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Examination of *Burkholderia renojensis*, *Streptomcyes avermentilis*, and *Bacillus firmus* to management of *Meloidogyne incognita* on corn

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Abstract:

Growers continue to look for new innovative ways of boosting corn yields. Seed treatments can be an effective way of protecting early seedlings from disease and nematode. For this study, the objective was to evaluate various corn seed treatments for plant growth, and management of *Meloidogyne incognita* population. Experiments were conducted to determine the efficacy of selected potential biological control products (Untreated seeds, *Burkholderia renojensis*, *Streptomcyes avermentilis*, and *Bacillus firmus)* for the management of the root-knot nematode (*M. incognita*) on corn. Test was conducted in the greenhouse at the R.R. Foil Plant Science Research Center at Mississippi State University. The study included the effect of these treatment on plant growth and nematode development in greenhouse. Plant growth parameters measured included fresh weights, height, and root weight. Nematode parameters included gall formation on the roots, number of egg mass, number of juveniles in the soil, and eggs produced. A statistically significant difference ($p \leq 0.05$) was obtained regarding the growth rate of plant development for corn with treatments *B. renojensis* alone and *B. renojensis* +*S. avermentilis*+ *B. firmus* to improve plant growth also, no negative effects were recorded on plant growth. Nematode development included gall formation on the roots, number of egg mass, number of juveniles in the soil, and eggs produced was reduced by *B. renojensis* alone and *B. renojensis* +*S. avermentilis*+ *B. firmus* compared to control treatment. The mean amounts of J2 population was expressed with corn plant with Root-knot nematode in treatments (*B. renojensis* alone and *B. renojensis* +*S. avermentilis*+ *B. firmus*) respectively were 1482.8 and 515 J2 respectively compared to the control with not nematicide was 10815. Also, the same treatments high significant to reduce number of eggs, galls, and egg mass compared with control. Also, there is not significant differences on plant growth for soybean plants with Root-knot.

INTRODUCTION:

Corn *(Zea mays* L.) is important crop in all the world. (*Zea mays* L.) is one of the most important cereals crops used in the human diet in large parts of the world and an important feed component for livestock. Global production exceeds 600 metric tons (1), with about 60% produced in the developed countries, particularly by the United States of America, China produces 27% of the world's maize and the rest is grown in countries of Latin America, Africa and Southern Asia with a large proportion being produced in the tropics and subtropics. *Meloidogyne incognita* (Kofoid & White) Chitwood is commonly found associated with corn (Zea mays L.) in the southeastern United States (2; 3). Although corn is considered tolerant to *M. incognita*, yield losses in heavily infested fields may be 30% or greater (3). Corn included in cropping systems in fields infested with *M. incognita* can result in 10- to 20-fold greater nematode populations (2; 4, 5). This increase in *M. incognita* numbers may adversely affect yields of root-knot susceptible crops following corn. *M. incognita* is microscopic roundworms is found in a wide range of homeland and agroeco systems (6). *M. incognita* is a sedentary endoparasites pathogen feeding into the root and tuber cells. The egg mass is account 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. The J2 establishes a nutrition site primary in bark /or relative parenchyma cells. Those cells can gain a high metabolic activity after genetically modified by excretion of the root-knot (6). 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. Foliar symptoms include slight to severe stunting, chlorosis, and nutritional deficiency (7). Management practices for controlling *M. incognita* include cultural practices, resistance, and use of nematicides. Biological control is one of methods used to soil-borne pathogens by introduced microorganisms has been calculated for through 65 years (8), but over extreme of that time it has not been considered commercially practical. Biocontrol of nematodes was top studied via Duddington (9). The expansion of biological dominance agents is also considered an active stand by nematode control on the vegetables (10). Seed treatments are chemical or biological substances that are applied to seed or vegetative propagation materials to control disease organisms, insects or other pests. Although seed treatment has been a widely accepted practice in the United States for over 30 years, the practice has been recognized as beneficial for hundreds of years. Seed treatments include bactericides, fungicides, insecticides, nematicides and herbicides known as safeness (11; 12). Currently numerous biological compounds are being tested to determine their efficacy to the nematodes. *Burkholderia renojensis* plays a

