Kasetsart J. (Nat. Sci.) 43 : 239 - 244 (2009)

Evaluation of *Azadirachta indica* as a Soil Amendment for Controlling Bacterial Wilt of Tomato

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ABSTRACT

Azadirachta indica A.Juss. (Neem) was evaluated as a possible alternative to soil treatment under greenhouse conditions, for the control of bacterial wilt in tomato plants caused by *Ralstonia solanacearum*. Thirty-day-old tomato seedlings were planted in 10-inch plastic pots that had been filled with infested soil amended by 5% crushed neem seed. The wilting symptom percentage of plants was determined weekly. The inclusion of the crushed neem seed in the soil significantly reduced the incidence of bacterial wilt on the susceptible cultivar, Seedathip3. Disease incidence in the untreated, infested soil (control) reached 100%, while in the neem-treated samples only 50% disease incidence occurred. Neem affected the bacterial count of *R. solanacearum* in the soil at 28 and 56 days after planting (DAP). Populations of *R. solanacearum* decreased at 28 DAP and no bacteria were detected in the neem treatment at 56 DAP. The observed reduction in the pathogen population and the increase in healthy plants in the greenhouse indicated that neem could have an important role in biologically based management strategies for the control of bacterial wilt disease.

Key words: *Azadirachta indica*, bacterial wilt, *Lycopersicon esculentum*, neem, *Ralstonia solanacearum*

INTRODUCTION

Bacterial wilt caused by *Ralstonia solanacearum* (Yabuuchi *et al*., 1995) is a serious disease of tomatoes (*Lycopersicon esculentum* Mill.) in the tropics and subtropics. In Thailand, bacterial wilt disease is the most important bacterial disease of tomatoes because it has been found in almost all tomato-cultivated areas where it causes substantial yield reduction. Bacterial wilt disease is considered the most difficult disease to control due to several factors, such as the bacterium's wide host range, its long survival period in the soil and the lack of effective chemical controls. Planting resistant varieties is the most effective and simplest method for controlling the disease. However, a major problem is the instability of the resistant plant lines. A recent study showed that extracts of various medicinal plants exhibited antibacterial activity against *R. solanacearum*. Several plant extracts were investigated as possible alternatives to control bacterial wilt in tomatoes by soil drenching. Crude extracts have been trialed from *Artabotrys hexapetalus* (Twongsin, 2001), *Sphaeranthus indicus* (Suprapta *et al*., 2003) and *Zanthoxylum rhetsa* (Yimyaem, 2005). *Azadirachta indica* A.Juss. (neem) has been used for more than 4,000

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Received date : 27/06/08 Accepted date : 05/01/09

years in India and Africa as a medicine as well as for pest control. Although to date, neem's effect on insects has been the best known by far, the various products from neem can influence other pest organisms as well. Neem has demonstrated antifungal activity and neem products affect various types of nematodes. The ethanol extract of neem seed also has bactericidal activity and reduced the ability of *Xanthomonas axonopodis* pv. *citri* to colonize lime leaves (Leksomboon, 2007). Soil amendment is acceptable to farmers as a means of controlling soil pathogens. Therefore, the objective of this research was to evaluate the efficacy of crushed neem seed as a soil amendment to control bacterial populations and wilt development in tomatoes.

MATERIALS AND METHODS

Inoculum preparation

The virulent *Ralstonia solanacearum* (RS) strain T161 was isolated from tomatoes in Nakhon Pathom province. A spontaneous nalidixic acid-resistant (Nal^r) derivative of the RS strain T161 was obtained by spreading 0.1 ml of a water suspension of RS strain T161 onto NGA amended with 25 μg/ml of nalidixic acid (Nal-NGA). The mutant strain T161 Nal^r appeared identical to the wild type in pathogenicity and morphological characteristics. For inoculum preparation, the strain T161 Nal^r was grown at 28°C on Nal-NGA. After 24 h of incubation, cells were harvested in sterile distilled water and the suspension was adjusted to 10^8 colony forming unit/ml (CFU/ml).

