RESEARCH ARTICLE Agricultural Science Digest, Volume 45 Issue 6: 1011-1017 (December 2025)

Effect of Biological Factors in Physiological and Growth Indicators of Okra Plant [*Abelmoschus esculentus* (L.) Moench] and Percentage of (NPK) Elements with Root-Knot Nematode (*Meloidogyne javanica*)

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ABSTRACT

Background: Okra, *Abelmoschus esculentus* L., is an important crop in Iraq because of its nutritional and industrial uses. Diseases and pests have severe problems for okra farming, chiefly root-knot nematodes (*Meloidogyne javanica*), which lower yield. By evaluating the efficiency of biological control agents, such as Abamectin, in reducing the effects of root-knot nematodes, improving okra growth and increasing nutrient uptake (NPK) as an alternative to dangerous chemical pesticides, this study aims to improve sustainable agricultural practices.

Methods: To evaluate biological agents against *M. javanica* in okra, a field experiment was conducted at the University of Kufa utilizing a completely randomized block design (CRD) with three replications. Sterilized soil was used to sow okra seeds and each pot was given with 2500 nematode eggs. Streptomyces *Paecillomyces variotii*, avermitilis and Celest 10% FS were among the seed treatments. By removing and counting second-instar larvae from egg masses, nematode populations were assessed and growth parameters such as plant height, weight, fruit yield and nodes were measured.

Result: In comparison to controls, *S. avermitilis* and *P. variotii* considerably decreased *M. javanica* populations, enhancing plant growth indices (weight, fruit yield, height and nodes). Furthermore, okra plants' absorption of phosphorus (P), nitrogen (N) and potassium (K) was improved by biological treatments. Abamectin was one of the combination treatments that successfully reduced the quantity of nematode eggs per root. Significantly, the development of okra plants was unaffected by these biological agents, demonstrating their potential as long-term nematodes.

Key words: NPK, Okra, *Paecillomyces variotii*, *Streptomcyes avermen.*

INTRODUCTION

Okra (*Abelmoschus esculentus* L.) is considered an herbaceous plant belonging to the Malvaceae family, which is one of the most important and widespread plant families and has high importance, whether it is food, such as okra, or industrially, for the sake of fiber, such as cotton and others are cultivated to benefit from it medicinally (Miead and Aljuaifari, 2023). Okra in Iraq, which is considered one of the subtropical countries and its cultivation is widely spread, especially in the western, central and southern regions (ICSO, 2016). They are eaten after cooking, drying, freezing or canning for the purpose of consumption in other seasons such as winter because they contain some nutritional elements such as potassium, calcium and phosphorus (Iqbal *et al*., 2011). This vegetable crop is grown in all governorates of Iraq, according to ICSO (2017). It also recorded a decrease in production quantities due to infection with many pathogens and pests that affect okra plants (FAO, 2018). The cultivation and production of okra (*A. esculentus* L.) is increasing in Najaf Governorate, as is the case in most governorates of Iraq, from one season to another and from year to year. The productivity of one dunum (1/4 hectare) was estimated at 950, 1149, 3175 and 2312 kg/dunum in the growing seasons of 2010, 2012, 2015 and 2018, respectively. However, the total productivity of okra in Iraq in 2011 increased by 4.7% from 2010 and decreased by more than 85% for 2017 (ICSO, 2018).

As well as the effect of different environmental conditions and the sensitivity of the plant to infection with pathogens such as fungi, bacteria and nematodes (Khan *et al.*, 2002; Aljuaifari *et al*., 2019) and other pests have a major role in reducing production (Hail *et al*., 2007). Root-knot nematode. (*Meloidogyne javanica*) which is the most

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How to cite this article: Sheerali, S.M.R., Aljuaifari, W.A.R. and Alyasiri, H.I. (2025). Effect of Biological Factors in Physiological and Growth Indicators of Okra Plant [*Abelmoschus esculentus* (L.) Moench] and Percentage of (NPK) Elements with Root-Knot Nematode (*Meloidogyne javanica*). *Agricultural Science Digest*. **45(6):** 1011-1017. doi: 10.18805/ag.DF-735.