good role as a biocontrol agent related to the enzymes that have produced by this genus of bacteria. These enzymes are included lipolytic, proteolytic, and hemolytic which have activity as toxin or antibiotics. (13). Some strains of *B. renojensis* produced some of antifungal products, that can be used as antibiotics for pathogens as management. (14). *B. renojensis* has been shown activity in the fixing atmospheric nitrogen, potential uses as biocontrol, and help to plant growth encouragement. (15). Abamectin (*Streptomcyes avermentilis*) is nematocidal to *M. incognita* and exposure for 1hr to concentrations greater than 0.39 µg/ml for *M. incognita* inhibits tomato root infection (16). Applying abamectin near plant-parasitic nematodes has been effective in suppressing infection (17). In 2005, an abamectin formulation (Avicta 500 FS) was marketed by Syngenta Crop Protection as a seed treatment for early season control of nematode damage to cotton seedlings. However, abamectin seed treatment variable in suppressing *M. incognita* and increasing cotton yields in nematode-infested fields (18; 19). The main objective of this study is to find out the best way to management the disease that causes by plant parasitic nematodes. Using different bacteria to control on the diseases on corn plants with biological control to *M. incognita*.

MATERIALS AND METHODS:

Plant Growth/Inoculation with Root Knot (*Meloidogyne incognita*)

Nematode cultures: *M. incognita* was originally isolated from corn and maintained in the greenhouse on corn *(Zea mays* L.). Eggs were collected from the plants with age eight to ten weeks -old of *M. incognita* cultures with NaOCl (20). Second-stage juveniles (J2) were collected in hatching chambers with a 20-µm pore screen that allows only hatched J2 to migrate into the collection dish. Seeds were previously treated with the appropriate experimental biological compound (Table 1). Seeds were planted into 15 cm dia. clay pots filled with an autoclaved freestone fine sandy loam sand: soil mix (1:1, v/v) (11; 12). Plants were grown first by sowing one seed directly in pots filled with 500 cm3 of the sterilized soil-sand mixture under greenhouse conditions. A suspension of 2000 from nematodes (Root-knot) was pipetted into each pot at the time of planting. All experimental treatments are arranged in a RCBD with 5 replications in the greenhouse at approximately 30°C with artificial light 12 hours/day. Data acquired analyzed using a statistical test system (SAS) version 9.4. and the standard of significant was collection at 5%. Least significant difference (LSD) tests at $P = 0.05$ employed to the differences among treatments for the parameters measured. Plants watered every day around 4 times and fertilized weekly. At 60 days, tests were harvested. Root-Knot nematode to set the nematode extraction, roots were located into a beaker with sufficient 10% chlorine bleach solution to coating the roots. The roots were mad for 4 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. (Sieve: No. 200, USA standard test sieve. Fisher scientific Company, 75 micrometers. Sieve: No. 500. USA standard test sieve. Fisher Scientific Company, 25 micrometers). 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. (Sieve No. 60, USA standard test sieve. Fisher Scientific Company, 250 micrometers. Sieve No. 325, USA standard test sieve, 45 micrometers. Fisher Scientific Company, USA.) 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 30-40 ml juvenile egg extract was collected by washing the 325-mesh sieve extract into a 150 ml beaker. The beaker content could settle for 2 hours. After 2 hours, 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 from International Equipment Company (Model 120 Size 2 50/60 Hertz, 7.3 amps). 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 microscope (Japan Model C35AD-4) at 40X magnification (11; 12).

Table (1) Bacteria *Burkholderia renojensis, Streptomcyes avermentil and Bacillus firmus* that used in the experiments treated with corn seeds infested with *M. incognita*.

Treatment	Product	Rate
1	Untreated seeds (Control)	
2	*Burkholderia renojensis*	6 floz/cwt
3	*Burkholderia renojensis*	8 floz/cwt
4	*Streptomcyes avermentilis (Abamectin)*	4 floz/cwt
5	*Bacillus firmus + Burkholderia (renojensis*	3 + 6 floz/cwt
6	*Burkholderia renojensis + Bacillus firmus + Streptomcyes avermentilis*	8 + 3 + 4 floz/cwt

Measurements and Parameters: Plant growth and nematode life stages development

Plants were harvested for evaluation of fresh weight, height of plants, and root weight. All these experiments with different treatments of biological seeds treatments (Table1) 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. Egg Masses Staining: Egg masses of *M. incognita* was stained by dipping the roots in 0.015% Phloxine B solution for 20 minutes as described by (20) and then washing the stained

roots with tap water to remove the residual Phloxine B (11).