Preparation of infested soils

Seeds of *Lycopersicon esculentum* Mill. 'Seedathip 3' susceptible to bacterial wilt were sown in sterile soil. Each seedling was transplanted into a 6-inch plastic pot. Forty plants were inoculated with the strain T161 Nal^r 28 days after transplanting. Inoculation was made by cutting the lateral roots with a scalpel along one side of the plant to a depth of approximately 3 cm and pouring 30 ml of 10^8 CFU/ml bacterial suspension over the severed roots. At the end of 21 days, all plants inoculated were either dead or dying. The soil containing plant debris was used as the infested soil.

Treatments and evaluations

The experiment was conducted in the greenhouse at the Department of Plant Pathology, Kasetsart University, Kamphaeng Saen campus, Nakhon Pathom province. Experimental treatments were: i) uninfested soil; ii) uninfested soil with crushed neem seed; iii) infested soil; and iv) infested soil with crushed neem seed. Thirty-day-old plants of *L. esculentum* Mill. 'Seedathip 3' were transplanted from 4-inch plastic pots into 10-inch plastic pots containing 2 kg of soil. Crushed neem seed was mixed thoroughly into the soil (5% g/g soil) before transplanting. Twenty single-plant replications were used for each treatment. Plants were placed in the greenhouse to allow symptom development. The number of plants that showed bacterial wilt symptoms was observed weekly, while the populations of RS in the soil were determined at 28 and 56 days after planting (DAP) using Nal-NGA. At the end of trial, the effect of the neem treatment on total bacterial population and tomato yield were determined. RS and total bacterial populations (the number of colony-forming units (CFU) per g soil) were transformed (logarithmic (x+1)) for statistical analysis.

RESULTS

Treatment of infested soil with crushed neem seed before planting resulted in a reduction of bacterial wilt incidence compared with the untreated infested soil. Wilt symptoms did not occur in the neem treatment at 7 DAP. The incidence of disease was 40 and 50% in the neem treatments at 14 and 21 DAP, respectively

Table 1 Percentage bacterial wilt of the 'Seedathip 3' tomato variety over time following soil amendment with crushed neem seed (CNS).

Treatment	Days after planting(DAP)			
	7	14	21	28
Uninfested soil	0	0	0	0
Uninfested soil + 5% CNS	0	0	0	0
Infested soil(untreated)	60	100	-[a]	-
Infested soil + 5% CNS	0	40	50	50

[a] All plants died.

Table 2 Soil population densities of *Ralstonia solanacearum* and healthy tomato plants growing in the infested and uninfested soil following soil amendment with crushed neem seed (CNS).

Treatment	Population density of *R. solanacearum*[a]		Healthy plants [b](%)
	28 DAP	56 DAP	
Uninfested soil	-[c]	-	100
Uninfested soil + 5% CNS	-	-	100
Infested soil(untreated)	5.80 a[*]	2.43	0
Infested soil + 5% CNS	2.25 b	0	50

[a] Mean values are $\log_{10}$ transformation of CFU/g of soil. Average soil population density at 0 day (before planting) was 6.58

[b] Percent healthy plants at 56 days after planting(DAP)

[c] Not determined

[*] Mean values with different letters are significantly different at $P = 0.05$ according to Student's t-test.

(Table1). At 28 DAP, 50% of the tomato plants had survived in the infested soil with neem treatment. In the untreated, infested soil treatment, the wilt symptoms were first observed at 7 DAP with 60% wilt and plants had 100% wilt within 14 DAP and had died at 21 DAP.

The treatment of crushed neem seed resulted in significant reductions in the population density of RS. At 28 DAP, soil populations of RS were reduced 61.2 % compared with the untreated, infected soil. No population of *R. solanacearum* was detected in the neem- treated, infested soil at 56 DAP. The neem treatment resulted in differences for both the disease development and population density of RS. At 56 DAP, 50% of the tomato plants had survived in the infested soil with neem treatment and 100% in the control treatments without the pathogen (Table 2).

