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Use of *Pseudomonas aeruginosa* in the control of root-knot disease complex in tomato: the effects of different inoculum levels of *Meloidogyne javanica* and *Rhizoctonia solani*

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Summary

The potential impact of *Pseudomons aeruginosa* strain IE-6 as a biological control agent against *Meloidogyne javanica* at four inoculum densities (0, 250, 500 and 1000 eggs/plant) and *Rhizoctonia solani* at three inoeulum levels (0, 1 and 3 ml culture suspension/kg of soil) was examined on tomato in the greenhouse experiments. The biocontrol bacterium suppressed root infection caused by *R. solani* and *M. javanica* on tomato in both sterilized and non-sterilized soils. Root-rot infection increased with the increase in pathogen(s) concentration. *P. aeruginosa* showed better biocontrol effects at low population levels of *M. javanica* and *R. solani* than at higher population densities of the pathogen(s). Root-rot disease severity was more pronounced in sterilized soil compared to the non-sterilized one. Soil infested with high population densities of *R. solani* (3 ml /kg of soil) and *M. javanica* (2000 eggs/pot) resulted in complete mortality of tomato seedlings in sterilized soil, whereas some plants were found to survive in non-sterilized soil. There seems to be a correlation between population density of *M. javanica* and root colonization by *R. solani*. Root colonization by other three root-infecting fungi including *Macrophomina phaseolina, Fusarium oxysporum* and *Fusarium solani* was also lower in the presence of *P. aeruginosa* in non-sterilized soil. *P. aeruginosa* enhanced plant growth in both types of soil.

Key words: *Rhizoctonia solani, Meloidogyne javanica, Pseudomonas aeruginosa,* biological control

INTRODUCTION

Rhizoctonia solani has long been recognized as a destructive pathogen on a wide variety of crop plants throughout the world. The pathogen exists as active mycelium in soil and attacks over 2000 species of plants (P a r m e t e r, 1970) and has been found to infect more than 68 hosts in Pakistan (G h a f f a r, 1988). Physiological alterations in plant roots induced by *Meloidogyne* spp., or exudates emanating from infected roots could be of major importance in the predisposition of plants to infection by other soilborne organisms (G o l d e n and V a n G u n d y, 1975). There are reports where disease incidence of *R. solani* on cotton increased with the association of root-knot nematode, *Meloidogyne incognita* (R e y n o l d s and H a n s e n, 1957). A severe root-rot of tomato caused by *M. incognita* and *R. solani* was associated with nutrient mobilization into gall tissue and root exudation (V a n G u n d y et al., 1977). Some non-pathogenic bacteria can induce physiological changes throughout entire plants, making them more resistant to pathogens. Saprophytic bacteria applied as seed or tuber treatment were shown to inhibit early root penetration by *Heterodera schachtii* and *Globodera pallida* in sugarbeet under greenhouse and field conditions (O o s t e n d o r p and S i k o r a, 1989). Of the various rhizosphere bacteria, *Pseudomonas aeruginosa* (Schroeter) Migula has been reported to control root-rot and root-knot diseases in chili and tomato (S i d d i q u i et al., 1999; S i d d i q u i et al., 2000; S i d d i q u i and E h t e s h a m u l-H a q u e, 2000). An experiment was therefore carried out to determine the effects of *P. aeruginosa* in the control of damping off root-knot disease complex in tomato (*Lycopersicon esculentum* Mill.).

MATERIALS AND METHODS

Field soil (Sand: Silt: Clay, 70 : 19 : 11%) of pH-8.1 with moisture holding capacity (MHC) of 40% was obtained from the Department of Botany, University of Karachi. The soil was naturally infested at the rate of 2-9 sclerotia g^{-1} of soil of *Macrophomina phaseolina*, as found by a wet sieving and dilution technique (S h e i k h and G h a f f a r, 1975); 5.5 % colonization of *Rhizoctonia solani* on sorghum seeds was used as baits (N a s h and S n y d e r, 1962); and 3000 cfu g^{-1} of soil of mixed population of *Fusarium* spp., as estimated by soil dilution technique (W i l h e l m, 1955). The soil was pasteurized for 30 min at 115°C and 15 psi to destroy the microorganisms. Five-day-old culture of *Pseudomonas aeruginosa* strain IE-6 originally isolated from the rhizosphere of sunflower and maintained on King's B medium was used in the present study. *Rhizoctonia solani* isolated from infected tomato roots was maintained on Potato Dextrose Agar medium for 5 days before use. The effectiveness of *P. aeruginosa* strain IE-6 as a biological control agent against *R. solani* was tested in pot experiments according to the method described by P l e b a n et al., (1995). The fungus was grown in 100-ml PD-broth at 30°C without shaking for 3-4 days, weighed and 2 g was macerated in 30-ml tap water with sterile scalpel blade. This suspension was used to infest the soil (sterilized and non-sterilized).