Root image acquisition and analysis:

The root scanned to determine acquired images analyzed for the cumulative root length, surface area, average root diameter, root volume, number of tips. number of Forks, and number of crossings with using winRHIZO Pro software (Version 2009c, Regent Instruments, Inc.). Roots cut and separated from the stems and washed thoroughly avoiding any disturbance to the root system. The cleaned individual root systems will be floated in 5 mm of water in a 0.3 × 0.2 m Plexiglas tray. Greyscale root images acquired by setting the parameters to ''high'' accuracy (resolution 800 by 800 dpi), (11; 12).

RESULTS:

All the experiments with different seed treatments (*Burkholderia renojensis, Streptomcyes avermentilis,* and *Bacillus firmus)* that was including different rates of biological control, there was no negative effect on plants growth and development occurred by *Meloidogyne incognita* when using these treatments compared with control without treatment (Figure1). Results referred had been shown a positive effect to improve plant growth of corn plants with these tratments (Table 1). Results were a significant effect on weight of plant and roots weight with treatment (*Burkholderia renojensis at rate* 8 floz/cwt and *Burkholderia renojensis + Bacillus firmus + Streptomcyes avermentilis* at rates 8 + 3 + 4 floz/cwt) were 37.9 and 40.98 grams compared to 24.6 grams in the control treatment. In the same treatment did not show any significant different with height of corn plants compared to control treatment. However, there was a significant effect on weight of roots with these treatments compared to control treatment (Figure 1).

In the end of the experiment, nematode life stages development of *M. incognita* was effect by treatments (*Burkholderia renojensis, Streptomcyes avermentilis,* and *Bacillus firmus).* Results had been shown a significant effect to reduce number of juveniles and eggs of *M. incognita* with treatments (*Burkholderia renojensis* at rate 6 floz/cwt and *Burkholderia renojensis + Bacillus firmus + Streptomcyes avermentilis* at rates 8 + 3 + 4 floz/cwt) were 1482. 8 and 515 juveniles per 500 cm^3 soil and 4326 and 1965 eggs per 500 cm^3 soil in both treatments respectively compared to untreated treatment was 10815 juveniles per 500 cm^3 soil and 11433 eggs respectively in control treatment. Other treatments also were significant with different effect to reduce number of juveniles and eggs compared to control (Figure 2).

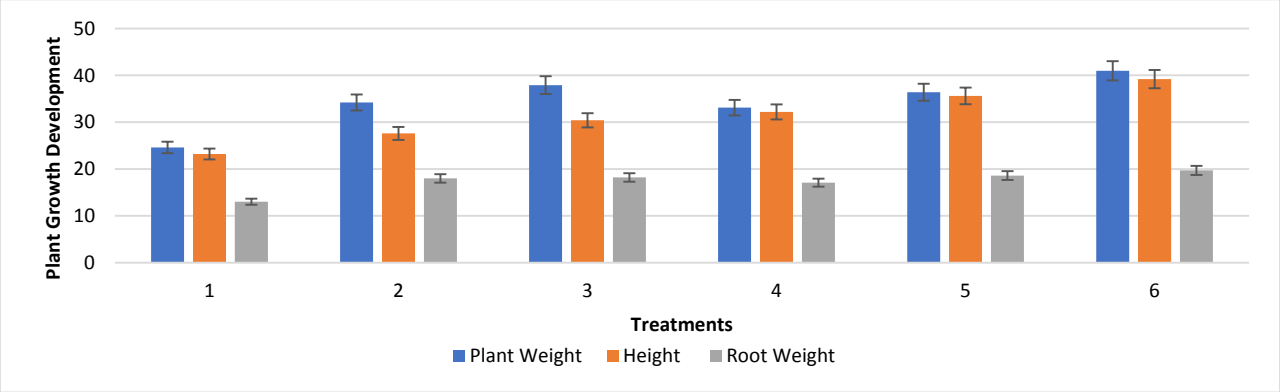


Figure 1: Effect of *Burkholderia renojensis , Streptomcyes avermentil and Bacillus firmus* on corn plants growth (plant weight, plant height, and roots weight) infested with *M. incognita.* Data are means of the 5 replicates for each treatment 60 days. Means compared by using (LSD) at 0.05. P-Value, Plant/ Weight grams, 0.00341, LSD 0.05= 3.032, Plant/ Height/cm3, 0.0821, LSD 0.05= 8.768, Roots weight/grams, 0.046, LSD 0.05= 1.43

