new products that prevent and combat the pathogen.

This paper aimed to test the effect of ginger extract in different concentrations, on seed germination and seedling emergence and also to estimate by seed tests the effectiveness of *Zingiber officinale* in different concentrations, against the pathogen *Alternaria alternata*.

MATERIAL AND METHODS

The research was carried out in the chemistry laboratory within the Research and Development Station for Fruit Tree Growing Baneasa, Bucharest.

Plant material and pathogenic fungus. In order to test and estimate the effectiveness of ginger - *Zingiber officinale* in different concentrations, both on the germination and emergence of plants and against the pathogen *Alternaria alternata*, Romanian variety Barbara (Agrosel) of pepper seeds was used.

The ginger extract was obtained by pressing the fresh rhizome of *Z. officinale* followed by obtaining different concentrations (1%, 5% and 10%).

Alternaria alternata isolated from diseased fruits and seeds was the pathogenic species used to estimate the efficacy of ginger in different concentrations by seed tests.

The substance with fungicidal action tested was in the form of fresh ginger extract.

The blotch test method, recommended by International Seed Testing Association was used to test the three experiments: the pepper seeds were placed between the folds of blotting paper moistened with sterile distilled water, followed by their incubation at temperatures between 22-25ºC for 3-6-9 days (Malone and Muskett, 1964; Hulea, 1969; Rădulescu and Negru, 1966).

Method used to study the influence of ginger treatment in different concentrations on pepper germination and emergence. A number of 60 seeds for each variant (1%, 5%, 10%) were washed with sterile distilled water, dried in an oven, and then moistened by immersion for 15 minutes with ginger (Figure 1). The control group was composed of 60 seeds, washed with sterile distilled water and then dried (Baicu, 1968; Constantinescu, 1974). Observations were performed in dynamics (three, six and nine days) and followed seed germination and the emergence of pepper plantlets.

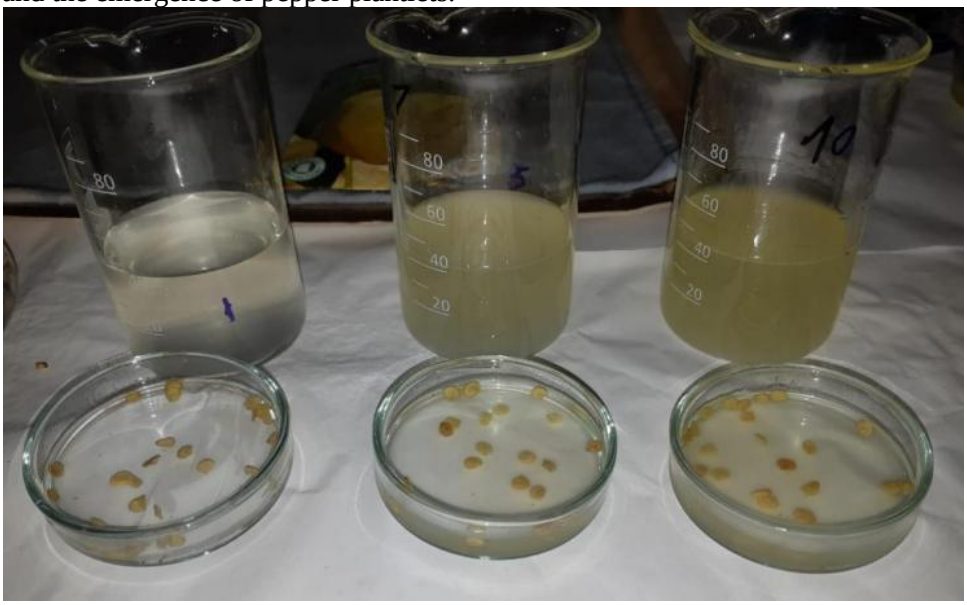

Figure 1. The appearance of ginger-treated seeds in different concentrations

Method used to estimate the effectiveness of ginger treatments in different concentrations in order to prevent and control the pathogenic species *Alternaria alternata in vitro* by seed tests. The tested pathogen was isolated directly from the plant material. The plant material was passed through distilled water twice. Inoculum harvesting was performed by detaching small portions of mycelium from the affected areas with pathogens that had the

characteristic aspect of mycelium. The detached mycelium was then placed on PDA culture medium (Potato-glucose-agar, Difco, prepared according to the recipe, sterilized by autoclave: 1.2 atmospheres, 20 minutes). The harvesting operation was carried out in the sterile chamber-hood with laminar flow; with the scalpel, flamed and cooled, the inoculum was passed with the help of a transplanting needle on an isolating medium, sinking slightly.