The experiment was planned as a 3 x 5 x 2 x 2 factorial design. The factors

included 3 inoculum levels of *R. solani*, 5 population densities of *M. javanica*, two levels of *P. aeruginosa* (with or without application) and two soil conditions (sterilized and non-sterilized). Steam-sterilized soil artificially infested with i) the inoculum of *R. solani* at 0, 1 and 3 ml culture suspension/kg of soil was transferred in 8-cm-diam., plastic pots at 350 g/pot; ii) the eggs of *M. javanica* obtained from aubergine (*Solanum melongena* L.) roots were inoculated at 0, 250, 500, 1000 and 2000 eggs/pot by making three holes in the soil around the tomato seedlings; the same densities of *M. javanica* were applied at all inoculum levels of *R. solani*; iii) a 25-ml aqueous cell suspension of *P. aeruginosa* having 2.5 x 10^8 cfu/ml was drenched in pots at all the levels of combinations of *M. javanica* and *R. solani*. One similar set of pots remained without the bacterial application; and iv) the same treatments were also applied in non-sterilized soil. After soil treatment 3-week-old tomato cv SUN 6002 (PVP) seedlings raised in sterile soil were planted in each pot at 3 seedlings/pot. The treatments were replicated three times and pots were kept randomized on the greenhouse bench. The temperature of the upper soil surface throughout experiment was ranged between 18-24°C. Each treatment was replicated three times and pots were randomized on the greenhouse bench. Number of dead plants were recorded every week after nematode inoculation up to six weeks.

Plants were uprooted 45 days after the addition of nematodes, and plant growth parameters (plant height and fresh weight of shoot) and infections by the root-infecting fungi and root-knot nematodes were recorded. Number of galls induced by *M. javanica* on the entire root system was counted with the aid of a low power microscope (x6). To determine the incidence of fungi, 5-mm-long root pieces after surface sterilization were plated onto PDA plates supplemented with penicillin (100,000 units/l.) and streptomycin sulfate (0.2g/l.). The plates were incubated at 28°C for 1 week and incidence of root-infecting fungi was recorded as follows:

Infection % = (Number of plants infected by a fungus / total number of plants) x 100

RESULTS

Soil condition (sterile and non-sterile) showed no significant effects on gall formation due to *M. javanica*. The bacterium, inoculation levels of *R. solani*, and population densities of *M. javanica* showed significant ($p<0.05$) differences on the root-knot infection in tomato (Fig. 1). An increase in nematode population increased gall formation either in the presence or absence of *R. solani* and/or *P. aeruginosa*. Root damage caused by the root-knot nematode was lower in *P. aeruginosa*-treated plants compared with untreated controls. The bacterium was found more effective in the suppression of *M. javanica* at low nematode and *R. solani* populations. Root-knot infection was greater in sterilized soil compared to that in non-sterilized soil.

Soil condition, inoculum levels of *R. solani* and population densities of

M. javanica showed significant ($p<0.05$) effects on the incidence of *R. solani* and subsequent root-rot. A correlation between population levels of *M. javanica* and colonization of *R. solani* on tomato roots was also observed. Root-rot infection was more severe in the absence of *P. aeruginosa*. Similarly, disease symptoms were more pronounced in sterile soil compared to non-sterile soil. *R. solani* at 3-ml/kg of soil in the presence of *M. javanica* at all the inoculum levels resulted in 100% *R. solani* infection. *P. aeruginosa* was found ineffective at the highest inoculum level of *R. solani* (3-ml/kg of soil) and 2000 eggs/pot of *M. javanica* (Fig. 2).
Inoculum levels of *R. solani*, population densities of *M. javanica* and *P. aeruginosa* showed non-significant differences on the incidence of *M. phaseolina*, *F. oxysporum* and *F. solani*. Infection of *M. phaseolina*, *F. oxysporum* and *F. solani* was dependent on the population of *M. javanica*. The incidence of root-infecting fungi was much lower in *P. aeruginosa*-treated plants as compared to untreated controls (Fig. 3).

