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**Biological control of lettuce root-knot disease by the used of *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Paecilomyces lilacinus***

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The effects of *Pseudomonas aeruginosa*, *Bacillus subtilis* and antagonistic fungus *Paecilomyces lilacinus* (provitan), on the growth and gall development of lettuce infected by root-knot nematodes *Meloidogyne* spp. was studied both in greenhouse and field environments. In field experiments, lettuce seedlings were cultivated in nematode infested soil, and *P. aeruginosa* and *B. subtilis* were applied every week prior to harvesting. *Paecilomyces lilacinus* was mixed with nematode infested soil two weeks prior to, and again two weeks after planting the lettuce. The results showed that the weight of lettuce planted in nematode infested soil, containing these three tested organisms, was higher than those cultivated in nematode infested soil with no control agents. *Bacillus subtilis*, *P. aeruginosa* and *P. lilacinus* (provitan) also decreased nematode population densities and suppressed nematode infection. As a result fewer galls were developed within the roots.

The effects of three tested organisms, *B. subtilis*, *P. aeruginosa* and biocontrol agent *P. lilacinus*, along with bacterial culture supernatants of *B. subtilis* and *P. aeruginosa* on root-knot nematodes were examined while being grown in pots. The results show that the average weight of lettuce planted in nematode infested soil and controlled with *P. aeruginosa* 30 ml was significantly higher ( $P=0.05$ ) when compared to those treated with other control agents, as well as those grown without control agents. Also shown in this study, *B. subtilis*, *P. aeruginosa* and both *B. subtilis* culture supernatant 50 ml and *P. aeruginosa* culture supernatant 10 ml, 30 ml and 50 ml significantly suppressed root-gall development within the root system ( $P=0.05$ ) when compared to those cultivated in nematode infested soil with no control agents. As a result of using these control agents, fewer galls were developed within the roots. Even though, *B. subtilis*, *P. aeruginosa* and *P. lilacinus* (provitan) decreased nematode population densities, the nematode population level was still much higher than the economic threshold level. The supernatants of *B. subtilis* 50 ml and *P. aeruginosa* 10 ml, 30 ml and 50 ml significantly decreased nematode population densities ( $P=0.05$ ). These bacterial supernatants are potentially

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effective agents for controlling root knot nematodes; however further investigation of their use, as well as the development of field application methods for these control agents are needed.

**Key words:** root-knot disease, *Meloidogyne* spp., *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Paecilomyces lilacinus*, biological control

## Introduction

Root-knot disease, which is caused by *Meloidogyne* spp., is one of the most significant diseases currently impacting Thai farmers. This disease causes massive economic crop losses. Northern provinces, which supply much of Thailand's industrial lettuce production, have been especially hard hit by this disease. Due to the soil texture in these areas, *Meloidogyne* spp. can complete 2-3 life cycles in lettuce plants, resulting in the huge epidemics of the disease. The symptoms of lettuce root-knot disease include stunting, wilting, yellowing and poor lettuce quality, thus not meeting market standards for size and weight. Failure to achieve these minimum requirements prohibits selling of the affected crops. Such symptoms are the result of the root-system being destroyed by the organism which causes galls and swelling of the roots. If the disease occurs in lettuce seedling cultivating fields, the typical result is the death of the seedlings. This destructive pathogen also negatively affects a wide array of vegetable crops throughout the Highland areas of Thailand.

