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Different Soil Improvers Enhance Cucumber Productivity by Suppressing Root-Knot Nematodes (*Meloidogyne javanica* L.)

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Abstract: Root-knot nematodes (RKNs) severely infest cucumber crop; however, limited is known about the effect of different soil improvers on RKNs suppression and subsequent improvement in cucumber productivity. Therefore, the current study was conducted to infer the role of different soil improvers (i.e., humic acid, commercial dry yeast, rhizobium and Nemakey) in suppressing RKNs and improving cucumber productivity. The soil improvers were applied at the time of sowing and data relating to growth (leaf area, chlorophyll contents, root and shoot biomass), yield related traits (fruit width and length, number of aborted flowers, days to harvest and yield) and number of egg sacs and nematode larvae in soil were recorded. All soil improvers suppressed the number of egg sacs and larvae compared to control treatment, and the highest suppression was recorded with Nemakey compared to the rest of the soil improvers. Similarly, growth and yield related traits were improved by all soil improvers compared with the control treatment and the highest improvement was noted with Nemakey. It is concluded that Nemakey can effectively be used to suppress RKNs and improve cucumber productivity in nematode contaminated soils. Moreover, the efficacy of Nemakey should be tested for other crops.

Keywords: Nemakey, root-knot nematodes, cucumber, growth, productivity

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1. Introduction

Cucumber, (*Cucumis sativus* L.) is the most important cucurbitaceous crop grown around the world (Asadi et al., 2018; Zhang et al., 2018). Several biotic and abiotic factors adversely affect the productivity of cucumber (Anwar et al., 2018; Wei et al., 2015). The biotic factors include the diseases caused by fungi, bacteria, viruses and nematodes. The root-knot nematodes (*Meloidogyne* spp.) are the most prevalent cause of yield losses in cucumber (Abu-Gharbieh et al., 2005; Karajeh, 2013).

The root-knot nematodes (RKNs) infest several crops globally (Ntidi et al., 2016; Shigueoka et al., 2016), including vegetable crops (Naz et al., 2016; Podesta et al., 2016; Zhou et al., 2016). Over 100 species of *Meloidogyne* have been described from different parts of the world (Karssen and Moens, 2006); however, only four species (*M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla*) are responsible for economic losses and 95% infestations globally. The global distribution of these species is as follows: *M. incognita* 47%, *M. javanica* 40%, *M. arenaria* 7% and *M. hapla* 6% (Sasser, 1980). These RKNs adversely affect uptake of nutrients and water, and translocation of photosynthates and minerals (Williamson and Hussey, 1996) resulting in altered root: shoot ratio (Anwar and Van Gundy, 1989), which has a negative effect on the yield (Orr and Robinson, 1984).

The management of RKNs is more difficult than that other pests as they mostly inhabit the soil and usually attack the underground parts of the plants (Stirling, 2018). Although nematicides are effective,

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easy to apply and show rapid effects; however, they are withdrawn from the market in some developed countries because of their concerns about public health and environmental safety (Schneider et al., 2003). Besides, nematicides' use to control RKNs is an expensive measure and may have high environmental risk to air, water, non-target organisms and the applicators. Moreover, excessive and continuous use of pesticides to control different pests and diseases resulted in public awareness against their environmental and health impacts. Thus, there is a need to develop alternative control measures to manage plant-parasitic nematode under field conditions.

The main approaches of alternative control are: (1) genetic modification of the biocontrol agent towards gaining disease suppression mechanisms efficient against as many as pathogens (2) environmental manipulation favoring the biological control agent, while suppressing the competitive microflora and (3) developing a mixture of strains with enhanced bio control activity (Helmberger et al., 2018; Janisiewicz, 1988; Learmount et al., 2016; Warnock et al., 2017). Developing a mixture of strains of biological control agents is the most easy and quickest way of alternative control. Several strategies can be considered for obtaining the biocontrol mixtures such as mixing different organisms with various plant colonization mechanisms to control targeted pathogens mixtures or mixtures of antagonists show an optimum performance at various temperature, pH, and moisture conditions for plant colonization.