Plant growth was progressively suppressed with an increase in pathogen(s) concentration in both sterilized and non-sterilized soils. No symptom of wilting was observed the first 3 weeks of nematode inoculation in both sterilized and non-sterilized soils. After 4 weeks, a higher inoculum level of *R. solani* (3-ml/kg of soil) and population density (2000 eggs/pot) of *M. javanica* in the absence of *P. aeruginosa* showed 100% mortality in tomato seedlings in sterilized soil, whereas few plants were able to survive in non-sterilized soil at the same rate. *P. aeruginosa*-treated plants showed delayed root-rot disease in both sterile and non-sterile soil (data not presented). Soil treatment with *P. aeruginosa* strain IE-6 stimulated plant growth. Plant height and fresh weight of shoots in bacterized plants were higher ($p<0.05$) compared to the untreated controls. *P. aeruginosa* used with *M. javanica* at 500 eggs/pot resulted in maximum plant height in sterile soil whereas in non-sterile soil, the bacterium used in the absence of *M. javanica* and/or *R. solani* gave maximum plant height. *P. aeruginosa* used in the absence of *M. javanica* and/or *R. solani* showed the greatest shoot growth in both sterilized and non-sterilized soils (data not presented).

DISCUSSION AND CONCLUSIONS

In the present study, root-rot infection caused by fungi was more severe in *M. javanica* – infested soil than in non-infested soil. Some species of *Pythium* (*P. aphanidermatum*, *P. ultimum*), *Rhizoctonia* (*R. solani*, *R. bataticola*) and *Fusarium* (*F. solani*) are prominent amongst the root-rot fungi that are known to interact with different plant-parasitic nematodes (K h a n, 1993). Tomatoes have frequently been reported to suffer the effects of disease complexes, with *M. incognita* interacting with *R. solani* (C h a h a l and C h a h b r a, 1984; H a s a n and K h a n, 1985).

Like wilt-inducing fungi, root-rot fungi are capable of causing root diseases on their own and have their own inherent mechanism of root penetration. The role of the nematode in root-rot diseases in general is related to assisting the fungal pathogen in its pathogenesis and increasing host susceptibility. Nematodes, by wounding, provide the fungal pathogen access to root tissue (I n a g a k i and P o w e l l, 1969).

The lesions caused by the lesion or burrowing nematodes or invasion tract by penetrating juveniles of root-knot or cyst nematodes provide a suitable location for establishment and colonization of the fungal pathogens (B o o t h and S t o v e r, 1974; P o l y c h r o n o p o u l o s et al. 1969). Further, physiological alterations by the nematode improve the nutrient status of the host for the fungal pathogen. *Rhizoctonia solani* preferred *Meloidogyne hapla*-induced galls on radish, the mycelium accumulated over the galls showing vigorous growth and abundant sclerotia formation. Extensive necrosis of the galls occurred and the root became obliterated. Non-galled regions of roots did not show sclerotial formation (K h a n and M ü l l e r, 1982). Pre-penetration studies showed that the *R. solani* was specially attracted to nematode gall tissue and the sclerotia were selectively formed on the galls. The fungus responded to stimuli that originated from the nematode-infected roots and passed through semi-permeable cellophane membranes, by producing sclerotia on the membrane just opposite to the galls. The leakage of nutrients from the roots attracted the fungus to the galls and initiated sclerotial development (G o l d e n and v a n G u n d y, 1975). Therefore, physiological alterations that ensure better nutrient availability to the penetrated fungal pathogen are key factors of the synergistic action faced by the host.