The use of chemicals to control root-knot disease has provided good results. However, because of environmental toxicity and the cost of these chemicals, alternative means of control have also been investigated. Use of resistant cultivars and bio-control agents such as manure, to increase microorganisms is effective in decreasing nematode population in soil. (McSorley, 2001) Also, botanical nematocides, the cultivation of nematode suppressing plants such as marigolds (*Tagetes species*) can be used to control root-knot disease. (Toida *et al.*, 1990) Moreover, previous studies have reported that many antagonistic fungi such as *Arthrobotrys haptotyla*, *A. oligospora*, *A. thaumasa*, *Dactylella leptospora*, *Harposporium anguillulae*, *Meristacrum* sp. have been proven effective in controlling root-knot nematodes (Siddiqui and Mahmood 1996; Li *et al.*, 2000; Nordbring-Hertz *et al.*, 2000). Many studies have indicated that rhizobacteria, *P. aeruginosa* and *B. subtilis*, not only enhance plant growth but also suppress root-knot infection and nematode density in the soil (Siddiqui *et al.*, 1999; Siddiqui, 2000; Siddiqui, 2001). Prakob *et al.* (2007) investigated the effects of mixed arbuscular mycorrhizal fungi (AM), rhizobacteria *Pseudomonas aeruginosa*, *Bacillus subtilis*, and antagonistic fungus *Paecilomyces lilacinus* (provitan) on growth and gall development of tomatoes infected by root-knot nematodes *Meloidogyne* spp. under greenhouse conditions.

Tomato seeds were planted in soil inoculated with AM, *P. aeruginosa* and *B. subtilis*. The tomato plants were cultivated using infested soil containing either 10 or 100 of the second stage juvenile (J2) of *Meloidogyne* spp. *Paecilomyces lilacinus* was mixed with nematode infested soil prior to and again after planting tomatoes. These tested organisms not only enhanced the growth of tomato plants but also significantly suppressed root-knot infection and nematode population densities resulting in less gall development in the root system. (Prakob *et al.*, 2007).

This study was investigated the use of antagonistic fungus *P. lilacinus* (biocontrol), two rhizobacteria, *P. aeruginosa* and *B. subtilis*, and also the supernatants of these bacteria in controlling lettuce root-knot disease caused by *Meloidogyne* spp. both in greenhouse and field environments.

## **Materials and methods**

### ***Strains and culture conditions***

Antagonistic fungus *Paecilomyces lilacinus* (Provitan) was provided by Prof. Dr. Johne, (Germany). The application of biocontrol was conducted as suggested. One isolate of *B. subtilis* BT/714, previously isolated from the rhizosphere of tomatos, was provided by Dr. Ampan Promsri, Faculty of Agriculture, Chiang Mai University. One isolate of *P. aeruginosa* 27853 used in this study was provided by the department of Microbiology, Faculty of Associate Medical Sciences, Chiang Mai University. Two isolates of bacteria used in this study were maintained in Tryptic Soy media and kept at 4 °C for stock culture.

### ***Preparation of supernatants***

Tryptic Soy broth (TSB) was prepared, sterilized, and inoculated with a fresh batch of test bacteria. The culture flasks were incubated at 37°C until reaching the concentration of  $2.5 \times 10^8$  cfu/ml. After the incubation period, the cultures were centrifuged at 5000 rpm for 10 minutes and their supernatants were collected and frozen at -20°C until being used during future experiments. The supernatants were completely thawed prior to drenching soil and it should be done in the evening in order to prevent the degradation of some active ingredients by sunlight.

### ***Preparation of nematode infested soil***

Soil infested with root-knot nematodes was collected from a lettuce cultivated area with a serious root-knot disease epidemic. This area is located in

the Northern Maehae, Maejam district, within the province of Chiang Mai. The soil was then examined and the quantity of juvenile staged *Meloidogyne* spp. counted, by using the Cobb sieving and Beermann funnel methods.

### ***Effects of microorganisms on root-knot nematodes in a greenhouse environment***

Plastic Pots (10 inches in diameter) were filled with infested soil, containing the second stage juvenile of *Meloidogyne* spp. (10 J2 in soil 500 cm<sup>3</sup>). The 30 day old lettuce was then transplanted in the pots and organized into 6 treatment groups as follows:

Treatment 1: non-inoculated (negative control)

Treatment 2: Furadan + *Meloidogyne* spp.

Treatment 3: *P. lilacinus* + *Meloidogyne* spp.

Treatment 4: *P. aeruginosa* + *Meloidogyne* spp.

