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Original scientific paper

Protection of organic cereals from insect and rodent pests in a warehouse by combined use of traps and sticky tapes

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SUMMARY

Several options of protection of organic cereals (winter and spring wheat, rye and maize) from insect and rodent pests, using a combination of traps (with or without pheromone/attractant) and sticky tapes and no chemicals, were tested in a warehouse over the summer-spring season of 2019/2020. Temperature in the warehouse was 14-29°C and humidity around 50%. The average grain moisture of winter and spring wheat and rye was 10-11%, while it was 12-14% in maize grain, and the average grain temperature of all cereals was 13-27°C. Regarding stored-product insect pests, five coleopteran, two moth and one Psocoptera species were detected, and the coleopterans predominated (98.5%) along with secondary pest insects (94.0%). Regarding rodents, only specimens of *Mus musculus* were found throughout the test period, their maximum monthly frequency being 72 (in January 2020). A combination of traps (with or without pheromones) and sticky tape barriers was found to provide an effective tool for trapping insects. Also, snap traps and trapping boxes for killing rodents, when used simultaneously with sticky tape barriers, were found to provide good protection of cereals from house mice. The pest control effect was also confirmed by collecting samples of organic cereal grain, which showed no significant presence of stored-product insects or grain damage (0.94% and 0.96% in spring wheat and rye, respectively) at the end of the test period. The results showed a great potential of combined application of traps and sticky tapes for protecting organic cereals in horizontal bulk storages, but the use of chemicals approved for organic food production would be required under extended storage periods.

Keywords: cereals, insects, rodents, traps, sticky tapes, warehouse

INTRODUCTION

Plant products such as organic winter and spring wheat, rye and maize come under attack by various organisms during storage, namely insects, mites, microorganisms, rodents and birds (Hill, 1990; Meyer, 1994; Rees, 2004; Almaši, 2008; Stejskal et al., 2015). Some 15% of grain products are believed to be lost globally each year, 80% of which by insect and some 10% by rodent and bird infestation (Reichmuth et al., 2007).

Insects are able to damage stored products by feeding, and their destructive power may be total (Rees, 2004). Insect presence in food has a negative impact on human health, primarily through product contamination with body hair and feces, which may cause allergic reactions and other effects on humans, and change environment conditions in storage (temperature and humidity) so that fungi or some other harmful microorganisms may ultimately develop in plant products (Hubert et al., 2018; Stejskal et al, 2018). Stored-product insects may be primary pests that damage whole grain, such as the coleopterans *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), *Sitophilus granarius* (L.), *Sitophilus oryzae* (L.) and *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae), the moth *Sitotroga cerealella* (Oliv.) (Lepidoptera: Gelechiidae), or pests that feed on damaged grain as secondary pests, including the coleopterans *Tribolium castaneum* (Herbst), *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae), *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Cucujidae) and *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae), and moths *Plodia interpunctella* (Hubner) and *Ephestia kuehniella* Zeller (Lepidoptera: Phycitidae) (Hill, 1990; Rees, 2004; Almaši, 2008, Nayak & Daglish, 2018).

Two types of rodents are the most frequent and equally destructive storage pests: the brown rat *Rattus norvegicus* (Berck.) and the house mouse, *Mus musculus* (L.). They are cosmopolitan species with exceptional adaptability to human environments, which is why they have been termed commensal rodents (Frantz & Davis, 1991). Storages provide them shelter and a choice of available food throughout the year, which is especially evident in storages of plant products over periods of unfavorable weather outside, e.g. when winter temperatures are low (Lund, 1994). Owing to their great reproductive potential, and favourable conditions existing inside warehouses, they produce copious offspring over the year, which causes great damage and control problems (Meyer, 1994). Estimation of damage that rodents are able to cause in storage facilities is very complex as they are able not only to spoil stored products and ruin installations in storages but they also pose a great threat to the health of humans and domestic animals because they are hosts and vectors of many infectious diseases (Battersby et al., 2008). A single rodent is known to require daily rations of food in the amount of 10% of its body weight, but contamination with its urine, feces and hair is an even greater concern (Meyer, 1994; Timm, 1994; Pimentel et all., 2001) as one house mouse is able to excrete up to 50 fecal pellets in a day, and a brown rat up to 40 (Meyer, 1994).

Only a very small number of control products based either on synthetic chemicals or natural preparations are allowed to be used for protection of organic plant products in storages, and they have different modes of action and levels of effectiveness. Some EU countries (Kljajić et al., 2019), as well as Canada (OMRI, 2020) and the US (Grieshop et al., 2012; USDA NOP, 2020) allow organic plant products to be protected in storages either by physical means (ventilation, hot or cool airing) or by applying boric acid, azadirachtin, pyrethrin or diatomaceous earth (without synthetic substances), while spinosad-based products are allowed to be used but they have not yet been registered for protection of stored products. The situation in Serbia is similar as its Organic Production Act (2010), and the related Code on Control and Certification in Organic Production and Methods of Organic Production (2011), which includes a list of plant protection products registered for use in organic production, do not yet specify any registered chemical as adequate for protecting plants and plant products from harmful insects and rodents in storage facilities.

The modern concept of protection of stored products from insect and rodent pests relies predominantly on two approaches: integrated and biorational, and mandatory sanitary measures are implicit as a primary form of protection, followed then by other means and methods of optimized monitoring and control that involve low risks to human health and the environment. Traps with or without pheromones/food baits make an important tool, as well as insect sampling during product upload and storage (Kljajić et al., 2016; Hagstrum & Phillips, 2017; Morrison et al., 2020). Snap traps and sticky tapes are also often used for monitoring and controlling rodents (Buckle & Smith, 1994; Hubert et al., 2018).

The objective of this study was to examine the effects of combined use of traps (with or without pheromones/attractants) and sticky tapes for the protection of organic cereals (winter and spring wheat, rye and maize) from stored-product insects and rodents in a horizontal bulk shed (warehouse) during an extended period of storage.

MATERIALS AND METHODS

Storage facility and organic commodities

Testing was performed in Kikinda, Serbia (N45°49'217", E20°28'469") from the summer 2019 to spring 2020. The trial was performed in a warehouse

that was 60 m long, 30 m wide and 6 m high, and having concrete floor and sides, and roof constructed of metal panels.

Organic cereals, consisting of 2019 harvests of wheat (winter and spring), rye and maize, were stored as bulk grain along one side of the warehouse over an area 40 m long and 10 m wide and separated within metal boxes constructed of 1.2 m high panels fixed to metal pillars which were set at 2 m distance. Maximum height of cereal bulk did not exceed 1 m. The initial amounts of organic cereals were: 33.000 kg winter wheat, 18.000 kg spring wheat, 5.000 kg rye, and 60.000 kg maize grain.

Storage conditions for the organic cereals we measured by mini meteorological stations Kestrel 3000 and 4000 (Environmental Meter, USA). Air temperature mostly did not exceed 29°C, while relative humidity was predominantly up to 50%, except in late December 2019 when temperature was lower (14.3°C) and humidity higher (52%). Moisture content and temperature of all organic cereals were measured during sampling by a Dickey-John Mini GAC (Dickey–John Co., USA). Parameters were determined as the sum for collective samples (two) based on minor samples which were collected at different locations/depths of grain per type of commodity at the beginning (summer 2019), in the middle (winter 2019/20) and at the end of storage period (spring 2020). The average grain moisture content of all organic cereals over the entire storage period was 10-11% for winter and spring wheat and rye, and 12-14% for maize grain. The average grain temperature of organic cereal samples varied significantly, depending on the season, i.e. indoor and outdoor air temperature, but it never dropped below 13°C or exceeded 27°C.

Measures applied

In order to increase the efficacy and reliability of monitoring, and to achieve the "pest control effect" by reducing their populations, based on principles specified by Toews and Nansen (2012) and guidelines provided by producers of traps and tapes, a great number of traps was laid throughout the warehouse, more than it is practiced conventionally (20 per warehouse) and at smaller distance than the usual 10 m approximately. Moth traps were set at 2-4 m distance, traps for coleopterans at 5 m and pitfall cone traps at < 2 m distance. Coleopteran traps were laid just around the boxes, while pitfall cone traps were thrust into the bulk grain. Pheromone traps for moths were set up on pillars around the grain boxes. Sticky tape barriers were laid around the boxes and along the entire length of walls and inside the bulk grain.

Snap traps for small rodents, hidden in bait boxes, and sticky tapes were laid along the internal of facility walls. Trapping boxes containing rodenticide baits were laid around the external side of the facility. Sticky tape barriers were laid around the grain boxes, and in places where introduction of insects or rodents was possible during grain handling, and around the main and secondary entrances/exits.

All sets of equipment (traps and sticky tapes) were emptied or replaced with new ones in keeping with manufacturer guidelines and depending on the state they are in and findings made during warehouse inspection.

We used a total of: 35 pheromone traps for moths (Tip: AF DEMI DIAMOND), 10 coleopteran traps containing pheromone/attractant (Type: Xlure MST), 35 pitfall cone traps for setting inside cereal grain to monitor coleopterans, 20 rodent trapping boxes (12 AF-RAT bait boxes installed around the facility and 8 AF SNAPPA boxes containing AF NO ZONE tape inside the facility), 32 rodent boxes containing snap traps (SNAP-E-MOUSE inside AF SNAPPA boxes), and 300 m of 30 cm wide sticky barrier tapes for insects and rodents (AF NO ZONE). All types of traps and the sticky tape were provided by the company Sanus-M d.o.o. of Novi Sad, Serbia.

Monitoring in storage facility

Insect frequency in traps (pheromone traps for moths, for coleopterans and pitfall cone traps) was determined as recommended by trap manufacturers or more often depending on the findings during each inspection of the warehouse. After counting, insects in traps were destroyed, while specimens that could not be determined immediately were taken to the laboratory to be more closely inspected and determined. Lethal trap boxes and sticky barrier tapes allowed direct monitoring of rodent species presence and animal frequency, as well as their seasonal dynamic in the facility. Rodent specimens caught on sticky tapes were removed during shed inspection, and the tape was replaced at such points with new tape. Storage insects were also noted on the tapes but their frequency was not determined.

The presence and frequency of stored-product insects in organic cereals during storage was determined based on instructions given in a manual for public grain storage operation (Mastilović et al., 2011). Samples were collected with a probe at different points and depths of bulk grain and two samples of 6 kg were formed for each type of organic cereal grain.

After determining moisture content and temperature, samples were sieved through 1, 2 and 3 mm sieves (Haver & Boecker, Germany) in order to check insect presence and frequency. A 6 × magnifying glass with lighting was used for the procedure. Besides determining the presence of insects, samples were also checked for rodent feces and hair.

After inspection, the samples collected in the warehouse were packed in plastic bags and transferred to the laboratory for further analyses.

Laboratory examination of effects

Grain samples were collected and tested in the Laboratory of Applied Entomology of the Institute of Pesticides and Environmental Protection, Belgrade, Serbia. After arriving in the laboratory, the samples were first subsampled into two working samples, each of 1 kg, then poured into glass jars (2.5 l) and covered with cotton cloth and fixed with rubber band. The samples rested in the laboratory at the temperature of 25±1°C and relative humidity of 60±5% for 60 days of incubation. After incubation the samples were sieved through 1, 2 and 3 mm sieves (Haver & Boecker, Germany), depending on the type of grain. Detected insects were determined under a stereo microscopes MSZ 5400 (Kruss, Germany) and SZX 122 (Olympus, Germany). Grain that remained after sieving and removal of insects was poured back into jars, lidded and hand mixed for 1 min to achieve regular dispersion of dust and tiny particles of grain. After mixing, portions of 25 g of wheat or rye, and 50 g of maize grain from each jar were poured with a plastic cup into plastic containers (50 cm x 20 cm), providing three replicates (3 × 2 per cereal type). Winter and spring wheat and rye samples were then sieved through 0.8, 1 and 2 mm sieves, and maize grain through 0.8, 1 and 3 mm sieves. Several categories of grain were separated in each subsample: undamaged grain, broken grain, infested grain and dust with impurities. The grain of each category was weighed to determine its proportion in each subsample. Undamaged and infested grains were also determined in order to calculate weight loss (FAO, 1992).

The samples were examined for the presence of insects, as well as rodent feces and hair in the process of sieving.

Data analysis

All data were processed in StatSoft version 7.1 (StatSoft Inc., 2005, Tulsa, OK, US). Frequency count and proportion data are presented as exact values or means. In tests resulting in means, data were subjected to one-way ANOVA and the means were separated by the Tukey-Kramer (HSD) test at P=0.05 (Sokal & Rohlf, 1995).

RESULTS AND DISCUSSION

Effects of measures applied to stored-product insects

Temperature and relative humidity data show that conditions in the warehouse storing organic cereals were good over the trial period from the summer of 2019 to the spring of 2020 but they were also good for storage insects (coleopterans and moths), as well as rodents. Insects are known to be organisms whose body temperature shows the highest dependence on external temperature, and optimal temperatures of stored-product insects range from 25-33°C, and suboptimal from 13-25°C and 33-35°C when their activity decreases and metabolism and development slow down (Fields et al., 2012). The temperature of 14.3°C recorded in December 2019 evidently had a negative impact on the activity and development of insects in the warehouse.

Eight species of harmful arthropods were collected and determined in the trial: two stored-product insect species in the order Lepidoptera, five species of storage beetles (order Coleoptera) and one booklouse species (order Psocoptera). Table 1 shows the captured species of stored product insects classified based on specific methods of their collection, i.e. by trapping or by sieving. The results show that the storage beetles *R. dominica*, *O. surinamensis*, *C. ferrugineus* and *T. confusum* were most successfully detected by pitfall cone traps, the moths *S. cerealella* and *P. interpunctella* by pheromone traps, and the booklouse *Liposcelis bostrychophila* Bad. by sieving.

Samples of organic cereals examined after 60 days of incubation in the laboratory were found to contain secondary stored-product insects (*T. confusum, C. ferrugineus, O. surinamensis, P. interpunctella*) as the most frequent (94.0%) of all detected insects, while primary storage pests (*S. zeamais, R. dominica, S. cerealella*) were far less frequent (6.0%) (Figure 1a). Besides, 98.5% of all insects were beetles, and storage moths made 1.5% (Figure 1b). Beetles were the secondary storage insects that caused no significant damage of organic cereals at any time during the period of storage in the warehouse.

Table 1. Types of stored-product insects collected by different types of traps and species identified after sampling organic cereals and sieving

Insect species	Sampling method			
	Pheromone traps for moths	Pheromone traps for wingless insects	Cone traps	Sampling/ Sieving
Order Lepidoptera - moths				
Sitotroga cerealella (Oliv.)	+	–	–	–
Plodia interpunctella (Hbn.)	+	–	–	–
Order Coleoptera – beetles				
Sitophilus zeamais Motsch.	–	+	+	+
Rhyzopertha dominica (F.)	–	–	+	–
Oryzaephilus surinamensis (L.)	–	+	+	+
Cryptolestes ferrugineus (Steph.)	–	–	+	–
Tribolium confusum DuVal	–	+	+	+
Order Psocoptera – booklouse				
Liposcelis bostrychophila Bad.	–	–	–	+

+ presence confirmed; - presence not confirmed

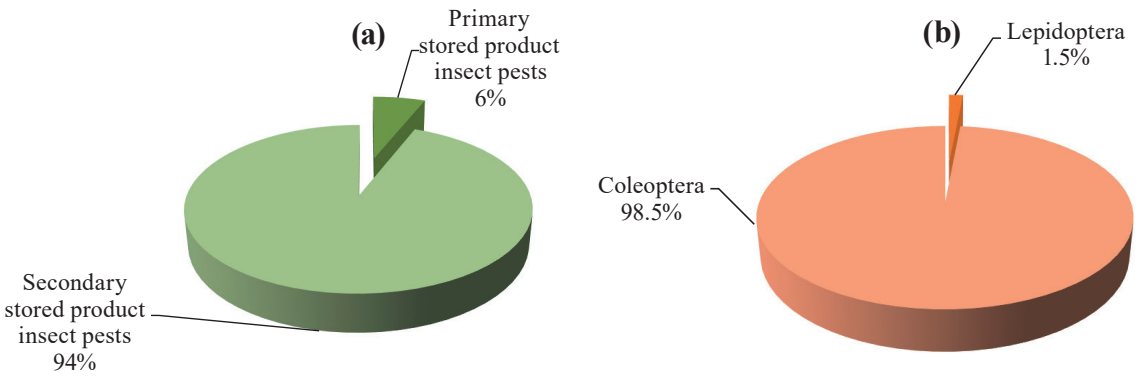

Figure 1. Primary and secondary stored-product insect pests (a), and Coleoptera and Lepidoptera insect pests (b) detected in organic cereals

Data in Figure 2a show that pheromone traps for Coleoptera, positioned around the boxes containing organic cereals, as well as pitfall cone traps pressed into grain, were highly effective in capturing beetles. The largest number of beetles was caught at the beginning of December 2019, i.e. 25-30 specimens/trap, and from the end of January to June 2020, when their frequency was 15 specimens/trap. Data presented in Figure 2b show that pheromone traps for Lepidoptera set around the boxes with organic cereals were highly effective in catching storage moths. The highest number of them were captured in May 2020, 10 moths/trap on the average, while their average number was 6-7 and 4.0 moths/trap in December and September of 2019, respectively. Pheromone and other traps for storage moths significantly impacted their mating, which resulted in a significant reduction in their numbers. When the starting number of moths in a storage is low, such approach to moth control is a very effective and cost-effective tool (Trematerra & Gentile, 2010; Trematerra et al., 2011; Toews & Nansen 2012; Trematerra & Colacci, 2020).