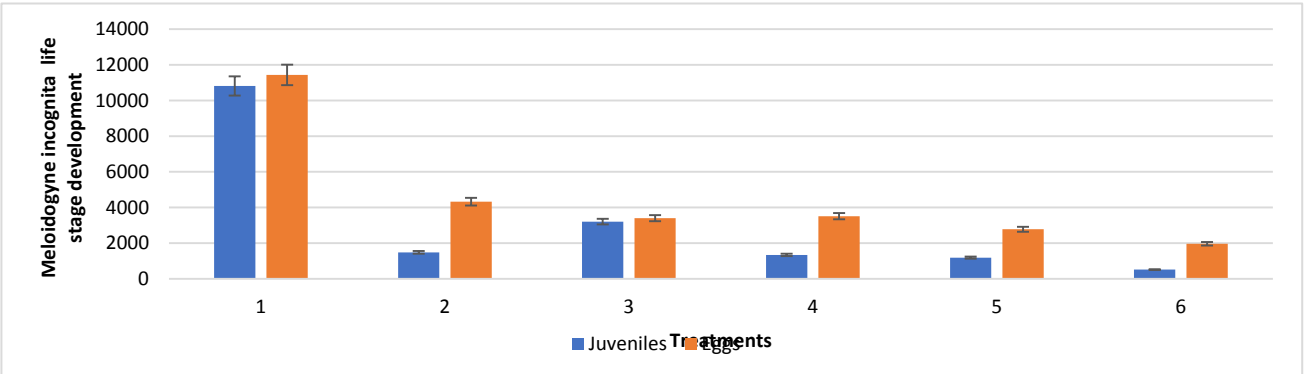


Figure 2: Effect of *Burkholderia renojensis , Streptomcyes avermentil and Bacillus firmus* on *M. incognita* life stage development (juveniles and eggs/ 500 cm^3 soil) on corn. Data are means of the 5 replicates for each treatment 60 days. Means compared by using (LSD) at 0.05. P-Value, Juveniles/ 500 grams soil, 0.00011, LSD 0.05= 3478.263, Eggs/500 grams soil, 0.00021, LSD 0.05= 2863.54