In the present study, the degree of response was related to the inoculum density of pathogens (*R. solani* and *M. javanica*) and type of soil (sterilized and non-sterilized soil). Application of *R. solani* at 3-ml/kg and population density of 2000 eggs of *M. javanica* caused 100% mortality in tomato seedlings in sterilized soil. No symptom of wilting in tomato plants was observed until three week of nematode inoculation in bacterized and non-bacterized plants. By the fourth week after nematode inoculation, non-bacterized *R. solani*-infected plants wilted and had no detectable growth in sterilized soil, whereas few plants were able to survive in non-sterilized soil. Infected *P. aeruginosa*-treated plants showed slower disease progression and continued to grow until the harvest time. Recently, endophytic *Pseudomonas fluorescens* was found as an effective biocontrol agent of phytopathogenic fungus *R. solani* on bean seedlings under growth chamber conditions (D o w l i n g and T h o m s o n, 2000). S h a r m a and N o w a k (1998), reported that the degree of protection afforded by *in vitro* bacterized plants depended on the inoculum density of *Verticillium dahliae*; the best and worst protection occurred at lowest (10^3 conidia/ml) and highest (10^6 conidia/ml) levels, respectively. *Pseudomonas aeruginosa*, a plant growth-promoting rhizobacterium has also been reported to control root rot-root knot disease complex in chili (S i d d i q u i et al. 1999).

A combination of *M. javanica* and *R. solani* showed retardation in the plant growth and resulted in mortality of tomato seedlings in both sterilized and non-sterilized soil. Root rot-root knot disease was more pronounced in non-sterilized soil as compared to sterilized soil. A decrease in root-rot severity may be due to the greater infection of *F. oxysporum* in non-sterilized soil. The fungus posses a higher competitive saprophytic ability than the other root parasites (S a b e t and K h a n, 1969). *F. oxysporum* is also known to parasitize the oospore of *Phytophthora cactorum* (S n e h et al. 1977). Similarly, O y a r z u n et al. (1994), reported that non-

pathogenic strain of *F. oxysporum* reduced *F. solani* infection which caused root-rot of red clover and pea, respectively.

The effective biological control of root-pathogens using *Pseudomonas aeruginosa* can be attained in those crop-fungus-nematode combinations where yield loss can be minimized by reducing nematode penetration within the first few weeks after germination of seeds or seedling transplantation.

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Zwalczanie zgnilizny i guzowatości korzeni przy użyciu *Pseudomonas aeruginosa*: bakterii ryzosferowej

Badano skuteczność 3 szczepów *P. aeruginosa* biologicznym zwalczaniu zgnilizny korzeni powodowanym przez *Macrophomina phaseolina*, *Fusarium solani* i *Rhizoctonia solani* oraz guzowatości korzeni przez nicienia *M. javanica* na roślinach chili i *V. mungo* w warunkach szklarniowych. Wszystkie trzy szczepy istotnie redukowały populację nicienia w glebie, jego inwazyjność, rozmnażanie i tworzenie guzów. Infekcja korzeni przez grzyby była także skutecznie ograniczana przez użycie *P. aeruginosa*. Antagonistyczne bakterie wykazywały większą skuteczność w biologicznym zwalczaniu chorób oraz korzystne oddziaływanie w przypadku roślin 15 dniowych niż zbieranych po 30 i 45 dniach. Populacja bakterii w ryzosferze zmniejszała się gwałtownie po 15 dniach po inokulacji nicieni. Bakteria *Pseudomonas aeruginosa* szczep Pa-5 wykazywała maksymalną nodulację w przypadku roślin 15 dniowych, a szczep Pa-7 – w przypadku 30 i 45 dniowych roślin *V. mungo*.