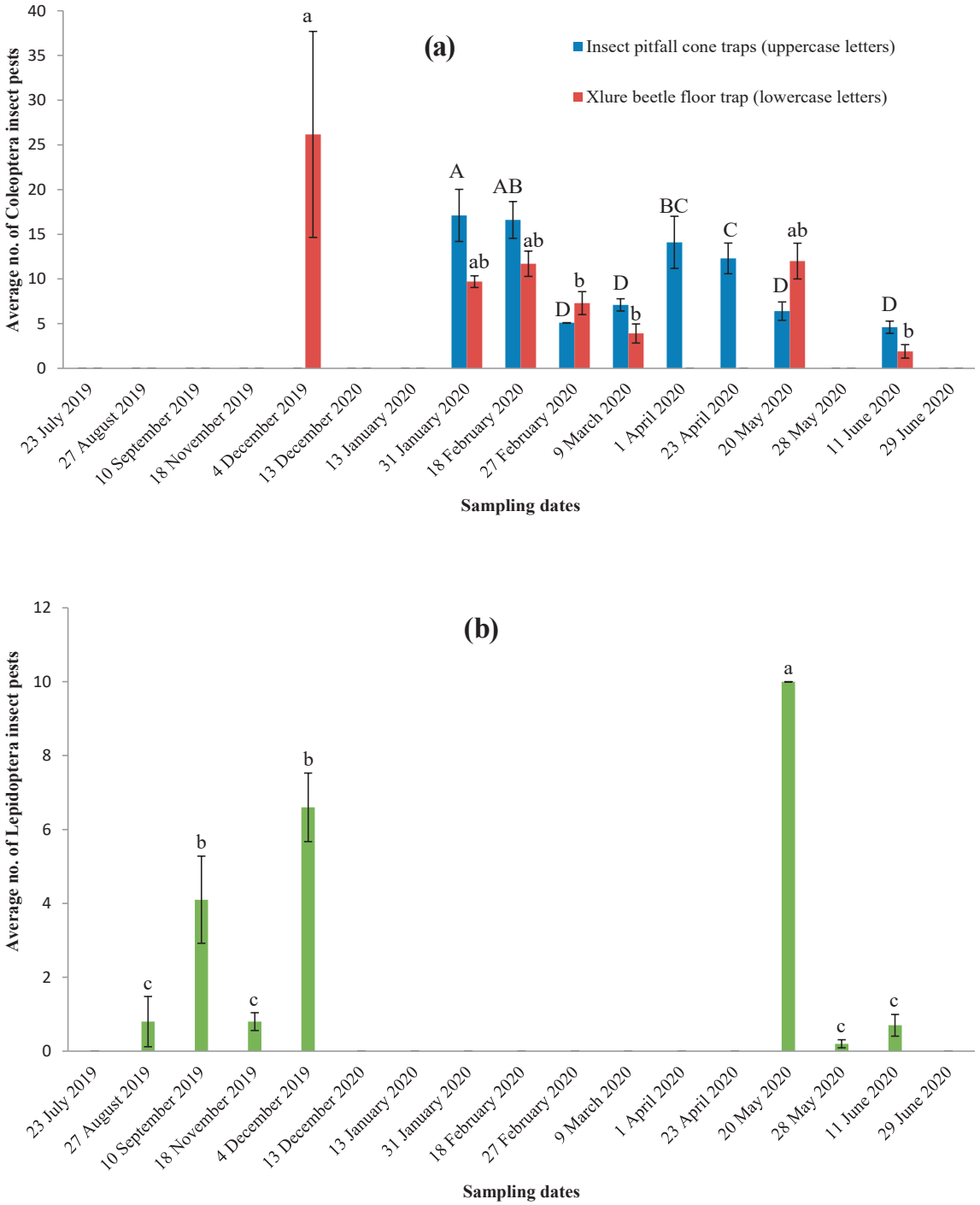

Figure 2. Average number of captured Coleoptera (a) and Lepidoptera (b) insect pests

Table 2 shows the average frequency of stored-product insects per 1 kg of organic cereal grain as determined immediately after sampling in the warehouse and after the incubation period of 60 days in the laboratory. Few insects were detected in the organic cereals at the moment of sampling, and the most abundant and frequent was the beetle *O. surinamensis* in winter wheat captured on May 19, 2020, namely 20 specimens/kg on

Table 2. The average number of insects/kg organic cereal grain, counted immediately after sampling in the warehouse and after 60 days of incubation in the laboratory

Cereals	Sampling date	Stored-product insects	Average no. of insects/kg cereal grain ($\bar{x}$±SE)	
			At sampling	After incubation
Winter wheat	23. 7. 2019.	*O. surinamensis*	0.5±0.5	218.0±1.9
	18. 12. 2019.	*S. zeamais*	1.0±1.0	31.0±9.0
		O. surinamensis	0.0±0.0	27.5±25.6
		C. ferrugineus	0.0±0.0	0.5±0.5
	19. 5. 2020.	*O. surinamensis*	20.5±1.5	764.0±77.2
		S. zeamais	0.0±0.0	35.5±7.5
		C. ferrugineus	0.0±0.0	154.5±59.7
Spring wheat	23. 7. 2019.	*O. surinamensis*	0.0±0.0	11.5±3.5
	18. 12. 2019.	*O. surinamensis*	0.0±0.0	61.0±31.1
		S. zeamais	1.0±1.0	7.0±4.0
		A. calandrae [a]	0.0±0.0	8.0±2.0
	19. 5. 2020.	/ [b]	/	/
Rye	23. 7. 2019.	*O. surinamensis*	0.0±0.0	0.5±0.5
	18. 12. 2019.	*S. zeamais*	0.5±0.5	57.5±25.6
		O. surinamensis	0.5±0.5	0.5±0.5
		S. cerealella	0.0±0.0	29.0±3.0
	19. 5. 2020.	*O. surinamensis*	8.5±0.5	436.0±156.4
		C. ferrugineus	0.0±0.0	515.5±85.2
		Ephestia sp.	0.0±0.0	13.5±2.5
Maize	23. 7. 2019.	/ [c]	/	/
	18. 12. 2019.	*O. surinamensis*	0.0±0.0	0.5±0.5
		S. cerealella	0.0± 0.0	3.5±0.5
	19. 5. 2020.	*O. surinamensis*	4.0±2.0	651.5±301.3
		S. zeamais	0.0± 0.0	13.5±5.5

[a] Parasitoid; [b] Wheat (spring) issued from storage; [c] Maize not yet loeaded in storage

average, then in rye with the average of 8.5 specimens/kg, and in maize with the average of 4.0 specimens/kg. Considering primary pests, the most frequent was the beetle *S. zeamais*, detected at the rate of 1.0 specimen/kg in winter and spring wheat at sampling on December 18, 2019, and then in rye, 0.5 specimens/kg on average.

After 60 days of incubation, insects were abundant in all organic cereals, and *O. surinamensis* was again the most frequent regarding specimen counts and its proportion in the samples collected on May 19, 2020 from winter wheat, was 764 specimens/kg on the average, and the average of 651 specimens/kg was found in maize samples. In this variant, the beetle *S. zeamais* was again the most frequent primary pest detected during sampling on December 18, 2019 in rye with the average of 57 specimens/kg, while in winter wheat the average was 31 specimens/kg.

Table 3 presents the results of sample analyses regarding the average proportion of undamaged grain, broken grain, impurities and dust, infested grain and weight loss of organic cereal grain as determined after 60 days of incubation in the laboratory at 25°C and 60% relative humidity. The results show that the proportion of undamaged and broken organic cereal grain, as well as impurities and dust, would change very little, especially for winter wheat and rye, if grain were stored under conditions that exist in the laboratory, i.e. under stable optimal temperature and relative humidity, while changes would be significant regarding infested grain and weight loss of grain. As different types of traps were laid in the warehouse throughout the experimental period and conditions for survival and development of harmful insects were unfavorable during winter and early spring, their development was slowed down and their numbers increased barely.

Generally, the results indicate a hidden infestation of organic cereals with stored-product insects from the beginning of storage, which could have originated from: 1) a facility in which the commodities were briefly stored before their transfer to the bulk grain warehouse in which the study was conducted, 2) transport or 3) inadequate maintenance of the storage facility so that insects were able to move from inaccessible corners into organic wheat (winter and spring), rye and maize grain. However, the combination of applied measures and methods ensured a high degree of efficacy of the applied traps and sticky tapes that were used for catching storage insects because only a low number of insects were detected in the cereal samples.

Under the conditions described, and based on the stored-product insects found and degree of their infestation in the warehouse, there was no need for undertaking any form of chemical protection of the organic cereals. On the other hand, if conditions for development of storage insects were to be favorable over an extended period of time, and especially if primary pests of the order Coleopteran were present, such an approach would be significantly less effective, and either control measures would have to be applied or the commodities processed or used over a brief period of time, which is consistent with a conclusion made by Bevan et al. (1997).

Table 3. Average percentage of undamaged and broken grain, impurities and dust, infested grain and loss of grain weight in samples of organic cereals after 60 days of incubation in the laboratory

Cereals	Sampling date	Average percentage (% ± SE)				
		Undamaged grain	Broken grain	Impurities and dust	Infested grain	Loss of grain weight
Wheat (winter)	23. 7. 2019.	96.2±0.2 a[a]	3.5±0.2 a	0.2±0.1 b	0.0±0.0 c	0.00±0.00 b
	18. 12. 2019.	96.0±0.3 a	3.2±0.2 a	0.4±0.1 b	0.1±0.0 b	0.05±0.03 b
	19. 5. 2020.	94.9±0.5 b	3.6±0.4 a	0.8±0.1 a	0.2±0.1 a	0.94±0.93 a
Wheat (spring)	23. 7. 2019.	95.1±0.6 a	4.0±0.5 a	0.9±0.5 a	0.0±0.0 b	0.00±0.00 b
	18. 12. 2019.	95.4±0.5 a	3.8±0.5 a	0.5±0.1 a	0.3±0.1 a	0.07±0.03 a
	19. 5. 2020.	/[b]	/	/	/	/
Rye	23. 7. 2019.	96.0±0.0 a	3.2±0.2 c	0.8±0.2 b	0.0±0.0 b	0.00±0.00 a
	18. 12. 2019.	94.5±0.1 b	4.7±0.1 a	0.6±0.1 b	0.1±0.0 a	0.22±0.15 b
	19. 5. 2020.	94.8±0.6 b	3.8±0.4 b	1.1±0.2 a	0.1±0.1 a	0.96±0.60 a
Maize	23. 7. 2019.	/[c]	/	/	/	/
	18. 12. 2019.	90.0±0.5 b	9.1±0.6 a	0.3±0.1 a	0.6±0.3 a	0.24±0.12 a
	19. 5. 2020.	92.7±0.6 a	6.6±0.9 b	0.2±0.1 a	0.6±0.3 a	0.01±0.01 b

[a] Values marked with different letters per cereal differ significantly (Tukey-Kramer HSD test, significant at P=0.05); [b] Wheat (spring) issued from storage; [c] Maize stored later

Effects of measures applied to rodents

Clear evidence, i.e. feces findings and direct catches on sticky tapes and in snap traps, indicated that only the house mouse, *Mus musculus,* was present in the warehouse. The results were consistent with the species biology and ecology, and with structural and spatial arrangements inside the warehouse. Figure 3 shows the catching results for house mice, comparing two trapping approaches, i.e. the use of rodent boxes for snap trapping, and sticky tapes. No hair or feces were found in the stored products during sampling and sieving in the facility or during sample inspection in the laboratory. The results infer that sticky tape barriers were more efficient in capturing rodents than snap traps, while their combined application enabled full protection of cereals stored in the warehouse.

Figure 4 shows the dynamic of house mouse catches over the season 2019/2020. During seasonal weather change from warm to cold periods of the year, rodents begin to enter storage facilities in significant numbers in search for shelter and accessible food. From November 2019, rodent catches in traps and especially in sticky tape barriers increased, and their highest numbers were recorded in January 2020 when the outside weather was most adverse. The fewest mice were caught at the beginning of spring, which is consistent with rodent reproduction biology and the achieved trapping efficacy over the preceding period. An increase in the number of captured animals in April 2020 was consistent with the dynamic of natural development of house mouse populations (Đukić et al., 2005) and the frequency of grain manipulation in the facility.

Based on the results shown in Figure 5, it can be inferred that the position of traps had a great impact on trapping rate because the highest number of mice were trapped in the vicinity of the entrance. Also, there is a clear difference in trapping rates between sticky tape barriers and traps positioned in the same location around the facility entrance. Barrier tapes were found to be more effective in capturing house mice in the warehouse than snap traps.

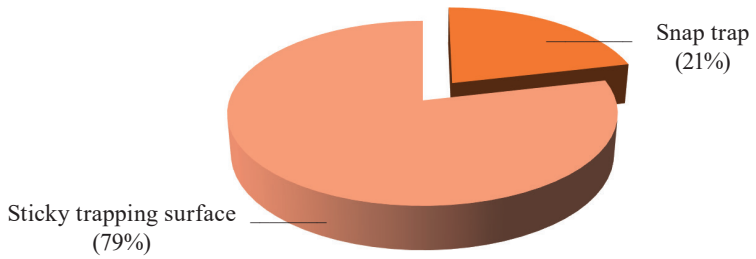

Figure 3. Comparison of methods for catching house mice

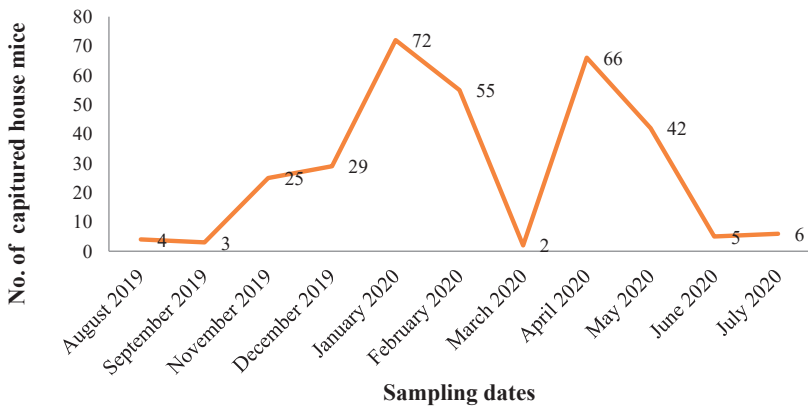

Figure 4. Dynamic of house mouse catches over the season 2019/2020

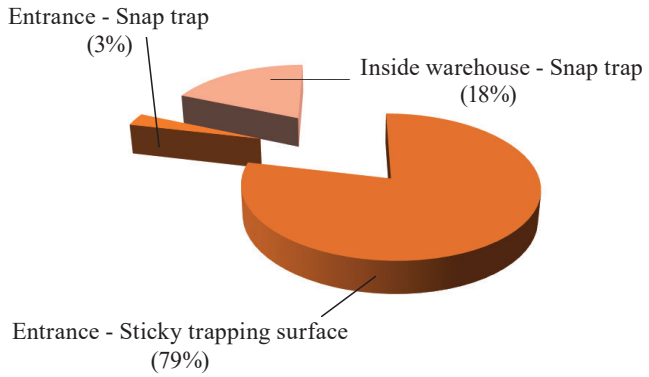

Figure 5. Effectiveness of rodent trapping in terms of trap location in warehouse

Based on the results of rodent frequency checks in the warehouse over the season 2019/2020, it is clear that the number of house mice increased after the warehouse was opened to enable grain handling. Snap trapping of house mice was shown to produce good results during bulk storage free of chemical treatments. Considering the efficacy of trapping and the fact that such means of rodent control are completely safe for humans and the environment, the combination of boxes with snap traps and sticky tape barriers proved effective in protecting stored products from house mice. Evidently, the approach to such activities should be expanded to include arrangements, maintenance and work organization inside the storage facility, as well as good knowledge of rodent biology (primarily reproduction and expansion) and sanitation measures to be undertaken in a wide area around cereal storages (Buckle & Smith, 1994; Đukić et al., 2005). The data on trapped rodents and those caught on sticky tapes around the entrance point evidently indicated a constant danger of rodents penetrating into the storage facility. Laying traps and sticky tapes (which proved the most effective) around the entrance prevents rodents from penetrating the facility and causing damage to stored grain.

In conclusion, a combination of various types of traps (with or without pheromones/attractants) for stored-product insects (beetles and moths) and for rodents (house mouse), applied in greater number and at smaller distance than it is usually practiced for storage monitoring, and sticky tape barriers for pests, provide successful protection of organic cereals, especially when it is practiced in the late autumn-early spring season. Such an approach is certainly a significant contribution to preserving the initial quality of organic cereals of wheat, rye and maize, and to overall improvement of the safety of plant food because chemical protection is avoided.

ACKNOWLEDGEMENT

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Efekti zaštite organskih cerealija od štetnih insekata i glodara u podnom skladištu kombinovanom primenom klopki i lepljivih traka

REZIME

U periodu leto 2019 – proleće 2020. godine je ispitana mogućnost zaštite organskih cerealija (ozime i jare pšenice, raži i kukuruza) od štetnih insekata i glodara u podnom skladištu bez upotrebe hemikalija, kombinovanom primenom klopki i lepljivih traka. Temperatura vazduha u skladištu je bila 14-29°C, a relativna vlažnost vazduha oko 50%. Prosečan sadržaj vode u zrnima svih organskih cerealija je tokom celog perioda skladištenja bio 10-11% u zrnima pšenice (ozime i jare) i raži, a 12-14% u zrnima kukuruza, a prosečna temperatura zrna 13-27°C. Od skladišnih insekata je primenom klopki (sa i bez feromona/atraktanata) i lepljivih traka zabeleženo prisustvo pet vrsta tvrdokrilaca, dve vrste leptira i jedne vrste prašnih vaši, a dominantni su bili tvrdokrilci (98,5%) i sekundarne vrste štetnih insekata (94,0%). Od glodara je tokom celog perioda zabeleženo samo prisustvo jedinki vrste *Mus musculus*. Utvrđeno je da je kombinovana primena klopki, sa i bez feromona/atraktanata, i lepljivih traka - barijera, vrlo efikasna mera u hvatanju skladišnih insekata. Takođe, konstatovano je da su mehaničke klopke i lepljiva traka u kutijama za deratizaciju, zajedno sa lepljivom trakom - barijerom, vrlo efikasne u zaštiti cerealija od domaćeg miša. Postignut je i efekat "suzbijanja" štetočina, jer u uzetim uzorcima nije detektovano brojno prisustvo skladišnih insekata i nije utvrđeno značajnije oštećenje zrna organskih cerealija. Dobijeni rezultati pokazuju veliki potencijal kombinovane primene klopki i lepljivih traka u zaštiti organskih cerealija u podnom skladištu, s tim da bi u slučaju dužeg perioda skladištenja bila neophodna primena hemikalija koje imaju dozvolu za primenu u organskoj proizvodnji hrane.

Ključne reči: cerealije, insekti, glodari, klopke, lepljive trake, skladište

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Original scientific paper

Application of different combinations of lactic acid, phototrophic bacteria and yeast mixtures in control of seed and seedlings pathogens of tomato and pepper

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SUMMARY

Application of three combinations of lactic acid bacteria (*Lactobacillus plantarum*, *Lactobacillus rhamnosus*), phototrophic bacteria (*Rhodopseudomonas palustris*) and yeast (*Saccharomyces cerevisiae*) with sugar cane molasses, marked as: EM1, EM5 and EM AGRO, against the phytopathogenic fungi of tomato and pepper: *Fusarium oxysporum, Alternaria alternata, Botrytis cinerea*, *Colletotrichum* sp., *Verticilium dahliae* and *Pythium aphanidermatum* was evaluated *in vitro* and *in vivo*. A combination of bacteria and yeast named EM5 showed the highest mycelium growth inhibition against *B. cinerea* (38.4%) in a double agar diffusion test. In a microdilution test, the combination EM1 showed the highest inhibitory effect on *B. cinerea* (MIC $1x10^{-3}$ µl/ml), while EM5 showed a similar inhibitory effect towards *F. oxysporum*, *A. alternata* and *Colletotrichum* sp. (MIC 10 µl/ml). The use of EM1 (in concentrations 10 and 100 µl/ml) and EM AGRO (10 µl/ml) is recommended for tomato seedling protection. EM1 (100 µl/ml), EM5 and EM AGRO (10 µl/ml) are recommended for pepper seedling protection.