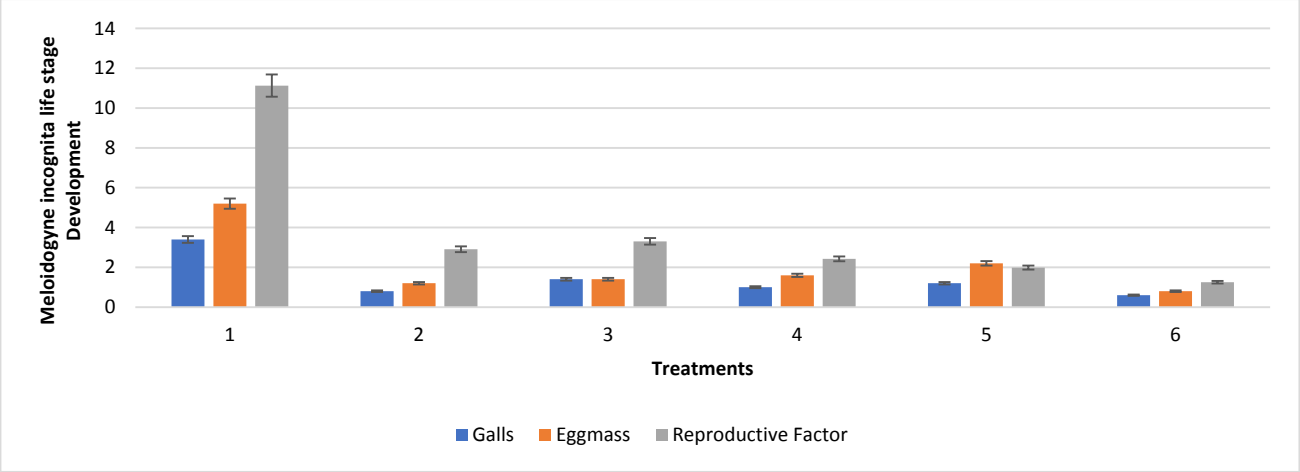


Figure 3: Effect of *Burkholderia renojensis , Streptomcyes avermentil and Bacillus firmus* on *M. incognita life stage development* (Galls, Eggmass, and reproductive factor) on corn. Data are means of the 5 replicates for each treatment 60 days. Means compared by using (LSD) at 0.05. Reproductive factor = Number of juveniles + number of eggs + number of galls + number of egg mass/ 2000 (number of eggs that infested with seeds). P-Value, galls/ roots plant, 0.0311, LSD 0.05= 1.02, Eggmass/ roots plant, 0.020, LSD 0.05= 1.45, reproductive factor/ plant, 0.0001, LSD 0.05= 3.023

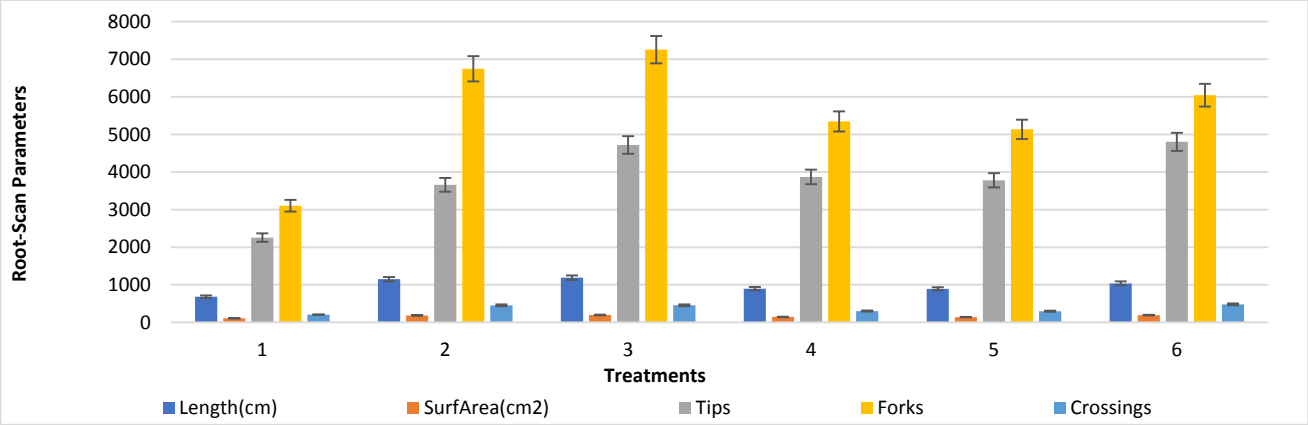


Figure 4: Effect of *Burkholderia renojensis , Streptomcyes avermentil and Bacillus firmus* on corn root development by root-scan by WinRHIZO optical scanner infested with *M. incognita.* Data are means of the 5 replicates for each treatment 60 days. Means compared by using (LSD) at 0.05. P-Value, Length (cm)/ roots plant, 0.0001, LSD 0.05= 168.98, Surfare (cm^2)/ roots plant, 0.0031, LSD 0.05= 32.45, Tips/ roots plant, 0.00051, LSD 0.05= 798.34, Froks/ roots plant, 0.0087, LSD 0.05= 2187.26, Crossings/ roots plant, 0.0341, LSD 0.05= 202.21.

