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EXAMINATION OF BIOLOGICAL SEED TREATMENTS TO MANAGEMENT OF ROOT-KNOT NEMATODE (*MELIODOGYNE* sp.) ON OKRA (*ABELMOSCHUS ESCULENTUS*)

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Abstract

One of the most important for Root-knot nematode (*Meliodogyne* sp.) management is biological control as seed treatment. In the test that was conducted in the field at Abbassiya city- at Najaf city in the summer season of 2019 to determine the efficacy of selected biological products. Biological seed treatment was including fungicide as basic treatment for all of the treatments in the test, the fungicide was Celest 100 Fs. Treatments included seeds treated with Abamectin (*Streptomcyes avermentilis*), Nemhat as organic nematicide and fungus (*Paecillomyces* sp.). Seed treatments could be an early method of protection seedling of Okra (*Abelmoschus esculentus*) in the field from disease and nematodes. The main objective of this study was to evaluate different seed treatments for plant growth and management of Root-knot nematode. Plant growth parameters measured included fresh weight, height, number of nodes, number of flowers and roots weight. Nematode parameters included gall formation on the roots, number of juveniles in the soil, number of eggs, number of eggs mass, number of adults. A statistically significant difference (p-value 0.05) was obtained regarding the growth range of plant development for Okra with treatments *Streptomcyes avermentilis* and *Paecillomyces* sp. to improved plant growth, also, there was no negative effect on plant growth was recorded with these treatments. Nematode development included gall formation on the roots, number of juveniles in the soil, number of eggs, number of eggs mass, number of adults was reduced by treatments *Streptomcyes avermentilis* and *Paecillomyces* sp compared to control treatment that have no treated and fungicide only. Also, there was no significant difference on plant growth for okra plants with Root-knot nematode (*Meliodogyne* sp.)

Key words: Okra, *Meliodogyne* sp, *Streptomcyes avermentilis, Paecillomyces* sp.

Introduction

Okra, *Abelmoschus esculentus* (L.) Moench, among all crops, is one of the summer vegetables crop in Iraq that almost grown in all over the country (ICSO, 2016). Although Iraq is one of the biggest okra producers in terms of total production, the per donum (1/4 hectare) productivity is not only fluctuating seasonally but also remains very low compared to India, Egypt, Jordan and Cyprus (FAO, 2016). In each year the yield damages from the okra crop because of the nematode have been evaluated for the nematode could be sitting in the fields without seeing any symptoms clear on the plants on top of ground, the yield damages caused by this nematode are oftentimes undervalued. The yield damages can be different from year to the year that causes by this nematode and are affected by the variety of the okra, the soil biotic factors and abiotic and climatological cases. The nematode can be more destroyed for the okra if it is spread in the fields. As in another vegetable crops, okra production in Iraq is suffering from yield loss because of facing many biotic and abiotic factors. Yield loss by the plant parasitic nematodes is one of the most important issues (Stephan and Abu-Gharbieh, 2010). The yield losses caused by root-knot nematodes are due to the build-up of inoculum of this pathogen (Kayani *et al.*, 2013) and continuous growing of similar okra varieties in the same field year after year (Hussain *et al.,* 2011). Root-knot nematode *Meloidogyne* spp. is the most prevalent nematode associated with vegetable crops in Iraq (Stephan *et al.*, 1977) and chronic losses usually occur due to the frequent and high population of this nematode (Stephan *et al.*, 1998). Root-knot nematodes are considered among the top 5 major plant pathogens and the first among the

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10 most important genera of plant parasitic nematodes in the world (Mukhtar, *et al*., 2013). *Meloidogyne* spp. are one of the most widespread and damaging plant-parasitic nematodes throughout the world and substantially affects growth and yield of okra (*Abelmoschus esculentus* L). *Meloidogyne* spp. attack different crop plants, including vegetables, causing severe growth retardation due to formation of typical galls. Okra (*Abelmoschus esculentus* L. Moench) is one of the most important vegetable crops of the world, being popular in many tropical and subtropical countries. It is mostly cultivated for human consumption and for industrial use as fiber attack different crop plants, including vegetables, causing severe growth retardation due to formation of typical galls. It is mostly cultivated for human consumption and for industrial use as fiber (Hussain *et al.,* 2012). Although the Root-knot nematode (*Meloidogyne* spp. includes over a hundred species, the four-wide distributed *Meloidogyne incognita, M. javanica, M. arenarea* and *M. hapla* are the most damaging on vegetables (Taylor *et al.*, 1982).