The search for novel and environment-friendly alternatives to manage RKNs has therefore become increasingly important. Extensive investigations have been conducted over the last twenty years to assess the potential of different biocontrol agents to manage RKNs. However, the biological control agents suffer narrowness of their target pathogens compared to commercial pesticides (Janisiewicz, 1996) and inconsistent performance (Wilson and Backman, 1998). Nonetheless, research efforts have found that nematophgous bacteria are widely distributed, possess diverse modes of action and have broad host ranges (Tian et al., 2007).

Biological control of different plant diseases was focused primarily using bacteria or filamentous fungi (Whipps, 2001). Therefore, application of yeast as biocontrol agents acts as a new method against different pathogens. Potential use of yeasts as biocontrol agents of soil-borne fungal plant pathogens and as plant growth promoters were recently investigated by El- Tarabily et al. (2006). Wide variety of yeasts has been used extensively for the biological control of post-harvest diseases of fruits and vegetables (Punja, 1997; He et al., 2003). Yeasts belonging to the genera *Rhodotorula*, *Crytococcus* and *Saccharomyces* are common components of the soil rhizosphere (Sampedro et al., 2004); thus, can be used for the control of RKNs.

Different organic materials are traditionally used to improve soil fertility and structure. Moreover, these materials are known to control RKNs (Houx et al., 2014). Different pests, including RKNs are being managed by different organic materials, such as animal and green manures and compost in the recent years (Rudolph and DeVetter, 2015; Forge et al., 2016). However, these materials have merely been tested for improving cucumber productivity and suppressing RKNs.

Therefore, the current study was designed to test the efficacy of different organic soil improvers for RKNs suppression and yield improvement of cucumber. It was hypothesized that different soil improvers will suppress the egg sacs and nematode larvae in the soil. Similarly, the soil improvers will improve the growth and yield due to suppressed activities of RKNs. The results of the study will help to find a sustainable management option for RNKs in cucumber.

2. Materials and Methods

2.1. Experimental Site Description

The current study was conducted in the plastic house of Bazian Research Station, Agricultural Research Directorate, Sulaimaniyah, Iraq (54.0745 latitude, 31.3635 longitude and 847 m above sea level).

Table 1. Physicochemical properties of the soil used in the study

Soil Property	Value
Texture	Clay loam
PH	7.3
E.C. (ds/m.)	0.28
N (ppm)	1000
P (ppm)	14.2
K (ppm)	2.34
Ca (ppm)	22
Mg (ppm)	33.6
Na (ppm)	2.3
Cl (ppm)	10.65
$CaCO_3$ (ppm)	270000
HCO_3^- (ppm)	103.7

The soil of the greenhouse was contaminated with *Meloidogyne javanica* nematode. The density of nematodes was recorded before the initiation of the experiment at three different soil depths, i.e., 0-10, 11-20 and 21-30 cm. Nematode larvae were isolated and counted from greenhouse soil by Baermann funnel. The observed larval density was 80, 121, and 93 larva/250 g of soil in 0-10, 11-20 and 21-30 cm soil depth, respectively. The observed nematode larval density was higher than the recommended density for cucumber (50 larva/250 g soil). Moreover, soil sample was collected and analyzed for physical and chemical properties. The physico-chemical properties of the soil are presented in (Table 1).

2.2. Experimental Treatments

Four different soil improvers, i.e., humic acid, commercial dry yeast that contain *Saccharomyces cerevisiae* fungi, rhizobium bacteria and vegetable extract called Nemakey were used in the study along with a control treatment for comparison. Distilled water was used in the control treatment of the study. Humic acid (2 ml plant $^{-1}$) was applied to the soil 10 days before sowing. Commercial dry yeast (*Saccharomyces cerevisiae*) was incorporated to the soil (5 g m^{-2}) at three different stages, i.e., 10 days before sowing, during mid-season and at the end of the season. Rhizobium bacteria was incorporated by planting bean seeds 15 days before the sowing of cucumber and bean plants where harvested at flowering stage from the stem near the soil and removed. Nemakey is plant extract used for strengthening the crops against pathogens in addition to their organic substitutes for nitrogen and potassium. It also helps plants to absorb nutrients. Nemakey was applied to the soil before sowing (1 ml m^{-2}), during first fruiting and in the middle of season.

