THE EFFECTS OF *BACILLUS SUBTILIS* BACTERIA ON *MELOIDOGYNE JAVANICA (NEMATODE)* INFECTION AND TOMATO PLANT GROWTH

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ABSTRACT

Root-knot nematodes (*Meloidogyne* spp.) are important pests of many cultivated plants. Recently the most efficient chemical control products of nematodes, (e.g. methyl bromide); have been restricted due to their toxic characteristics. This study was conducted in the area of tomatoes (*Lycopersicun esculentum Mill)*, which have been grown commercially . in order to isolate *Bacillus subtitles* bacteria from the soil to be used in biological control of root knot disease which caused by nematode *Meloidogyne javanica*. this to eliminate the use of agrochemical and their hazard on human health and environment. the results showed that the application of *Bacillus subtitles* bacteria reduced *Meloidogyne javanica* galls information and number of juveniles in the soil either as a seed treatment, root dipping or as a soil drench application but seeds treatment showed a little better result than the other application methods.

Keywords: Bio-control , *bacillus subtilis M. javanica.*

Introduction

Root knot nematodes (*Meloidogyne spp*), are worldwide in their distribution , attack a wide variety of crops ,and more than 3000 host species. .The four common root-knot nematode species, namely *Meloidogyne incognita,M. javanica,M. arenaria,and M. hapla* are the most abundant and damaging nematode of vegetables , (Maqbool and Shahina, 2001). Various species of *Meloidogyne* induce major morphological and physiological changes within roots, not only yield is greatly affected but quality is also reduced. (ZA.Siddiquiand Mahmood,(1999).

Control of plant parasitic nematodes is difficult because of the enormous variety of suitable hosts. (Stirling, 1991) Plant parasitic nematodes, are small microscopic roundworms that live in the soil and attack the roots of plants. Crop production problems induced by nematodes, therefore, generally occur because of root dysfunction, reducing rooting volume ,and foraging and utilization efficiency of water and nutrients. In many cases, a mixed community of plant parasitic nematodes is present in a Field, rather than having a single species occurring alone. In addition to the direct crop damage caused by nematodes, many of these species have also been shown to predispose plants to infection by fungal or bacterial pathogens or to transmit virus diseases, which contribute to additional yield reductions, (J. W. Noling, 2012).

Plant parasitic nematodes living belowground are difficult to control by chemical means because of large quantities and repeated applications required to treat the entire soil volume occupied by plant roots. A number of antagonistic bacteria have been reported in suppressing

soil-borne pathogens and enhancing plant growth. An advantage of targeted introduction of antagonists to the plant is that microbial populations can grow from a small quantity of inoculums and colonize the rhizosphere and root (Sikora, 1992).

A promising alternative is the use of microbial antagonists against plant parasitic nematodes which are ecofriendly and economically feasible approaches and does not allow the nematodes to develop into new races or biotypes. In addition, they are amenable for the mass production, formulation and easy delivery in the field. In recent years, Plant Growth Promoting Rhizobacteria (PGPR), viz. *Pseudomonas spp* ,and *Bacillus subtilis* (Oostendorp and Sikora, 1989) have been reported to be effective in boosting the plant vigor and also found to be deleterious to the plant pathogens and nematodes (Rodriguez Kabana et al., 1965; Singh et al., 1990).. The low level of control consistency of many biocontrol agents against soil-borne pathogens under field conditions is most likely due to the complexity and variability of the soil physics, chemistry and microbial activity in the soil as well as due to environmental factors (Weller, 1988).. The objective of the present studies were to study the effect of *Bacillus subtilis* bacteria on nematode population in the soil and gall formation of *M. javanica* and their effects on the plant growth.

MATERIALS AND METHODS

Collection of root Samples and survey

A total number of two sites were survey. From which fifteen samples of tomatoes infected with root knot nematodes (7 to 8) samples from each site, were collected rely on above ground symptoms.

Sampling from tomato plants.

Sampling from tomato plants done by uprooting the whole plant from soil using spade. Effort made to remove the entire root system by digging carefully around the roots. After excising the aerial portion ,and removing soil from the root system of the uprooted plants, the roots placed in bags. All The bags tied, and labeled . Nematodes extracted by the use of Whitehead and Hemming tray methods (Whitehead 1986).In this method, the infested roots with egg masses washed thoroughly under tap water. The roots along with soil were kept in the tray lined with tissue paper having sufficient water that roots and soil should dipped in water ,and after 24 hours, the water was poured off in a beaker ,and allowed to settle for one hour. When the juveniles had settled, the excess of water siphoned off until about 100 ml remained. The suspensions of juveniles (J-2s) taken with a pipette and three replicates of 2 ml of aliquots of J-2s were counted in a counting dish.