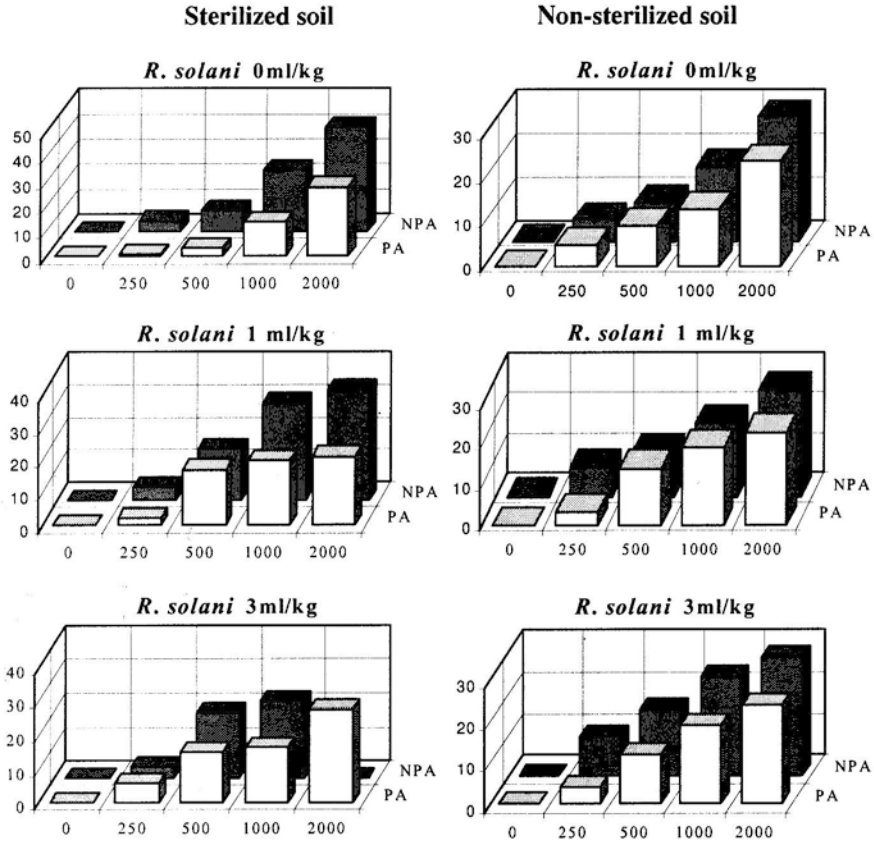

Fig. 1. Effects of *Pseudomonas aeruginosa* on the development of root-knot infection in sterilized and non-sterilized soil containing different population levels of *Meloidogyne javanica* and *Rhizoctonia solani.*

NPA = Without *Pseudomonas aeruginosa.*
PA = With *Pseudomonas aeruginosa.*

LSD $p<0.05$
Soil conditions (sterile and non-sterile soil) = 0.83; *P. aeruginosa* = 0.83;
Inoculum levels of *R. solani* = 1.02 and Population densities of *M. javanica* = 1.32.

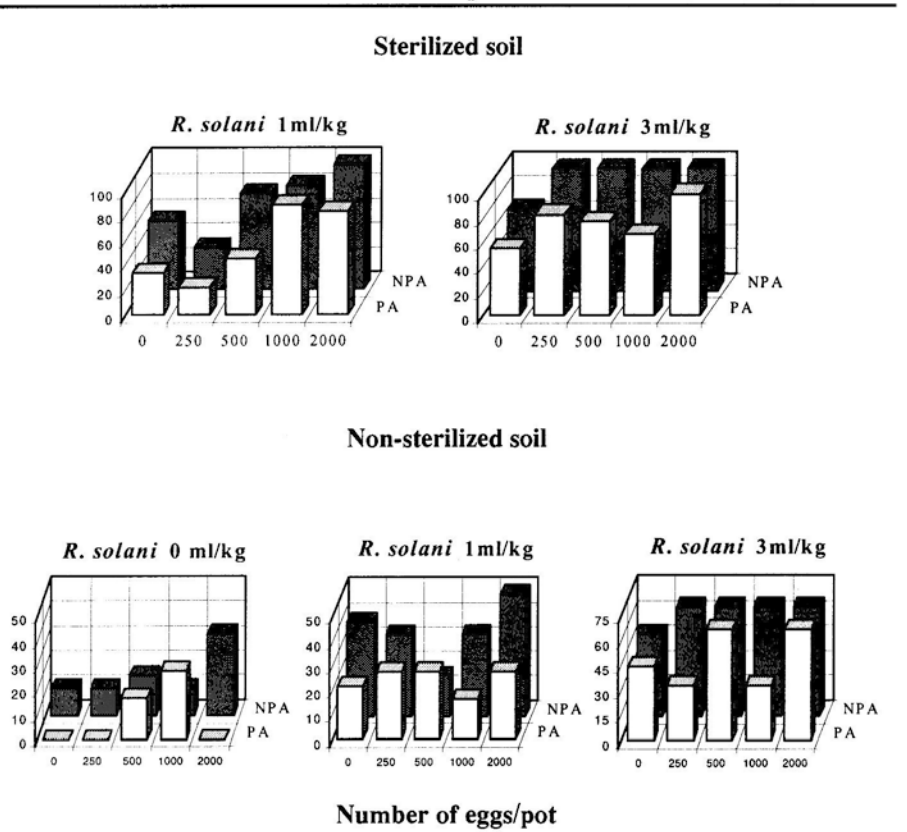

Fig. 2. Effects of *Pseudomonas aeruginosa* in the control of *Rhizoctonia solani* in sterilized and non-sterilized soil artificially infested with different population levels of *Meloidogyne javanica* and *Rhizoctonia solani*