Treatment 5: *B. subtilis* + *Meloidogyne* spp.

Treatment 6: only *Meloidogyne* spp. (positive control)

\*Each treatment was replicated 5 times and the pots were kept in a randomized complete block design in field conditions.

### ***Effects of microorganisms' culture supernatants on root-knot nematodes in a greenhouse environment***

Plastic Pots (10 inches in diameter) were filled with infested soil containing the second stage juvenile of *Meloidogyne* spp. (10 J2 in soil 500cm<sup>3</sup>). The 30 day old lettuce was then transplanted in the pots and organized into 9 treatments as follows:

Treatment 1: *Meloidogyne* spp + Supernatant of *B. subtilis* 10 ml

Treatment 2: *Meloidogyne* spp + Supernatant of *B. subtilis* 30 ml

Treatment 3: *Meloidogyne* spp. + Supernatant of *B. subtilis* 50 ml

Treatment 4: *Meloidogyne* spp. + Supernatant of *P. aeruginosa* 10 ml

Treatment 5: *Meloidogyne* spp. + Supernatant of *P. aeruginosa* 30 ml

Treatment 6: *Meloidogyne* spp. + Supernatant of *P. aeruginosa* 30 ml

Treatment 7: Furadan + *Meloidogyne* spp.

Treatment 8: only *Meloidogyne* spp. (positive control)

Treatment 9: non-inoculated (negative control)

\*Each treatment was replicated 5 times and the pots were kept in a randomized complete block design in field conditions.

***Effects of microorganisms on root-knot nematodes in a field environment:***

This experiment was conducted in a root knot nematode infested area (1X4 m) located in Northern Maehae, Maejam district, Chiang Mai province. The 30 day old lettuce was transplanted in the regions of the field organized into 6 treatment groups as follows:

Treatment 1: non-inoculated (negative control)

Treatment 2: Furadan + *Meloidogyne* spp.

Treatment 3: *P. lilacinus* + *Meloidogyne* spp.

Treatment 4: *P. aeruginosa* + *Meloidogyne* spp.

Treatment 5: *B. subtilis* + *Meloidogyne* spp.

Treatment 6: only *Meloidogyne* spp. (positive control)

\*There were 48 lettuce plants in each treatment group. Each treatment group was replicated 4 times in a Randomized Block Complete Design (RBCD).

\*This experiment was done on two consecutive crops in order to provide a more conclusive result.

***Data collection and analysis***

The lettuce was collected (except those planted as guard rows) and was weighed after harvesting and the yield of lettuce per cultivated area was calculated. The nematode gall index was rated after harvesting on a scale of 0–5, with 0 = no gall formation, 1 = modest gall developed within the root system, 2 = gall development affecting less than 25% of the root system, 3 = gall development affecting 25-50% of the root system, 4 = gall development affecting 50-75% of the root system, 5 = >75% of the root system affected with gall development. The number of second stage juveniles of *Meloidogyne* spp. in the soil was also counted. The collected data was subjected to analysis of variance (anova), followed by a least significant difference (LSD) test at P=0.05.

**Results**

***The effects of tested microorganisms on lettuce root-knot disease in a green - house environment***

The effects of microorganisms on root-knot nematodes in a greenhouse environment were studied with the used of plastic pots (10 inches in diameter) filled with infested soil containing the second stage juvenile of *Meloidogyne* spp. (10 J2 in soil 500 cm<sup>3</sup>). The 30 days old lettuce was then transplanted in the pots and organized into 6 treatments groups as described in materials and methods. The results showed that the lettuce planted in nematode infested soil

containing the three tested organisms, *B. subtilis*, *P. aeruginosa*, and biocontrol *P. lilacinus*, weighed more than the lettuce grown in inoculated soil which was controlled with the chemical Furadan, and less than the weight of those grown in non-inoculated soil (control) (Table 1). However, there was not a significant difference of weight between each treatment group. This research also revealed that *B. subtilis*, *P. aeruginosa* and *P. lilacinus* (provitan) not only suppressed root-gall development within the root but also decreased the nematode density in the soil. However, only *P. aeruginosa* showed significant root-gall suppression ( $P=0.05$ ) and only *P. aeruginosa* and *B. subtilis* significantly decreased the nematode population densities ( $P=0.05$ ) when compared to those cultivated in nematode infested soil with no control agents. (Table 1)

**Table 1.** Effects of *P. aeruginosa*, *B. subtilis*, *P. lilacinus* and bacterial supernatants on weight and root gall development of lettuce grown in a greenhouse environment and nematode population (J2) found in the soil after harvesting.