At the end of the trial (65 DAP), crushed neem seed had not reduced the total bacterial population and yield (Table 3). In the infested soil treated with crushed neem seed, the total bacterial population ($\log_{10}$ (CFU/g soil) = 7.29) was not significantly different from the population in the untreated, infested soil ($\log_{10}$ (CFU/g soil) = 7.37), the untreated, uninfested soil ($\log_{10}$ (CFU/g soil) = 7.14) and the treated, uninfested soil ($\log_{10}$ (CFU/g soil) = 7.42). The average yield per tomato plant was 80.3 and 81.5 g in the infested and uninfested soil, respectively, amended with 5% crushing neem seed compared with 78.4 g in the uninfested soil that had not been amended (Table 3, Figure 1).

DISCUSSION

Several plant extracts were shown to be effective in reducing the incidence of bacterial wilt. Aqueous extracts of nine plants: guava (*Psidium guajava*), eucalypt (*Eucalyptus globulus*), painted

Table 3 Tomato yield grown in 10-inch plastic pots with and without crushed neem seed amendment and total bacterial population in soil at the end of trial.

Treatment	Yield of tomato [a](g/plant)	Bacterial population density [b]
Uninfested soil	78.4	7.14
Uninfested soil + 5% CNS	81.5	7.42
Infested soil(untreated)	0	7.37
Infested soil + 5% CNS	80.3	7.29

[a] Mean values of total fresh fruit per plant at 65 days after planting

[b] Total bacterial population in soil was determined using a dilution plate technique onto nutrient glucose agar at 65 days after planting. Mean values are $\log_{10}$ transformation of CFU/g of soil.

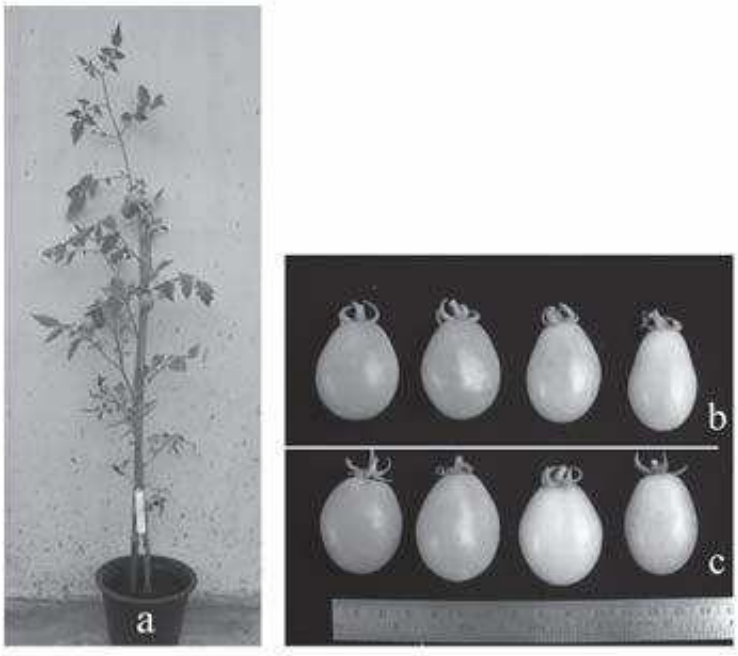

Figure 1 (a) A symptom-free tomato plant grown in soil infested with *Ralstonia solanacearum* and amended with 5% crushed neem seed; (b) tomato fruit from plants grown in soil infested with *R. solanacearum* and amended with 5% crushed neem seed; (c) tomato fruit from plants grown in uninfested soil and not amended.

