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Effects of Irrigation and Bioproducts of Microbial Origin on Nematode Community and Mycorrhizal Root Colonization in Soybean

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Abstract

Soybean (*Glycine max* L. Merr) is the most important legume and threaten by diverse pests and diseases. Complex interactions among rhizosphere organisms are found in all agro-ecosystems. Results of these interactions can be positive and/or negative in terms of plant production. Soil nematode community consists of different trophic groups of nematodes. Nematodes are the most abundant soil invertebrates. Several nematode species penetrate soybean roots as parasites, and can cause loss in yields. Arbuscular mycorrhiza fungi are obligate plant symbionts that colonize soybean roots naturally. The aim of the study was to evaluate effects of irrigation and amendments of bioproducts containing beneficial soil microorganisms (ABM) on nematode community and mycorrhizal root colonization in soybean. Field experiments were conducted in soybean in 2013 in Osijek, Croatia. The plots were either rain fed or irrigated to 60-100% field water capacity (FWC). We tested soil amendments and soil + foliar amendments of three commercial products containing beneficial organisms. Average number of nematodes per soil sample varied from 186,67 (soil ABM in non-irrigated plots) to 297,57 (soil+foliar ABM in plots with 60-100% FWC), and there were no significant differences between the treatments. Bacterial feeding nematodes were the most abundant, while plant parasitic genus *Pratylenchus* was the most abundant among other plant parasitic nematodes. There was no clear influence of any of the treatments on soil nematode community. Amendments of the bioproducts increased mycorrhizal root colonization in rain fed plots, while it decreased the mycorrhizal root colonization when soybeans were irrigated. Irrigation increased mycorrhizal root colonization in plots without amendments of the bioproducts, and mycorrhizal colonization differed significantly between the sampling dates. Further research is needed to determine if irrigation alters the potential of mycorrhiza to colonize the roots.

Keywords: soybean, nematodes, *Pratylenchus*, arbuscular mycorrhiza fungi, irrigation, soil and foliar amendments, beneficial microorganisms

1. Introduction

Soybean (*Glycine max* L. Merr) is economically the most important legume. The largest production area under soybean is in North and South America (USA, Brasil, Argentina), China and India [1]. Soybean is used for food and feed because of the rich nutrition profile, proteins and oil. For that reason, it is widely used in different industry branches such as food, oil, pharmaceutical, textile and chemical industries [2]. An abiotic stress is a major constraint in crop production. Drought can reduce soybean yield over 50% annually. Drought stress is also transmitted from parental plants to F1 generation and reduces the seed germination rate, therefore optimal water supply is a must for the best seed quality [3]. In temperate climatic regions where natural precipitation during the growing season is lower than 300 mm, irrigation is necessary [4]. Adaptation of soybean to abiotic conditions in a site, along with plant interactions with other living organisms, represents principal factors for successful crop production [5]. Inoculation of soybean plants with beneficial bacteria and mycorrhizal fungi can facilitate water stress and increase yield, as much as other parameters like seed fat content [6].

Rhizosphere or soil near the soybean root zone is the most dynamic environment of microbe-plant interaction [7]. Soil organisms depend on each other for carbon and energy, and represent major component for assessment of soil health. Several groups of organisms are distinguished in rhizosphere, mainly saprophytes and plant symbionts. Multi trophic interactions in soil directly influence the biodiversity of soil organisms and indirectly promote plant growth and ability to withstand pathogen attack [8].