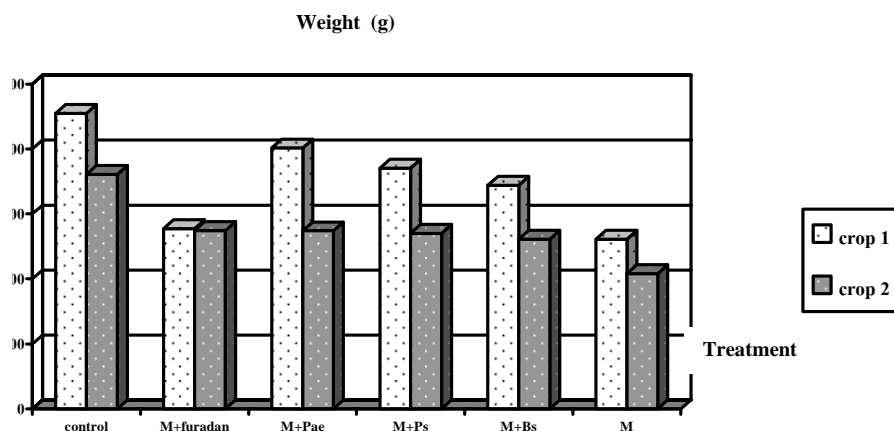
Treatment	Weight (g)	Root galling index	Nematode population J2 (soil 500 cm <sup>3</sup> )
<i>Paecilomyces lilacinus</i>	380.0 <sup>abc1</sup>	1.80 <sup>bc</sup>	14 <sup>bcd</sup>
<i>Bacillus subtilis</i>	308.0 <sup>c</sup>	1.60 <sup>bc</sup>	11 <sup>def</sup>
<i>Pseudomonas aeruginosa</i>	378.0 <sup>abc</sup>	1.00 <sup>c</sup>	9 <sup>f</sup>
Supernatant of <i>B. subtilis</i> 10 ml.	354.0 <sup>bc</sup>	1.80 <sup>bc</sup>	15 <sup>bc</sup>
Supernatant of <i>B. subtilis</i> 30 ml.	350.0 <sup>bc</sup>	1.60 <sup>bc</sup>	13 <sup>cde</sup>
Supernatant of <i>B. subtilis</i> 50 ml.	390.0 <sup>abc</sup>	1.60 <sup>bc</sup>	10 <sup>ef</sup>
Supernatant of <i>P. aeruginosa</i> 10 ml.	394.0 <sup>abc</sup>	1.20 <sup>c</sup>	11 <sup>def</sup>
Supernatant of <i>P. aeruginosa</i> 30 ml.	488.0 <sup>a</sup>	1.20 <sup>c</sup>	9 <sup>f</sup>
Supernatant of <i>P. aeruginosa</i> 50 ml.	451.0 <sup>bc</sup>	1.40 <sup>c</sup>	10 <sup>ef</sup>
- ve control (sterile soil)	414.0 <sup>abc</sup>	0 <sup>d</sup>	0 <sup>g</sup>
+ve control ( <i>Meloidogyne</i> spp.)	338.0 <sup>c</sup>	2.4 <sup>ab</sup>	17 <sup>b</sup>
furadan	358.0 <sup>bc</sup>	3.00 <sup>a</sup>	21 <sup>c</sup>
CV%	112.71	0.8043	3.2441