Incubation was performed at 24°C for 10 days; the developed colonies were purified by transferring to another vessel with sterile culture medium. Isolates were identified based on spore morphology, then transplanted and stored in pure cultures at 24°C (Manole and Ciocoiu, 2011).

In order to estimate the effectiveness of treatments with ginger in different concentrations in the control of *Alternaria alternata*, two variants were used:

Pre-treatment. Overall, 60 seeds (for each variant - 1%, 5%, 10%) were disinfected with 70% ethyl alcohol, washed with sterile distilled water, dried in an oven, then moistened by immersion for 15 minutes with a mixture of ginger and artificially contaminated by immersion in a calibrated spore suspension (5×10^6 spores / ml) for one hour. The contaminated seeds were incubated at 25°C.

Treatment. A number of 60 seeds (for each variant - 1%, 5%, 10%) were disinfected with 70% ethyl alcohol, washed with sterile distilled water, dried in an oven, then artificially contaminated, by bathing in a calibrated suspension of spores (5×10^6 spores / ml) for one hour. The seeds were then moistened, by immersing them, for 15 minutes, with ginger in different concentrations. The contaminated seeds were incubated at 25°C. The observations followed both the development of seedlings and the appearance of necrosis and alternariosis symptoms (Iacomi et al., 2004).

RESULTS AND DISCUSSION

Influence of ginger treatment (concentration 1%, 5%, 10%) on pepper germination and germination. In the control group (Figure 2) the germination was registered as 65% (on day 9), the seeds developed root/hypocotyl/cotyledons, but the seedlings were covered with *Rhizopus* sp. and *Aspergillus* sp.

The ginger variant - *Zingiber officinale* - concentration 1% allowed a germination, on day 9, of 70% (at 61% the root and the hypocotyl have developed, the rest presenting the root, hypocotyl and cotyledons - Figures 3, 4).

The ginger - *Zingiber officinale* - in a concentration of 1% did not influence the germination of seeds and did not allow the development of saprophytic or pathogenic fungi.

Figure 2. The untreated control group

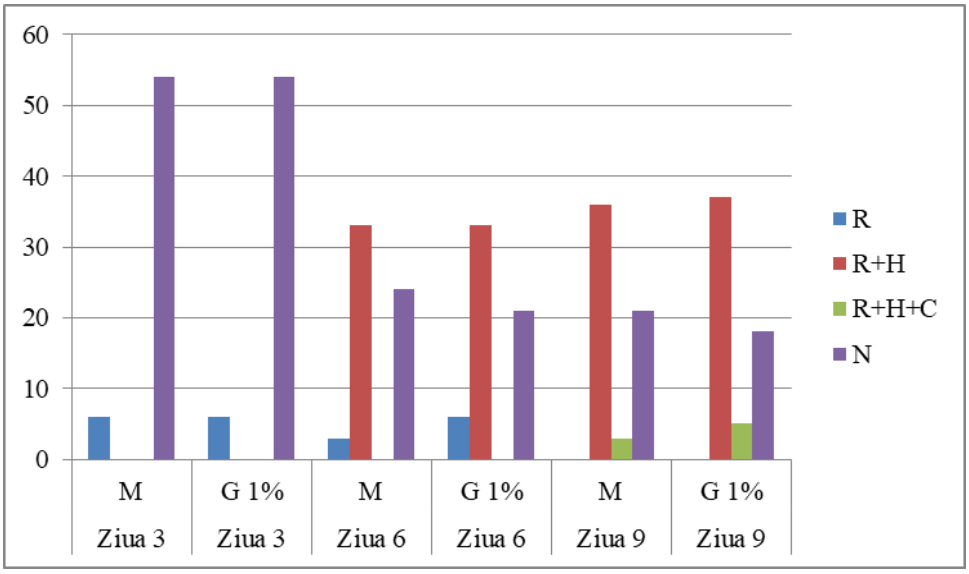

Figure 3. The influence of ginger treatment - Zingiber officinale - 1% concentration, on pepper germination and emergence M- control group, G 1% - ginger extract 1%, Ziua - Day, N=ungerminated, R = radicule, H = hypocotyl, C = cotyledons

Figure 4. The influence of ginger treatment - *Zingiber officinale* - 1% concentration, on pepper germination and emergence

The influence of ginger treatment - *Zingiber officinale* - 5% concentration, on pepper germination and emergence. In the control group, the seeds germinated in 65% on day 9 (Figure 5), they developed root/hypocotyl/cotyledons but the seedlings were covered with *Rhizopus* sp., *Aspergillus* sp.