Identification of root knot nematode based on perineal pattern:

Galls with mature females selected and placed in a Petri-dish with tap water; root tissues torn apart with forceps and half spear to remove adult females. Necks of females were cut-off with the help of a half spear to remove the interiors. The cuticle then placed in to a drop of 45% lactic acid on a plastic Petri dish. Similarly, 5-10 cuticles collected in the drop and allowed them to stand for 30 minutes. The Cuticle cut in half (equatorially) with the help of modified common blade and a portion of cuticle with perineal pattern to square shape. The trimmed perineal pattern placed back in the 45% lactic acid and cleaned free from debris, using the pulp canal file. After cleaning, the perennial pattern was transferred to a drop of glycerin on a

clean micro slide and aligned in such a way that anus was oriented downward. A warm cover slip placed on the glycerin drop sealed with nail polish and labeled.

Mass culturing of root-knot nematodes

To multiply the culture of root-knot nematodes, the most susceptible variety of Tomatoes used. Three weeks old seedlings transplanted in pots containing 2.5 kg, 1:2 sandy: loam soil, sterilized with hot steam of water, one plant/pot. One week after transplanting, these plants were inoculated freshly hatched second stage juveniles of *Meloidogyne javanica*. Tap water used to irrigate young seedlings throughout out the period of study. The temperature range 20-30 °C recorded. All above steeps concern nematodes done in Ministry of Agriculture Research Center of Saudi Arabia.

Re-culture of Isolates of bacteria provided.

The isolate of bacteria, provided by King Fahd University microbiology laboratory where isolated ,purified and identified . All these isolates of bacteria re-cultured in Royal Commission laboratory. By suspend 28 g powder of nutrient agar in one litter distilled water and bring to the boil to dissolve completely. Sterilized by autoclaving at 121^0c for 15 minutes. After the liquid cool poured in Petri dishes, and sterilized under UV light for 10 minutes. Bacteria striped on the solid agar-using loop. The dishes covered by cellophane. Moreover, incubated in 37^oc for 48 hours until colony development observed. Five days later bacteria used in experiments.

Seeds treatment method of bacteria application experiment

The bacteria were pre-cultured on nutrient agar medium, after 48 hours
bacteria germinated. Five loops of bacteria suspended in sterilized distilled water. The tomatoes seeds were soaked in the bacterial suspension for 3 minutes using 1% gum Arabic as a sticking agent, and then seeded into pots containing a sterilized sand/loam mixture (1:2, v/v). Each pot received three seeds. Two weeks, after seeds germination plants thinned to one plant per pot. After 2 weeks, the plants were inoculated suspended juveniles of *M. javanica*. The inoculation of nematodes was carried out by drenching 5ml inoculums volume with the juveniles (2000 juveniles), into the soil around the roots. Seed sterile with 1% without Ca $(ocl)_2$ without bacteria served as control. Each treatment replicated 3 times. The experiment terminated 6 weeks after nematode inoculation. Tomato plant roots were wash free of adhering soil particles using tap water. The following measuring made for both treated and control

1- Fresh shoots and roots weight
2- The numbers of galls of *M. javanica* recorded,
3- Plant height measured
4- Nematodes population in the soil
5-Isolation of nematodes from 25 grams of treated and control soil and
Make account of nematodes

Root dipping method of bacteria application experiment.

Roots of three-week-old tomato plants dipped for 3 min into the bacterial suspension and then planted into pots containing a sterilized soil (sand /loam) mixture (1:2, v/v). After 2 weeks, the plants were inoculated with5ml (2000 juveniles) of *M. javanica* per pot. Roots without

bacteria served as control used. Each treatment replicated 3 times and terminated. 6 weeks after nematode inoculation. The same measuring made for both treated and control were recorded..

Soil drench method of bacteria application experiment:

Five ml bacterial suspensions pipette onto the soil surface around 3 week old tomato plants. Plants inoculated with 2000 juveniles of *M. javanica* 14 days after bacterial application. The inoculation of nematodes carried out by drenching 5 ml inoculums volume with the juveniles into the potted soil around the roots. Soil without bacteria served as control used. Plants harvested after 6 weeks after nematode inoculation. The same previous measuring made for both treated and control.

RESULTS AND DISCUSSION

Table (1) Effect of *Bacillus subtilis* bacteria on plant growth and on *Meloidogyne javanica* control when applied to tomato as seeds treatment

Treatment	Plant height (cm) % Increased /decreased	Fresh weight of shoot(g) %Increased /decreased	Fresh weight of root(g) %Increased /decreased	Dry weight of shoot (g) %Increased /decreased	Dry weight of root (g) %Increased /decreased	No. of Juveniles /250gram of soil	Galls No % Increased Decreased
Bacillus Subtilis	25.64% Increased	56.5% increased	27.95% decreased	28.9% increased	24.46% decreased	30% decreased	56.71% decreased
Nematode alone	40.33	17.67	37	10.66	*23.2*	500	499

Table (2) Effect of *Bacillus subtilis* bacteria on plant growth and on *Meloidogyne javanica* control when applied to tomato as soil drench

Treatment	Plant height (cm) % Increased /decreased	Fresh weight of shoot(g) %Increased /decreased	Fresh weigh of root(g) %Increased /decreased	Dry weight of shoot (g) %Increased /decreased	Dry weight of root (g) %Increased /decreased	No. of Juveniles /250gram of soil	Galls No % Increased Decreased
Bacillus subtilis	23.98% Increased	54.67% Increased	57.65% decreased	6.14% Increased	28.76% decreased	27.8% decreased	57.92% decreased
Nematode alone	40.33	17.67	37	10.66	23.2	500	499