Root-knot: (*Meloidogyne incognita*) is microscopic roundworms is found in a wide range of homeland and agroeco systems. Those parasites infect gourd roots of plant, wherein they mash and full their life cycles. When left unlimited, those pathogens can be caused severe plant harm and yield lack to cucurbits and other popular vegetables in everywhere the Pacific. Two species of the nematodes hit gourd in all the world *Meloidogyne incognita* and *M. javanica* (Westerdahl and Becker, 2011). Meloidogyne contains around 100 species. Some of these species are considered globally necessary and can be caused severe loss on economic crops. *Meloidogyne* is a sedentary endoparasitic pathogen feeding into the root and tuber cells. The egg mass is accoune outside the female body. Under appropriate conditions, eggs hatch to allow second phase juveniles (J2). J2s shift in search on the host plants to full their life cycle. Once the J2 reach the plant root, the J2 penetrate the closest root tips in the extension area (Lohar and Bird, 2003). The J2 establishes a nutrition site primary in bark /or relative parenchyma cells (Viglierchio, 1991). Five to six cells serve as a nutrition site. Those cells can gain a high metabolic activity after genetically modified by excretion of the root-knot (Hussey, 1989). The cells undergo hypertrophy meaning undergoing mitotic splip without cell split (or cell wall formation) and form a dense likable multinuclear cytoplasmknown as "giant cell". These giant cells extend mostly into the stele of plant tissues. Adjacent cells around the nutrition site will undergo hypertrophy (abnormal of cell enlargement) and hyperplasia (abnormal lead to increase of cells numbers) response in all directions, shape of the root gall (Viglierchio, 1991). Management practices for controlling *M.* sp. include cultural practices, resistance variety, nematicides, and biological control. The biological control is considered to cover control that outcome from the work of soil microorganisms and the soil micro fauna and is intercede through technique such as, predation, parasitism, competition and antagonism (Stirling, 1991). Seed treatment nematicides are available in the market since 2005 and management practices have been changed from the standard granular in-furrow applications to seed treatments, such as Avicta Complete Cotton, (abamectin), and Votivo, a biological strain of the *Bacillus firmus*. Seed treatments have simplified the growing process and reduced producer's exposure to chemicals. There are some examples of bionematicides as seeds treatments including abamectin (Syngenta) has shown activity against soybean cyst nematode (*H. glycines*) and Root-knot nematode (*M. incognita*). (Qiao *et al*., 2012; Muzhandu *et al.,* 2014; Aljaafri, 2017; Aljaafri *et. al*., 2018). *Paecillomyces lilacinus* has been used a biological agent against Meloidogyne incognita for okra plants. *P. lilacinus* showed positive effect in the biological control for nematodes. The main objective of this study was to find out the best way to management the disease that causes by plant parasitic nematodes. Using different biological products was used to determine the effect on okra seeds as seed treatments with biological control.

Materials and Methods

Field of experiment and plant Growth/Inoculation with Root Knot (*Meloidogyne* sp.)

The field for the experiment was in Abbassiya city of Najaf. The field was 10 meters in long and 10 meters in wide. This area divided to 10 rows, each row with 1 meter in wide and 8 meters in long. Each row divided to 5 species with take half meter between each meter that used as replicate including 5 holes in each hole put 3 seeds that treated with biological products before planting**.** Nematode cultures: *M. incognita* was originally available in the field and planted with field that already infested with this nematode. From the field before study took sample of soil by randomly way from different area in the field to see the *Meloidogyne* sp. Second-stage juveniles (J2) were collected in hatching chambers with a 200-µm pore screen that allows only hatched J2 to migrate into the collection dish. Seeds were previously treated with the appropriate experimental biological products (Table 1). Seeds (3 seeds in each hole) were planted into 15 cm^3 in the long and 15 cm^3 in the wide as

Table 1: Biological products as seed treatments *for okra seeds* (*Abelmoschus esculentus*) grew in the field direct that infested with Rot-knot nematode (*Meloidogyne* sp.*)*.