Submitted: 08-03-2025 **Accepted:** 12-05-2025 **Online:** 02-07-2025

widespread and harmful types of plant parasitic nematode to various vegetable crops in Iraq (Stephan and Abu-Gharbieh, 2010; Rani and Kumary, 2021) and this is due to the large losses they cause due to their increasing numbers and spread in the soil (Stephan *et al*., 1998). This type of nematode infects approximately 100 different plants. Four genera of root-knot *nematodes Meloidogyne incognita, M. javanica, M. arenarea* and *M. hapla* were discovered and they are the most harmful to vegetables (Taylor *et al*., 1982).

One of biological agent is *Paecilomyces variotii* that have used against root-knot nematode, *P. variotii* was discovered at the International Potato Center in Peru by Jatala *et al.* (1979). *P. variotii* is considered among the most commonly used fungi to combat plant parasitic nematode. *P. variotii* is distinguished by its global spread and high growth speed. The genus includes 31 species (Inglis *et al*., 2006; Anver, 2023).

Another agent of biological control is Abamectin product that have development taking place in the manufacture of chemical pesticides, which has played a major role in controlling a wide range of pests, has found that many of these pesticides have a harmful effect on humans and non-target organisms, which negatively affects the environmental balance (Ishaaya, 2012). Abamectin is one of the biocides produced from the fermentation of a bacterium found in the soil called *Streptomyces avermitilis*, which affects boring, sucking insects and nematodes parasitic on plant roots (Aljaafri, 2017). NPK plays good roles to improved plant growth development (Nagegowda and Senthivel, 2020; Jagadesh, 2022) as have seen in many studies before for that reason this study came to saw effect of biological agents to against and management Root-knot nematode (*M. javanica*) and study effect of these agents on NPK percentage in okra plant that had grown in the greenhouse.

MATERIALS AND METHODS

Okra seeds

Seeds used in the study: Okra seeds of the Betraa variety, with a germination rate of 97% and purity of 100%, which are characterized by high sensitivity to root-knot nematodes (Kandouh, 2019) and millet seeds were used to load the fungi on them. *P. variotii*, a purified isolate of the *P. variotii* was obtained from the Plant Pathology Laboratory in the Department of Plant Protection at the College of Agriculture - University of Kufa. The method of Dewan (1988) was followed in using millet, *Panicum miliaceum,* seeds to load the inoculum of the fungus *P. varioti* (Hnoosh and Aljuaifari, 2020).

Extraction of root-knot nematode (*M. javanica*)

Extraction of root-knot nematodes from the soil and estimating the numbers of juveniles in them, collect 500 grams of soil surrounding the roots of the infected okra plant in the field and place it in a 4-liter bucket to fill it with water, then mix it well by hand and pour it into a 60 mμ sieve with another bucket underneath it to get rid of parts of the roots and large impurities. Then the mixture is taken in the last bucket to be poured through another sieve 325 mμ and gently apply a stream of running water to it to remove the clay and collect the sediments at the bottom of the sieve Collect it in a 50 ml tube and leave it for one hour to ensure that all the plankton goes to the bottom to get rid of the excess water. The tube is then shaken again to mix its contents and distribute it to the tubes of the centrifuge and put it at a speed of 1500 rpm for 6 minutes. Gently, ensuring that the sediment remains in the tube, add the sugar solution (which was prepared previously). By dissolving 225 grams of table sugar in 500 ml of distilled water, the sediment is then stirred to homogenize it with the sugar solution inside the tube, then it is returned to the centrifuge again at the same speed and for one minute. The solution is poured into a 500 mμ sieve and washed with sterile distilled water to remove the sugar solution and remaining impurities then collected juveniles in the sieve after pouring water Gently rub it and place it in a 50 ml plastic cup. Take 20 ml of it in a graduated Petri dish to estimate the number of juveniles and count it (Aljaafri, 2017; Hnoosh and Aljuaifari, 2020).