Plant growth-promoting microorganisms (PGPM) include bacteria, and fungi, that live in soil and rhizosphere and stimulate plant growth by synthesizing phytohormones, producing siderophores, fixing atmospheric nitrogen, dissolving inorganic forms of elements such as phosphorus, and increasing plant resistance to stress and abiotic biotic environmental conditions [9–11]. The most commonly used inoculant of PGPM in the soybean crop belongs to rhizobium bacteria that colonize the root creating nodules which supplies plant with biologically fixed atmospheric nitrogen. Mixed cultures of microorganisms such as *Bradyrhizobium* with *Azospirillum*, *Bacillus*, *Pseudomonas* and *Glomus* are considered valuable and used in soybean production [10, 12, 13]. This type of co-inoculation shows great efficiency especially in soils where stressful environmental conditions such as low phosphorus content prevail [10]. The use of mineral fertilizers can be minimized when soybeans are inoculated with PGPM, and this measure is desirable since it is environmentally sustainable [13]. Higa and Parr [14]. isolated group of beneficial microorganisms from the soil and named them effective microorganisms. This group included approx. 80 species, mostly photosynthetic bacteria, lactic acid bacteria, yeasts, actinomycetes, and fermenting fungi such as *Aspergillus* and *Penicillium*.

2. Interactions of nematodes and mycorrhizal fungi in rhizosphere

Nematodes are the most abundant soil invertebrates, with beneficial and detrimental role in agriculture. They serve as good bioindicators of the effect of agricultural practices and contaminants on the functioning of the soil food web [15]. Mainly, five nematode trophic groups are commonly found in agricultural soils: plant parasitic, bacterial feeding, fungal feeding, omnivorous and predaceous [16]. Soybean is an important oilseed crop and source of high quality protein. Plant parasitic nematodes are economically important plant pathogens for all agricultural

plants, and pose treat to production of globally demanding high yield soybeans. The most important soybean nematodes are soybean cyst nematodes (*Heterodera glycines* Ichinohe) and root lesion nematodes (*Pratylenchus* spp.). In Croatia, *Pratylenchus* is the most frequent and abundant genus of plant parasitic nematodes found in soybean, however economically important yield reductions due to root lesion nematodes damage have not been reported [17].

Rhizosphere microorganisms are often antagonistic to plant parasitic nematodes. By decomposing organic matter in rhizosphere, microorganisms release nematicidal compounds in surrounding soil. Their derivatives are often toxic and negatively affect nematodes. Soil microorganisms also compete with nematodes for the same source of food, or feed upon the nematodes. Lowering the amount of space for living and food source, the microorganisms could suppress nematode population. This interaction occurs in both directions. Nematophagous fungi (e.g. *Paecilomyces* spp., *Pochonia* spp., *Verticilium* spp., *Trichoderma* spp. etc.) and antagonistic bacteria (eg. *Pasteuria penetrans*, *Pseudomonas fluorescens* etc.) are soil-borne microorganisms that are very useful bioagents against plant parasitic nematodes.

Efficacy of biocontrol agents often depends on ability to adopt to different cropping techniques and soil conditions. Bioproducts containing beneficial microorganisms are mostly registered as fertilizers or plant growth promoters, and claim to enhance plant tolerance and defense system, and finally increase yields by suppressing plant parasitic nematodes by associating with mycorrhiza [18]. Interactions among beneficial soil organisms and plant parasitic nematodes are mainly evaluated under laboratory or greenhouse conditions [19]. Nematode trophic groups other than plant parasitic are beneficial, since they contribute to nitrogen mineralization by feeding on and by dispersing beneficial bacteria, also they are regulating rates of decomposition [20].

Arbuscular mycorrhiza fungi (AMF) are obligate symbionts that colonize the roots of most cultivated plant species. The most plant species form mycorrhizal symbiosis naturally [21]. Association of plants with AMF increase the absorptive surface of the plant root system, enhance plant access to immobile soil minerals, and increase plant growth rates, respectively. Mycorrhizal symbiosis provides soybean with nutrients, mitigates abiotic stress such as draught and improves host plant resistance against pests and diseases [21]. It induces a variety of physiological and molecular biological changes in the host plant and may improve plant resistance and tolerance to the most important plant parasitic nematodes [22]. Direct and indirect effects of AMF on rhizosphere organisms are observed as results of altered plant exudation. Direct and indirect effects on the soil biota may include altered plant exudation, and via competition and mutualism [23]. The aims of the study were to evaluate effects of irrigation and amendments of beneficial soil microorganisms nematode community and mycorrhizal root colonization in soybean.