Treatment	Products	Rate
1	Fungicide only (Celest 10% FS) used as basic treatment with all treatments almost.	1.8 MG/LITTER
2	Fungicide + Nemahit (Organic nematicide)	100cm^3/ 100 litter water + Fungicide
3	Fungicide + Abamectin (*Streptomcyes avermentilis)*	50cm^3/ 100 litters water + fungicide
4	Fungicide + *Paecillomyces* sp.)	10^7 from fungus + fungicide
5	*Paecillomyces* sp.	10^7 from fungus
6	Untreated seeds (Control)	4 ML Water only
7	Fungicide + Abamectin + Nemahit	100cm^3/ 100 litter water + Fungicide + 50cm^3
8	Abamectin (*Streptomcyes avermentilis)*	50cm^3/ 100 litters water
9	*Paecillomyces* sp. + Nemahit (Organic nematicide)	10^7 from fungus + 100cm^3/ 100 litter water
10	*Paecillomyces* sp. + Abamectin (*Streptomcyes avermentilis*)	10^7 from fungus + 50cm^3/ 100 litters water

square after putting seeds covered by some of soil. (Aljaafri, 2017, Aljaafri *et al*., 2018). All experimental treatments are arranged in a RCBD with 5 replications in the field at environment situations. Data acquired analyzed using a statistical test system (SAS) version 9.4. and the standard of significant was collection at 5%. (0.05) Least significant difference (LSD) tests at P = 0.05 employed to the differences among treatments for the parameters measured. Okra seeds watered every day in the first few days from planting and fertilized weekly after three weeks from planting. At 75 days, tests were the final harvested. Also, at 10 days did counting the percentage of germination for okra seeds. At 30 days of planting take samples of soil to count Root-Knot nematode-juveniles (J2) and see the effect on plant growth including different parameters for plant growth (fresh weight, height of plants and roots, plant and root weight, number of leaves and weight of fruits in end of the experiment at 75 days). *Meloidogyne* sp. set to extraction, roots were located into a beaker with sufficient 10% chlorine bleach solution to coating the okra roots. The okra roots were mad for 3 minutes by thrilling with a scapula in the 10% chlorine bleach solution. The 10% chlorine bleach/ nematode extract was teeming from the beaker and sieved through a 200-mesh sieve nested on top of a 500-mesh sieve. Nematodes were counted on a grated petri dish under the Olympus BH2 B071 microscope (Japan Model C35AD-4) at 40X magnification. Soil and water contents of the bucket set singly as substantive over (bucket 1) were teeming through a 60-mesh sieve till bucket 2. The contents of bucket 2 were sieved over the sink used a 325-mesh sieve. Renew rinsing was done through the 325-mesh sieve with a gentle flow of water till 20 ml soil or minus remained on under most of the 325-mesh sieve. A 20-60 ml juvenile egg extract was collected by washing the 325-mesh sieve extract into a 120 ml beaker. The beaker content could settle for 2 hours. After 1 hour, water was rejected. A timer was set to 10 minutes. The sugar-nematode hang was leaving into 50 ml centrifuge tube and centrifuged for 1 min at 1500 rpm using a centrifuge. After centrifugation, the supernatant was teeming off onto a 500-mesh sieve grasped up the sink. The bead soil layer of the centrifuge tube was discarded. Examination and count of eggs and juveniles on grated Petri dishes were done using the Olympus BH2 B071

Fig. 1: Number of juveniles' nematodes for plant-parasitic nematode (*Meliodogyne* sp.) and Not parasitic nematodes that were available in the field before planting okra plants. Data are means of the 5 replicates for each sample. Means compared by using (LSD) at 0.05. P-Value, 0.1264, LSD 0.05= 3836.4.

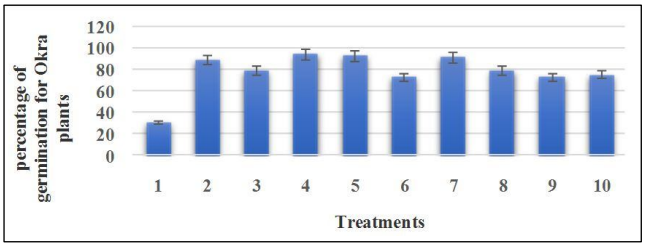

Fig. 2: Effect of Biological products as seed treatments on percentage germination of okra plants (*Abelmoschus esculentus*) infested with *M.* Sp. Data are means of the 5 replicates for each treatment 10 days. Means compared by using (LSD) at 0.05. P-Value, 0.020, LSD 0.05= 4.866.