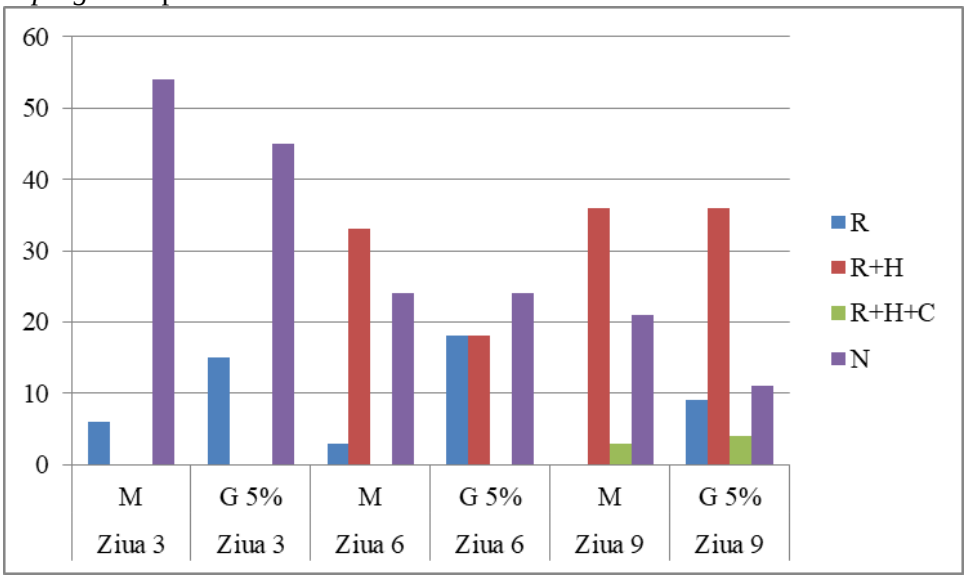

Figure 5. The influence of ginger treatment - Zingiber officinale - 5% concentration, on pepper germination and emergence M- control group, G 1% - ginger extract 1%, Ziua - Day, N=ungerminated, R = radicule, H = hypocotyl, C = cotyledons

The ginger - *Zingiber officinale* - in a concentration of 5% allowed a germination of 81.66%, on day 9 (Figure 6). This variant has favorably

influenced the germination and development of the seedlings, compared to the control group, and did not allow the development of saprophytic or pathogenic fungi.

Figure 6. The influence of ginger treatment - Zingiber officinale - 5% concentration, on pepper germination and emergence

The influence of ginger treatment - *Zingiber officinale* - 10% concentration, on pepper germination and emergence. In the control group the rated of germination was 65% in day 9 (Figure 7), they developed root/hypocotyl/cotyledons and the plantlets were covered with *Rhizopus* sp. and *Aspergillus* sp.

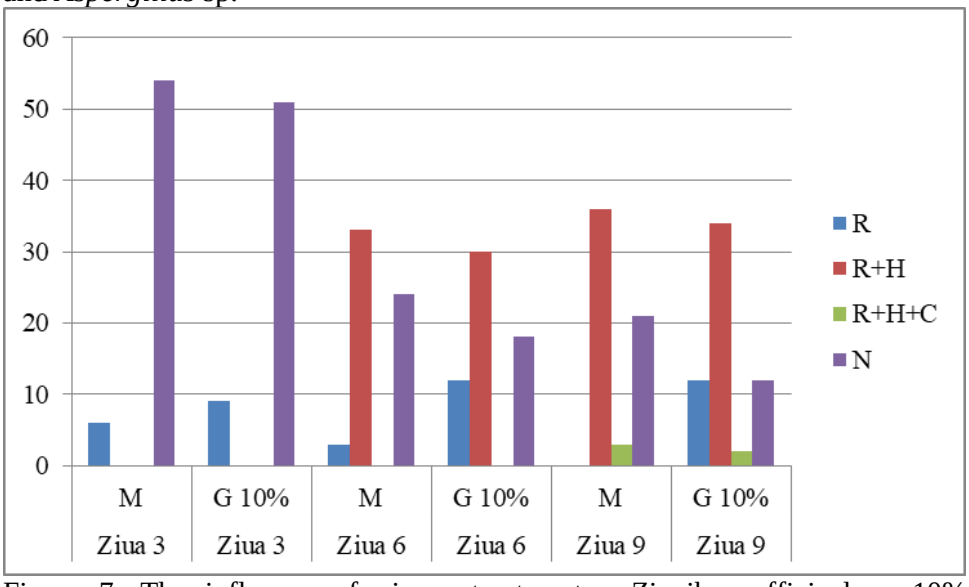

Figure 7. The influence of ginger treatment - Zingiber officinale - 10% concentration, on pepper germination and emergence M- control group, G 1% - ginger extract 1%, Ziua - Day, N=ungerminated, R = radicule, H = hypocotyl, C = cotyledons

The ginger variant - *Zingiber officinale* - 10% concentration allowed a germination of 80%, on day 9 (Figure 8). This variant did not allow the development of saprophytic or pathogenic fungi either.