3. Experimental design

Field experiments were conducted in soybean in 2013, at Agricultural Institute Osijek, Croatia (45°32' N and 18°44' E, altitude 90 m). Size of the experimental field was 405 m². The field has a history of a long-term soybean-maize rotation. The soil is characterized as eutric non-calcareous brown soil developed on calcareous loess substrate middle gleyed and silt/clay loam texture. Soybean (cultivar Ika) was grown and maintained by conventional farming practices. To examine effects of amendments of bioproduct of microbial origin on soil nematodes and mycorrhizal colonization, plots were assigned to three types of treatments: control,

soil amendments and soil + foliar amendments in irrigated and rain fed plots. The experiment was set according to randomized block design in three replicates. Three commercial bioproducts were used in experiment: EM Aktiv (Multikraft), Nourivit and Nourivit plus (Nourivit Technologies GmbH). These products contain more than 40 different species of beneficial soil microorganisms (mainly lactic acid bacteria, photosynthetic bacteria, and yeasts) and sugarcane molasses, claimed by the manufacturer. In plots with soil amendments, EM Aktiv was applied in dosage 30 L ha⁻¹ prior sowing of soybean to enable activation of microorganisms. In plots with soil + foliar treatment, soil amendment of EM Aktiv (30 L ha⁻¹) was applied