Table 2: Effect of Biological products as seed treatments on plant growth development of okra plants (Number of leaves/ plant, Weight of roots/ plant grams, Weight of plants/ plant grams, Roots Long/ plant cm^3, and Plant Long/ plant cm^3) infested with *M.* sp. After 30 days from planting in the field.

Treatments	Number of leaves / plant	Weight of roots / plant grams	Weight of plants / plant grams	Roots Long / plant cm^3	Plant Long / plant cm^3
1	5.4	1.295218	4.18592	8.2	10.8
2	6.8	3.3148	7.49984	11.3	12.6
3	5.8	3.586	8.05696	9.88	10.76
4	6.6	4.98	10.64004	11.4	11.2
5	7.2	2.71918	7.96152	11.6	13.6
6	7	1.85038	5.98372	11.54	13.34
7	6.8	4.41458	9.77344	12.32	13.72
8	6.6	4.494	8.20516	11.44	12.78
9	6	3.90544	7.06648	12.8	14.84
10	6	3.5	6.63848	11.54	11.76
P-Value	0.659	0.0069	0.061	0.028	0.124
L.S.D 0.05	1.925	1.909	3.677	2.391	3.033

Data are means of the 5 replicates for each treatment 30 days of planting. Means compared by using (LSD) at 0.05.

microscope (Japan Model C35AD-4) at 40X magnification (Aljaafri, 2017, Aljaafri *et al*., 2018). Egg mass staining method, the entire root system of each okra plant that had been infested with *Meloidogyne* sp. subjected to egg masses staining procedure. After removing the okra roots from soil, each root was thoroughly washed, blotted dry and placed in a 150 ml beaker with 100 ml of the stain solution (prepared as formerly described). After 15 minutes of soaking in stain, root was rinsed in tap water and blotted dry. Egg mass was observed, counted and photographed under the stereomicroscope (Alti 122) at 2X magnification (Damasceno *et al.*, 2016). All these experiments with different treatments of biological seeds treatments (Table 1) counted number of juveniles, eggs, average of galls that was examined for galling and rated according to the following method. Each group of root plant materials for these treatments were laid on the lab counter top and observed for root knot galls. Root galling is recorded on a scale as follows: 0 = no galling, 1 = 25% galling, 2 = 50% galling, 3 = 75% and 4 =100% galling. (Aljaafri, 2017).

Results

The isolation of nematodes for the field before study showed there was a lot of numbers of Root-note nematode (Meloidogyne sp.) available in the field was 30% (2191.2) and 70% (5019.2) free living nematodes also were found it in the field beside plant- parasitic nematodes. (Fig. 1).

All the experiments with different products of biological as seed treatments (fungicide, Nemahit (Organic nematicide, *Streptomcyes avermentilis* and *Paecillomyces* sp.), there was no negative effect on plants germination and development occurred by *Meloidogyne* sp. when using these treatments compared with control without treatment and fungicide only also on plant germination. Almost, most of treatments were significant to improve okra seeds germination compared to control treatments (untreated seeds and fungicide only). (Fig. 2).

There was no negative effect on plants growth and development after 30 days from planting including (Number of leaves/ plant, Weight of roots/ plant grams, Weight of plants/ plant grams, Roots Long/ plant cm^3, and Plant Long/ plant cm^3) infested with *M.* sp. After 30 days from planting in the field) occurred by *Meloidogyne* sp. when using these treatments compared with control without treatment (Table 2). Results referred had been shown a positive effect to improve plant growth of okra

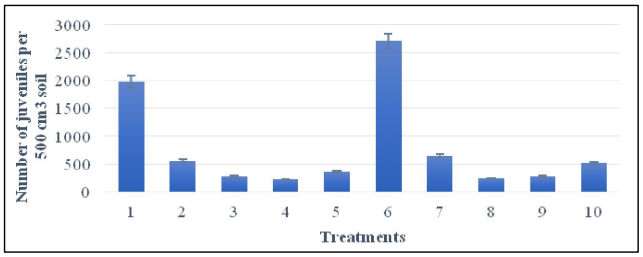

Fig. 3: Effect of *Biological products as seed treatments* on *Meliodogyne* sp. development on okra plants (number of juveniles per 500 cm^3 soil). Data are means of the 5 replicates for each treatment 30 days of planting. Means compared by using (LSD) at 0.05. P-Value, Juveniles, 0.0001, LSD 0.05= 796.67.