Method for extraction of eggs and preparing root-knot worm inoculum *M. javanica*

The infected okra seedlings were uprooted and their roots were gently washed with water to get rid of the dust stuck to them. They were then cut into small pieces approximately 1-2 cm long and 40 grams of them were placed in a half-liter baker and 200 ml of NaOCl (minor) sodium hypochlorate solution (minor) was added to it at a concentration of 10%. Shake and rub the roots by hand for 3 minutes, then pour the brewer's content into a 200 mμ sieve. To prevent root pieces from falling, place a 450 mμ sieve under it to collect the nematode eggs. Then, quickly wash the eggs while they are in the sieve with running water well to get rid of the remaining NaOCl (Hussey and Barker, 1973). To obtain second-instar larvae, the eggs were incubated in dishes containing sterile water at room temperature for five days. Then the juveniles are collected using a 500 mμ sieve, then their numbers are estimated by taking 20 ml of the suspension and placing it in a petri dish that has been prepared in advance to count the worms, then the number of juveniles in the total volume of the solution is estimated. In the same way, the number of eggs is calculated before hatching, which were then added to the soil that was to be contaminated with root-knot nematode, at a rate of 2500 eggs (Aljaafri, 2017; Kandouh, 2019).

Leaves content of nutrients N, P, K (%)

Leaf samples were collected from each green manure, leaves 4 and 5 were taken from each branch and each experimental unit and dried in an electric oven at 65°C until the weight was constant. Then they were ground and 0.2 grams of the ground plant sample were taken. The samples

were digested by adding 4 ml of concentrated sulfuric acid and 2 ml of concentrated perchloric acid then estimate nitrogen using Kjeldahl device. Total phosphorus was determined using ammonium molybdate and measured with a spectrophotometer at a wavelength of (880) nm. As for potassium, it was determined using a flame photometer (Al-hadrawi, 2020).

Measurements growth of plants and development and nematode development

This study had taken more than two years, it was starting from spring 2022 until summer 2024. Plant growth development which was including (plant height/ cm^3, weight of plant, weight of roots, weight dry of plant, weight dry of roots, weight of fruits/ grams, number of fruits per plant, number of nodes) were measured after 60 days of planting for okra plants in the greenhouse. Root-knot nematode has been counted, number of juveniles and eggs for root-knot nematodes (Aljaafri, 2017). Statistical Analysis The experimental design was a random complete design. Data were analyzed using SAS (Version 9.2 SAS Institute Inc., Cary, NC). (Aljaafri, 2017).

RESULTS AND DISCUSSION

The effect of the biological seeds treatments that have used in the greenhouse test with rot-knot nematode (*M. javanica*) on the severity of infection with okra plants in the greenhouse on plant growth development after 60 days of planting

The treatments given in Table 1 obviously demonstrate the efficiency of biological control agents used in this research. The superior performance of the combined treatment (*S. avermitilis* + *P. variotii*) suggests a synergistic effect, that targeting both nematode eggs and juveniles simultaneously provides comprehensive root protection, leading to significantly improved plant vigor and nutrient uptake compared with the individual applications. Results became clear from the study as mentioned in the (Table 2) that the infection severity in the treatment of the interaction between Root-knot nematode (*M. javanica*) and biological agents that were including *P. variotii* and *S. avermentilis* was highest in the improved plant growth development that infected with root-knot nematode *M. javanica*, which reached to 87.34 cm^3, 42.12 grams.30.98 grams, 19.23 grams, 6.99 grams, 11.43 grams, 4.09 per plant 7.90 per plant by the treatment *P. variotii* + Abamectin (*S. avermentilis*) + *M. javanica* compared to untreated treatment (*M. javanica* only) which was 38.98 cm^3, 17.34 grams, 10.45 grams,7.46 grams, 2.03 grams, 4.03 grams, 3.03 per plant, 5.08, per plant in the plant height/ cm^3, weight of plant, weight of roots, weight dry of plant, weight dry of roots, weight of fruits/ grams, number of fruits per plant, number of nodes) respectively. It was also clear from the study that all treatments of the biological seeds' treatments improved plant growth development of okra that had been grown in the greenhouse. The improved plant growth of okra that had used with biological treatments because of activity of these products to increased plant growth development as had been showed in previously studies by (Aljaafri, 2017, Hnoosh and Aljuaifari, 2020). As for the effect of root-knot nematodes, *M. javanica* as showed in previously study showed decreased plant growth development on okra plant

Table 1: Biological seeds treatments that have used in the greenhouse test with rot-knot nematode (*M. javanica)*.