2.3. Experiment Setup and Design

The experiment was conducted in greenhouse. The seedbed was prepared, and different soil improvers were added to soil as per treatment explained above. The row × row and plant × plant distance was kept 50 and 40 cm, respectively. The experiment was laid out according to completely randomized design with four replications. The crop was kept free from weeds by manual weeding.

2.4. Observations

Data related to growth, allometric traits, yield and quality was recorded. Plant height of ten randomly selected plants from each experimental unit was measured at maturity with the help of measuring tape and averaged. Leaf area was measured at 30 days after sowing with the help of leaf area meter (DT Area meter, Model MK2, Delta T Devices, Cambridge, UK). Fruit length and width of ten randomly selected fruits was measured and averaged to record fruit length and width. Biomass production was recorded at maturity. Five randomly selected plants were harvested, weighed fresh, dried in an oven at 65 ± 5 °C until constant weight and then dry weight was recorded. The total chlorophyll content of fresh leaves was estimated following the method suggested by Barnes et al. (1992). Total chlorophyll was calculated according to the following formula;

$$\text{Total chlorophyll (mg } g^{-1}) = (20.2 \times A_{645}) + (8.02 \times A_{663}) \times \frac{V}{1000 \times W}$$

where, A = absorbance at specific wavelength; V = final volume of chlorophyll extract and W = fresh weight of sample.

Number of egg sacs were computed from three different locations in each experimental unit 30 days after sowing and averaged. Similarly, number of nematode larvae were counted 60 DAS from three different locations and averaged. The number of root nodes were computed from ten randomly selected plants in each experimental unit and averaged. Number of aborted flowers from five randomly selected plants were counted at maturity and averaged. Total yield harvested during the season was computed and averaged.

2.5. Statistical Analysis

The collected data was tested for normality by Shapiro-Wilk normality test. The variables having non-normal distribution were transformed by Arcsine transformation. Fischer's Analysis of Variance (ANOVA) technique was used to test the significance in data. Least significant difference test at 99% probability was used to separate treatments' means where ANOVA indicated significance. Finally, correlation was tested among all measured variables.

Table 2. Analysis of variance of different soil improvers for different yield and quality parameters of cucumber

Parameters	Sum of squares	Mean squares	F Value	P Value
Chlorophyll Contents	19.93	4.98	14.58	0.0004*
Fruit Width	2.20	0.55	22.34	0.0001*
Fruit Length	39.35	9.84	25.44	0.0001*
Number of Larvae	299534.40	74883.60	746.35	0.0001*
Number of Egg Sacs	165.33	41.33	68.89	0.0001*
Number of Aborted Flowers	22.27	5.57	30.48	0.0001*
Number of Days to Harvest	197.60	49.40	6.56	0.0074*
Number of Root Nodes	374.27	93.57	73.87	0.0001*
Peel Thickness	1060.00	265.00	9.91	0.0017*
Total Leaf Area	0.44	0.11	50.83	0.0001*
Total Soluble Solids	7.62	1.90	34.86	0.0001*
Shoot Biomass	3.91	0.98	273.00	0.0001*
Root Biomass	1.08	0.27	140.32	0.0001*
Yield	206.87	51.72	17.70	0.0002*

*=significant at $p \leq 0.01$

3. Results

Different soil improvers significantly influenced all measured variables during the experiment (Table 2). The highest and the lowest leaf area was recorded for Nemakey and control treatments (Table 3). The longest fruits were harvested from Nemakey and Rhizobium treatments, whereas humic acid produced shortest fruits. Rhizobium and humic acid observed the highest fruit width, whereas the lowest fruit width was recorded for commercial dry yeast. The highest and the lowest root biomass was recorded for Nemakey and Rhizobium treatments, respectively. Similarly, Nemakey and commercial dry yeast observed the highest and the lowest shoot biomass, respectively. The highest chlorophyll contents were noted for Nemakey, whereas Rhizobium and commercial dry yeast had the lowest chlorophyll contents during the study (Table 3).

The highest number of egg sacs and larvae were recorded in the control treatment, whereas Nemakey observed the lowest number of egg sacs and larvae (Table 4). Similarly, the highest number of root nodes were noted for Nemakey, whereas the lowest number of root nodes were recorded for control. The highest number of aborted flowers were recorded for control, humic acid and Rhizobium, whereas commercial dry yeast observed the lowest number of aborted flowers. The lowest number of days to reach harvest stage were observed for Nemakey, whereas rest of the treatments taken same number of days to reach harvest. The highest and the lowest yield was recorded for Nemakey and control treatments, respectively (Table 4).