Keywords: tomato, pepper, lactic acid bacteria, phototrophic bacteria, yeasts, antifungal potential

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) and pepper (*Capsicum annuum* L.) are two very important vegetable crops in Serbia. In 2019, tomato production in Serbia was 111.649 tonnes on 7.880 ha (FAO, 2021), while Gvozdenović (2010) reported over 150.000 tonnes of peppers that were harvested from 21.000 ha.

Tomato and pepper crops are exposed to many phytopatogenic fungi, such as: *Alternaria alternata*, *Colletotrichum* spp., *Fusarium* spp. (Mannai et al., 2018; Rezaee et al., 2018), *Pythium* spp. (Whipps & Lumsden, 1991), *Botrytis* spp. (Williamson et al., 2007), *Rhizoctonia* spp., *Septoria lycopersici* and *Verticilium* spp., which are able to cause severe economic losses. Some of these phytopatogenic fungi can produce toxins that have harmful consequences for human health. Frequent application of synthetic pesticides, as control measures in the management of seed and seedlings diseases, is associated with resistance of these pathogens to synthetic pesticides (Rosslenbroich & Stuebler, 2000; Hahn, 2014), which increases production costs and polluting the environment.

Biological control is one of the most important alternative strategies (Karimi et al., 2012). The issues of fungal resistance, environmental pollution, and negative effects on human health can be significantly reduced by applying biological plant protection products. Several bacterial antagonists are used in plant protection, but as they live in nature close to pathogens, they need to be identified, isolated, amplified and correctly applied.

Important groups of microorganisms used in the biological control of fungal diseases are lactic acid bacteria (LAB) (Dalie et al., 2010; Laref & Guessas, 2013; Zebboudj et al. 2014). The application of plant growth promoting bacteria (PGPB), to control phytopathogens, has gained increasing attention, for example purple nonsulfur bacteria (PNSB) *Rhodopseudomonas palustris* strains have been mentioned as possible biocontrol agents (Nookongbut et al., 2019). Therefore they may be considered as commercial alternatives to chemical pesticides to manage plant diseases, provide food security and contribute to a sustainable agrosystem (Stamenković et al., 2018).

The objective of this study was to determine the antagonistic capacity of PGPB by evaluating the antifungal power of three combinations of lactic acid bacteria, a phototrophic bacterium and yeast *in vitro* and *in vivo* against the phytopathogenic fungi of tomato and pepper: *Fusarium oxysporum, Alternaria alternata, Botrytis cinerea*, *Colletotrichum* sp., *Verticilium dahlia* and *Pythium aphanidermatum*.

MATERIALS AND METHODS

Antagonistic activity of investigated mixtures

Double agar diffusion test. To evaluate the efficiency of three combinations of lactic acid bacteria (*Lactobacillus plantarum*, *Lactobacillus rhamnosus* >10^3CFU/g), phototrophic bacteria (*Rhodopseudomonas palustris* >10^3CFU/g) and yeast sugar molasses (*Saccharomyces cerevisiae* >10^3CFU/g), marked as: EM1, EM5 and EM AGRO (property of LUMAX - doo, Belgrade, products registered commercially as soil conditioners), an *in vitro* assay was performed on potato dextrose agar (PDA) to observe mycelial development of *F. oxysporum, A. alternata, B. cinerea*, *Colletotrichum* sp. (from a collection of the Institute for Plant Protection and the Environment, Belgrade), *V. dahliae*, and *P. aphanidermatum* (from a collection of the Institute of Pesticides and Environmental Protection, Belgrade), originating from tomato and pepper seeds. Mycelial disks (5 mm diameter) from 15-day old pure cultures of the investigated fungi were placed on PDA dishes. Twenty-four hours later, different bacterial combinations were added at 3 cm distance. Petri dishes with mycelia disks alone served as the positive control (K+). The dishes were incubated at 25°C. Three replicates were used for each treatment. After 7 days, mycelia diameter was measured in two directions. The percentage of inhibition of radial growth (PIRG) was calculated following the method of Al-Al-Hetar et al. (2011):

$$PIRG\% = [(R1-R2)/R1] \times 100\%$$

where R1= radial micelial growth on the control plate, and R2 = radial micelial growth on treated plates.

The results were statistically analysed using STATISTICA v. 6 (StatSoft, Inc.).

Microdilution test in vitro. Minimum inhibitory concentrations (MIC) of three combinations of lactic acid, phototrophic bacteria and yeast, marked as: EM1, EM5 and EM AGRO against *F. oxysporum, A. alternata, B. cinerea*, *Colletotrichum* sp., *V. dahliae*,. and *P. aphanidermatum,* were determined by microdilution using 96-well microtitre plates according to Balouiri et al. (2016) in a concentration range of 10 µl/ml - 1×10^{-9} µl/ml of each mixture. Fungal spores were washed from the surface of potato dextrose (PD) plates with sterile 0.85% saline solution containing 0.1% Tween 80 (v/v). Spore suspension was adjusted to a concentration of approximately 5×10^4 in the final volume of 100 µl per well with different dilutions of bacterial suspension. Microtitar plates were incubated for 5 days at 25°C. The experiment was repeated four times. Fluconazole (0.8 mg/ml) was used as a positive control. The lowest concentrations without visible growth were defined as the minimum concentrations inhibiting fungal growth.

Effects of tested mixtures on seed and seedling infection percentage

Filter paper test. Effects of two concentrations (100 µl/ml and 10 µl/ml) on the percentage of infection of tomato and pepper seed on filter paper were examined. Sixty seeds (20 in each of three repetitions) were soaked in the two concentrations and transferred to wet filter paper for two exposure periods lasting 3 h and 4 h. The percentage of infection was assessed 7 days after treatment. Seeds soaked in sterile water were used as a negative control.

In vivo (soil test). Untreated seedlings of tomato and pepper were planted in soil substrate, watered with 3 ml of tested mixtures at concentrations of 100 µl/ml and 10 µl/ml every 4 days during three weeks. The experiment was set up in three replications with 20 plants in each variant. An untreated control was watered with the same amount of water. The presence of disease was recorded after 15 days. The results were analysed using the statistical analysis package STATISTICA c. 6 (StatSoft, Inc.).

RESULTS

Antagonistic activity of investigated mixtures

Double agar diffusion test. All tested pathogens except *P. aphanidermatum* were inhibited by the mixtures investigated (Figure 1A,B). The investigated mixtures demonstrated the highest level of inhibition against the fungus *F. oxysporum* (30.3-38.4%), followed by *A. alternata* (28.0-30.4%), while no inhibition was observed against *P. aphanidermatum*. The mixture EM 5 showed the highest degree of inhibition of micelial growth of *F. oxysporum*, *A. alternata*, *B. cinerea* and *Colletotrichum* sp., and moderate inhibition of *V. dahlia*. The degree of interactions between the tested mixtures and pathogens was high (R=0.838).

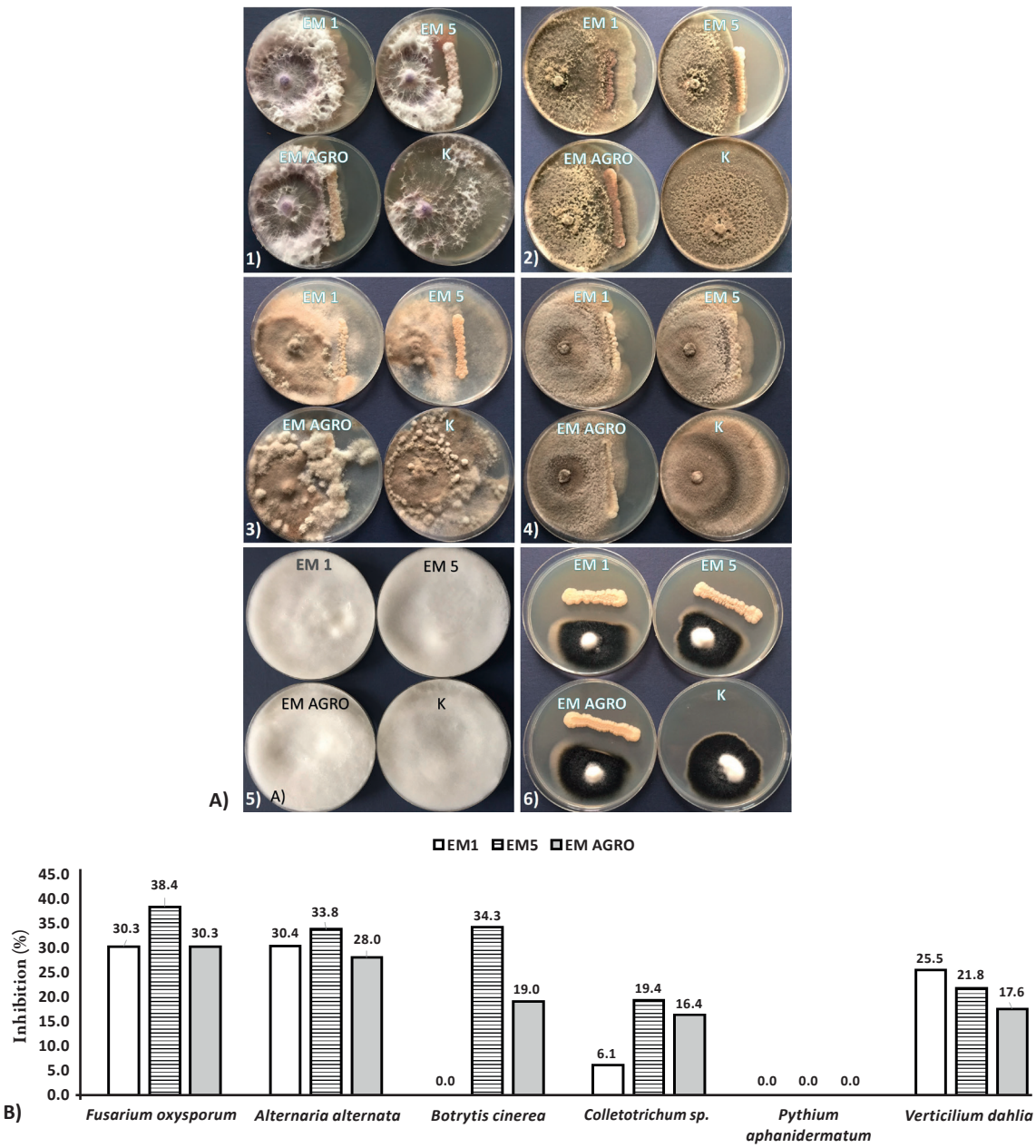


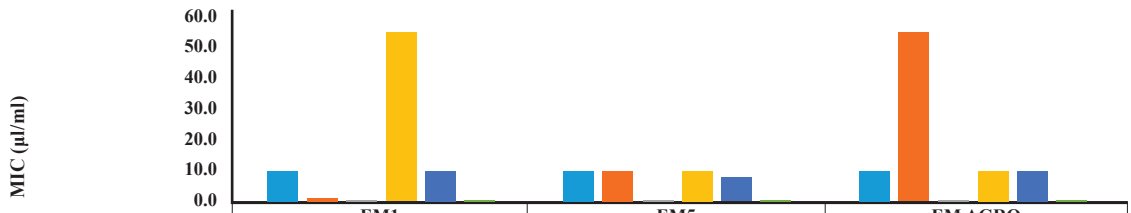
Figure 1. The effect of combinations of bacteria and yeast on mycelial growth inhibition of: 1) *Fusarium oxysporum*, 2) *Alternaria alternata*, 3) *Botrytis cinerea*, 4) *Colletotrichum* sp., 5) *Pythium aphanidermatum* and 6) *Verticilium dahlia* (A), and the percentage of micelial inhibition growth in dual cultivation test (B)

Microdilution test - minimum inhibitory concentration (MIC). The tested combination EM1 (Figures 2 and 3) showed its highest inhibitory effect on *B. cinerea* (MIC 1×10^{-3} µl/ml), *V. dahliae* (MIC 3×10^{-3} µl/ml), and *A. alternata* (MIC 1µl/ml); moderate against *F. oxysporum* and *P. aphanidermatum* (MIC 10 µl/ml), and the lowest on *Colletotrichum* sp. (MIC 55 µl/ml).

EM5 showed a uniform inhibition capacity against *F. oxysporum*, *A. alternata* and *Colletotrichum* sp. (MIC 10 µl/ml), slightly lower against *P. aphanidermatum* (MIC 7.75 µl/ml), and the lowest against *B. cinerea* (2.5×10^{-2} µl/ml) and *V. dahliae* (MIC 2.8×10^{-1} µl/ml).

EM AGRO inhibited the mycelial growth of *B. cinerea* and *V. dahliae* with its lowest concentration (MIC 1×10^{-1} µl/ml). A slightly higher concentration was observed to inhibit *F. oxysporum, Colletotrichum* sp. and *P. aphanidermatum* (MIC 10 µl/ml), and the least effect was observed towards *A. alternata* (MIC 55 µl/ml).

This experiment demonstrated the highest susceptibility of *V. dahlia and B. cinerea* (<1 µl/ml) to all tested mixtures, while *F. oxysporum* and *P. aphanidermatum* showed



	EM1	EM5	EM AGRO
Fusarium oxysporum	10.000	10.000	10.000
Alternaria alternata	1.000	10.000	55.000
Botrytis cinerea	0.001	0.025	0.100
Colletotrichum sp.	55.000	10.000	10.000
Pythium aphanidermatum	10.000	7.750	10.000
Verticilium dahliae	0.003	0.280	0.100

Figure 2. Minimum inhibitory concentration (MIC) for three combinations of bacteria and yeast determined for phytopatogenic fungi of tomato and pepper seed and seedlings

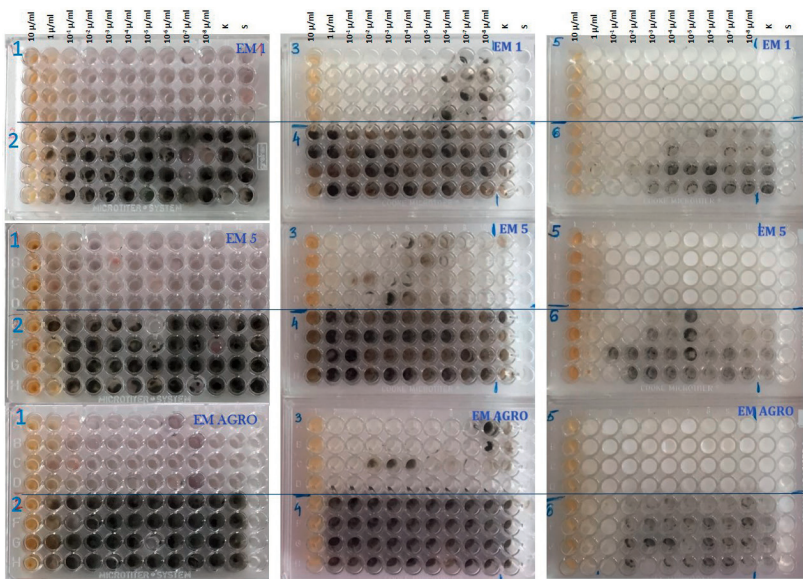


Figure 3 Minimum inhibitory concentration (MIC) of combinations of bacteria and yeast against phytopatogenic fungi: 1) 1) *Fusarium oxysporum*, 2) *Alternaria alternata*, 3) *Botrytis cinerea*, 4) *Colletotrichum* sp., 5) *Pythium aphanidermatum* and 6) *Verticilium dahlia*

satisfactory susceptibility to EM5 and EM AGRO. *A. alternata* and *Colletotrichum* sp. did not show satisfactory susceptibility to the tested combinations (EM AGRO, EM 1) (Figures 2 and 3).

Influence of tested combinations on infection percentage of seeds and seedlings of tomato and pepper

Effects of tested combinations on the percentage of infected tomato and pepper seeds (on filter paper). Experiment analysis showed that 15 of 20 tomato plants in the non-treated experiment were asymptomatic on average, while 19-20 of 20 plants (per repetition) were asymptomatic in the treated plates (Figure 4).

An analysis based on concentration and exposure time of seedlings to combinations revealed that an average of 15 pepper seedlings were asymptomatic in the non-treated control, while the number ranged from 15-20 (20 seedlings per repetition) in treatments. Only seedlings treated with EM AGRO at 100µl/ml concentration were infected as high as control seedlings, while all other treatments showed a significant decrease in infection (Figure 5).