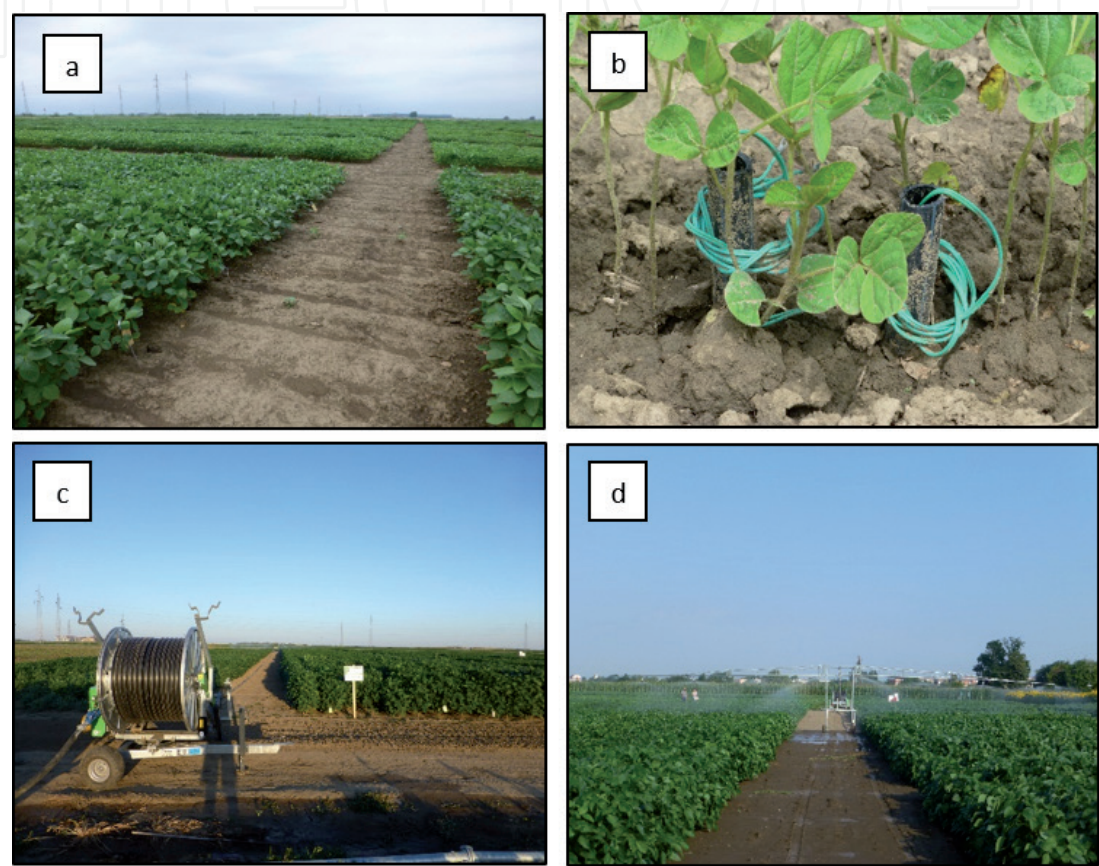


Figure 1.
The experimental field setup: a) soybeans in the experimental plots, b) the watermark sensors of soil moisture, c) self-propelled sprinkler (typhon), d) boom irrigation systems.

Treatment	Quantities of the irrigated water (mm) and date of the treatments		Grain yields (kg ha ⁻¹)
	mm	date	
Control	0	—	3000
Irrigated plots	35	June 3-6	4050
	35	July 12-14	
	35	July 21-23	
	35	August 2-4	

Treatments: control – 0 mm of added water by irrigation; irrigated plots – maintenance of soil water content from 60 to 100% field water capacity (FWC).

Table 1.
Irrigation schedule at the experimental site.

prior sawing and two foliar treatments of Nourivit (4,5 kg ha⁻¹) and Nourivit plus (4,5 L ha⁻¹) were applied during vegetation.

Self-propelled sprinkler (typhon) was used to irrigate plots 60–100% of the field water capacity (FWC) (**Figure 1**). Irrigation depended of the soil water content, the weather characteristics, mainly precipitations (**Table 1**).

Quantities of water and frequency of irrigation are presented in **Table 1**. Irrigation rate was 35 mm, and following measures of soil moisture were done at root depth of 30 cm. The method consisted of Watermark sensors and hand-held field meter. Sensors were buried in the soil after sowing the soybeans at two depths: 15-20 cm and 25-30 cm and removed after the harvest. Measurements were taken twice a week or after the significant rainfall and irrigation regime.

Long-term mean (LTM, 1961-1990) of precipitations is 368 mm during the growing season (April–September) in Osijek (**Figure 2**). Investigated area has a semi-humid and drought prone climate. In 2013, the environmental conditions were moderate, with minimal deviations from total precipitation and air temperature of LTM.

Soil and root sampling for nematode and mycorrhizal fungi analysis was done twice during the vegetation, in July and September. Extraction of nematodes from soil was done following modified Baermann funnel method [24]. Nematodes were counted and separated according to their feeding habit to trophic groups [16]. and according to the morphological characteristics plant parasitic nematodes were identified to the genus level [25, 26]. Ten soybean roots were excavated in three replications from each and subjected to microscopic analysis for mycorrhizal colonization. Soybean root and mycorrhizal preparation, and staining was done according to the method described by Vierheilig et al. [27]. The presence of mycorrhizae was determined according to the method described by Trouvelot et al. [28]. and following parameters were determined: mycorrhizal frequency in the root system (F), intensity of mycorrhizal colonization in the root system (M), arbuscule abundance in the root system (A), intensity of mycorrhizal colonization in the root fragments (m) and arbuscule abundance in mycorrhizal parts of root fragments (a). The data were log(n+1) transformed prior analysis of variance (PROC GLM). The means are back-transformed and presented in Tables. The means were separated by Tukey test (P<0,05) (SAS 9.2; SAS Institute, Carey, NC, USA).