Table (3) Effect of *Bacillus subtilis* bacteria on plant growth and on *Meloidogyne javanica* control when applied to tomato as root dipping

Treatment	Plant height (cm) % Increased /decreased	Fresh weigh of shoot(g) %Increased /decreased	Fresh weight of root(g) %Increased /decreased	Dry weight of shoot (g) %Increased /decreased	Dry weight of root (g) %Increased /decreased	No. of Juveniles /250gram of soil	Galls No % Increased Decreased
Bacillus subtilis	27.27% Increased	109.39% increased	34.24% decreased	6.29% increased	30.4% decreased	28.2% decreased	42.1% decreased
Nematode alone	40.33	17.67	37	10.66	23.2	500	499

In this study bacteria *Bacillus subtilis*, test against the nematode *Meloidogyne javanica* showed greatest increase in plant height which reach 27.27% over control when *Bacillus subtilis*, added as root dipping Table (3) . This may be Because of phytohormones contents increased by bacteria (IAA and GA_3) and the contents of N, P, K^+, Ca^{2+}, and Mg^{2+} (Mohamed, H.I. and Gomaa, E.Z. ,2012) , production of IAA, siderophores, (Khan. 2010).and . Also Phosphate solubilization ,amino acid, and hormones produced by *Bacillus spp*,(Debora *et al*,2007). Moreover of bacteria population increasing in the soil. this agreed with Kloepper J W, Schroth M.N, (1978). and , Nasima *et al.*, (2002). The study revealed that the fresh weight of shoot showed greatest increasing which reach 109.39% over control when *Bacillus subtitles* applied as root dipping . Table (3). This is due to good property of acetic acid production by *Bacillus subtilis*. Also Proteases have potential role to reduce nematode effect on plant. The dry weight of shoot increased by 57.65% when bacteria applied as soil drench. Table (2). The study indicated that the treatment with *Bacillus subtilis* decreased fresh weight of root by 56.75% when applied soil drench ,Table (2). and dry weight of root by 30.4% when applied as root dipping , Table (3). because of less galls information , (kloepper et al , 1999).

Effects of *Bacillus subtilis* on Nematodes population in soil showed significant results. decreased by 30% under control when added as seeds treatment ,Table (1). *Bacillus subtilis* showed more reduction in Nematodes population which means it has more effects on nematodes control.

Effects of *Bacillus subtilis* on galls formation revealed reduction of galls on plant roots by 57.92% under control when *Bacillus subtilis* applied as soil drench , which showed highly significant results regarding root knot disease control by reducing number of juveniles in soil and reduced the gall numbers on the roots. This results agreed with Javed Asghar Tariq, (2008). *Bacillus* is the large group of bacteria that have shown diversified effects on plant-parasitic nematodes. *Bacillus spp*. that demonstrated nematicidal effects include *Bacillus. subtilis* (Gokte and Swarup, 1988), many previous studies showed that *Bacillus subtilis* can used as bio-agent to control soil borne diseases as well as plant parasitic nematodes.

Khan et al.,(2001) fined *that Bacillus . subtilis* , caused greater reduction in nematode multiplication. Numerous *Bacillus* strains have been found to express activities that suppress pests and pathogens including nematodes, (Siddiqui and Mahmood, 1999). Beneduzi Aziz, (2013) study indicated that the .Effects of PGPR can occur via local antagonism to soil-borne pathogens and nematodes or by induction of systemic resistance against pathogens .

The findings of this study confirmed that *Bacillus subtilis* can be used as bio-agent to control root knot disease of tomatoes and thus reduce dependence on the synthetic nematocides and their hazards. This agrees with Kloepper ,et ,al. (1991), Kloepper et al. (1999), Siddiqui et al. (2001), Ali et al. (2002), Siddiqui ,and Shaukat (2002) ,and Munif et ,al, (2000). Study results showed that application of bacterial endophytes significantly reduced *M. incognita* infestation on tomato either as a seed treatment, root dipping or as a soil drench application. The results of the present study supported by the study of Munif, A., Hallmann J. and R.A. Sikora. (2000) which concluded that *Bacillus subtilis.* is able to reduce the number of penetrating nematodes and root galls of tomato when applied as a root dipping ,soil drench. And Seed treatment. Present study agreed with Samaraj Subramanian Thiyagarajan (2014).

This results agreed with Javed Asghar Tariq, (2008). who concluded from his studies that *Bacillus subtilis* used against *Meloidogyne javanica* to control root knot disease of tomatoes.

All the previous studies agreed with present study in using bacteria as bio-agent to control root knot diseased of tomato caused by plant parasitic nematode as well as other disease of tomatoes.

CONCLUSIONS:

bacillus subtilis bacteria is able to control root Knot disease on Tomato caused by *Meloidogyne javanica* nematode , by reduction of galls number on the roots and reduction of nematode population in soil . when applied as a root dipping , soil drench and seed treatment . *Bacillus subtilis* bacteria can uses as bio- agent to control Nematode .

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