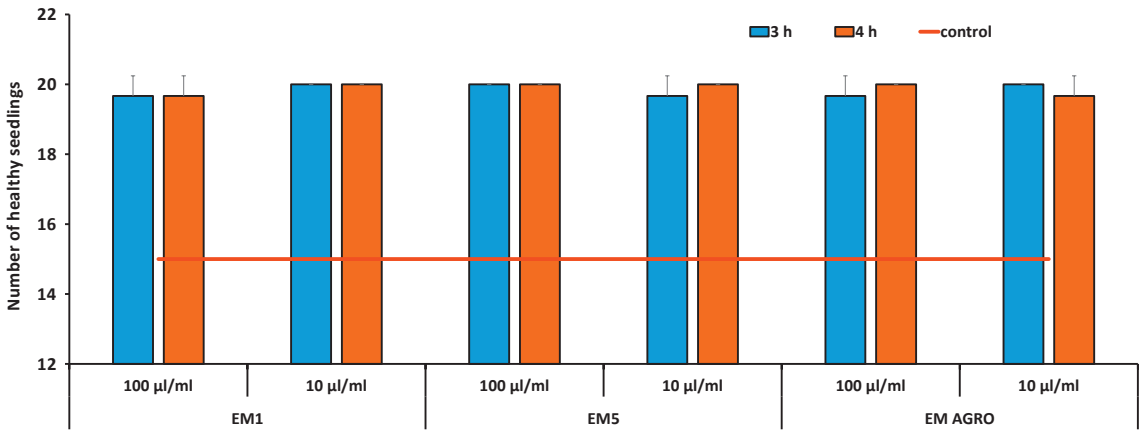


Figure 4. Effects of bacteria and yeast combination, treatment concentration, and exposure time on the number of asymptomatic tomato seedlings

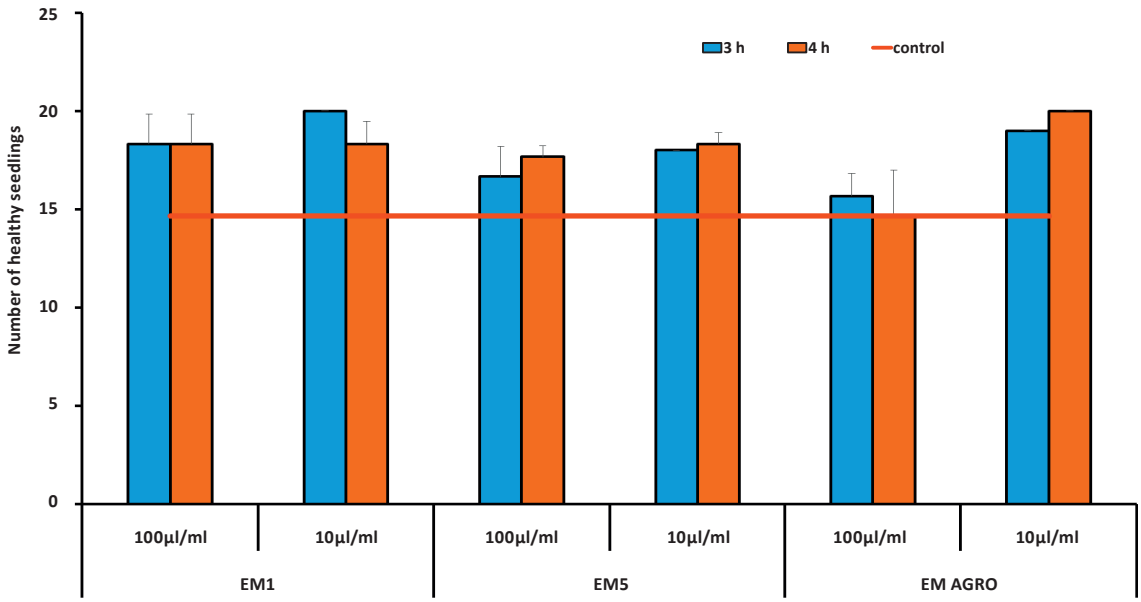


Figure 5. Effects of treatment, concentration, and time of exposure on frequency of asymptomatic pepper seedlings

Effects of tested combinations on the percentage of infection of tomato and pepper seedlings (soil test). The EM1 and EM5 treatments applied at both concentrations completely suppressed the occurrence of *Fusarium* sp. The treatment with EM AGRO completely suppressed the occurrence of *Pythium* sp. in tomato seedlings (Figure 6A). The most effective treatment was EM1 at both concentrations as it managed to suppress the occurrence of fungi of the genus *Fusarium*, as well as fungi of the genus *Pythium,* which appeared in 5% of the samples, while 20% appeared in control samples). Data analysis (Figure 6B) showed a statistically significant increase in the number of asymptomatic plants (14-18) treated with any of the three combinations, while an average of 9 asymptomatic plants were observed in the non-treated control.

The infection rate of *Fusarium* in non-treated control was 85%, while treatments with EM1 and EM5 at 100 µl/ml concentration completely suppressed these phytopatogenic fungi in pepper seedlings (Figure 7A). The highest efficacy in suppressing fungi of the genus *Pythium* was observed in the treatments EM1 and EM AGRO at 10 µl/ml concentration. Data analysis showed that pepper seedlings treated with any of the three combinations showed statistically significant 17-18 asymptomatic plants of the 20 tested, while an average of 4 asymptomatic plants were observed in the control treatment (Figure 7B).

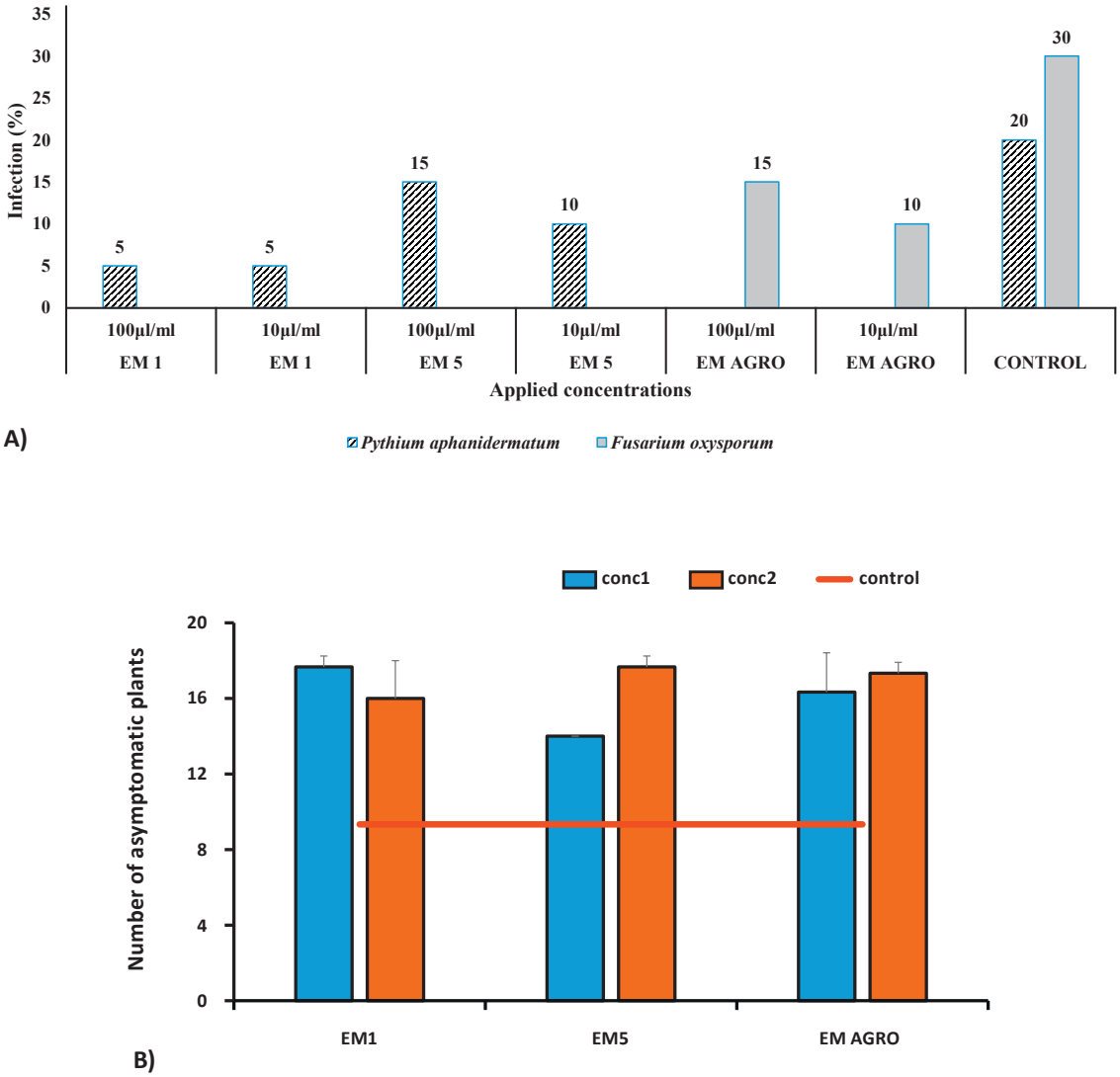


Figure 6. Effects of combinations on infection rate of tomato plants (A) and the number of asymptomatic plants (B)

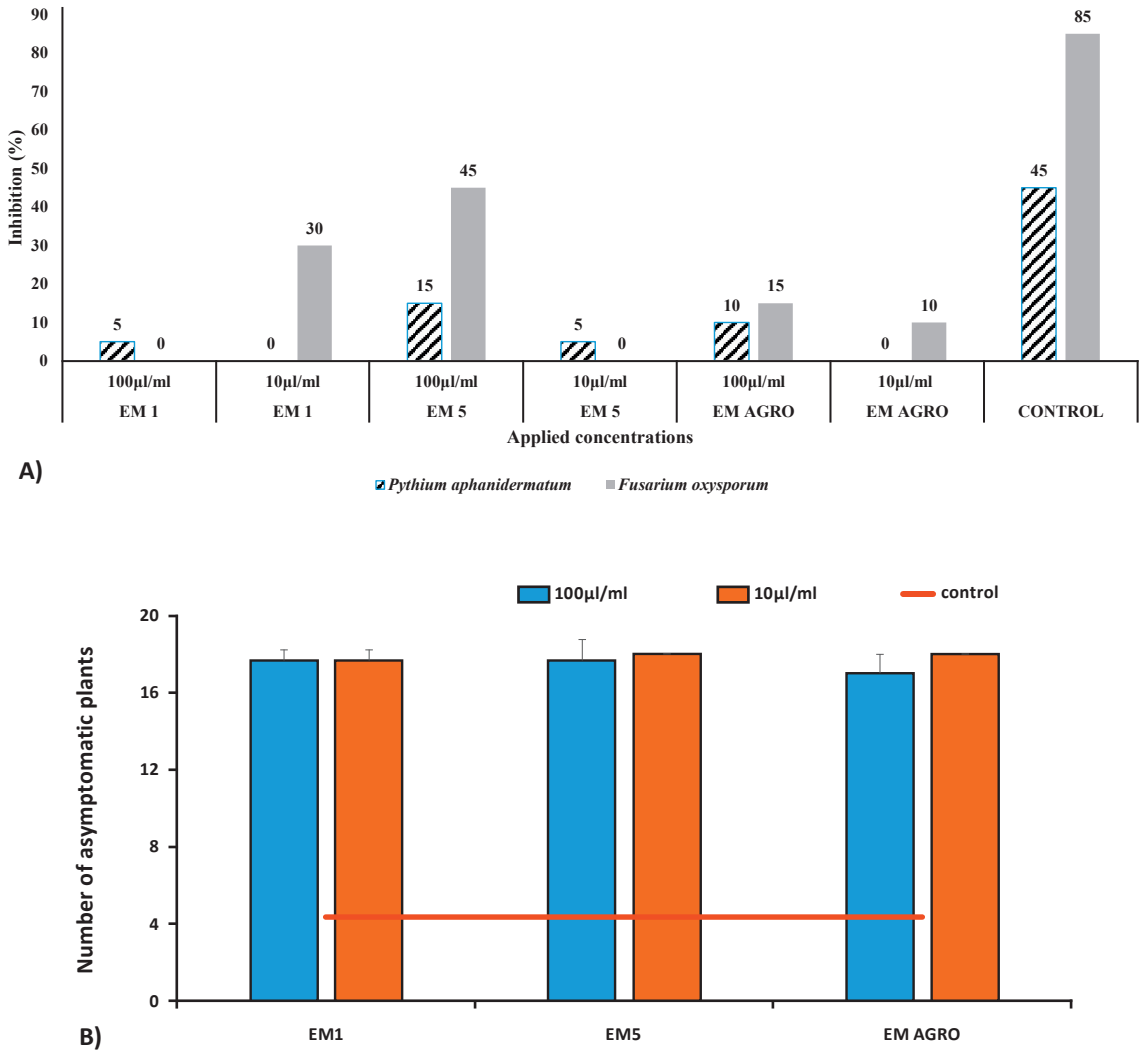


Figure 7. Effects of tested concentrations on the infection rate of pepper plants (A) and number of asymptomatic plants (B)

DISCUSSION

New, alternative strategies for biological control using lactic acid bacteria have been explored to understand the relation between pathogens and antagonistic bacteria in order to control many phytopatogenic casual agents, for example: *Fusarium* spp. (Lavermicocca et al., 2000), *A. alternata* (Zabouri et al., 2021), *B. cinerea*, *Monilinia laxa,* and *Penicillium expansum* (Trias et al., 2008).

Elsewere, the application of *R. palustris* as a plant growth promoting bacteria (PGPB), was shown to influence plant growth and combat plant patogens, such as *Magnaporthe oryzae* (Nookongbut et al., 2020).

Nally et al. (2012) published important data about the antifungal activity of yeast, *S. cerevisiae,* against *B. cinerea* on grapes, while Chand-Goyal and Spotts (1997) and Spadaro et al. (2004) examined it on apples not only at room temperature, but also in a refrigerated chamber. Several reports have mentioned the potential use and applications of different genera and species of antagonist yeasts to control *B. cinerea* on grape tissues (Lima et al., 1999; Castoria et al., 2001; Zahavi et al., 2000; Schena et al., 2000; Masih et al., 2001; Sesan et al., 1999). Other researchers have also reported biocontrol potentials of *S. cerevisiae* against *Penicillium roqueforti* in stored wheat (Petersson & Schnurer, 1995), *Macrophomina phaseolina* and *Fusarium solani* in tomato (Attyia & Youssry, 2001),

Monilia fructicola in apples (Zhou et al., 2008) and *A. alternata* in *Pinus silvestris* (Payne et al., 2000).

There are no reports in literature about combined antagonistic effects of lactic acid, phototrophic bacteria and yeast. The results of this study showed that a combination of different lactic acid bacteria (*L. plantarum*, *L. rhamnosus*), phototrophic bacteria (*R. palustris*) and yeast (*S. cerevisiae),* marked as EM5, demonstrated a strong antifungal effect against *F. oxysporum, A. alternata* and *B. cinerea.* The combination EM5 showed the highest rate of spore inhibition towards *F. oxysporum, A. alternata, B. cinerea*, *Colletotrichum* sp. and *P. aphanidermatum*. All three combinations, at both concentrations and exposure times, showed significant decrease in infection of tomato seeds on filter paper. The treatment EM1 applied at 10 µl/ml concentration over 3 h exposure time, and EM AGRO concentration of 10 µl/ml and 4 h exposure time achieved complete symptom suppression on pepper seeds on filter paper. Both concentrations of all three tested combinations reduced the percentage of tomato and pepper infection with the phytopatogenic fungi *Fusarium* sp. and *Pythium* sp.

Both concentrations of EM1 treatment showed significant efficacy on tomato, and 100 µl/ml concentration on pepper, as well as the lower concentration (10 µl/ml) of EM AGRO on tomato, and the lower concentration (10 µl/ml) of EM5 and EM AGRO on pepper.

Determination of efficacy of biological agents is of paramount importance for preserving ecosystem and human health, and represents the first step towards implementation of alternative, non-pesticide methods in plant protection.

A combination of bacteria and yeast named EM5 stood out in our current *in vitro* experiments as the combination with the highest antifungal potential.

In situ experiments on tomato and pepper seedlings showed a high potential of all combinations used, especially the lower concetrations (10 µl/ml), while the lowest rate of seedlings infection was achieved by applying the combination of EM1 (10 µl/ml-3 h) and EM AGRO (10 µl/ml-4 h).

The use of EM1 (at both concentrations) and EM AGRO (10 µl/ml) is recommended for tomato seedling protection. EM1 (100 µl/ml), EM5 and EM AGRO are recommended to be used at lower concentration (10 µl/ml) for pepper seedling protection.

The results obtained from *in situ* and *in vitro* experiments represent the basic principles for synthesizing biological plant protection products based on the tested combinations of bacteria and yeast, which could safely reduce the infection potential of important phytopathogenic fungi: *F. oxysporum*, *A. alternata*, *B. cinerea*, *Colletotrichum* sp. and *P. aphanidermatum*.

ACKNOWLEDGEMENT

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Primena različitih kombinacija smeša mlečno kiselinskih, fototrofnih bakterija i kvasaca u suzbijanju patogena semena i klijanaca paradajza i paprike

REZIME

U radu je ispitivan antifungalni uticaj tri kombinacije smeša mlečno kiselinskih bakterija (*Lactobacillus plantarum*, *Lactobacillus rhamnosus*), fototrofnih bakterija (*Rhodopseudomonas palustris*) i kvasaca (*Saccharomices cerevisiae*) sa melasom šećerne trske označenih kao: EM1, EM5 i EM AGRO, *in vitro* i *in vivo* na fitopatogene gljive paradajza i paprike: *Fusarium oxysporum*, *Alternaria alternata*, *Botrytis cinerea*, *Colletotrichum* sp., *Verticilium dahliae* i *Pythium aphanidermatum*. Kombinacija bakterija i kvasca EM5, je u eksperimentima dvojne kultivacije ispoljila najviši stepen inhibicije porasta micelije *B. cinerea* (38.4%). U mikrodilucionom testu, kombinacija EM1 ispoljila je najveći inhibicioni efekat na *B. cinerea* (MIC 1×10^{-3} µl/ml), dok je EM5 pokazala ujednačen efekat inhibicije prema *F. oxysporum*, *A. alternata* i *Colletotrichum* sp. (MIC 10 µl/ml). Za zaštitu rasada paradajza preporučuje se upotreba EM1 (u koncentracijama 10 i 100 µl/ml) i EM AGRO (10 µl/ml). Za zaštitu rasada paprike preporučuje se upotreba EM1 (100 µl/ml), EM5 i EM AGRO u nižoj koncentraciji (10 µl/ml).

Ključne reči: paradajz, paprika, mlečno kiselinske bakterije, fototrofne bakterije, kvasci, antifungalni potencijal

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Original scientific paper

A large-scale study on the effectiveness of a *Bacillus subtilis* Ch-13-based biofungicide against green mould disease and mushroom yield improvement

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SUMMARY

The aim of this study was to test a biofungicide based on *Bacillus subtilis* Ch-13 and its effectiveness in the control of green mould disease of cultivated mushroom in comparison with the fungicide prochloraz. Biofungicide effectiveness in disease control and impact on yield were evaluated on *Agaricus bisporus* after its natural infection with *Trichoderma aggressivum* in a commercial mushroom growing facility. An assay for testing the microbial efficacy of the biofungicide was conducted in two different procedures involving either three or two split doses. The highest statistically significant effectiveness in green mould control was shown by the fungicide prochloraz (71.43%), followed by the biofungicide applied in tree split doses (53.57%), and finally its two doses (45.46%). The biofungicide significantly improved yield in comparison with an untreated control and the fungicide prochloraz. Three split applications of *B. subtilis* strain Ch-13 enhanced mushroom yield to a larger extent than its two split doses, although the same final amount was used in both procedures. Biofungicide application in three split doses increased the total mass of harvested mushrooms 8.41% compared to the untreated control, and 10.53% compared to the fungicide prochloraz. These results implied that the biofungicide should be applied in three split applications: 30 ml (second day after casing) + 15 ml (two weeks after casing) + 15 ml (after first flush, 20-25 days after casing). The biofungicide *B. subtilis* Ch-13 should be further investigated regarding its joint usage with chemical fungicides in different application procedures, as it showed remarkable characteristics both in terms of promoting mushroom yield and inhibiting the spread of mycopathogenic *T. aggressivum*.