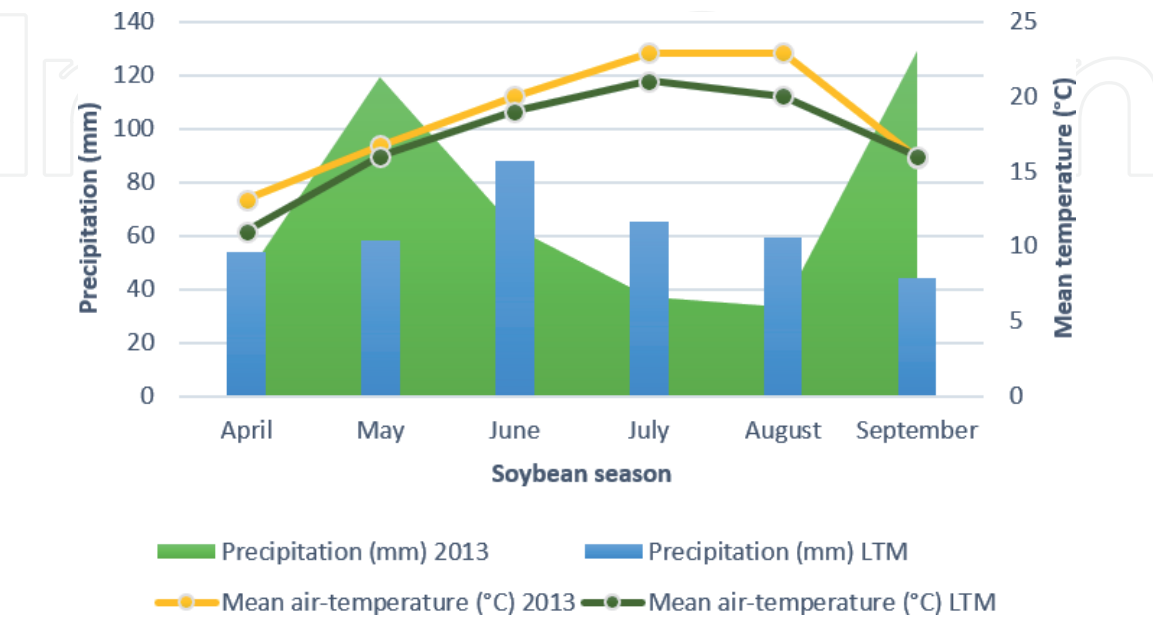


Figure 2.
Precipitation and air-temperature in growing season 2013 and long-term means (LTM) (Osijek weather bureau).

4. Results

4.1 Influence of irrigation and amendments of beneficial organisms on soil nematode community

Soil samples in soybean were taken twice to estimate the effect of irrigation and amendments of bioproducts of microbial origin on nematode trophic groups (**Table 2**). According to F-statistics for group of plant parasitic nematodes irrigation level ($F=11,51$, $P<0,001$), month of sampling ($F=14,95$, $P<0,001$) and interaction treatment*month was found as statistically significant ($F=5,56$, $P<0,001$). Population density of bacterial feeding nematodes significantly changed only when affected simultaneously by two variables treatment*month ($F=4,58$, $P<0,05$). Omnivorous nematodes were significantly affected only by the month of sampling ($F=4,16$, $P<0,05$), while significant response of predators was found only in simultaneous effect of two variables irrigation*month ($F=14,14$, $P<0,05$). Group of fungal feeding nematode did not respond significantly to any of the tested variables. Statistics revealed that interaction of treatment with bioproducts and month of sampling significantly affects total nematode community ($F=3,47$, $P<0,05$).

	Nematode trophic group					Total
	PP	F	B	O	P	
Irrigation	11,51**	0,95	1,91	0,38	0,45	1,84
Treatment	1,78	0,66	1,68	1,95	1,20	1,51
Month	14,95**	0,03	1,37	4,16*	0,45	0,85
Irrigation*Treatment	0,00	0,64	0,04	1,45	2,49	0,02
Irrigation*Month	0,31	0,42	0,14	1,52	14,14*	0,03
Treatment*Month	5,56**	0,44	4,58*	0,46	2,49	3,47*

Data are F-values; PP – plant parasitic, F – fungal feeding, B – bacterial feeding, O – omnivorous, P – predator.
* $P<0,05$.
** $P<0,001$.