Treatments	Measured that used
Celest 10% FS + *M. javanica*	1.8 MG/litters + 2500 eggs of *M. javanica*
P. variotii + *M. javanica*	*P. variotii* + 2500 eggs 10^7 of *M. javanica*
Untreated seeds (Control) + *M. javanica*	2500 eggs of *M. javanica*
Abamectin (*S. avermentilis)* + *M. javanica*	50 cm^3/100 litters water+ 2500 eggs of *M. javanica*
P. variotii + Abamectin (*S. avermentilis*) + *M. javanica*	*P. variotii* + 50 $cm^3/10^7$ 100 litters water + 2500 eggs of *M. javanica*

Table 2: Effect of the biological seeds treatments that have used in the greenhouse test with rot-knot nematode (*M. javanica*) on the severity of infection with okra plants in the greenhouse.

Treatments	Height plant /cm^3	Weight of plant /grams	Weight of roots /grams	Dry weight of plant /grams	Dry weight of roots /grams	Weight of fruits /grams	Number of fruits per plant	Number of nodes per plant
1	59.34	23.54	15.43	9.56	4.76	5.88	4.6	6.80
2	74.43	34.23	17.73	13.78	3.98	6.34	3.12	6.66
3	38.98	17.34	10.45	7.46	2.03	4.03	3.03	5.08
4	80.34	38.09	28.54	18.09	6.12	7.98	5.09	7.24
5	87.34	42.12	30.98	19.23	6.99	11.43	4.09	7.90
P-Value	0.0021	0.0010	0.0043	0.060	0.050	0.065	0.043	0.056
L.S.D 0.05	8.798	12.021	8.43	5.32	3.12	4.87	0.91	1.34

that planted in the field infected with root-knot nematode by (Hnoosh and Aljuaifari, 2020). In other hand most of treatments did not have any negative effect on plant growth development that treated with biological products as had seen by these results mentioned in (Table 2). The general name of the pesticide is Fludioxonil and it belongs to the chemical group Phenylpyrrole. Its molecular formula is $C_{12}H_6F_2N_2O_2$. It is used to protect against many fungal pathogens that infect seeds and foliage The soil or storeroom is treated with it before planting and these pathogens were including many pathogens and 1.5 ml/kg for many vegetable crops such as potatoes, okra and cucumbers, which encourages and stimulates healthy plant growth throughout the season and thus this is reflected positively in increasing in the quantity and quality of the crop (Mahmood and AL-Abedy, 2021; Hnoosh and Aljuaifari, 2020).

The effect of the biological seeds treatments that have used in the greenhouse experimental with rot-knot nematode (*M. javanica*) on the severity of infection with okra plants in the greenhouse on nematode life stages development after 60 days of planting

The results of the experiment showed in (Fig 1) that treated the seeds of okra with some biological agents that were including *P. variotii* and *S. avermentilis* and fungicide product all of them led to reduced number of juveniles for rot-knot nematode in 500 grams soil that planted in plastic bag for period of time. While the treatments with these agents gave positive results in reducing juveniles of rot-knot nematode (*M. javanica)* especially in treatment *P. variotii* and *S. avermentilis* together with *M. javanica* which was 133.333 juveniles per 500 cm^3 compared to untreated treatment that was 2106.666 juveniles per 500 cm^3.