Keywords: *Bacillus subtilis*, biofungicides, edible mushroom, *Trichoderma aggressivum*, mushroom disease control

INTRODUCTION

The most devastating pathogen of cultivated mushroom (*Agaricus bisporus* L.) is *Trichoderma aggressivum* Samuels & W. Gams (Samuels et al., 2002) and, unlike other casing mycopathogens, it colonizes the substrate of *A. bisporus* and causes crop losses of between 60 and 100% (O'Brien et al., 2017). Kosanović et al. (2020) revealed that the concentration of *T. aggressivum* conidial suspension of 10^{-4} conidia per ml decreased mushroom yield 29-56%, while an inoculum of 10^{-3} conidia per ml caused 68-100% yield decrease. The fungicides prochloraz and metrafenone are allowed to be used in edible mushroom cultivation in the EU (Carrasco et al., 2017). These two fungicides have been registered in Serbia for other crops but not yet approved for use in mushrooms cultivation (Team of editors, 2020). Furthermore, prochloraz decomposes due to microbial degradation in casing soil, and its effectiveness in disease control is so rapidly lost after application (Grogan et al., 2000).

A good alternative to chemical control of mushroom diseases is the application of antagonistic microorganisms, primarily *Bacillus* species (Savoie et al. 2001). A biofungicide based on the most frequently used Canadian strain of *Bacillus velezensis* (Ehrenberg) Cohn, QST713, registered against many plant pathogens and mycopathogens (Védie & Rousseau, 2008; Pandin et al., 2018; Potočnik et al., 2018), is not available on the Serbian market at present. The Russian strain *Bacillus subtilis* (Ehrenberg) Cohn Ch-13 (Chebotar et al., 2009; Kayin et al., 2015), which has recently become available in Serbia, was compared with the chemical fungicide prochloraz and *B. velezensis* QST713 in a recent small-scale study in the experimental mushroom growing room (Potočnik et al., 2019). The biofungicide *B. subtilis* Ch-13 showed higher effectiveness against the compost pathogen *T. aggressivum,* and also increased mushroom yield more with its lower concentration than *B. velezensis* QST713 (Potočnik et al., 2019). Large-scale experiments with edible mushroom disease control are rather scarce. One of the few was conducted by Regnier and Combrinck (2010), establishing a suitable application regime (40 µl l^{-1}) for non-formulated essential oils of lemon, verbena, thyme and lemongrass, as well as two of their main components (nerol and thymol), against *M. perniciosa* in commercial growing facility under conditions of natural infection.

Based on the promising results of the previous small-scale experiment (Potočnik et al., 2019), the aim of this study was to compare the biofungicide based on *B. subtilis* Ch-13 and the fungicide prochloraz regarding green mould disease control under conditions of natural infection. The impact on mushroom yield was also estimated during this large-scale experiment in a commercial mushroom growing facility.

MATERIAL AND METHODS

Antifungal agents

The biofungicide Ekstrasol F SC (BioGenesis d.o.o., Belgrade, Serbia), based on *Bacillus subtilis* Ch-13 (1×10^8 CFU ml^{-1}), was tested as a potential antifungal agent for the control of *T. aggressivum* in natural infections of casing soil. The experiment was conducted in B8 growing chamber of the mushroom production facility of Delta Danube d.o.o., Kovin, Serbia. The biological efficacy and effectiveness of the biofungicide was evaluated by comparing it with the commercial fungicide prochloraz (Mirage® EC, ADAMA Agricultural Solutions UK Ltd., UK; content of a.i. 450 ml l^{-1}).

Tests in mushroom growing room

Treatments of casing soil in the mushroom growing chamber were carried out according to standard PP 1/270 (1) methodology (EPPO, 2010), using the biofungicide based on *B. subtilis* Ch-13 and the commercial prochloraz-based chemical fungicide.

Mushroom substrate packed in plastic bags sized 0.4 × 0.6 × 0.25 m ($l \times w \times h$), filled with 18 kg of compost and spawned with 0.7% of grain spawn of *A. bisporus* (Italspwan, Onigo di Pederobba, Italy), was provided by the compost producer Champicomp d.o.o., Pločica, Kovin, Serbia. Five plastic bags provided a casing surface of 1 m^2 which was used for treatment calculation. Compost was cased with 7 kg of black peat casing soil (Pešter peat soil, Dallas Company, Tutin, Serbia), and disinfected with peracetic acid 0.02% (Peral-S 15%, Vetprom, Belgrade, Serbia), 90 ml per m^2 of casing. Casing soil was cased in a 50 mm layer and incubated at 25°C for 8 days (case-run). The day of casing was regarded as day one. Over the next seven days air temperature was reduced in stages to 17°C. The fungicide prochloraz and the biofungicide were

repeatedly applied using an automatic "fir" sprayer with 10 full cone nozzles. Prochloraz was applied at the standard product application rate registered in the EU in two split applications, each treatment consisting of 1.5 ml in 1.8 l H_2O per 1 m^2 of casing surface on the fourth day after casing and after the first flush (approximately 20-25 days after casing). The biofungicide *B. subtilis* Ch-13 was used in two different application procedures in the same total amount of 60 ml per m^2 of casing surface: (1) three times: 30 ml (second day after casing) + 15 ml (two weeks after casing) + 15 ml (after first flush, approximately 20-25 days after casing); and (2) twice: 30 ml (second day after casing) + 30 ml (after first flush, approximately 20-25 days after casing). Each volume was diluted in 1 l of water and applied per m^2 of casing surface. Untreated control plots within groups were sprayed with tap water.

Each treatment and untreated control was repeated twice in a randomized block design experiment with casing area of 56 m^2 per block consisting of 224 bags of mushroom substrate (repetitions). The average values from both trials are presented. The fruiting bodies were hand-picked in two successive production flushes: the first from day 14 to 22 after casing, the second from day 23 to 35. The harvested mushrooms were weighed and divided into two groups based on visual observation, i.e. either with or without symptoms of green mould disease. Fungicide effectiveness was calculated by Abbott's formula (Abbott, 1925):

$$\% \text{ effectiveness} = (Ic - It)/Ic \times 100$$

where Ic - disease incidence in inoculated control; It - disease incidence in treated samples. Disease incidence was recorded as a percentage of fruiting bodies with symptoms compared with those without symptoms.

The effect of fungicides on mushroom productivity was evaluated as biological efficiency (BE), calculated as the ratio of fresh weight of total fruiting body yield and weight of dry spawned substrate, and expressed as percentages (Chrysayi-Tokousbalides et al., 2007) according to formula:

BE = (fresh total fruiting body yield/dry spawned substrate mass) × 100.

Statistical analyses

Data were examined using the one-way analysis of variance (ANOVA), including the comparison of means by the *F*-test. The test was used to compare the significance of differences among data for the average biological efficacy and effectiveness of different bio/fungicide treatments against *T. aggressivum* in the mushroom growing chamber. In all analyses, the level of significance was at least $P<0.05$ (Sokal & Rohlf, 1995). Statistical data analysis was performed using the software Statistica for Windows 6.0 (Stat Soft Italia, 1997).

RESULTS AND DISCUSSION

Dark green colonies were observed on the sides of compost surface eight days after casing, corresponding to first symptoms of green mould disease caused by *T. aggressivum* (Milijašević-Marčić et al., 2017).

Suppression of green mould disease incidence by using bio/fungicides is shown in Figure 1. The biofungicide *B. subtilis* Ch-13 significantly decreased disease incidence after natural *T. aggressivum* infection of cultivated mushrooms, compared to the chemical fungicide prochloraz and untreated control. The effectiveness of disease control was presented in two ways: in comparison with the standard fungicide prochloraz (E_{st}) set to 100%, and in relation to untreated control (E_k) (Table 1). The highest effectiveness in green mould control was shown by the fungicide prochloraz (71.43%), followed by the biofungicide *B. subtilis* Ch-13 applied in three split doses (53.57%). *B. subtilis* Ch-13 used in two split applications was the least effective against the pathogen (46.45%). Despite the same final concentration, the effectiveness of *B. subtilis* Ch-13 in green mould disease control was significantly higher when it was applied three times than in two applications. *B. subtilis* Ch-13 used in three split applications demonstrated effectiveness which was 17.86% lower than that of the standard chemical fungicide but still exceeded 50% in comparison with untreated control.

In the previous small-scale experiment, Potočnik et al. (2019) reported that *B. subtilis* Ch-13, applied at the concertation of 10^8 CFU per m^2, achieved 23% effectiveness when used in the amount of 10 ml m^{-2}; 27% in 20 ml m^{-2}, and 35% in 30 ml m^{-2} against *T. aggressivum*. The strain Ch-13 applied at the dose of 2×10^8 CFU per m^2 showed better efficacy (27.4%) than *B. velezensis* QST713 (23%) used at its higher concentration (5×10^9CFU per m^2). Prochloraz showed the highest effectiveness in disease control in both our experiments, 71% in the current large-scale study after natural infection, and 77% in the earlier small-scale assay after artificial infection with *T. aggressivum* 10^6 conidia per m^2 of casing soil (Potočnik et al., 2019).

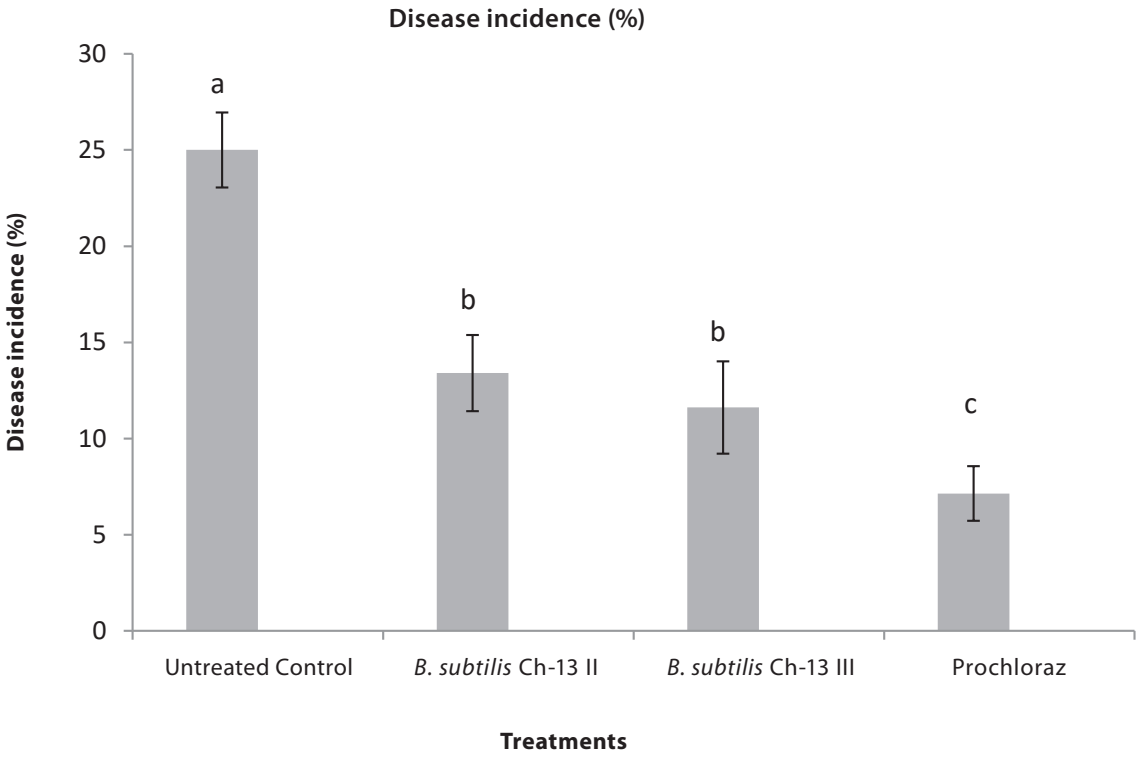

Figure 1. Suppression of disease incidence using bio/fungicides against naturally infected *Trichoderma aggressivum* on *Agaricus bisporus* in a large-scale assay; data are means of two trials, each including 224 replicate experimental bags ± SE, standard error of means; standard error of differences = 9.41; df, degrees of freedom = 3; $F = 70.22$; P-value = 0.001. Values within series marked with the same letters are not significantly different according to F-test ($P<0.05$).

Table 1. The effectiveness of biofungicide treatments in disease control on *Agaricus bisporus* naturally infected with *Trichoderma aggressivum* in a large-scale assay, as related to a standard fungicide (E_{st}) and untreated control (E_k)

Treatments	Bio/fungicide application rate (ml m^{-2})	E_{st}[1] (%)	E_k[2] (%)	SE
Bacillus subtilis Ch-13 1×10^8 CFU ml^{-1}	$1 \times 30 + 2 \times 15$	75.00 b[3]	53.57 b	4.79
Bacillus subtilis Ch-13 1×10^8 CFU ml^{-1}	2×30	65.03 c	46.45 c	3.95
Prochloraz 450 ml a.i. l^{-1}	2×1.5	100.00 a	71.43 a	2.85

Data are means of two trials, each including 224 replicates of experimental bags ± SE, standard error of means; Effectiveness (E)% in disease symptoms control, when standard fungicide effectiveness [1](E_{st}) is set to 100% or when effectiveness is related to untreated control [2](E_k); SEDs, standard error of differences=9.41; df, degrees of freedom=3; F=70.22; P-value=0.001. [3]Values within series marked with the same letters are not significantly different according to F-test ($P<0.05$).

A statistically significant increase in mushroom yield was noted when the biofungicide *B. subtilis* Ch-13 was used in two and three split doses, in comparison with the untreated control and prochloraz fungicide (Figure 2). The chemical fungicide (standard) did not significantly improve mushroom yield compared to the untreated control. Furthermore, impact on mushroom yield was shown as a biological efficiency coefficient (BE) when either the impact of untreated control (BE_{st}) or the standard fungicide prochloraz (BE_k) were set to 100 % (Table 2). The biofungicide increased mushroom yield more when it was used frequently, i.e. in three split applications, than only twice, although the same final amount was used in both treatments.

The strain *B. subtilis* Ch-13 used in three split applications improved the total mass of harvested mushrooms compared both with the untreated control (8.41%) and prochloraz fungicide (10.53%).

The previous small-scale experiment showed that treatments with *B. subtilis* Ch-13, used at concentrations 1-3 × 10^8 CFU ml^{-1}, resulted in considerably enhanced mushroom yield (72-76%), compared to all uninoculated treatments: control plots (66%), fungicide prochloraz plots (68%), and biofungicide *B. velezensis* QST713 plots (58-68%) applied at higher concentrations of 5 × 10^9 CFU ml^{-1} and 1 × 10^{10} CFU ml^{-1} (Potočnik et al., 2019).

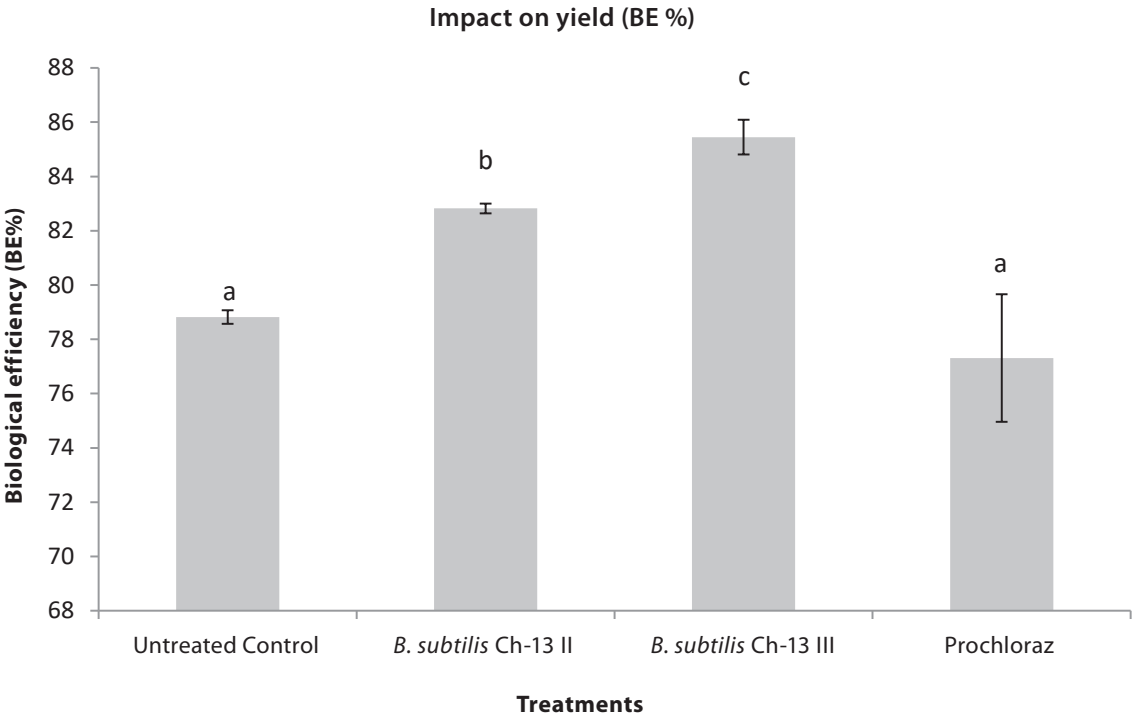

Figure 2. Impact of different bio/fungicides on the yield of cultivated mushroom (*Agaricus bisporus*) naturally infected with *Trichoderma aggressivum* in large-scale assays. Data are means of two replicates and each included 224 replicate experimental bags ± SE, standard error of means; BE% - Biological efficiency = ratio of the fresh weight of total mushroom yield and weight of dry spawned substrate; SEDs, standard error of differences=48; df, degrees of freedom=3; *F*=25; *P*-value=0.001. Values within series marked with the same letters are not significantly different according to *F*-test (P<0.05).

Table 2. Impact of bio/fungicides on the yield of cultivated mushroom (*Agaricus bisporus*) naturally infected with *Trichoderma aggressivum* in a large-scale assay

Treatments	Bio/fungicide application rate (ml m^{-2})	BE_{st}[1] (%)	BE_k[2] (%)	SE
Bacillus subtilis Ch-13 1 × 10^8 CFU ml^{-1}	1 × 30 + 2 × 15	110.53 a[3]	108.41 a	1.29
Bacillus subtilis Ch-13 1 × 10^8 CFU ml^{-1}	2 × 30	107.13 b	105.07 b	0.36
Prochloraz 450 ml a.i. l^{-1}	2 × 1.5	100.00 c	98.08 c	4.70
Untreated control	–	101.95 c	100.00 c	0.51

Data are means of two trials, each including 224 replicate experimental bags ± SE, standard error of means; Biological efficiency (BE) % = ratio of the fresh weight of total mushroom yield and weight of dry spawned substrate, when standard fungicide impact [1](BE_{st}) or untreated control impact [2](BE_k) is set to 100 %; SEDs, standard error of differences=48; df, degrees of freedom=3; *F*=25; *P*-value=0.001. [3]Values within series marked with the same letters are not significantly different according to *F*-test (P<0.05).