Table 2.
GLM analysis of effect of irrigation level and amendment of bioproducts on nematode trophic groups densities.

Plant parasitic nematodes	Effects		
	Treatment	Month	Treatment*Month
<i>Ditylenchus</i>	1,71	9,27**	1,41
<i>Filenchus</i>	0,32	4,29*	1,25
<i>Malenchus</i>	1,63	0,20	3,20*
<i>Merlinius</i>	0,60	2,16	0,17
<i>Pratylenchus</i>	3,58*	3,44	1,87
<i>Tylenchorynchus</i>	0,83	4,36*	1,72
<i>Tylenchus</i>	0,28	3,68	1,65

Data are F-values.
* $P<0,05$.
** $P = 0,001$.

Table 3.
GLM analysis of the effects of treatments with bioproducts, month of sampling, and their interaction on plant parasitic nematodes in soybean.

Seven plant parasitic nematode genera were identified from soil samples of each treatment (**Table 2**). Treatment of soybean with bioproducts significantly affected plant parasitic genus *Pratylenchus* ($F=3,58$, $P<0,05$) (**Table 3**). Month of sampling significantly affected population of *Ditylenchus* ($F=9,27$, $P<0,001$), *Filenchus* ($F=4,29$, $P<0,05$), and *Tylenchorynchus* ($F=4,36$, $P<0,05$). Simultaneous effect of treatment and month of sampling was statistically significant for the population of *Malenchus* ($F=3,20$, $P<0,05$).

Nematodes belonging to the genus *Pratylenchus* were the most abundant among all other plant parasitic nematodes (**Table 4**). In the treatment with a soil amendment of bioproducts, populations of *Pratylenchus* were significantly higher compared to the treatment with two amendments of bioproducts (i.e. soil+foliar). The lowest population density of *Pratylenchus* spp. (31,86% of plant parasitic nematodes per soil sample) was found in plots with soil and foliar amendment. However, the treatments did not significantly differ from the control plots, where on average 42,94% *Pratylenchus* spp. of total plant parasitic nematodes per soil sample was identified. In previous studies from Croatia, *Pratylenchus* was also the most dominant genera in soybean [17, 24, 29, 30]. In Brasil, one of the world's leading soybean production area plant parasitic nematodes are major constrain and the most dominant nematode trophic group [31]. In the same study, *Pratylenchus*, *Helicotylenchus* and *Meloidogyne* were found as the most important plant parasitic nematode genera in soybeans. Total population of plant parasitic nematodes in our study did not significantly differ when comparing types of amendments of bioproducts.

Another study tested long term amendments of effective microorganisms, compost and mineral fertilizers on soil nematode community [32]. The results of the cited study showed that effective microorganisms applied together with compost increased the abundance of total bacterial and plant parasitic nematodes compared to the plots with mineral fertilizer, compost and control. Plant parasitic nematodes were the most dominant trophic groups in their study, and increased in relative abundance by 34.33% in effective microorganisms' plots compared to the mineral fertilizer plots. Wheat biomass in the cited study was also increased by amendments of effective microorganisms, which could be the reason for increase in plant parasitic nematodes populations, since more food was available. Amendments of manure, a source rich with diverse species of microorganisms, to soil increase abundance of nematode community [33].

Plant parasitic nematodes	Treatments		
	Control	Soil	Soil+foliar
<i>Ditylenchus</i>	1,86 a	2,92 a	5,20 a
<i>Filenchus</i>	16,04 a	8,33 a	19,36 a
<i>Malenchus</i>	7,29 a	0,83 a	2,50 a
<i>Merlinius</i>	1,25 a	0,90 a	2,91 a
<i>Pratylenchus</i>	42,94 ab	71,67 a	31,86 b
<i>Tylenchorynchus</i>	9,38 a	20,08 a	16,25 a
<i>Tylenchus</i>	8,96 a	11,25 a	11,67 a

Data are percentage of relative nematode abundance; Values in rows with different letters are statistically significant at $P<0,05$.