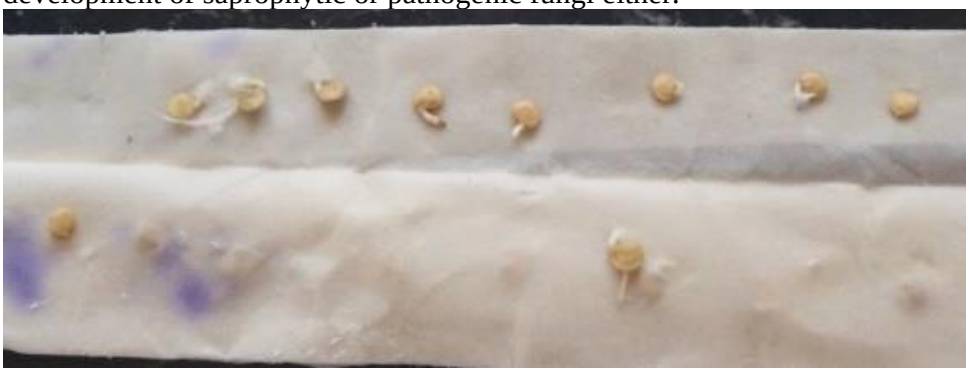

Figure 8. The influence of ginger treatment - Zingiber officinale - 10% concentration, on pepper germination and emergence

Compared to the control group, where the germination was 65% and the seedlings were well developed (root, hypocotyl, cotyledons), the variants 1%, 5%, 10% of ginger - *Zingiber officinale*, positively influenced the germination, reaching up to 81.66% in variant 5%.

Efficiency of preventive treatment with ginger - *Zingiber officinale* - 1% concentration in combating the pathogen *Alternaria alternata* in peppers. The infected, untreated control group germinated in a percentage of 33.33%, on day 9 having necrotic roots, hypocotyl and cotyledons, the seeds (Figures 9, 10) being covered with a black mycelium (*A. alternata*).

Due to the efficiency of the action of ginger - *Zingiber officinale*, in the 1% concentration variant, the mycelium of the pathogenic fungus *Alternaria alternata* did not develop, and the seeds germinated in a proportion of 60%.