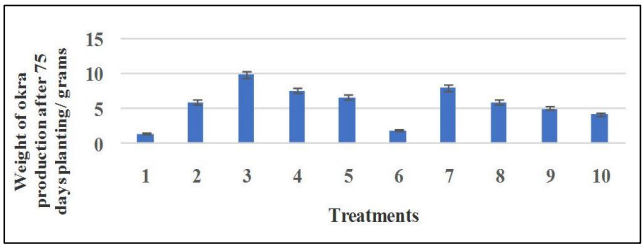

Fig. 4: Effect of *Biological products as seed treatments* on okra plants production (weight of production fruits for each plant) infested with *M.* sp. in the field. Data are means of the 5 replicates for each treatment 75 days. Means compared by using (LSD) at 0.05. P-Value, fruits/ weight grams, 0.0001, LSD 0.05= 2.422.

Table 3: Effect of Biological products as seed treatments on plant growth development of okra plants (Number of leaves/ plant, long of roots/plant/ cm^3, long of plants/plant/cm^3, Weight of roots/ plant grams, and Weight of plants/ plant/ grams) infested with *M.* sp. After 75 days from planting in the field.

Treatments	Number of leaves / plant	Long of roots / plant cm^3	Long of plants / plant cm^3	Weight of Roots / plant/grams	Weight of Plants/ plant grams
1	5.667	6.957	12.33	2.59	5.837
2	10.67	12.67	26.7	12.2	45.27
3	18	16.67	35	12	66.1
4	12.67	15.67	35.87	8.7	50.87
5	8.667	19	33.33	10.27	58.42
6	5.667	17.67	16.43	5.6	9.893
7	8.667	16.33	25.67	10.07	41.01
8	10.67	16.33	26.5	7.633	28.11
9	10.67	12.67	26.7	9.567	35.9
10	15.33	15.5	39.67	10.33	64.9
P-Value	0.139	0.0001	0.0041	0.023	0.042
L.S.D 0.05	8.684	3.021	12.531	5.140	39.45

Data are means of the 5 replicates for each treatment 30 days of planting. Means compared by using (LSD) at 0.05.

plants with these treatments (Table 2). Results were significant effects on okra plants growth parameters with treatment (fungicide, Nemahit (Organic nematicide, *Streptomcyes avermentilis,* and *Paecillomyces* sp.), compared to control treatments. In the same treatment did not show any negative significant effect on plant growth with *Streptomcyes avermentilis* and *Paecillomyces* sp. (Table2).

At 30 days of planting, nematode life stages development of *M.* sp. was effect by treatments (fungicide, Nemahit (Organic nematicide, *Streptomcyes avermentilis* and *Paecillomyces* sp.), compared to control treatments. Results had been shown a significant effect to reduce number of juveniles of *M.* sp. with treatments (Nemahit (Organic nematicide, *Streptomcyes avermentilis*

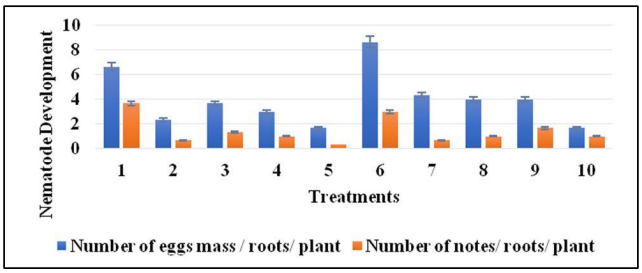

Fig. 5: Effect of Biological products as seed treatments on nematode development on okra plants (number of eggs mas and number of notes per roots / plant) infested with *M.* Sp. Data are means of the 5 replicates for each treatment 75 days. Means compared by using (LSD) at 0.05. P-Value, number of eggs mass, 0.0071, LSD 0.05= 3.462, number of notes (galls), 0.0011, LSD 0.05= 1.425.

and *Paecillomyces* sp.), compared to control treatments. Other treatments also were significant with different effect to reduce number of juveniles compared to control (Fig. 3).

At 75 days from the planting, seed treatments (fungicide, Nemahit (Organic nematicide, *Streptomcyes avermentilis* and *Paecillomyces* sp.) that was including different rates of biological control, there was no negative effect on okra plants production and development infested by *Meloidogyne* sp. when using these treatments compared with control without treatment (Fig. 4). Results referred had been shown a positive effect to improve plant production of okra plants with these treatments (Fig. 4).