<sup>1</sup> Means that data within the same column followed by the same letter are not significantly different by Least Significant Difference (LSD) ( $P=0.05$ )

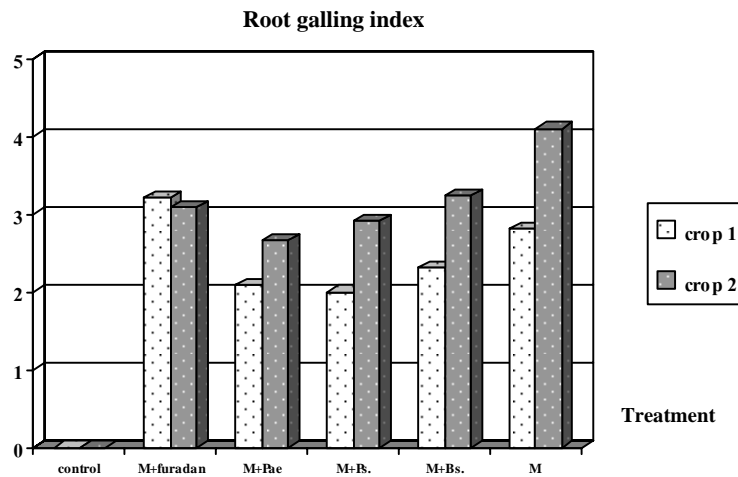
### *In a field environment*

The effects of microorganisms on root-knot nematodes, cultivated in a field environment, were conducted in a root knot nematode infested area. This experiment was conducted on two consecutive crops in order to provide a more conclusive result. The results showed that *B. subtilis*, *P. aeruginosa*, and biocontrol agent *P. lilacinus*, significantly ( $P=0.05$ ) weighed more than the weight of those grown in inoculated soil controlled with the chemical Furadan and less than the weight of those grown in non-inoculated soil (Fig. 1) In addition, *B. subtilis*, *P. aeruginosa* and *P. lilacinus* (provitan) significantly

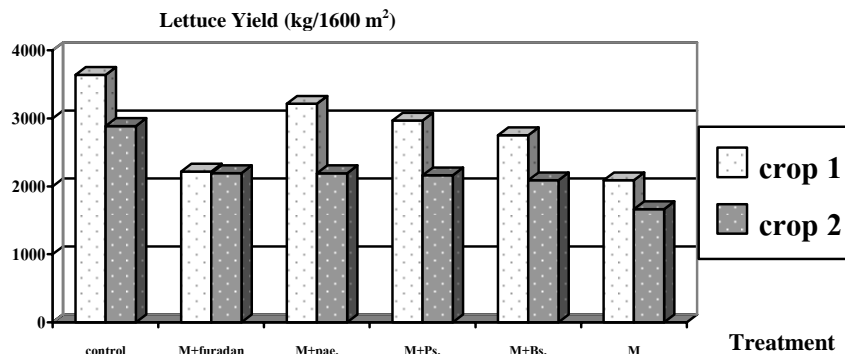
suppressed nematode infection ( $P=0.05$ ), resulting in fewer galls within the roots. (Fig. 2) Lettuce yields from the first crop increased by 31.77%, 41.72% and 59.33% in the fields controlled with *B. subtilis*, *P. aeruginosa* and *P. lilacinus*, respectively and 29.71%, 25.33% and 31.57%, respectively in the following crop. (Fig. 3) This research illustrates the substantial benefits of *B. subtilis*, *P. aeruginosa* and *P. lilacinus* (proviton) in decreasing nematode population densities; however, the resulting nematode population level was still much higher than the accepted economic threshold level (Fig. 4).