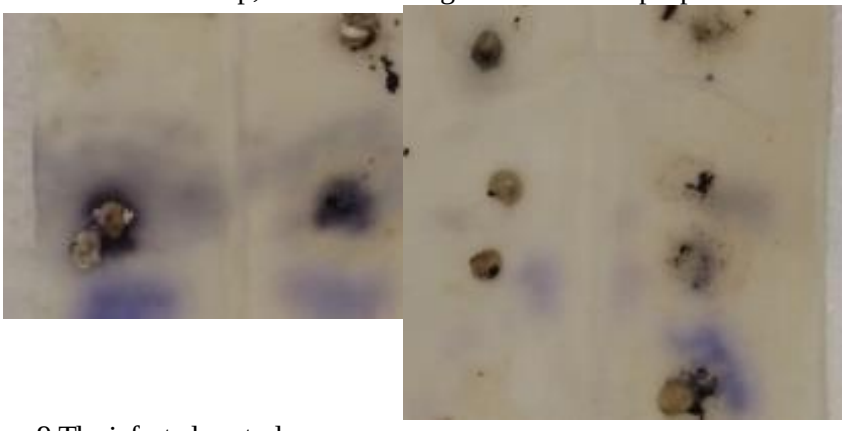

Figure. 9 The infected control group

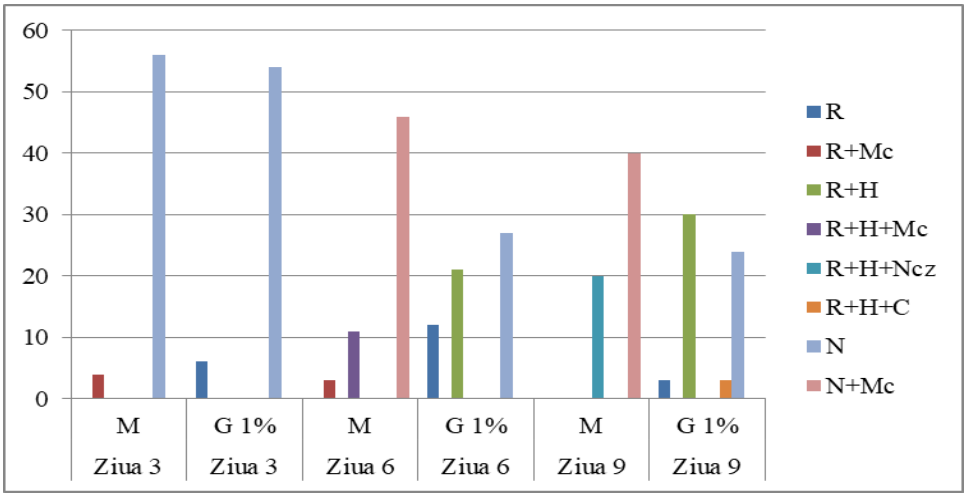

Figure 10. The efficiency of preventive treatment with ginger - Zingiber officinale - 1% concentration in the control of the pathogen Alternaria alternata in peppers (M – control group, G 1% - ginger extract 1% (Zingiber officinale), R = radicule, Mc = the presence of mycelium, H = hypocotyl, Ncz = necrosis, C = cotyledons, N =ungerminated seeds)

Efficiency of preventive treatment with ginger - *Zingiber officinale* - 5% concentration in combating the pathogen *Alternaria alternata* in peppers. The untreated, untreated control germinated in a percentage of 33.33%, on day 9 having necrotic roots, hypocotyl and cotyledons, the seeds (Figure 11) being covered with black mycelium (*Alternaria alternata*).

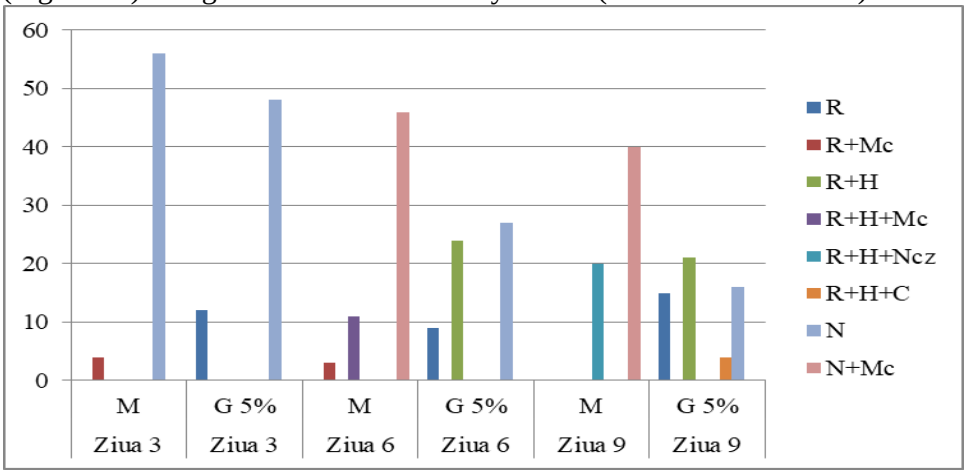

Figure 11. The efficiency of preventive treatment with ginger - Zingiber officinale - 5% concentration in the control of the pathogen Alternaria alternata in peppers (M – control group, G 5% - ginger extract 5% (Zingiber officinale), R = radicule, Mc = the presence of mycelium, H = hypocotyl, Ncz = necrosis, C = cotyledons, N =ungerminated seeds)

Due to the efficiency of the action of ginger - *Zingiber officinale*, in the 5% concentration variant, the mycelium of the pathogenic fungus *Alternaria alternata* did not develop, and the seeds had a germination proportion of 73.33%

Efficiency of preventive treatment with ginger - *Zingiber officinale* - 10% concentration in combating the pathogen *Alternaria alternata* in peppers. The infected, untreated control germinated in a percentage of 33.33%, on day 9 having necrotic roots, hypocotyl and cotyledons, the seeds (Figure 12) being covered with black mycelium (*Alternaria alternata*).