Different growth, allometric and yield related traits exhibited significant positive/negative correlations with each other (Table 5). Chlorophyll contents had negative correlation with number of days to reach harvest, whereas had positive correlation with root biomass, shot biomass and yield. Fruit width was positively correlated with number of aborted flowers. Similarly, fruit length was positively correlated with total leaf area. The number of nematode larvae were positively correlated with the number of egg sacs, days to harvest and root nodes, whereas had negative correlation with total leaf area, root biomass and yield.

Table 3. Effect of different soil improvers on growth, allometric and biochemical traits of cucumber

Treatments	Total Leaf Area (cm^2 plant^{-1})	Root Biomass (g plant^{-1})	Shoot Biomass (g plant^{-1})	Chlorophyll Contents (mg g^{-1})	Fruit Length (cm)	Fruit Width (cm)
Control	1.34 c	2.55 d	4.68 b	41.70 b	15.03 b	3.83 ab
Humic acid	1.23 d	2.78 c	4.75 b	41.55 b	13.33 c	4.03 a
Commercial dry yeast	1.49 b	2.88 b	4.45 c	40.47 c	15.73 b	3.00 c
Rhizobium	1.46 b	2.41 e	4.43 c	39.80 c	17.07 a	4.03 a
Nemakey	1.74 a	3.19 a	5.82 a	43.17 a	18.00 a	3.67 b

Table 4. Effect of different soil improvers on number of egg sacs, number of larvae of Meloidogyne *javanica*, number of root nodes, number of aborted flowers, number of days to harvest and yield of cucumber

Treatments	Number of Egg Sacs of nematode	Number of Larvae of nematode	Number of Root nodes	Number of Aborted Flowers	Number of days to Harvest	Yield ($kg/5m^2$)
Control	12.33 a	446.67 a	3.33 d	8.50 a	62.33 a	50.40 c
Humic acid	7.00 b	156.67 c	6.33 c	9.27 a	61.33 a	55.48 b
Commercial dry yeast	4.67 c	108.67 d	6.00 c	5.80 c	59.67 a	49.37 c
Rhizobium	7.00 b	278.67 b	11.67 b	8.70 a	64.33 a	51.25 c
Nemakey	2.33 d	48.67 e	17.33 a	7.50 b	53.67 b	59.32 a

The number of egg sacs were positively correlated with days to harvest and rot nodes, whereas negatively correlated with total leaf area, root and shoot biomass. Yield was positively correlated with chlorophyll contents, root and shoot biomass, whereas had negative correlation with nematode larvae, days to harvest and root nodes (Table 5).

4. Discussion

The application or manipulation of nematode antagonistic microbes is one area being investigated to find out the alternative to nematicides (Meyer, 2003; Qureshi et al., 2011). Fungi are known to possess a huge diversity of metabolic pathways and they provide several large classes of commercial compounds, including many antibiotics used in medicines. Several compounds with nematicide activity have also been reported from fungi (Li et al., 2007). However, no major commercial product based on these natural fungal compounds has been developed yet (Li et al., 2007). Secondary metabolites from fungi associated with rhizosphere and rhizoplane of crop plants offer an exciting area of research for the discovery of potential nematicide compounds.

The results indicated that different soil improvers suppressed the number of egg sacs and larvae. The suppression in nematode larvae resulted in improvement of growth and yield related traits. However, huge differences were noted among the soil improvers for RKNs suppression and yield improvement. The possible reason of these differences is different nature of the organic soil improvers used in the current study. Several earlier studies have also reported significant differences among soil improvers for suppressing nematode activity (Tejada, 2009; Tabarantab et al., 2011). The results of the current study also contradict with several earlier studies where addition of organic compounds increased the number of nematode larvae in soil. The increase in the activity of nematodes in these studies was related to improvement in root surface area and biomass with the addition of organic manures. These improvements in root biomass and surface area provided more feeding sites for nematodes (Li et al., 2010; Roth et al., 2015; Wang et al., 2006).