As for the inoculated treatments in the same experiment, the biofungicide *B. subtilis* Ch-13 used at the concentration of 1×10^8 CFU ml^{-1} increased yield (68%) more than *B. velezensis* QST713 (63%) used at its higher concentration of 5×10^9 CFU ml^{-1} (Potočnik et al., 2019). In the current large-scale experiment, the biofungicide *B. subtilis* Ch-13 concentration of 1×10^8 CFU ml^{-1}, and its dose of 60 ml m^{-2} of casing soil, improved yield 83-85%, while the biofungicide was applied in the small-scale experiment at lower doses and achieved proportionately lower yield: at the concentration of 10 ml m^{-2} - 68%, 20 ml m^{-2} - 72% and at 30 ml m^{-2} - 74% (Potočnik et al., 2019). Similar yields (79%) were obtained in untreated control plots in both experiments, i.e. in the previous small-scale trial (artificial infection) (Potočnik et al., 2019) and the current large-scale trial under conditions of natural infection.

The mode of action of *Bacillus* spp. biofungicides is based on competition for nutrients, substrate colonization (Chen et al., 2013), synthesis of antibiotics, iron chelators, antifungal volatile organic compounds and cell wall degrading enzymes (Manjula & Podile, 2005). Competition could also be responsible for the inhibition of *T. aggressivum* growth. Furthermore, *B. subtilis* strains are considered safe for the environment and harmless to human health and are generally recognized as safe (GRAS) organisms (FDA, 2020). Additionally, *Bacillus* spp. strains form endospores which ensure their survival and persistence in the environment (Cawoy et al., 2011). The current investigation of different procedures for the application of *B. subtilis* Ch-13 revealed benefits from applying three split doses to suppress the growth of *T. aggressivum,* an aggressive compost pathogen and causal agent of green mould disease, and to promote *A. bisporus* production.

CONCLUSION

The biofungicide based on *B. subtilis* Ch-13 showed better efficacy in green mould disease control and the highest positive impact on mushroom production when it was used in three split applications, rather than two. It suggests that the biofungicide should be applied three times: 30 ml (on the second day after casing) + 15 ml (two weeks after casing,) + 15 ml (after the first flush, approximately 20-25 days after casing). The microbial biofungicide *B. subtilis* Ch-13, which is harmless to the environment and non-target organisms, should be further investigated regarding its combinations with chemical fungicides in order to achieve better efficacy in disease control as it showed remarkable characteristics both in inhibiting the spread of the mycopathogen *T. aggressivum,* the causal agent of the most serious mushroom disease, and in promoting mushrooms production.

ACKNOWLEDGEMENT

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Ispitivanje primene biofugicida na bazi *Bacillus subtilis* Ch-13 u suzbijanju prouzrokovača zelene plesni i pospešivanju prinosa šampinjona u industrijskim razmerama

REZIME

Cilj rada je ispitivanje mogućnosti primene biofungicida na bazi *Bacillus subtilis* Ch-13 u suzbijanju prouzrokovača zelene plesni i povećanju prinosa šampinjona. Biofungicid je testiran nakon prirodne zaraze *Trichoderma aggressivum* u komercijalnom gajilištu šampinjona i poređenjem sa fungicidom prohlorazom. Testirana je efikasnost mikrobiološkog biofungicida kroz dva postupka višestruke primene, u tri i u dve ponovljene doze. Najveću statistički značajnu efikasnost u suzbijanju prouzrokovača zelene plesni je ispoljio fungicid prohloraz 71,43%, zatim biofungicid primenjen u tri doze 53,57% i najmanju primenjen u dve doze 46,45%. Efikasnost *B. subtilis* Ch-13 u suzbijanju prouzrokovača zelene plesni je bila veća od 50% kada je primenjen u tri doze, za razliku od niže efikasnosti u dvokratnoj primeni. Testirani *B. subtilis* Ch-13 je značajnije povećao prinos šampinjona primenjen u tri podeljene doze nego u dve, iako sa istom ukupnom primenjenom količinom preparata. Biofungicid je znatno poboljšao prinos u poređenju sa netretiranom kontrolom i fungicidom prohlorazom. Soj *B. subtilis* Ch-13 je pokazao izuzetno pozitivan uticaj na prinos šampinjona primenjen u tri doze, sa povećanjem ukupne količine ubranih šampinjona 8,41% u odnosu na netretiranu kontrolu i 10,53% u odnosu na fungicid prohloraz. Ovi rezultati pokazuju da bi biofungicid na bazi *B. subtilis* Ch-13 trebalo primeniti u tri podeljene doze: 30 ml (drugi dan nakon stavljanja pokrivke) + 15 ml (dve nedelje nakon stavljanja pokrivke) + 15 ml (nakon prvog talasa plodonošenja, 20-25 dana nakon pokrivanja). Biofungicid *B. subtilis* Ch-13, neškodljiv za životnu sredinu i neciljne organizme, bi trebalo dalje ispitati u zajedničkoj primeni sa hemijskim fungicidima u različitim načinima primene da bi se obezbedila bolja efikasnost u suzbijanju prouzrokovača bolesti, jer je pokazao zadovoljavajuće osobine i u sprečavanju širenja mikopatogena *T. aggressivum* i povećanju prinosa.

Ključne reči: *Bacillus subtilis*, biofungicidi, šampinjon, *Trichoderma aggressivum*, suzbijanje bolesti pečuraka

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Original scientific paper

A study on the allelopathic tolerance of garden pea varieties to *Sorghum halepense* (L.) Pers. extracts

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SUMMARY

The present research study identified garden pea varieties with pronounced tolerance to the allelopatic action of *Sorghum halepense*. Nine varieties were selected to study the effects of shoot and root weed biomass, applied in three extract concentrations (1, 5 and 10%), on seed germination and initial germ growth. A variance analysis of data revealed significant influence ($p < 0.05$) of three studied factors (variety, type and concentration of extracts) on the investigated parameters. Statistically insignificant was only the influence of extract type (root/aboveground biomass) on seed germination. Based on absolute values of the allelopathic indicator RI, which determines the degree of allelopathic inhibition in terms of germination, germ length and weight in different varieties, the variation was as follows: from -0.30 to -0.04, from -1.31 to -2.96 and from -0.47 to 0.02. The total effect of allelopathic action of *S. halepense* on all studied parameters, presented as a GGE-biplot analysis, defined Pulpudeva and Puldin varieties as exhibiting higher tolerance, in contrast to Denitsa and Vyatovo, which were sensitive. Ran I, Mira, Musala and Vechernitsa occupied an intermediate position. Cultivation of allelopathic tolerant varieties is a promising addition to the current weed control strategy, especially in organic production.

Keywords: allelopathy, weeds, *Pisum sativum, Sorghum halepense*

INTRODUCTION

Allelopathy is a biological phenomenon of chemical interactions between organisms in an ecosystem and it should be taken into consideration when solving problems related to pest and weed control in sustainable agriculture (Macías et al., 2019). Allelopathic interactions of plants were observed back in the 4th century BC, but they received necessary attention of the scientific community and farmers only in recent years. In modern agriculture, allelopathy plays an important role in maintaining agroecosystem sustainability through the application of various environmentally-friendly strategies, such as cover crops, crop rotation, incorporation of plant residues, mulching, bioherbicides (Scavo et al., 2018), tolerant cultivars (Bakhshayeshan-Agdam & Salehi-Lisar, 2020; Khatri et al., 2020), etc. Moreover, with an increasing importance of organic farming and environmental protection, more and more attention will be paid to research of allelopathy, and physiological and ecological mechanisms of allelopathy are gradually becoming clearer (Cheng & Cheng, 2015).

Crops possessing allelopathic properties are numerous: they include arboreal and herbaceous species, as well as many weeds (Scavo et al., 2018). The ability to synthesize and release allelopathic compounds in the environment or to tolerate the presence of allelochemicals released by other plants may determine a species' ability to survive and reproduce (Trezzi et al., 2016). The most important allelochemicals include glucosinolates, terpenes, phenolic compounds, alkaloids, benzoxazinoids, sorgoleon, and momilactones (Jabran, 2017).

The allelopathic potential of crops may be used for weed control. This is possible by channeling the allelopathic activity of crops by several techniques. These techniques may include the cultivation of varieties that have allelopathic potential (Jabran, 2017) or varieties with high tolerance to weed species (Cheema & Ahmad, 1992; Cheema et al., 2002). Studies have been conducted to identify varieties with increased allelopathic tolerance to major weeds in crops such as wheat (Shao et al., 2019), corn (Baličević et al., 2014), lupine (Georgieva, 2019), vetch (Georgieva et al., 2018) and others.

The present study aimed to establish the allelopathic effect of different concentrations of *Sorghum halepense* extracts on the initial growth of garden pea varieties and to identify those with increased allelopathic tolerance.

MATERIAL AND METHODS

A laboratory study was carried out as a three-factor experiment at the Institute of Forage Crops (Pleven) in 2021. The first factor (A) included nine varieties of garden pea (*Pisum sativum* L.): Ran I, Pulpudeva, Musala, Denitsa, Skinado, Puldin, Mira, Vyatovo and Vechernitsa. The second factor (B) was *S. halepense* biomass (shoot or root), while three concentrations of weed extracts (1.0, 5.0 and 10.0%) were the third factor (C). Distilled water was used in control Petri dishes.

Shoot and root biomass of *S. halepense* was collected at the flowering stage. It was dried to constant dry weight at 60 °C and ground (Chon & Nelson, 2001). To prepare the extracts, an amount of 100 g of ground plant material was suspended in 1 l of distilled water at 24 ± 1 °C for 24 hours. The obtained extracts were filtered and brought to final concentrations of 1.0, 5.0, and 10.0%. Thymol as a preservative was added in the amount of 1 g/l to each extract. One hundred and five seeds of each pea variety were portioned out into Petri dishes (9 cm diameter) containing filter paper. Each Petri dish received 8 ml of pipetted aqueous extract. The dishes were placed in a thermostat at 22 °C ± 1 °C for 7 days. The following parameters were reported: germination (%), germ length (root and stem) (cm), germ weight (root and stem) (g), and inhibition (%). Germination percentage was calculated using the formula: % germination = (germinated seeds/total number of seeds) × 100, and the inhibition percentage (I, %) was determined using a formula of Chung et al. (2003): % inhibition = [(control-extracts)/control] × 100. The following equations were used to calculate allelopathy indicators: RI=1-C/T(T≥C) and RI=T/C-1(T≤C), where C is the control value, T is the processing value, RI>0 indicates promotion, and IR<0 indicates inhibition. The absolute values are consistent with the intensity of allelopathy action (Zhang et al., 2015). Tolerance index (TI) was determined by an adapted formula of Tahseen and Jagannath (2015). The received data were analyzed using GGEbiplot (PBSTAT 1.2), and the software product Statgraphics Plus for Windows Ver. 2.1.

RESULTS

The data variance analysis revealed significant influence ($p < 0.05$) of the three studied factors (excluding the type of extract on germination) on seed germination rate, and germ length and weight of nine garden pea varieties (Table 1). The "variety" factor was decisive for seed germination (47.6% of total variation), while weed extract concentration had the strongest influence in terms of germ growth and biomass accumulation, 59.8 and 61.0% of total variation, respectively. The interaction of factors A × B, A × C, and A × B × C was statistically significant for all considered parameters, as the A × C interaction had the highest effect. For all studied parameters, the B × C interaction was the weakest and statistically insignificant.

Germination

Aqueous extracts of *S. halepense* showed a general tendency to inhibit seed germination of the tested pea varieties (Figure 1). Also, it was observed that the increasing concentrations of extracts also increased their suppressive effect. Exceptions were found in Pulpudeva and Puldin varieties, in which none of the three concentrations of aboveground biomass (1, 5 and 10%) had negative impact on seed germination, and it also occurred in some other varieties (Denitsa, Skinado, Mira) to which the lowest concentration of 1% also had no pronounced negative effect. Based on the calculated average effects of six weed extracts on the germination process, decrease in germination in

Table 1. Analysis of variance for seed germination and germ growth in garden pea varieties

Causes of variation	Degrees of freedom	Sum of squares	Mean square	Influence of factors	Sum of squares	Mean square	Influence of factors	Sum of squares	Mean square	Influence of factors
Parameters		Germination, %			Germ length, cm			Germ weight, cm		
Total	287	82336.2		100.0	3640.4		100.0	1.59332		100.0
Factor A- variety	8	39179.1	4897.4	47.6*	466.5	58.31	12.8*	0.19192	0.0240	12.0*
Factor B - type of extract	1	121.2	121.2	0.1	10.2	10.21	0.3*	0.01557	0.0156	1.0*
Factor C - concentration of extracts	3	17070.1	5690.0	20.7*	2175.4	725.11	59.8*	0.97129	0.3238	61.0*
A×B	8	3500.6	437.6	4.3*	69.5	8.69	1.9*	0.01990	0.0025	1.2*
A×C	24	14587.9	607.8	17.7*	290.5	12.10	8.0*	0.15830	0.0066	9.9*
B×C	3	86.7	28.9	0.1	16.6	5.54	0.5	0.00526	0.0018	0.3
A×B×C	24	4910.7	204.6	6.0*	147.9	6.16	4.1*	0.06426	0.0027	4.0*
Error	216	2880.0	133.3	3.5	463.9	2.15	12.7	0.16600	0.00077	10.4

LSD at 0.05 probability level

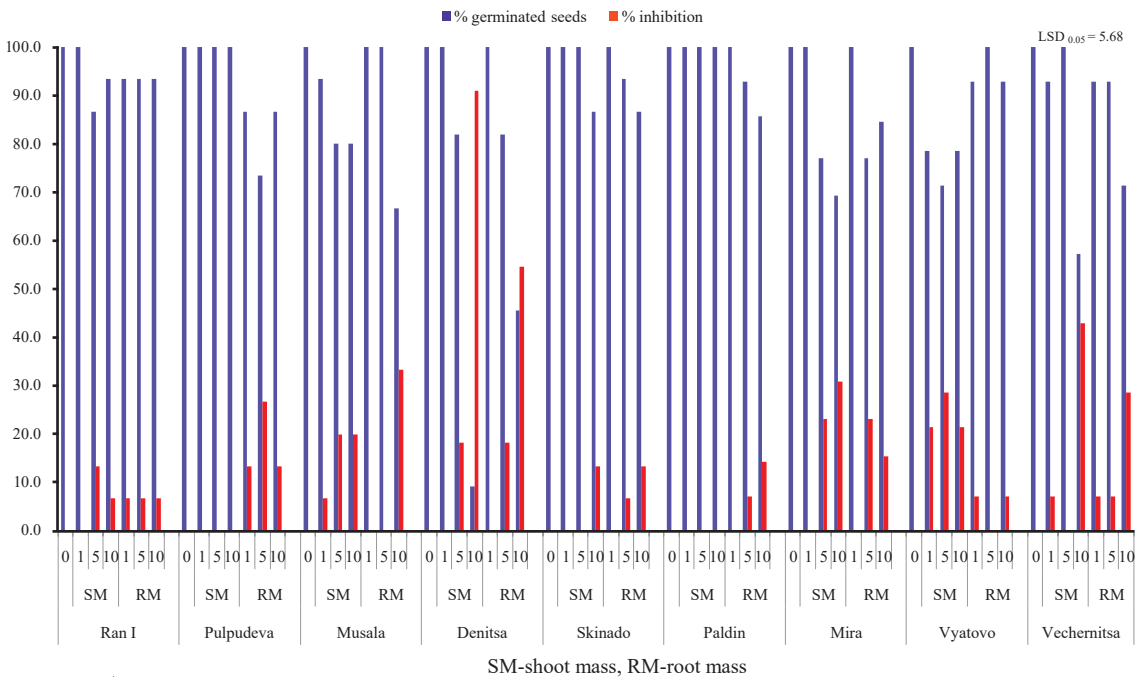


Figure 1. Influence of water extracts of *Sorghum halepense* on seed germination in pea garden varieties

different varieties varied from 6.7 to 90.9%. As a result of concentrations increasing (from 1 to 5 to 10%), the decrease was on average 3.5, 9.9 and 20.6%, while extract type (aboveground or root biomass) caused no significant difference, and it was 12.1 and 10.5%, respectively. The allelopathic indicator RI, which determines the degree of allelopathic inhibition, ranged from -0.067 to -0.909 (Table 1). The lowest average values of variety RI were demonstrated by Puldin, Skinado and Pulpudeva (-0.036, -0.056, -0.089), and the highest by Denitsa (-0.303).

Germ length

As a whole, data in Table 2 show the greatest germ length in the control variants of all garden pea varieties. Maximums were noted for Puldin and Vyatovo varieties, and minimums for Denitsa and Vechernitsa. Compared to control data, the differences in all treated variants were statistically significant, except for the 1% concentration of shoot biomass on Pulpudeva, Musala and Vechernitsa, and 1% concentration of root biomass on Vechernitsa.