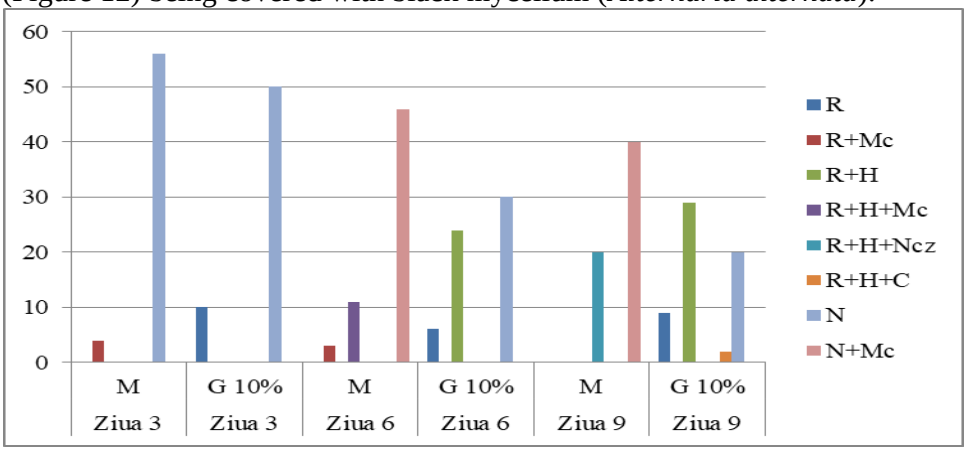

Figure 12. The efficiency of preventive treatment with ginger - Zingiber officinale - 10% concentration in the control of the pathogen Alternaria alternata in peppers (M – control group, G 5% - ginger extract 10% (Zingiber officinale), R = radicule, Mc = the presence of mycelium, H = hypocotyl, Ncz = necrosis, C = cotyledons, N =ungerminated seeds)

Due to the efficiency of the action of ginger, in the 10% concentration variant, the mycelium of the pathogenic fungus *Alternaria alternata* did not develop, and the seeds germinated in proportion of 66.66%.

Efficiency of curative treatment with ginger - *Zingiber officinale* - 1% concentration in combating the pathogen *Alternaria alternata* in peppers. The infected, untreated control germinated in a percentage of 33.33%, on day 9 having necrotic roots, hypocotyl and cotyledons, the seeds (Figure 13) being covered with black mycelium (*Alternaria alternata*).

The curative treatment with ginger - *Zingiber officinale*, in a concentration of 1%, in order to control the pathogen *Alternaria alternata* in peppers allowed a germination of 83.33%, and no development of the pathogenic fungi was registered (Figures 13, 14).

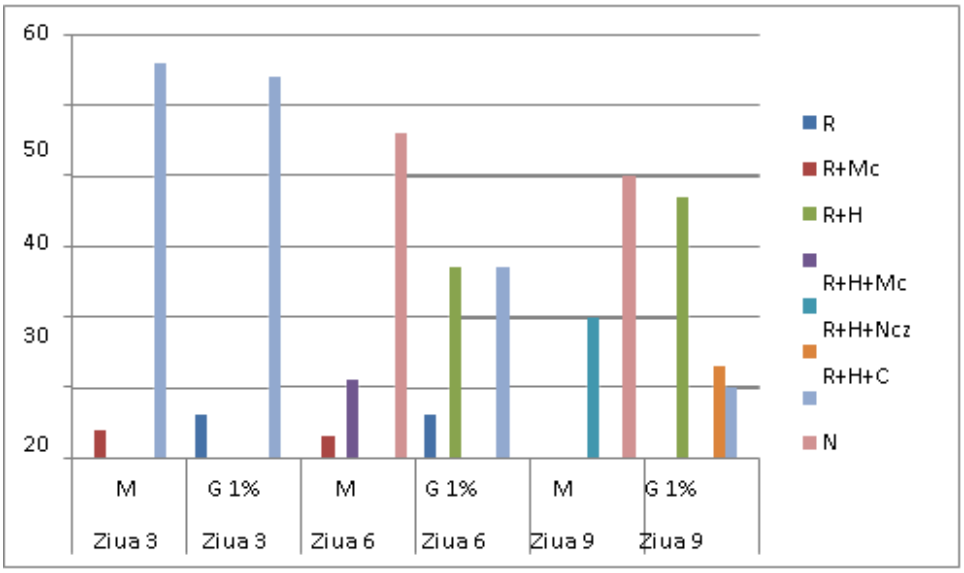

Figure 13. The efficiency of curative treatment with ginger - *Zingiber officinale* - 1% concentration in the control of the pathogen *Alternaria alternata* in peppers (M – control group, G 1% - ginger extract 1% (*Zingiber officinale*), R = radicule, Mc = the presence of mycelium, H = hypocotyl, Ncz = necrosis, C = cotyledons, N =ungerminated seeds)

Figure 14. The efficiency of curative treatment with ginger - *Zingiber officinale* - 1% concentration in the control of the pathogen *Alternaria alternata* in peppers (day 9)

The efficiency of curative treatment with ginger - *Zingiber officinale* - 5% concentration in the control of the pathogen *Alternaria alternata* in peppers (day 9) (Figures 15-16).