Table 4.
Analysis of variance for the effects of amendments of bioproducts on plant parasitic nematodes.

4.2 Influence of irrigation and amendments of beneficial organisms on mycorrhizal root colonization

Statistical analysis revealed significant influence of irrigation (F=34,95, P<0,001), month of sampling (F=94,70, P<0,001), and interaction irrigation*treatment and treatment*month (F=14,29, P<0,001; F=13,16, P<0,001) on mycorrhizal frequency in the root system (**Table 5**). Intensity of mycorrhizal colonization in the root system and in root fragments is under a significant influence of irrigation (F=38,17, P<0,001; F=34,16, P<0,001), treatment (F=4,48, P<0,05; F=6,00, P<0,05), month (F=145,99, P<0,001; F=61,87, P<0,001), interaction irrigation*treatment (F=17,29, P<0,001; F=13,40, P<0,001) and treatment*month (F=11,43, P<0,001; F=16,78, P<0,001). Arbuscule abundance in the root system and in root fragments was significantly affected by irrigation (F=10,99, P<0,01; F=21,92, P<0,001) and all interactions: irrigation*treatment (F=14,73, P<0,001; F= 6,04, P<0,05), irrigation*month (F=68,83, P<0,001; F=95,42, P<0,001) and treatment*month (F=10,11; P<0,001; F=3,33, P<0,05).

Effects of commercial AMF products on growth, nutritional, and physiological responses of soybean in another study reveal the difference between the products with regard to their response to water deficit [34]. Inoculation of plants with AMF was found more important than soil moisture in improving plant growth to overcome drought stress [35]. We found month of sampling and irrigation as the most important factor for mycorrhizal root colonization. However, treatments with bioproducts were similarly important only in interaction with date of sampling.

4.3 The importance of irrigation on the effect of different amendments of beneficial organisms

In previous study, irrigation and nitrogen fertilization increased significantly soybean grain yields [36]. The grain yields in this study were also considerably increased in irrigated plots with more than 1000 kg ha⁻¹ difference between control and irrigated plots (**Table 1**). Average number of nematodes per soil sample varied from 186,67 (soil treatment in non-irrigated plots) to 297,57 (soil+foliar treatment in plots with 60-100% FWC), and there were no significant differences between

	Root colonization (%)				
	F	M	A	a	m
Irrigation	34,95***	38,17***	10,99**	21,92***	34,16***
Treatment	2,06	4,48*	2,56	0,99	6,00*
Month	94,70***	145,99***	0,08	1,17	61,87***
Irrigation*Treatment	14,29***	17,29***	14,73***	6,04*	13,40***
Irrigation*Month	1,09	0,70	68,83***	95,42***	3,45
Treatment*Month	13,16***	11,43***	10,11***	3,33*	16,78***

Data are F-values; F – mycorrhizal frequency in the root system, M – intensity of mycorrhizal colonization in the root system, A – arbuscule abundance in the root system, a – arbuscule abundance in mycorrhizal parts of root fragments, m – intensity of the mycorrhizal colonization in the root fragments.
*P<0,05.
**P<0,01.
***P<0,001.

Table 5.
GLM analysis of effects of treatments with bioproducts, month of sampling, irrigation level, and their interaction on mycorrhizal colonization of root system.