Table 2. Influence of *Sorghum halepense* extracts on germ length and fresh biomass accumulation in garden pea varieties

Variety	Type of extract	Concentration, %	GL, cm	GW, g	RI			TI	
					GR	GL	GW	GL	GW
Ran I		Control	11.14	0.281					
	Shoot mass	1.0	8.32	0.261	0.00	−1.22	−0.07	62	66
		5.0	5.11	0.135	−0.133	−1.98	−0.52		
		10.0	3.81	0.104	−0.067	−2.66	−0.63		
	Root mass	1.0	14.29	0.312	−0.067	0.28	2.31		
		5.0	5.58	0.173	−0.067	−1.82	−0.38		
		10.0	4.63	0.120	−0.067	−2.19	−0.57		
Pulpudeva		Control	10.42	0.301					
	Shoot mass	1.0	10.35	0.323	0.00	−0.91	2.16	72	68
		5.0	5.76	0.155	0.00	−1.63	−0.48		
		10.0	5.29	0.167	0.00	−1.78	−0.45		
	Root mass	1.0	14.88	0.268	−0.133	0.43	−0.11		
		5.0	4.14	0.146	−0.267	−2.27	−0.52		
		10.0	4.87	0.165	−0.133	−1.93	−0.45		
Musala		Control	10.13	0.235					
	Shoot mass	1.0	9.49	0.231	−0.067	−0.96	−0.02	56	75
		5.0	5.51	0.167	−0.200	−1.66	−0.29		
		10.0	3.97	0.141	−0.200	−2.30	−0.40		
	Root mass	1.0	6.39	0.220	0.00	−1.43	−0.06		
		5.0	6.43	0.221	0.00	−1.42	−0.06		
		10.0	2.22	0.071	−0.333	−4.12	−0.70		
Denitsa		Control	6.98	0.221					
	Shoot mass	1.0	4.38	0.151	0.00	−1.37	−0.32	60	55
		5.0	3.91	0.148	−0.182	−1.53	−0.33		
		10.0	0.50	0.003	−0.909	−11.96	−0.98		
	Root mass	1.0	8.88	0.192	0.000	0.27	−0.13		
		5.0	3.50	0.118	−0.182	−1.71	−0.47		
		10.0	4.05	0.115	−0.545	−1.48	−0.48		
Skinado		Control	9.29	0.221					
	Shoot mass	1.0	6.20	0.175	0.00	−1.34	−0.21	43	53
		5.0	3.49	0.111	0.00	−2.38	−0.50		
		10.0	2.15	0.056	−0.133	−3.87	−0.75		
	Root mass	1.0	5.85	0.143	0.00	−1.42	−0.35		
		5.0	3.42	0.116	−0.067	−2.43	−0.47		
		10.0	2.94	0.104	−0.133	−2.82	−0.53		
Paldin		Control	13.88	0.216					
	Shoot mass	1.0	11.22	0.211	0.00	−1.15	−0.02	54	86
		5.0	8.56	0.207	0.00	−1.50	−0.04		
		10.0	4.70	0.101	0.00	−2.74	−0.53		
	Root mass	1.0	9.37	0.238	0.00	−1.37	4.17		
		5.0	8.23	0.245	−0.071	−1.56	3.54		
		10.0	3.11	0.108	−0.143	−4.14	−0.50		
Mira		Control	9.97	0.199					
	Shoot mass	1.0	7.69	0.182	0.00	−1.17	−0.09	57	80
		5.0	5.53	0.138	−0.231	−1.62	−0.30		
		10.0	3.16	0.092	−0.308	−2.84	−0.54		
	Root mass	1.0	8.94	0.280	0.00	−1.00	4.56		
		5.0	5.25	0.161	−0.231	−1.71	−0.19		
		10.0	3.59	0.104	−0.154	−2.50	−0.48		
Vyatovo		Control	12.30	0.288					
	Shoot mass	1.0	8.76	0.201	−0.214	−1.29	−0.30	44	54
		5.0	4.12	0.108	−0.286	−2.74	−0.62		
		10.0	4.00	0.101	−0.214	−2.82	−0.65		
	Root mass	1.0	8.46	0.252	−0.071	−1.34	−0.12		
		5.0	4.21	0.150	0.00	−2.68	−0.48		
		10.0	3.25	0.123	−0.071	−3.48	−0.57		
Vechernitsa		Control	8.45	0.262					
	Shoot mass	1.0	8.75	0.233	−0.071	0.04	−0.11	71	62
		5.0	5.27	0.169	0.00	−1.41	−0.36		
		10.0	2.48	0.062	−0.429	−3.00	−0.76		
	Root mass	1.0	8.92	0.252	−0.071	0.06	−0.04		
		5.0	7.76	0.173	−0.071	−0.96	−0.34		
		10.0	2.90	0.091	−0.286	−2.57	−0.65		
LSD at the 0.05 probability level A×B×C			0.72	0.014					

GL - germ length, GW - germ weight, RI - allelopathy indicator, TI - tolerance index

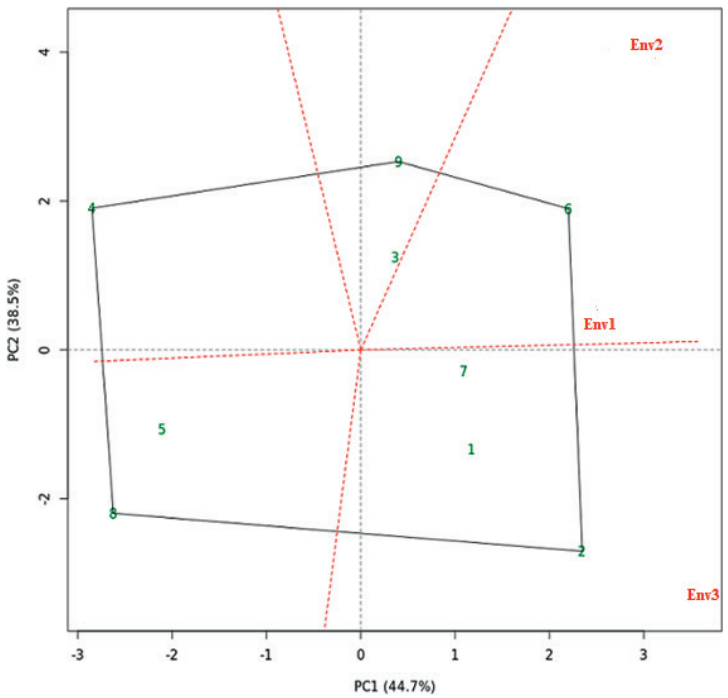
Similar to the previous parameter, treatment with weed extracts of aboveground and root biomass at 1, 5 and 10% concentrations resulted in average inhibition of germ length by 18.8, 48.9, 67.5%, and 7.1, 47.6, 65.9%, respectively. It is obvious that the shoot biomass extracts of *S. halepense* had significantly stronger suppressive effect than those from root biomass (Table 1), with inhibition values of 45.1 and 40.2%, respectively. The average data based on the „variety" factor, regardless of concentration and type of extract, showed that the least affected were Vechernitsa and Pulpudeva, in which the allelopathic indicator RI was -1.31 and -1.35, and the tolerance index (TI) 71 and 72%, respectively. In some varieties (Ran I, Pulpudeva, Denitsa, Vechernitsa), 1% concentration of root biomass had a weak stimulating effect (RI from 0.06 to 0.43). According to TI data regarding the considered parameter „germ length", the studied genotypes Ran I, Pulpudeva, Musala, Denitsa, Puldin, Mira and Vechernitsa can be defined as tolerant (TI <75%), and Skinado and Vyatovo as sensitive (TI <50%).

Germ weight

The analysis of data concerning the germ weight parameter shows that pea cultivars exhibited different degrees of sensitivity to the action of aqueous extracts of *S. halepense* (Table 2). Denitsa, Skinado and Vyatovo demonstrated greater sensitivity, as their reduction in seed weight ranged from 45.2 to 46.8%. The lowest sensitivity was shown by Puldin and Mira (with reductions of 14.2 and 20.0%), while Ran I, Pulpudeva, Musala and Vechernitsa occupied an intermediate position. Similar to the results for previous parameters, increasing concentrations of weed extract enhanced the inhibitory effect on germ weight with values of 7.2, 36.1 and 58.9%, respectively, while the type of extract (shoot or root biomass), although it was a factor with less influence, reduced germ weight, and average values were 38.0 and 30.1%. The mentioned percentages of inhibition of biomass accumulation in different varieties were in accordance with RI and TI values, which identified Puldin and Mira as highly tolerant (TI> 75%), and the other varieties as tolerant (TI <75%).

GGE-biplot analysis

The GGE-biplot method and PBSTAT 1.2 software product were used to assess the total effect of weed extracts on seed germination and initial germ development in pea varieties. Figure 2 clearly demonstrates essential differences in the susceptibility of studied genotypes to the allelopathic stress of *S. halepense*,



1 - Ran I, 2 - Pulpudeva, 3 - Musala, 4 - Denitsa, 5 - Skinado, 6 - Paldin,
7 - Mira, 8 - Vyatovo, 9 - Vechernitsa
Env1 (1.0% concentration of water extract of *S. halepense*), Env2 (5.0%), Env3 (10.0%)

Figure 2. GGE-biplot of allelopathic tolerance in garden pea varieties

as well as the degree of suppressing impact of Env 1 (1.0% concentration of *S. halepense*), Env 2 (5.0% concentration) and Env 3 (10.0% concentration). Among the nine tested varieties, the highest tolerance was demonstrated by Pulpudeva and Puldin, which are located at the terminal points on the right side of the graph. Denitsa and Vyatovo are positioned on the left side of the coordinate system, i.e. at the farthest points from the center, which defines them as varieties with low tolerance. Skinado, located on the left side of the coordinate system, can also be included in this group. Ran I, Mira, Musala and Vechernitsa occupy intermediate positions.

DISCUSSION

The results of this study showed that seed germination and initial growth parameters in garden pea varieties were negatively affected by aqueous extracts of *S. halepense*. According to the reported parameters, the studied varieties exhibited greater sensitivity during the period of initial germ growth and biomass accumulation (with inhibition rates from 27.5 to 56.9% and from 14.2 to 46.8%, or corresponding RI values from -1.31 to -2.96 and from 0.02 to -0.47), and less sensitivity during seed germination (from 3.6 to 30.3%, or RI from -0.036 to -0.155). It is known that *S. halepense*, which is one of the most common and harmful weeds in the country (Vasilakoglou et al., 2005; Hristoskov, 2013), has a pronounced allelopathic potential. Allelopathic substances have been found to inhibit cell division and elongation, respiration and photosynthesis, water and nutrient uptake, protein synthesis and metabolism, activity of antioxidant enzymes, etc. (Cheng & Cheng, 2015). Major allelochemicals that *S. halepense* contains include chlorogenic acid, phenolic compounds, *p*-hydroxybenzeldehyde, *p*-coumaric acid, „sorgoleone" and „dihydrosorgoleone" (Movahedpour et al., 2010; Butnariu, 2012; Zohaib et al., 2016). For example, chlorogenic acid in *S. halepense* inhibits the key enzyme λ-phosphorylase, which participates in seed germination (Einhellig, 1995). Sorgoleone and p-coumaric acid inhibit H-ATPase activity, which is associated with water and nutrient uptake, and the activity has been found in various legumes (peas, soybeans) (Hejl & Koster, 2004). Sorgoleone also reduces the number of cells in each period of cell division, damaging the tubules and resulting in the appearance of polyploid nuclei (Hallak et al., 1999). Phenolic compounds can reduce the activity of phenol-b-glucose transferase, thus inhibiting root growth (Cheng & Cheng, 2015), and disrupt the integrity of DNA and RNA, which in turn adversely affects protein biosynthesis and cell growth (Li et al., 2010).

Reactions to allelochemicals were species-specific and depended on concentration (An et al., 2008). Species specificity to allelochemical action has also been reported by Bakhshayeshan-Agdam et al. (2015), who observed stronger resistance to the action of *Amaranthus retroflexus* extracts in wheat and cucumber than in common bean and alfalfa. In our previous study (Georgieva et al., 2015), considerable differences were found in the sensitivity of *Lupinus albus* and *Lupinus luteus* to *S. halepense* extracts. For example, fresh biomass accumulation in the primary germ of *L. luteus* was inhibited 3.8-40.3% at weed concentrations of 2.5, 5.0 and 10.0%, which determined the species as more sensitive to *S. halepense* extracts. On the other hand, *L. albus* was resistant as no allelopathic effect of the extracts was detected. The results of the present study proved that the response to allelochemicals may also be varietal specific. The varieties exhibiting high tolerance were Puldin and Pulpudeva, in which the suppressive effect of weed extracts on germination and initial growth, presented in total, was the least pronounced (63.5 and 68.5%, respectively). In contrast, Denitza and Skinado sustained the most pronounced effect. This was confirmed by the GGE-biplot method evaluation However, further studies are needed to assess the allelopathic tolerance of pea varieties under field conditions. Cultivation of allelopathy tolerant species and varieties is a promising addition to the existing weed control strategy, especially in organic production. The varietal response of chickpeas to extracts of *Xanthium strumarium* and *Parthenium hysterphorus* was reported by Khan et al. (2019). The authors found high tolerance to the phytotoxic action of invasive weeds in Karak-II variety, followed by Karak-I, Karak-III, Fakhr-e-Thal and Chattan. In a similar experiment with alfalfa and birdsfoot trefoil genotypes, Valcheva et al. (2018) indicated the alfalfa variety Multifoliate and local birdsfoot trefoil populations LP1 and LP2 were characterized by increased tolerance to the allelopathic action of aqueous extracts of *Cuscuta epithymum*. In a comprehensive study, Shao et al. (2019) investigated the allelopathic effects of four weed species (*Descurainia sophia, Galium tricorne, Avena sativa,* and *Vicia sativa*) on seed germination, germ length and weight of ten wheat cultivars (Yannong 19, Yannong 21, Jimai 22,

Lunong 116, Kaimai 18, Zhengmai 366, Wanmai 19, Wankenmai 1, Wanmai 50 and Wanmai 54). Based on a cluster analysis of allelopathic indicators (RI), the inhibition rate in Wanmai 19 was the weakest, and its resistance to weeds was the highest. Therefore, growing varieties with increased tolerance to allelopathic weed stress can reduce weed damage (Shahrokhi et al., 2011).

Regarding the concentration of weed extracts and their effects on recipient plants, it should be noted that high concentrations were usually inhibitory and low concentrations were stimulating, a phenomenon known as hormesis (Hadacek et al., 2010). Regarding the experimental conditions, the concentrations of 5 and 10% had significant negative effects on the initial growth parameters, while 1% concentration of root biomass in Ran I, Pulpudeva and Denitsa varieties had a distinct stimulating action (in the range 27.3-2.8%) on germ length, and the action was statistically significant. The effect of 5% concentration of root biomass in Ran I, Puldin and Mira in relation to germ weight was similar as the stimulating effect in that case ranged from 10.4 to 40.4%.

CONCLUSIONS

The present study demonstrated inhibitory effects of shoot and root aqueous extracts of *Sorghum halepense* on seed germination and initial germ growth in garden pea varieties. The suppressing action increased with increasing extract concentration (1, 5, 10%).

The variance analysis of data showed significant influence ($p < 0.05$) of the three studied factors (variety, type and concentration of extracts) on the investigated parameters. Statistically insignificant was only the influence of extract type (root/aboveground biomass) on seed germination. Comparing data regarding the type of extracts it was found that the weed shoot biomass had a more pronounced inhibitory effect than root biomass.

The allelopathic indicator RI, which determines the degree of allelopathic inhibition regarding germination, germ length and weight, varied in different varieties as follows: -0.30 to -0.04, -1.31 to -2.96, and -0.47 to 0.02.

The total effect of *S. halepense* allelopathic action on all studied parameters, presented by GGE-biplot analysis, identified Pulpudeva and Puldin varieties as exhibiting higher tolerance, in contrast to Denitsa and Vyatovo, which were sensitive. Ran I, Mira, Musala and Vechernitsa occupied an intermediate position.

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Proučavanje otpornosti različitih sorti graška na alelopatsko delovanje ekstrakta *Sorghum halepense* (L.) Pers.

REZIME

U istraživanju su identifikovane sorte graška sa izraženom otpornošću na alelopatsko delovanje *Sorghum halepense*. Odabrano je devet sorti kako bi se ispitao uticaj ekstrakta biomase izdanka i korena, primenjenih u tri koncentracije (1, 5 and 10%), na klijanje semena i inicijalni rast klijanaca. Analiza varijanse je pokazala značajan uticaj ($p < 0.05$) tri ispitivana faktora (sorta, vrsta i koncentracija ekstrakta) na proučavane parametre. Kao statistički značajan pokazao se samo uticaj vrste ekstrakta (koren/nadzemna biomasa) na klijanje semena. Na osnovu apsolutnih vrednosti alelopatskog indikatora RI, kojim se određuje alelopatska inhibicija klijanja, kao i dužine i težine klijanaca kod različitih sorti, utvrđene su sledeće respektivne varijacije: od -0.30 do -0.04, od -1.31 do -2.96 i od -0.47 do 0.02. U okviru ukupnog alelopatski uticaja *S. halepense* na proučavane parametre, pokazanog GGE-biplot analizom, pokazalo se da sorte Pulpudeva i Puldin poseduju veću tolerantnost u odnosu na sorte Denitsa i Vyatovo, koje su osetljive. Ran I, Mira, Musala i Vechernitsa imale su srednje vrednosti. Gajenje alelopatski tolerantnih sorti predstavlja perspektivan doprinos postojećoj strategiji za suzbijanje korova, naročito u uslovima organske proizvodnje.

Ključne reči: alelopatija, korovi, *Pisum sativum*, *Sorghum halepense*

Instructions for Authors

About Journal

Pesticidi i fitomedicina (Pesticides and Phytomedicine) is dedicated to the following research fields: toxicology and ecotoxicology of pesticides; phytopathology; applied entomology and zoology; weed science; plant and food products protection; use of pesticides in agriculture, sanitation and public health.

The journal continues the title *Pesticidi*, which was published over the period 1986-2003.

Pesticidi i fitomedicina (Pesticides and Phytomedicine) publishes original scientific papers and review papers that have not been published previously.

Pesticidi i fitomedicina (Pesticides and Phytomedicine) is an Open Access journal.

Contributions to the journal must be submitted in English, with summaries in English and Serbian (Serbian-speaking authors only).

As of 2020, *Pesticidi i fitomedicina (Pesticides and Phytomedicine)* is issued triannually (three issues annually).

As of 2021, Pesticides and Phytomedicine (Pesticidi i fitomedicina) will be published **online only,** and paper copies of future issues will no longer be available. The primary platforms for journal publication will continue to be: Scindeks (http://scindeks.ceon.rs/journaldetails.aspx?issn=1820-3949) and the publisher's official web site (http://www.pesting.org.rs/).

The journal is indexed in: Chemical Abstracts, CAB International; DOAJ, EBSCO, AGRIS, Scindeks.

In 2011, the journal converted to an electronic online journal management system on the SCIndeks Assistant portal at **http://scindeks.ceon.rs/journaldetails.aspx?issn=1820-3949.** The system enables easy article submission and communication among the editorial staff, reviewers and authors. It also includes several quality control services: *CrossRef* for DOI assignment, *CrossChek* for plagiarism prevention and *KWASS* for equipping articles with keywords extracted from a dictionary/thesaurus. Electronic editing is in compliance with the Journal Editing Act of the Ministry of Education, Science and Technological Development of the Republic of Serbia, and provides record-keeping stipulated in the Act.

Manuscript submission

To be published in *Pesticidi i fitomedicina (Pesticides and Phytomedicine),* an article must be based on original scientific results that have not been previously published and are not under consideration for publication elsewhere. Review articles should contain a comprehensive survey of a particular subject based on referenced literature and published results of the author(s) own research. All contributions are peer reviewed in a double blind process.

A click on "submit a manuscript" on the left-hand side of the journal home page in SCIndeks Assistant will lead users to a registration page and further on into a guided process of electronic manuscript submission. Serbian authors are requested to fill out the application form in both English and Serbian. Each visual or graphic item (table, chart, diagram or photo) should be submitted as a separate (supplementary) file.

Authors need NOT specify keywords in their articles. They will be extracted and selected by the Editor-in-Chief from the *KWASS* thesaurus (dictionary), which will significantly improve article visibility. Authors are entitled to accept or change some of the keywords.

Manuscript preparation

The manuscript should be prepared in Microsoft Word (A4 format, all margins 25 mm, font Times New Roman 12 pt). Articles have to be written in the English language, and only the title and abstract in both English and Serbian (Serbian summary will be furnished by the copyeditor for foreign authors'manuscripts).