copperleaf (*Acalypha wilkesiana*), artabotrys (*Artabotrys hexapetalus*), otaheite apple (*Spondias cytherea*), garden spurge (*Euphobia hirta*), tamarind (*Tamarindus indicus*), pineapple (*Ananas comosus*) and pomegranate (*Punica granatum*) were investigated for the control of bacterial wilt in tomato plants in the greenhouse (Twongsin, 2001). The aqueous extract of artabotrys produced the lowest severity of infection and a high survival rate of tomatoes at 14 days after treatment. However, soil drenching with the plant extract did not decrease the population of RS in the soil or control the disease 21 days after treatment. Yimyaem (2005) applied antagonistic bacterial powder and chuang chia (*Zanthoxylum rhetsa*) extract for the control of bacterial wilt in tomato plants in the greenhouse. The application of antagonistic bacterial powder and chuang chia or

chuang chia extract alone was not effective in reducing bacterial wilt incidence in the susceptible cultivar Seeda. No tomato plants had survived after 6 weeks of treatment.

The results of this experiment indicated that soil amendment with crushed neem seed was effective in the control of bacterial wilt in tomato plants. Crushed neem seed amendment significantly reduced bacterial wilt incidence by 50% compared to plants grown in the infected soil at 56 DAP. The neem treatment reduced the population density of *R. solanacearum* in the soil by 61.2 % at 28 DAP compared with the untreated, infested soil. The populations of *R. solanacearum* in the neem-treated, infested soil were not detected at 56 DAP. These results indicated that the mode of action of crushed neem seed was associated with bacterial growth and acted directly on the pathogen in soil.

The efficacy of chemicals or a green chemical to amend the soil has been studied for the control of bacterial wilt disease. The addition of urea (200 kg/ha N) and CaO (5,000 kg/ha) to soil have been reported to be effective in reducing the bacterial population and tomato bacterial wilt at the Asian Vegetable Research and Development Center in Taiwan. However, the results were not consistent when applied to soil from three other sites in Taiwan (Michel *et al*., 1997). Kawabata *et al* (2005) reported the control of tomato bacterial wilt by the addition of sawdust coated with PBVP-*co*-ST (an equimolar copolymer of *N*-benzyl-4-vinylpyridinium chloride with styrene) prior to transplantation. In this experiment, the bio-pesticide, crushed neem seeds were used as soil amendment. Neem is a dicotyledon and a member of the family Meliaceae, widely grown in all parts of Thailand. The neem seeds supplied at least two compounds, azadirachtin and salanin, that have insecticidal activity. In preliminary tests (unpublished data), the crude ethanol extract of crushed neem seed had bactericidal activity against *R. solanacearum* isolated from tomatoes (*Lycopersicon esculentum*), peppers (*Capsicum annuum*), ginger (*Zingiber officinale*) and galanga (*Alpinia galanga*).

Crushed neem seed may have a wide biological effect on other soil microbial populations. At the end of the current study (65 DAP), the total bacterial population in the soil and tomato yields were measured. The results indicated that the crushed neem seed treatment had no effect on soil bacterial populations and yields. Thus, the crushed neem seed may have a role in bacterial wilt control strategies, as crushed neem seed is considered to be more compatible with the environment than chemical treatments. However, more research needs to be carried out under field conditions in different areas.

CONCLUSION

Crushed neem seed was evaluated as a soil amendment under greenhouse conditions for the control of bacterial wilt in tomato plants caused by *Ralstonia solanacearum*. The neem treatment was found to reduce the pathogen population and disease development on the tomato variety 'Seedathip 3'. Treatment of the infested soil with 5% crushed neem seed increased the percentage of healthy plants at 56 DAP in the greenhouse compared with the untreated, infested soil. These findings represent the first report on the use of neem for controlling bacterial wilt disease.

ACKNOWLEDGEMENTS

The research was supported by Kasetsart University Research and Development Institute (KURDI), Kasetsart University. The author appreciated their financial support.

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