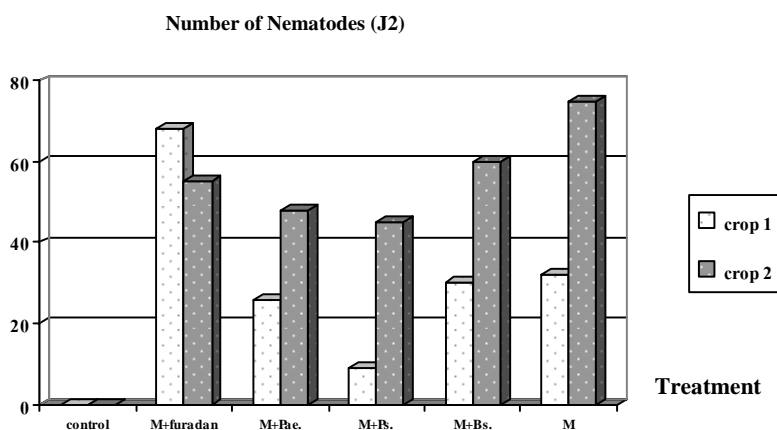
**Fig. 1.** Effects of *P. aeruginosa*, *B. subtilis* and *P. lilacinus* on the weight (g) of lettuce harvested from the first crop (crop 1) and the subsequent crop (crop 2) (Ps: *P. aeruginosa*, Bs: *B. subtilis*, Pae: *P. lilacinus*, and M: *Meloidogyne* spp.)



**Fig. 2.** Effects of *P. aeruginosa*, *B. subtilis* and *P. lilacinus* on gall development in the root system of lettuce harvested from the first crop (crop 1) and the subsequent crop (crop 2) (Ps: *P. aeruginosa*, Bs: *B. subtilis*, Pae: *P. lilacinus*, and M: *Meloidogyne* spp.)



**Fig. 3.** Effects of *P. aeruginosa*, *B. subtilis* and *P. lilacinus* on the yield of lettuce after harvesting from the first crop (crop1) and in the subsequent crop (crop 2) (Ps: *P. aeruginosa*, Bs: *B. subtilis*, Pae: *P. lilacinus*, and M: *Meloidogyne* spp.).



**Fig. 4.** Effects of *P. aeruginosa*, *B. subtilis* and *P. lilacinus* on nematode population after harvesting lettuce from the first crop (crop1) and the subsequent crop (crop 2) (Ps: *P. aeruginosa*, Bs: *B. subtilis*, Pae: *P. lilacinus*, and M: *Meloidogyne* spp.).

### ***Effects of microorganisms' culture supernatants on lettuce root-knot disease***

Effects of microorganisms' culture supernatants on root-knot nematodes were performed in a pot environment. The results showed that the average weight of lettuce planted in nematode infested soil gave the highest average weight when controlled with *P. aeruginosa* (30 ml) in comparison to those controlled with other control agents as well as those grown without control agents. (Table 2) In addition, *B. subtilis*, *P. aeruginosa*, along with the *B. subtilis* and *P. aeruginosa* culture supernatants 10 ml, 30 ml and 50 ml significantly suppressed root-gall development within the root system (P=0.05)



when compared to those cultivated in nematode infested soil with no control agents. As a result fewer galls were developed within the roots. Even though, *B. subtilis*, *P. aeruginosa* and *P. lilacinus* (provitan) decreased the nematode population densities, the nematode population level remained much higher than the accepted economic threshold level. Moreover, supernatants of *B. subtilis* 50 ml and *P. aeruginosa* 10 ml, 30 ml and 50 ml significantly decreased nematode population densities ( $P=0.05$ ). (Table 2)

**Table 2.** Effects of *P. aeruginosa*, *B. subtilis*, *P. lilacinus* and bacterial supernatants on weight and root gall development of lettuce grown in field environment and nematode population (J2) in soil after harvesting.