Title should be concise and refer to the subject. Full names and surnames of all authors, details of their respective affiliations and emails should be indicated below the title. If discrepancy in such data occurs between the textual document and submission metadata in Assistant, the former will be given precedence.

Abstract (not exceeding 300 words) should briefly state the main results and conclusions.

Articles should contain the following sections: Introduction, Material and Methods, Results, Discussion, Acknowledgement and References.

Introduction should present the state-of-the-art in a particular research field, as well as research intent.

Material and Methods should provide sufficient detail to allow the work to be reproduced. Conventional methods should only be referenced.

Results should be presented in a logical order, clearly and concisely, using adequate tables and graphics. Avoid repetition of the results in tables and graphics, or in the text.

Discussion should emphasize the importance of the results, as well as their place within the context of previous research. Wherever possible, Results and Discussion should be separate sections.

Acknowledgement should be collated at the end of the manuscript before References.

References cited in the text need to include the author's/ authors' surname(s) and year of publication:

- author, year;
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References mentioned in the manuscript must be listed in the References section at its end, in alphabetic order and using the **APA citation style** (see description at e.g. https://owl.english.purdue.edu/owl/resource/560/01/).

Journal references are required to contain the following information: name(s) of author(s), year of publication, title of article, title of journal, volume, issue number (unless pagination is continuous), pages (from-to) and DOI if available.

Dedić, B. (2012). Testing sunflower inbred lines for tolerance to phoma black stem. *Pesticides* & *Phytomedicine*, *27*(4), 299-303. doi:10.2298/PIF1204299D

Abbaspoor, M. & Streibig, J.C. (2005). Clodinafop changes the chlorophyll fluorescence induction curve. *Weed Science, 53*(1), 1-9. doi:10.1614/WS-04-131R

Abbaspoor, M., Teicher, H.B. & Streibig, J.C. (2006). The effect of root-absorbed PSII inhibitors on Kautsky curve parameters in sugar beet. *Weed Research, 46*(3), 226-235. doi:10.1111/j.1365-3180.2006.00498.x

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Timbrell, J. (2000). *Principles of biochemical toxicology* (3rd ed). London, UK: Taylor and Francis Ltd.

Frank, R. H. & Bernanke, B. (2007). *Principles of macroeconomics* (3rd ed.). Boston, MA: McGraw-Hill/Irwin.

Saari L.L. & Thill, D.C. (Eds.). (1994). *Resistance to acetolactate synthase inhibiting herbicides: Herbicide resistance in plants*. Boca Raton, FL, USA: CRC Press.

Dissertations: author's name, year of presentation, title, full name of the institution at which dissertation was defended.

Stepanović, M. (2012). *Osetljivost izolata Alternaria solani (Sorauer) iz različitih krajeva Srbije na fungicide i rizik rezistentnosti*. (Doktorska disertacija). Univerzitet u Beogradu, Poljoprivredni fakultet, Beograd.

Book chapters and articles in conference proceedings: author(s), year of publication, title of chapter/article/abstract, source title (with editors names), pages, place of publication and publisher.

Hammond, K. R. & Adelman, L. (1986). Science, values, and human judgment. In H. R. Arkes & K. R. Hammond (Eds.), *Judgement and decision making: An interdisciplinary reader* (pp. 127-143). Cambridge, England: Cambridge University Press.

Edwards, J.P., Fitches, E.C., Audsley, N. & Gatehouse, J.A. (2002). Insect neuropeptide fusion proteins – A new generation of orally active insect control agents. In T. Margini (Ed.), *Proceedings of the BCPC – Pests and diseases* (pp. 237-242). Brighton, UK: University of Brighton Press.

Internet references: author(s), year of publication, title, source title, link.

Graora, D., & Spasić, R. (2008). Prirodni neprijatelji *Pseudaulacaspis pentagona* Targioni-Tozzetti u Srbiji. *Pesticidi i fitomedicina*, *23*(1) 11-16. Retrieved from http://www.pesting.org.rs/media/casopis/2008/no.1/23_1_11-16.pdf

Radunović, D., Gavrilović, V., Gašić, K., Krstić, M. (2015). Monitoring of *Erwinia amylovora* in Montenegro. *Pesticides and Phytomedicine*, 30(3), 179-185. doi 10.2298/PIF1503179R or http://www.pesting.org.rs/media/casopis/2015/no.3/30-3_179-185.pdf

Kerruish, R.M. & Unger, P.W. (2010). *Plant protection 1 – Pests, diseases and weeds*. Retrieved from APPS at http://www.appsnet.org/Publications/Kerruish/PP1.pdf

Tables need to be numbered in Arabic numerals consecutively as they appear in text. Tables should be made exclusively in Word for Windows using the toolbar menu Table-Insert-Table, Times New Roman font, 12 pt, and single line spacing. Footnotes immediately below the table body should be given priority over other explanation in table header or in table cells, and text should be in Times New Roman font, 10 pt. Each table must have a header. Tables should be submitted as supplementary (separate) files, and their approximate location in the text marked.

Graphs should be processed in Microsoft Excel and all data in Times New Roman font. Explanations should be provided in captions, consecutively and marked with Arabic numerals. Graphs should be submitted as supplementary files, and their approximate location in the text marked.

Diagrams should be processed in Corel Draw (version 9 or later) or in Adobe Illustrator (version 9 or later) and all data written in Times New Roman font. Diagrams should be submitted as supplementary files and their approximate locations in the text marked.

Photos need to be taken by digital camera (resolution at least 150 dpi, photo dimension A4, file format JPG or TIFF). If authors are unable to submit original photos, those should be scanned in RGB mode (colour) or as Grayscale (black and white), with 300 dpi resolution in original size. Photos need to be marked with Arabic numerals in consecutive order. Provide each photo with a caption, mark its approximate location in the text and submit it as a supplementary file.

Authors are expected to use the accepted International System of Units (SI). Abbreviations should be defined in brackets at their first in-text mention. Provide full Latin names along with common names of organisms, and italicize only Latin names of genera and species, e.g. Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). After first mention, the Latin name can be abbreviated (e.g. *L. decemlineata*).

Review articles need to contain an introduction, appropriate subtitles and a reference list.

Publishing in *Pesticidi i fitomedicina (Pesticides and Phytomedicine)* is free of charge.

The editorial staff practice a policy of plagiarism prevention.

Uputstvo autorima

O časopisu

Časopis *Pesticidi i fitomedicina (Pesticides and Phytomedicine)* objavljuje naučne radove iz oblasti: toksikologije i ekotoksikologije pesticida; fitopatologije; primenjene entomologije i zoologije; herbologije; zaštite bilja i prehrambenih proizvoda; primene pesticida u poljoprivredi, komunalnoj higijeni i javnom zdravstvu.

Časopis *Pesticidi i fitomedicina (Pesticides and Phytomedicine)* predstavlja nastavak publikacije *Pesticidi*, koja je pod tim imenom izlazila u periodu 1986-2003.

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Radovi koji se prilažu moraju biti napisani na engleskom jeziku, sa rezimeom na engleskom i srpskom jeziku.

Od 2020. godine, časopis izlazi četvoromesečno (tri broja godišnje).

Od 2021. godine, časopis Pesticidi i fitomedicina (Pesticides and Phytomedicine) objavljuje sveske samo u elektronskom obliku, bez štampane verzije. Osnovne platforme na kojima se postavljaju sadržaji časopisa su: Scindeks (http://scindeks.ceon.rs/journaldetails.aspx?issn=1820-3949) i zvanični veb sajt izdavača (http://www.pesting.org.rs/).

Časopis se indeksira u sledećim bazama: Chemical Abstracts, CAB International; DOAJ, EBSCO, AGRIS, Scindeks.

Tokom 2011. godine, časopis je prešao na sistem onlajn uređivanja (Elektronsko uređivanje – e-Ur) na portalu SCIndeks Asistent **http://scindeks.ceon.rs/journaldetails.aspx?issn=1820-3949**. Ovaj sistem uređivanja omogućava funkcionalniju prijavu radova i komunikaciju uredništva sa recenzentima i autorima, a obuhvata i servise za kontrolu kvaliteta radova: *CrossRef* za dodelu DOI, *CrossChek* za prevenciju plagijarizma i *KWASS* za opremanje radova ključnim rečima ekstrahovanim iz rečnika/tezaurusa. Elektronsko uređivanje je usaglašeno sa Aktom o uređivanju časopisa Ministarstva prosvete, nauke i tehnološkog razvoja RS i obezbeđuje vođenje evidencije koje ovaj akt nalaže.

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Publikovanje u časopisu *Pesticidi i fitomedicina (Pesticides and Phytomedicine)* podrazumeva da rad sadrži rezultate originalnih istraživanja koji nisu objavljeni, odnosno nisu dostavljeni nekom drugom časopisu za objavljivanje. Pregledni radovi treba da sadrže sveobuhvatan prikaz određene teme zasnovan na referentnoj literaturi i publikovanim rezultatima sopstvenih istraživanja. Svi radovi se recenziraju, a recenzija je obostrano anonimna.

Klikom na "submit a manuscript" na levoj polovini početne stranice u SCIndeks Asistentu, dolazi se do opcije za registraciju i prijavu rukopisa i ulazi u vođeni postupak elektronske prijave rada. Obaveza srpskih korisnika je da prijavu popune na oba jezika (srpskom i engleskom). Svaki likovno-grafički prilog (tabela, grafikon, dijagram, slika) se prilaže kao zasebna (dopunska) datoteka.

Autori u radu NE NAVODE ključne reči. Njih će glavni urednik ekstrahovati iz *KWASS* tezaurusa (rečnika), što će značajno poboljšati vidljivost rada. Autori imaju pravo da dodeljene ključne reči prihvate ili da neke od njih zamene.

Priprema rada

Rad treba pripremiti u programu za obradu teksta Word (format A4, margine 25 mm, font Times New Roman 12 pt). Radovi treba da budu isključivo na engleskom jeziku sa naslovom i rezimeom na oba jezika (engleskom i srpskom).

Naslov treba da bude kratak i da upućuje na temu. Puna imena i prezimena svih autora, puni nazivi i adrese institucija svih autora i njihove email adrese treba navesti ispod naslova rada. U slučaju neslaganja ovih podataka u samom tekstu rada i u prijavi na platformi za uređivanje, prioritet će se dati podacima u samom tekstu rada.

Rezime (obima do 300 reči) treba da predstavi ono što je za rad najznačajnije.

Rad treba, po pravilu, da sadrži sledeća poglavlja: Uvod, Materijal i metode, Rezultati, Diskusija, Zahvalnica i Literatura.

Uvod treba da sadrži najnužniji pregled istraživanja u datoj oblasti i ciljeve istraživanja.

Materijal i metode treba opisati dovoljno detaljno da omoguće ponavljanje ispitivanja. Poznate metode i tehnike označiti samo odrednicom iz literature.

Rezultate predstaviti logičnim redosledom, jasno i precizno, koristeći prigodne tabele i grafičke prikaze. Izbegavati ponavljanje rezultata u tabelama i grafikonima, ali i u tekstu rada.

Diskusija treba da istakne značaj dobijenih rezultata, kao i njihovo mesto u kontekstu prethodnih istraživanja. Kad god je to moguće, diskusiju treba odvojiti od rezultata.

Zahvalnica se navodi na kraju teksta rada, pre literature.

Literatura se u tekstu rada citira navođenjem prezimena autora i godine:

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Dedić, B. (2012). Testing sunflower inbred lines for tolerance to phoma black stem. *Pesticides & Phytomedicine*, *27*(4), 299-303. doi:10.2298/PIF1204299D

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Stepanović, M. (2012). *Osetljivost izolata Alternaria solani (Sorauer) iz različitih krajeva Srbije na fungicide i rizik rezistentnosti*. (Doktorska disertacija). Univerzitet u Beogradu, Poljoprivredni fakultet, Beograd.

Poglavlja u knjigama i radovi u zbornicima: autor(i), godina publikovanja, naslov poglavlja/rada/apstrakta, naslov izvornika sa imenom (imenima) urednika, strane priloga, mesto publikovanja i naziv izdavača.

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Kerruish, R.M. & Unger, P.W. (2010). *Plant protection 1 – Pests, diseases and weeds*. Retrieved from APPS at http://www.appsnet.org/Publications/Kerruish/PP1.pdf

Tabele se obeležavaju arapskim brojevima prema predviđenom redosledu. Tabele se izrađuju isključivo u programu Word for Windows, kroz meni Table-Insert-Table, koristeći font Times New Roman, 12 pt i osnovni prored. Fusnotama neposredno ispod tabela treba dati prednost nad drugim objašnjenima u zaglavlju tabela ili u samim tabelama, a tekst se daje u fontu Times New Roman, 10 pt. Svaka tabela mora imati zaglavlje. Tabele se prilažu kao dopunske (zasebne) datoteke, a u samom tekstu se obeležava njihovo približno mesto.

Grafikoni treba da budu urađeni i dostavljeni u programu Excel, sa podacima u fontu Times New Roman. Potrebna objašnjenja daju se u legendama obeleženim arapskim brojevima prema redosledu. Grafikoni se prilažu kao zasebne (dopunske) datoteke, a u samom tekstu se obeležava njihovo približno mesto.

Dijagrami treba da budu urađeni i dostavljeni u programu Corel Draw (verzija 9 ili novija), ili u programu Adobe Illustrator (verzija 9 ili novija). Za unos podataka treba koristiti font Times New Roman. Grafikoni se prilažu kao zasebne (dopunske) datoteke, a u samom tekstu se obeležava njihovo približno mesto.

Fotografije treba da budu snimljene digitalnim fotoaparatom (rezolucija najmanje 150 dpi, dimenzija fotografije A4, a format zapisa JPG ili TIFF). Ukoliko autori nisu u mogućnosti da dostave originalne fotografije, treba ih skenirati u RGB modelu (ukoliko su u boji), odnosno kao Grayscale (ukoliko su crno-bele), sa rezolucijom 300 dpi u originalnoj veličini. Fotografije je potrebno obeležiti arapskim brojevima prema predviđenom redosledu. Za svaku fotografiju se daje legenda i obeležava njeno približno mesto pojavljivanja u tekstu. Svaka fotografija se prilaže kao zasebna (dopunska) datoteka.

Od autora se očekuje da koriste preporučene jedinice međunarodnog sistema (SI). Skraćenice je potrebno definisati u zagradama nakon prvog pominjanja u tekstu. Narodni nazivi organizama se daju uz pun latinski naziv, a kurzivom se obeležavaju samo latinski nazivi rodova i vrsta, npr. krompirova zlatica, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). Nakon prvog pojavljivanja, latinsko ime dalje treba pisati skraćeno (npr. *L. decemlineata*).

Pregledni radovi treba da sadrže uvod, odgovarajuće podnaslove i spisak literature.

Objavljivanje radova u časopisu *Pesticidi i fitomedicina (Pesticides and Phytomedicine)* je besplatno.

Uredništvo preduzima mere protiv grubog kršenja etičkih normi u vezi sa plagijarizmom.

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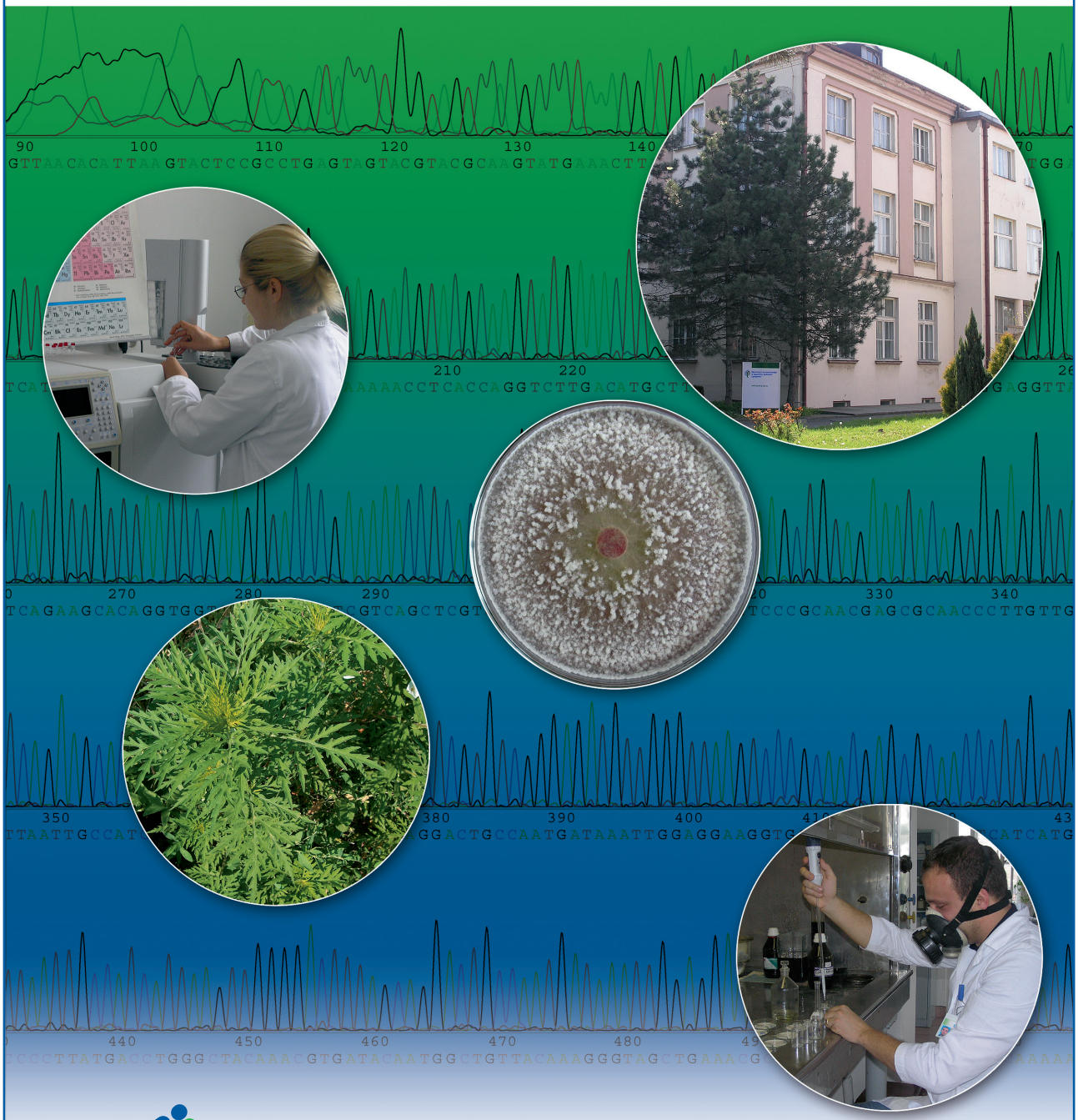

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