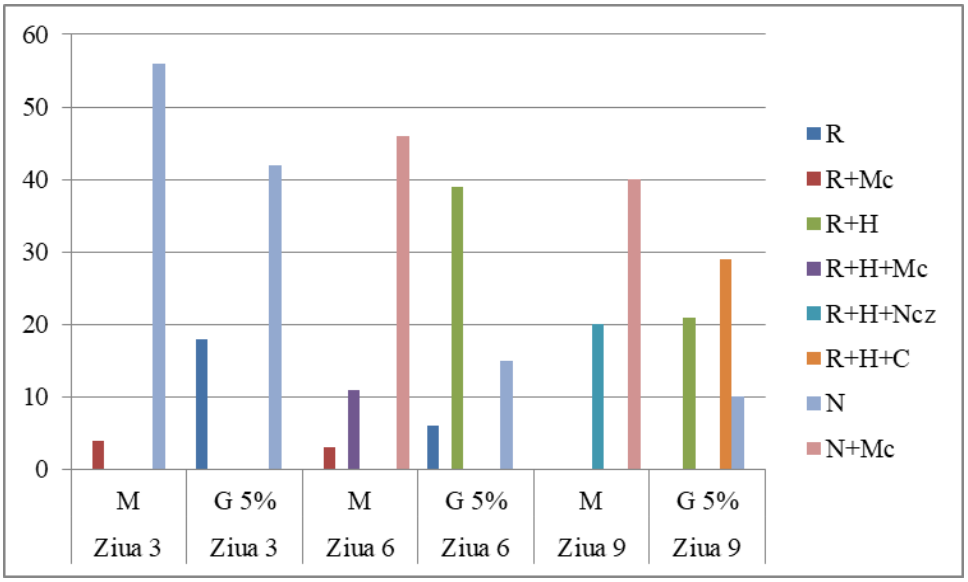

Figure 15. The efficiency of curative treatment with ginger - Zingiber officinale - 5% concentration in the control of the pathogen Alternaria alternata in peppers (M – control group, G 1% - ginger extract 5% (Zingiber officinale), R = radicule, Mc = the presence of mycelium, H = hypocotyl, Ncz = necrosis, C = cotyledons, N =ungerminated seeds)

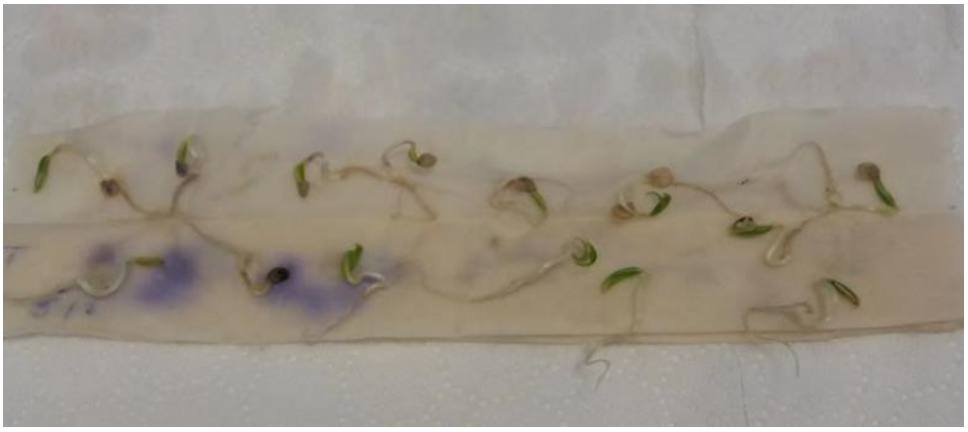

Figure 16. The efficiency of curative treatment with ginger - *Zingiber officinale* - 5% concentration in the control of the pathogen *Alternaria alternata* in peppers (day 9)

Efficiency of curative treatment with ginger - *Zingiber officinale* - 10% concentration in combating the pathogen *Alternaria alternata* in peppers. The curative treatment with ginger, in a concentration of 10%, in order to control the pathogen *Alternaria alternata* in peppers allowed a germination of 93.33%

(18.33% - root development, 61.66% - root and hypocotyl development, respectively 13.33% development of the root, hypocotyl and cotyledons) not allowing the pathogenic fungus to develop (Figures 17, 18).