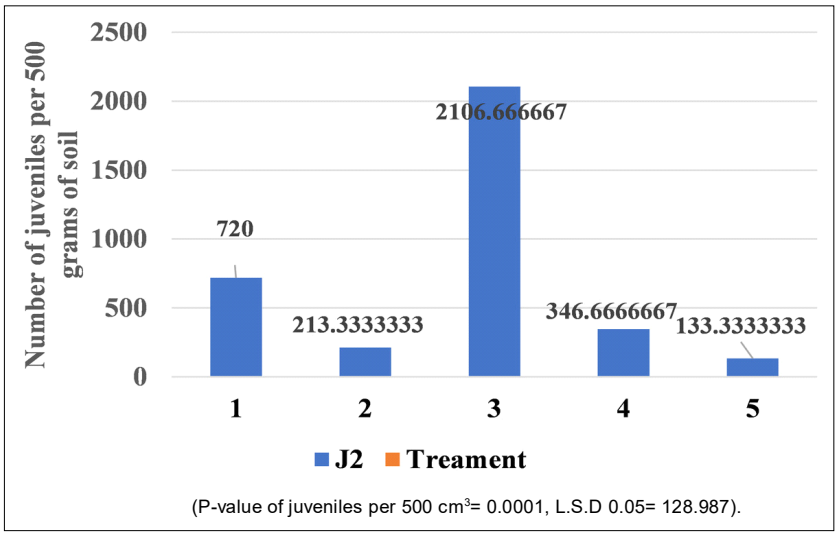


Fig 1: The effect of the biological agents that have used in the greenhouse test with rot-knot nematode (*M. javanica*) on nematode juveniles' development in 500 grams soil after 60 days of planting.

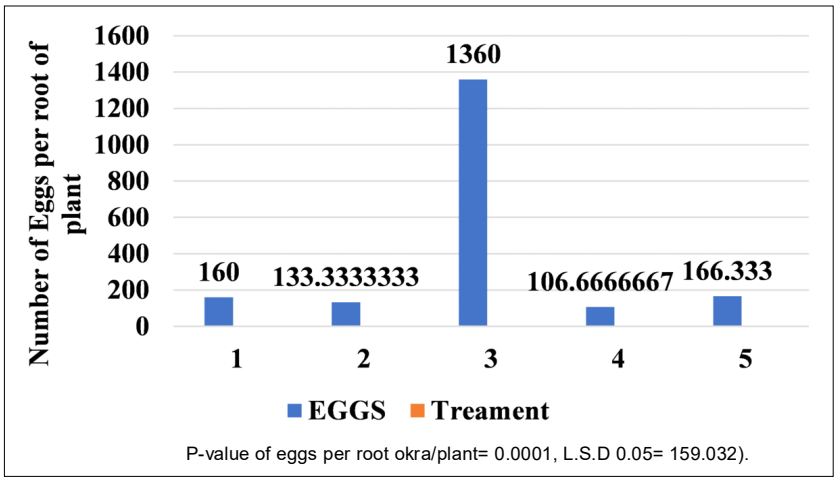


Fig 2: The effect of the biological agents that have used in the greenhouse test with rot-knot nematode (*M. javanica*) on nematode eggs development in each plant after 60 days of planting.

In addition, as mentioned in (Fig 1) most of treatments were positive to reduced number of juveniles for rot-knot nematode (*M. javanica*) compared to untreated treatment (control treatment). That reason for reducing life stage development for rot-knot nematode (*M. javanica*) because of all of these products including enzymes effect of nematode development as mentioned in previously study by Hnoosh and Aljuaifari, (2020). In addition, other researches showed activity for fungicide Celest is one of the most important pesticides used in fogging seeds and seeds before planting in the field to protect them from attack by pathogens endemic in the soil (Agrios, 2005), which allows the plant to germinate. Also, Aljaafri, (2017) showed that the use of the biopesticide Abamectin led to an increase in the growth and improvement of soybean plants grown in the greenhouse and reduced life stages development of rot-knot nematode after treating the seeds with this biopesticide. Abamectin is considered a biopesticide and is produced through the fermentation of microorganisms that live in the soil known as *Streptomyces avermitilis* if this pesticide is considered one of the most important biopesticides used in combating sucking insects and mites, as well as tunnel makers, which mainly interferes with the physiological activity of the insect and leads to killing and paralysis.