At the end of the experiment after 75 days of planting, all the treatments with different seed treatments (fungicide, Nemahit (Organic nematicide, *Streptomcyes avermentilis* and *Paecillomyces* sp.) that were including different rates of biological control, there was no negative effect on okra plants growth and development occurred by *Meloidogyne* sp. when using these treatments compared with control treatment (fungicide only and untreated seeds) (Table 3). Results had been shown a positive effect to improve okra plant with all biological control products compered to control treatments in this study. (Table 3).

Root-knot Nematode life stages development of *Meliodogyne* sp. was effect by treatments (Nemahit (Organic nematicide, *Streptomcyes avermentilis* and *Paecillomyces* sp.). Study had been shown a significant effect to reduce average of galls, eggs mass of

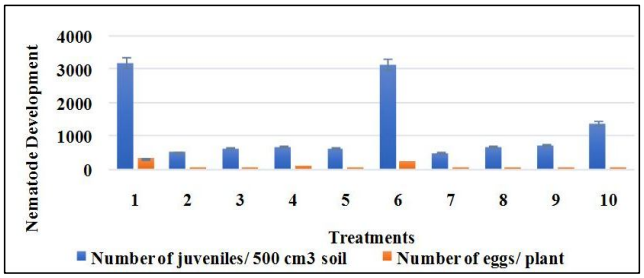

Fig. 6: Effect of Biological products as seed treatments on nematode development of okra plants (number of juveniles per 500 cm^3 soil and number of eggs per root / plant) infested with *M.* Sp. Data are means of the 5 replicates for each treatment 75 days. Means compared by using (LSD) at 0.05. P-Value, number of juveniles, 0.037, LSD 0.05= 582.57, number of eggs/ plant, 0.0004, LSD 0.05= 115.97.

Meliodogyne sp. with all treatments were significant reduced average of galls, egg mass compared to control treatments (Fig. 5). Egg mass was clear on the roots by taken images (Fig. 5).

Root-knot Nematode life stages development of *Meliodogyne* sp. was effect by treatments (Nemahit - Organic nematicide*, Streptomcyes avermentilis* and *Paecillomyces* sp.). Results had been shown a significant effect to reduce average of *M.* sp. (Juveniles and eggs) with almost all the treatments with different products of biological control. Most of treatments were significant reduced average of juveniles and eggs compared to control treatments (Fig. 6).

Discussion

None of the bacteria*, Streptomcyes avermentilis,* fungus (*Paecillomyces* sp.) *and* Nemahit-Organic nematicide had a negative effect on okra plants growth and development when challenged with *Meliodogyne* sp. All biological seed treatments had been shown significant difference to improve okra plant growth compered to control treatments. This result agreed with Aljaafri (2017) show activity for different seed treatment to improve plant growth for soybean with different biological seed treatments including abamectin (*Streptomcyes avermentilis)*. In addition, the experiments had shown activity to reduce number of life stages for *Meliodogyne* sp. That is related to abamectin is a blend of abamectin that is being used as a seed treatment to control plant-parasitic nematodes on cotton. Many of study had shown the toxicity of abamectin and its effectiveness as a seed treatment to control *M. incognita* on cotton and soybean are lacking. (Aljaafri, 2017, Abawi *et al*., 2003, Becker and Hofer, 2004). There are some examples of bionematicides as seeds treatments including abamectin (Syngenta) has shown activity against soybean cyst nematode (*H. glycines*) and Root-knot nematode (*M. incognita*). (Qiao *et al*., 2012, Muzhandu *et al*., 2014, Aljaafri, 2017, Aljaafri *et. al*., 2018). *Paecillomyces lilacinus* has been used a biological agent against Meloidogyne incognita for okra plants. *P. lilacinus* showed positive effect in the biological control for nematodes.

Conclusions

In summary, using different products included *Streptomcyes avermentilis,* fungus *(Paecillomyces* sp.*) and* Nemahit-Organic nematicide as seed treatments from biological control to management nematodes on okra plants. All the biological products performed statistically better than the control regarding improve plant growth and reducing Root-knot nematode (*Meliodogyne* sp.) life stages. All these different products of biological control, showed improving plants growth and make okra plants have some factors defense to the soil pathogen.

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