Table 5. Correlation among different growth parameters and yield attributes of cucumber

	Chlorophyll Contents	Fruit Width	Fruit Length	Nematode Larvae	Number of Egg Sacs	Number of Aborted Flowers	Days to Harvest	Number of Root Nodes	Total Leaf Area	Shoot Biomass	Root Biomass	Yield
Chlorophyll Contents	**1.00**											
Fruit Width	0.05	**1.00**										
Fruit Length	0.10	-0.11	**1.00**									
Nematode Larvae	-0.28	0.40	-0.25	**1.00**								
Number of Egg Sacs	-0.20	0.36	-0.51	**0.94**	**1.00**							
Number of Aborted Flowers	0.06	**0.89**	-0.33	0.44	0.50	**1.00**						
Days to Harvest	**-0.57**	0.29	-0.34	**0.62**	**0.57**	0.41	**1.00**					
Root Nodes	-0.33	0.36	-0.18	**0.98**	**0.90**	0.38	**0.62**	**1.00**				
Total Leaf Area	0.28	-0.32	**0.88**	**-0.52**	**-0.70**	-0.51	**-0.67**	-0.45	**1.00**			
Shoot Biomass	**0.85**	0.05	0.46	-0.49	**-0.52**	-0.04	**-0.75**	-0.50	**0.65**	**1.00**		
Root Biomass	**0.68**	-0.41	0.27	**-0.81**	**-0.74**	-0.48	**-0.82**	**-0.80**	**0.59**	**0.79**	**1.00**	
Yield	**0.74**	0.32	0.26	**-0.55**	-0.49	0.27	**-0.60**	**-0.58**	0.40	**0.83**	**0.64**	**1.00**

The figures in bold represent significant positive/negative correlation with respective variable(s)

Leaf yellowing, reduced plant height and biomass, decreased chlorophyll contents and low yield are the significant negative effects exerted by RKNs to different crops (Bora and Neog, 2006; Haider et al., 2003; Vovlas et al., 2008; Azam et al., 2011).

The RKNs feed on giant cells which ceases root growth and stops tips to swell, ultimately resulting in reduced root and shoot length (Siddiqui et al., 2014). Although, different soil improvers tended to improve the root and shoot biomass, severe reduction was noted in control treatment of the study. The decreased root and shoot biomass resulted in less nutrient uptake, eventually resulting in stunting and smaller plants. Several studies have reported the stunting of plants by different RKNs species (Ogbuji and Okarfor, 1984; Enopka et al., 1996; Haider et al., 2003; Khan et al., 2006).

Application of humic acid improved the growth and productivity of cucumber in the current study. Antioxidants such as α-tocopherol, α-carotene, superoxide dismutases, and ascorbic acid are improved by the application of humic acid (Sun et al., 2004). These antioxidants may play a role in the regulation of plant development and flowering, which ultimately improve the yield (Ziadi et al., 2001).

The highest chlorophyll contents were recorded with yeast during the study. This increase in photosynthetic pigments' formation could be attributed to the role of yeast cytokinins delaying the aging of leaves by reducing the degradation of chlorophyll and enhancing the protein and RNA synthesis (Shalaby et al., 2008). Antagonistic activity of the ascomycetous yeast strain *Pichia anomala* against *Fusarium spp.* contaminated barley grains was also reported by (Laitila et al., 2007).

Although yield was improved and nematode larvae were lowered by different soil improvers, Nemakey, a commercial organic fertilizer exhibited the highest RKNs suppression and yield improvement. Namakey is composed of plant extracts and oils. Root development is promoted by Nemakey which ensures the formation of healthy root hairs (Ismail et al., 2017). Improved root structure helped the plant to uptake more nutrients, which ultimately improved plant growth and yield. However, the actual mechanism of nematode suppression needs to be explored for Nemakey.

4. Conclusion

The yield improvement and suppression of RKNs indicates the potential of different soil improvers, especially Nemakey for controlling RKNs in cucumber and yield improvement. Nemakey can be efficiently utilized in RKNs contaminated soils for higher cucumber productivity. However, actual mechanism behind RKNs suppression with Nemakey needs to be thoroughly tested. Moreover, the potential for Nemakey for other vegetable crops should also be explored in future studies.

List of Abbreviations: RKNs: Root-knot nematodes, RNA: Ribonucleic acid, ANOVA: Analysis of variance

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