Treatment	Weight (g)		Root galling index		Nematode population (J2)	
	crop 1	crop 2	crop 1	crop 2	crop 1	crop 2
1	454.75 <sup>a</sup>	360.88 <sup>a</sup>	0 <sup>c</sup>	0 <sup>c</sup>	0 <sup>b</sup>	0 <sup>b</sup>
2	277.18 <sup>d</sup>	274.37 <sup>b</sup>	3.21 <sup>b</sup>	3.08 <sup>b</sup>	68 <sup>a</sup>	55 <sup>a</sup>
3	401.63 <sup>ab</sup>	274.00 <sup>b</sup>	2.09 <sup>b</sup>	2.66 <sup>b</sup>	26 <sup>ab</sup>	48 <sup>a</sup>
4	370.63 <sup>bc</sup>	270.13 <sup>b</sup>	2.00 <sup>b</sup>	2.91 <sup>b</sup>	9 <sup>b</sup>	45 <sup>a</sup>
5	344.25 <sup>c</sup>	261.00 <sup>b</sup>	2.31 <sup>b</sup>	3.23 <sup>b</sup>	30 <sup>ab</sup>	60 <sup>a</sup>
6	261.15 <sup>d</sup>	208.25 <sup>ab</sup>	2.82 <sup>a</sup>	4.10 <sup>a</sup>	32 <sup>ab</sup>	75 <sup>a</sup>
CV%	57.418	30.711	0.8814	0.5863	54.928	30.959

<sup>1</sup> Means within the same column followed by the same letter are not significantly different by Least Significant Difference (LSD) ( $P=0.05$ ).

## Discussion

In this study, three tested organisms, *B. subtilis* and *P. aeruginosa* and the biocontrol agent, *P. lilacinus*, were investigated for their effectiveness in controlling lettuce root-knot disease under greenhouse and field conditions. As showed in the results, the growth of lettuce planted in *Meloidogyne* spp. infested soil, being treated with these biocontrol agents, was higher growth than those planted in nematode infested soil with no control agents. The effects of *B. subtilis* and *P. aeruginosa* on growth was similar to those in a previous study by Siddiqui *et al.* (2001) in that *B. subtilis* and *P. aeruginosa* enhanced the growth of mungbean having complex diseases from both *Meloidogyne javanica* and other soil pathogens. The biocontrol agent *P. lilacinus* stimulated growth of tomatoes as well, and this finding validates the study by Goswami *et al.* (2006) in that *P. lilacinus* enhanced growth of tomatoes infected by *M. incognita* (Goswami *et al.*, 2006). The effects of these tested organisms on gall development within the lettuce root system were also examined with similar results in previously published work. In this study, the results showed that *B. subtilis*, *P. aeruginosa* and the biocontrol agent *P. lilacinus* suppressed root-

knot infections which resulted in fewer galls developing in the root system (Siddiqui, *et al.*, 2001, Linderman, 1992; Jaizme-Vega, 1997; Prakob *et al.*, 2007). Unexpectedly, Furadan did not produce a good result in controlling lettuce root-knot disease in either a greenhouse or field environment. The average weight and yield of lettuce controlled with Furadan was less than those cultivated in nematode infested soil while being controlled with either tested bio-agents or bacterial culture supernatants. In addition, gall development in lettuce controlled with Furadan was the highest average weight when compared to those controlled with other tested microorganisms, bacterial culture supernatants and those cultivated in nematode infested soil without any control agents. One possible reason for these is that Furadan might be toxic to other natural microorganisms including root-knot nematode antagonists within the soil. Therefore, the nutrient competition among these microorganisms was decreased. Furadan is toxic to *Meloidogyne* spp. and it has been used to control root-knot disease worldwide. However, *Meloidogyne* spp. might be less sensitive to Furadan than the other microorganisms in the area. It is possible that continued use of Furadan will eventually be able to control root-knot disease in future crops. To evaluate the population of *Meloidogyne* spp. in soil, second stage juveniles of *Meloidogyne* spp. was counted after harvesting the lettuce plants. The results showed that *B. subtilis* and *P. aeruginosa* suppressed the nematode population. As shown in many studies, *Bacillus subtilis* and *P. aeruginosa* were antagonistic to many soil pathogens. Siddiqui *et al.* (2001) demonstrated that *P. aeruginosa* and *B. subtilis*, when used as seed dressing or soil drench prevented root infection of mungbean by *M. phaseolina*, *F. solani* and *R. solani* both in field and greenhouse conditions. In addition to this suppressing root-infecting fungi, *P. aeruginosa* and *B. subtilis* also reduced nematode penetration and subsequent root-knot disease severity in mungbean. Root colonization by rhizosphere bacteria has been reported to reduce nematode invasion (Schroth and Hancock, 1982). *Pseudomonas aeruginosa* has been shown to reduce infection of *M. javanica* in various crops (Perveen *et al.*, 1998). Similarly, Siddiqui *et al.* (1999), found a significant reduction in gall formation induced by *M. javanica* when roots of chilies were treated with *P. aeruginosa*. *Bacillus thuringiensis* has also shown some toxic activity against plant-parasitic nematodes (Walker, 1971; Devidas and Rehberger, 1992). Perveen *et al.* (1998) demonstrated that *P. aeruginosa* prevented the infection by *M. javanica* in many crops. Root gall development in chilies infected by *M. javanica* was decreased when controlled with *P. aeruginosa* (Siddiqui *et al.*, 1999). It was also shown in the current study that the biocontrol agent *P. lilacinus* could control lettuce root-knot disease by suppressing nematode density in soil, resulting in a lower gall index when observed in root systems of