		Irrigation level					
		Control			60-100% FWC		
Bioproduct amendment		Soil	Soil+foliar	Control	Soil	Soil+foliar	Control
Nematode community	PP	7,92 a	6,14a	6,32a	11,87b	9,23a	9,65a
	B	152,50a	212,50a	206,25a	235,00 a	265,00 a	155,83a
	F	15,42a	28,95a	16,25 a	22,50 a	20,00a	19,58a
	O	9,17a	10,00a	12,50a	10,00 a	3,33a	3,33a
	P	1,67a	1,60a	0a	2,50a	0a	2,50a
Total		186,67a	259,27a	241,32a	281,87a	297,57a	190,90a
Mycorrhizal root colonization	F	25,78b	30,11b	16,28a	18,55a	6,89a	20,78b
	M	3,31b	3,93b	2,09a	1,99b	0,79a	4,62b
	A	0,37b	0,22ab	0,17a	0,02a	0,03a	0,39b
	m	11,70b	9,52ab	7,61a	5,23a	3,32a	12,66b
	a	11,05a	6,33a	7,23a	1,06a	1,23a	3,72a

Data are means of nematode population density and percentage of mycorrhizal root colonization; Values in rows marked with different letters are statistically significant at $P<0,05$; PP – plant parasitic, F – fungal feeding, B – bacterial feeding, O – omnivorous, P – predator; F – mycorrhizal frequency in the root system, M – intensity of mycorrhizal colonization in the root system, A – arbuscule abundance in the root system, m – intensity of the mycorrhizal colonization in the root fragments, a – arbuscule abundance in mycorrhizal parts of root fragments.

Table 6.
The effects of different amendments of bioproducts of microbial origin and irrigation level on nematode community and mycorrhizal root colonization.

the treatments (**Table 6**). Bacterial feeding nematodes were the most abundant in all treatments, ranging from 152,50 to 265 nematodes per sample, but no significant effects of treatments were found as well. Soil amendment of bioproduct in irrigated plots significantly increased the number of plant parasitic nematodes, where in average 11,87 nematodes were found per sample. Nematodes live in a film of water around the soil particles, so they respond quickly to any changes in environments and irrigation could affect the nematode survival. In another study, only the proportion of omnivores and the number of taxa identified was affected by irrigation [37]. Artificial irrigation could change soil physical and chemical properties, and abundance and diversity of nematode community is correlated with these changes [38]. In this study, there was no clear influence of irrigation on soil nematode community.

Mycorrhizal root colonization frequency in the root system was higher in the plots with bioproduct amendments in non-irrigated plots (**Table 6**). Significantly highest mycorrhizal frequency in the root system (30,11%) was observed in the treatment with soil+ foliar amendments of bioproduct in non-irrigated plot. This treatment had the greatest impact on root mycorrhiza. Irrigation also significantly affected the mycorrhizal colonization, since F was as low as 16.28% in non-irrigated control (without bioproduct amendments), compared to significantly high F (20,78%) in irrigated control. However, irrigation affected the mycorrhizal colonization in plots with bioproducts amendment. Bioproduct increased mycorrhizal root colonization for all tested parameters in non-irrigated plots. When applied twice in soybean vegetation, in soil and on foliar, bioproduct significantly decreased the mycorrhizal colonization (for all parameters, except parameter a) in irrigated regime 60-100% FWC. In other studies, higher mycorrhizal root colonization was observed during the dry comparing to the wet period [39]. but different results were also reported [40]. Difference in mycorrhizal root colonization also depend on the plant genotype [24].

5. Concluding remarks

Studies aiming to evaluate effect of commercial products containing beneficial microorganisms on soil nematode community, especially on plant parasitic nematodes and mycorrhizal root colonization are scarce. Nematode community structure respond quickly to changes in their environment resulting from agricultural practices and other changes in soil properties. The results we presented in this chapter reveal weak effect of irrigation and amendments of bioproducts containing beneficial organisms on abundance and structure of nematode community in soybean. However, the treatments we used had considerable effect on mycorrhizal root colonization. This result is positive for soybean production, since AMF could increase plant performance in drought stress and consequently impact on greater grain yields. Our results indicate that amendments of the bioproduct increase mycorrhizal root colonization in rain fed plots, while it decreased the mycorrhizal root colonization when soybeans were irrigated.

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Conflict of interest

The authors declare no conflict of interest.

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