Effect of the biological agents that have used in the greenhouse test with rot-knot nematode (*M. javanica*) on nematode eggs development in each plant after 60 days of planting

At 60 days of planting, the results of the statistical analysis (Fig 2) showed that there were highly significant differences in the reduction in the number of eggs of the root-knot nematode *M. javanica* present in the experimental that planted in plastic bag in the greenhouse compared to the comparison treatment. It was shown that treating the seeds with *P. variotii* + *M. javanica* and Abamectin (*S. avermentilis*) + *M. javanica* treatments were given good results to reduced number of eggs for Rot-knot nematode *M. javanica* which were 106.666 and 133.333 eggs of *M. javanica* / Rot of okra respectively compared to untreated treatment (control) that was 1360 eggs of *M. javanica* / root of okra. Other treatments also were significantly to reduced number of eggs for eggs of *M. javanica* / root of okra compared to control treatment. These results showed to reduced number of eggs of eggs of *M. javanica* / root of okra related to effect of biological agents against Root-knot nematode as shown in previously studies by (Hnoosh and Aljuaifari, 2020; Miead, 2023) to reducing number of eggs by biological seed treatments that had been done in the field experimental. The results that shown of reducing number of eggs by abamectin treatment in reducing the number of root-knot nematodes in field soil these also had been done by Khalil *et al.* (2012) demonstrated, by experimenting with pots in a greenhouse, the effect of Abamectin in reducing the number of egg sacs, the number of eggs in one bag, as well as the number of root knot nematode in the roots of tomato plants, as well as the nematodes community in the soil by a good percentage in reducing. It has been proven (Aljaafri, 2017) that the biopesticide Abamectin has an important role in reducing the numbers of eggs and larvae in some types of plant-pathogenic nematodes. There are also many studies that have proven that *P. variotii* has an important role in controlling some types of nematodes.

Table 3: Effect of the biological agents that have used in the-green house test with rot-knot nematode (*M. javanica*) on percentage of NPK elements that extracted of okra leaves which already infected by *M. javanica*.

Treatments	K	p	N
1	1.038	0.806	1.699
2	1.747	1.185	2.297
3	0.517	0.232	1.404
4	2.036	1.476	3.595
5	1.944	1.037	2.847
P-Value	0.0001	0.0021	0.0001
L.S.D 0.05	0.231	0.432	0.701

Effect of the biological agents that have used in the greenhouse test with Rot-knot nematode (*M. javanica*) on percentage of NPK elements that extracted of okra leaves which already infected by *M. javanica*

The results of the experiment showed in (Table 3) that treating the seeds with biological compounds and the *P. variotii* led to increase in the growth indicators of okra plants grown in the greenhouse at the end of the experiment. The results of the statistical analysis showed that there were clear significant differences in the increase in percentage of NPK in all the biological sed treatments of okra plants grown greenhouse compared to untreated treatment. As had been shown from (Table 3) the treatments with Abamectin (*S. avermentilis*) + *M. javanica* and *P. variotii*. + Abamectin (*S. avermentilis*) + M. javanica was high significantly to give high percentages of NPK which were 2.036, 1.476 and 3.595 and 1.944, 1.037, 2.847 NPK elements respectively in those treatments compared to control treatment that was 0.517, 0.232, 1.404 percentages of NPK of okra plants. Besides that, other treatments also were significant to increase NPK percentage compared to untreated treatment as had been shown in the (Table 3). Those results showed to increase percentages of NPK related to use biological products that have positive effect to increase availability of NPK elements in the plant as showed in previously study by (Hnoosh and Aljuaifari, 2020) showed improving growth of okra plants that grown in the field because used of (*S. avermentilis*) and *P. variotii* on okra as biological agents against rot-knot nematode. NPK fertilizer increased protein, ash, carbohydrates, mucilage N, P, K Ca and Mg contents of okra fruits compared with the control. Fat contents of okra fruits were reduced with different organic sources and NPK fertilizer compared with the control (Adekiya *et al*, 2020). The NPK fertilizer have been increased plant growth

development which was including plant height, number of leaves, stem girth and pod yield of okra plants compared with the control treatment that had been done by Adekiya *et al.* (2020).

CONCLUSION

Results *P. variotii* and *S. avermentilis* was highest in the improved plant growth development that infected with root-knot nematode *M. javanica*, also there was no negative effect on plant growth development. Besides that, there was good effect of these products to reducing number of juveniles and eggs of rot-knot nematode (*M. javanica*) compared to untreated treatment. The results of the statistical analysis showed that there were clear significant differences in the increase in percentage of NPK in all the biological sed treatments of okra plants grown greenhouse compared to untreated treatment.

Conflict of interest

All authors declared that there is no conflict of inerest.

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