lettuce planted in nematode infested soil containing the biocontrol agent. Khan *et al.* (2006) tested two fungi, *Monacrosporium lysipagum* and *P. lilacinus* individually and in combination against the root-knot nematode *Meloidogyne javanica* (Treub) Chitwood, cereal cyst nematode *Heterodera avenae* Wollenweber, and burrowing nematode *Radopholus similis* (Cobb) Thorne on tomatoes, barley and tissue cultured banana plants, respectively. In all cases, nematode populations were substantially reduced by both individual and combined applications of the fungi (Khan *et al.*, 2006). The mode and severity of infection of nematodes by a soil saprophyte *Paecilomyces lilacinus* (Thom) Samson was studied under laboratory conditions using microscopy. Infection of stationary stages of nematodes by *P. lilacinus* was studied with three plant-parasitic nematodes *Meloidogyne javanica* (Treub) Chitwood, *Heterodera avenae* Wollenweber and *Radopholus similis* (Cobb) Thorne. *Paecilomyces lilacinus* infected eggs, juveniles and females of *M. javanica* by direct hyphal penetration. The early developed eggs were more susceptible than the eggs containing fully developed juveniles. As observed by transmission electron microscopy, fungal hypha penetrated the *M. javanica* female cuticle directly (Khan *et al.*, 2006). As shown in this study, two types of bacteria, *B. subtilis* and *P. aeruginosa*, along with bio-control agent *P. lilacinus* and supernatants of *B. subtilis* and *P. aeruginosa*, not only decreased nematode population density, but also suppressed gall development in the root system, resulting in an increased yield of lettuce plants. Unfortunately, these tested organisms could not decrease nematode population to the level that is less than the economic threshold level. It is noticed that the yield of lettuce from the first crop was higher than those from the following crop because of lettuce diseases such as leaf spots and root rot which occurred during these experiments, especially in the subsequent crop. Therefore, the lettuce was harvested two weeks earlier than planned; causing the weight is less than expected. Having so many plant diseases can cause severe root-knot disease if there are no control methods. The occurrence of this disease was due to lack of knowledge especially the farmers knowledge regarding good cultural practices. Rather than removing and destroying diseased crops from the previous season, they normally incorporate these plant parts, leaves, stems and roots in the field to be used as a green manure. So the pathogen still remained in the field. Therefore, it is necessary to investigate the use of an integrated management, combined with application of these organisms / control agents, while also providing proper training and knowledge to the farmers in order to decrease nematode population density to avoid the nematode infestation that can cause economic loss. Further research to test whether these bacteria are environmental friendly is required. The results of this study demonstrate that the tested supernatants show promise and warrant

further investigation under field conditions for possible development as commercially viable control agents that are safe, effective, cost efficient and can be used in the future.

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