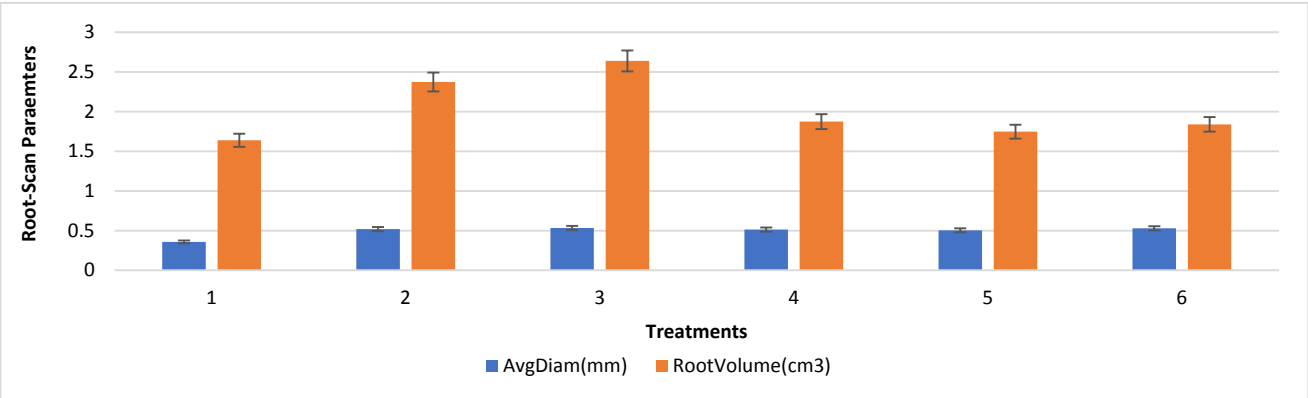
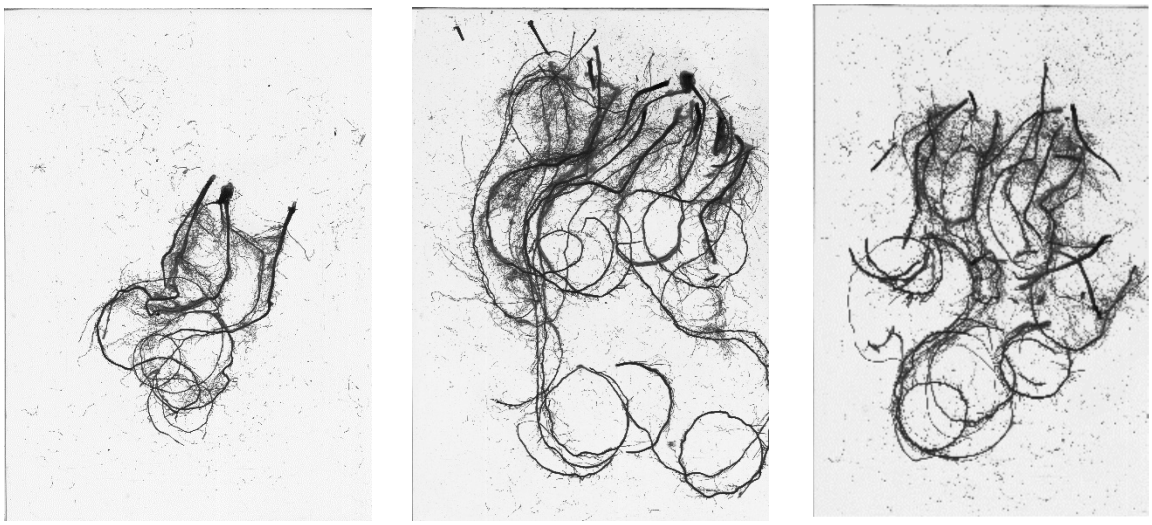
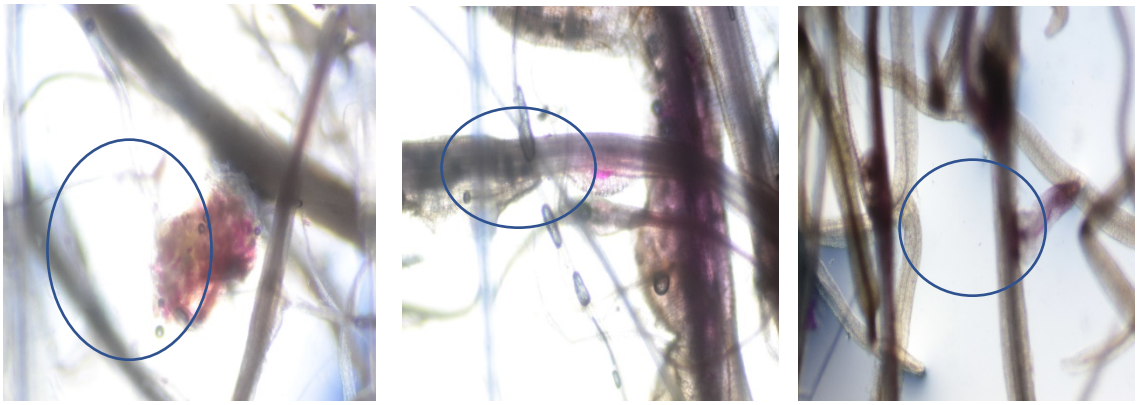