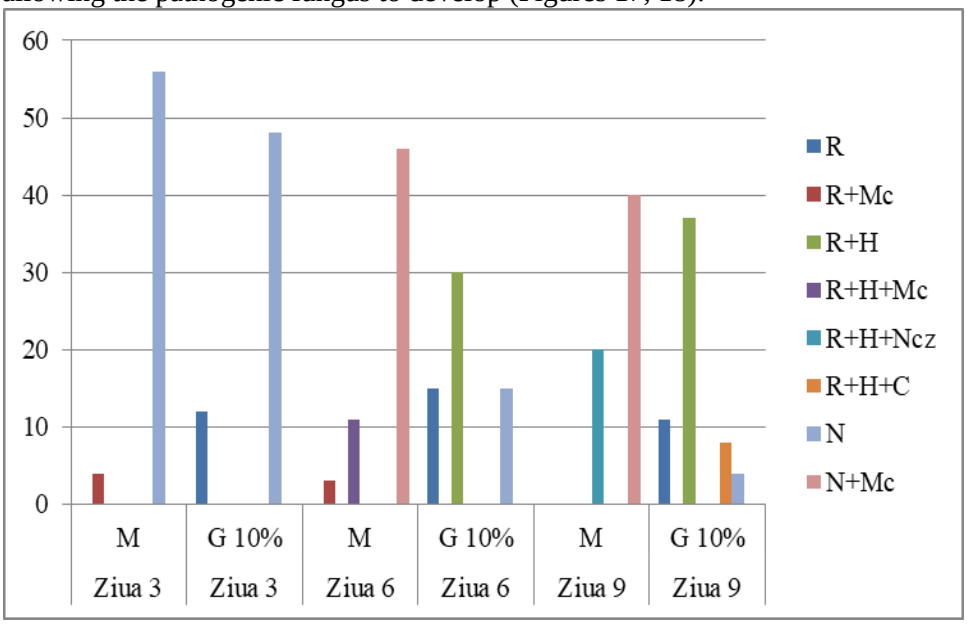

Figure 17. The efficiency of curative treatment with ginger - Zingiber officinale - 10 % concentration in the control of the pathogen Alternaria alternata in peppers (M – control group, G 1% - ginger extract 10 % (Zingiber officinale), R = radicule, Mc = the presence of mycelium, H = hypocotyl, Ncz = necrosis, C = cotyledons, N =ungerminated seeds)

Figure 18. The efficiency of curative treatment with ginger - *Zingiber officinale* - 10 % concentration in the control of the pathogen *Alternaria alternata* in peppers

Similar research (Sharma and Tiwari, 2013) highlighted the effectiveness of essential oil extracted from ginger (*Zingiber officinale* Roscoe.) in controlling *Alternaria alternata*, the minimum inhibitory concentration (MIC) of this oil showing results at 500 ppm for the fungus. Moreover, it was observed that essential oil extracted from ginger has a wide range of antifungal activities against *Aspergillus niger* van Tiegh., *Penicillium chrysogenum* Thom., *Alternaria alternata* (Fr.) Keissl. and *Fusarium roseum* Link., whereas MIC showed results at 1.0 mL/cm^{-3} of oil in respect to all fungi (Sharma *et al*., 2013).

The results of present work are consistent with other studies (Fawzi *et al*., 2009; Osman *et al.,* 2016; Ahmad and Qureshi, 2017), which proved that ginger extracts have antibacterial activity against *A. alternata* fungus.

CONCLUSIONS

The present study has shown the favorable influence of ginger extract - *Zingiber officinale* on the germination of pepper seeds. All three variants of concentration (1%, 5% and 10%) had higher germination percentages - 66%, 70% and 80% respectively compared to the control group that germinated in a rate of 65%.

The preventive treatments with the three concentration variants (1%, 5%, 10%) inhibited the development of the pathogen *Alternaria alternata*.

The curative treatments with the three concentration variants (1%, 5%, 10%) also inhibited the development of the pathogen *A. alternata*. In this case, a pronounced stimulation of seed germination was observed compared to the results obtained from investigations upon the influence of ginger on seed germination.

This research states that the ginger stimulates seed germination and has both preventive and curative action against the pathogen *Alternaria alternata*.

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