NPA = Without *Pseudomonas aeruginosa*
PA = With *Pseudomonas aeruginosa*

LSD $p<0.05$
Soil conditions (sterile and non-sterile soil) = 8.17; *P. aeruginosa* = 8.17; Inoculum evels of *R. solani* = 10.01 and Population densities of *M. javanica* = 12.92.

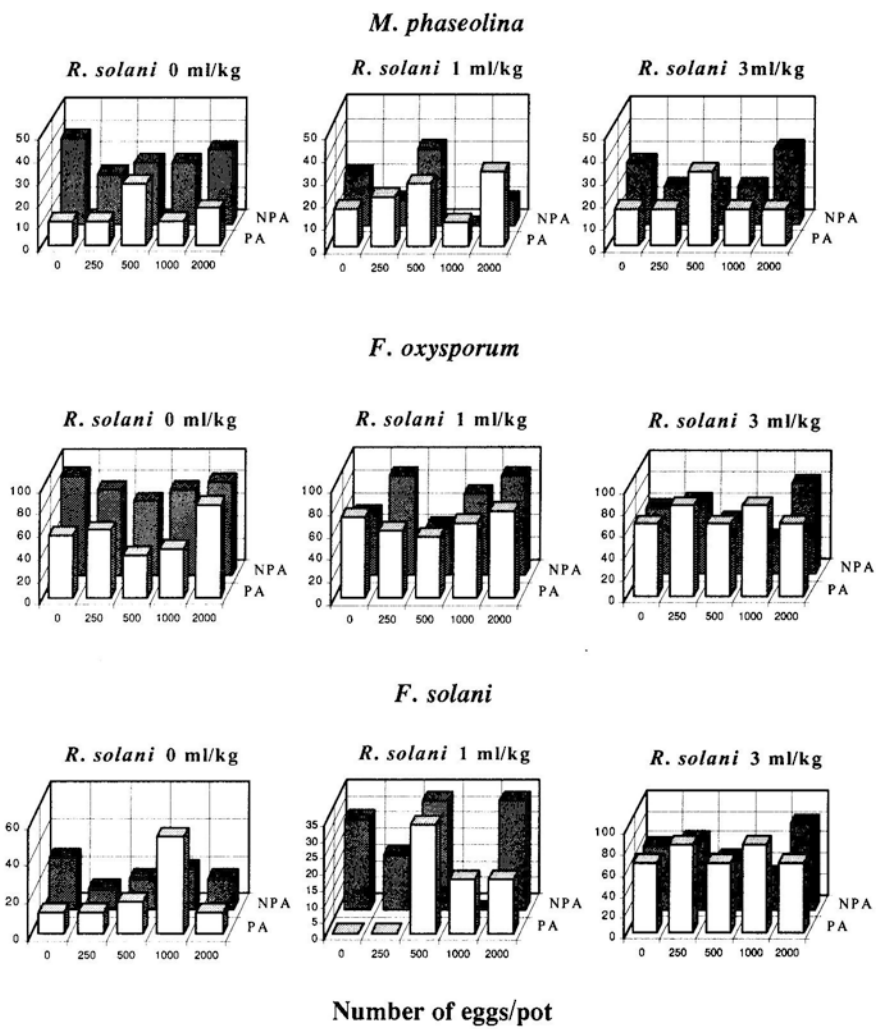

Fig. 3. Effect of *Pseudomonas aeruginosa* on infections of *Macrophomina phaseolina*, *Fusarium oxysporum* and *Fusarium solani* in soil containing different population levels of *Rhizoctonis solani* and *Meloidogyne javanica*

NPA= Without *Pseudomonas aeruginosa*
PA= With *Pseudomonas aeruginosa*

LSD $p<0.05$
P. aeruginosa = 8.17; Inoculum levels of *R. solani* = 10.01 and Population densities of of *M. javanica* = 12.92.