Figure 5: Effect of *Burkholderia renojensis , Streptomcyes avermentil and Bacillus firmus* on corn root development by root-scan by WinRHIZO optical scanner infested with *M. incognita.* Data are means of the 5 replicates for each treatment 60 days. Means compared by using (LSD) at 0.05. P-Value, AvgDiam (mm)/ roots plant, 0.0651, LSD 0.05= 0.365, Root Volume (cm^3)/ roots plant, 0.0421, LSD 0.05= 0.818



Control *Burkholderia renojensis* *Streptomcyes avermentil*

Figure (6) Root scanning of corn roots infested with *M. incognita* treated with *Burkholderia renojensis , Streptomcyes avermentil.*



(A) (B) (C)

Figure 7: *Meloidogyne incognita life stage* identified under microscope included (A) egg mass of *M. incognita* outside of the corn root, (B) adult female of *M. incognita* inside the root, (C) Juvenile second stage of *M. incognita* inside the root.

Nematode life stages development of *M. incognita* was effect by treatments (*Burkholderia renojensis, Streptomcyes avermentilis,* and *Bacillus firmus)*. Results had been shown a significant effect to reduce average of galls, eggs mass, and reproductive factor of *M. incognita* with treatments (*Burkholderia renojensis* at rate 6 floz/cwt and *Burkholderia renojensis + Bacillus firmus + Streptomcyes avermentilis* at rates 8 + 3 + 4 floz/cwt) were significant reduced average of galls, egg mass, reproductive factors compared to control treatment (Figure 3). Egg mass, juvenile second stage, and adult female were clear on the roots by taken images (Figure 7).

Figures (4, 5, 6) were the results of root-scan by WinRHIZO optical scanner had been shown different results most of them were significant to improve root growth compared to untreated seeds. The root scanned to determine acquired images analyzed for the cumulative root length, surface area, average root diameter, root volume, number of tips. number of Forks, and number of crossings with using winRHIZO Pro software had shown significant different compered to control treatment. In this study, root tips, forks, and crossings densities differed significantly with treatments (*Burkholderia renojensis* at rate 6, 8 floz/cwt and *Burkholderia renojensis + Bacillus firmus + Streptomcyes avermentilis* at rates 8 + 3 + 4 floz/cwt) compared to control Figures (4, 5).

DISCUSSION:

None of the bacteria *Burkholderia renojensis, Streptomcyes avermentilis,* and *Bacillus firmus* had a negative effect on plant growth and development when challenged with *Meloidogyne incognita.* All treatments had been shown significant difference to improve plant growth compered to control treatment. This result agreed with Aljaafri (11) show activity for different seed treatment to improve plant growth for soybean with different biological seed treatments. In addition, the experiments had shown activity to reduce number of life stages for *M. incognita*. Also, results for root scan had been shown significant effect to improve roots growth with *B. renojensis, S. avermentilis,* and *B. firmus* compared to control treatment. 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. In all field trials, incorporating abamectin into the soil was the method of choice to increase the probability of contact with plant-parasitic nematodes. Under controlled conditions, vegetable seed treated with abamectin provided protection against *M. incognita* (11; 21; 22). In addition, bacteria *B. renojensis* plays a good role as a biocontrol agent related to the enzymes that have produced by this genus of bacteria. These enzymes are included lipolytic, proteolytic, and hemolytic which have activity as toxin or antibiotics. (13). Some strains of *B. renojensis* has been produced some of antifungal products, that can be used as antibiotics for pathogens as management. (14). According to Wang, X. Q., et al (23) has been identified strain of *B. pyrrocinia* from tobacco rhizosphere shown significant effect as antifungal activities against fungi on plants and animal pathogens that was related to produce antifungal compounds as antibiotic and secondary metabolize by *B. pyrrocinia*. *B. renojensis* has activity in the fixing atmospheric nitrogen, potential uses as biocontrol, and help to plant growth encouragement. (15; 24; 25). *B. renojensis* has been described as biocontrol agent against mite and insect pests. (26). In summary, using different products included *Burkholderia renojensis*, *Streptomcyes avermentilis*, and *Bacillus firmus* as seed treatments from biological control to management nematodes on corn plants. All the biological products performed statistically better than the control regarding improve plant growth and reducing nematode (*M. incognita)* life stages. From of these different tests of biological control, results show improving plants make plants have some factors defense to the pathogen (27).

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