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Effect of BAU-Biofungicide and Plant Extracts against Root-Knot (*Meloidogyne javanica*) of Okra

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Abstract

The pot experiments were conducted from October 2009 to March 2010 in the Department of Plant Pathology, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh to study the effect of BAU-Biofungicide (*Trichoderma* spp.), Bermuda grass (*Cynodon dactylon*), Water spinach (*Ipomoea aquatica*) and Ivy leaf morninglory (*Ipomoea hederacea*) against root-knot of okra caused by *Meloidogyne javanica* using seed treatment and soil drenching methods. Among the treatments, BAU-Biofungicide and Bermuda grass leaf extract (S) gave superior result as it had increased length of shoot and root, weight of shoot and root, number of fruit and weight of fruit plant^{-1} correspondingly with the lowest galling incidence in okra in all methods. Better effect on plant growth characters with lower galling incidence and development of the nematode was observed with Water spinach extract (S) and Ivy leaf morninglory extract (S) compared to control. Lower effect of plant growth character was found with extract (S) of Ivy leaf morninglory followed by Water spinach leaf extract (S).

1. Introduction

Okra (*Hibiscus esculentus* L.) is a common and important vegetable crop in Bangladesh. During the year 2006-2007, the cultivated land of okra was 23,000 acres and production was 39,000 metric tons and the average yield was 1.69 metric ton acre^{-1} in Bangladesh (BBS, 2008). The soil and climate of Bangladesh are very favorable for the cultivation of okra but sometimes it is hampered by the attack of several species of nematodes (Timm and Ameen, 1960 and Mian, 1986). It is reported that 15 genera of plant parasitic nematodes are associated with commercial crops in Bangladesh, where *Meloidogyne* spp. are more abundant and widespread (Timm and Ameen, 1960). Among the nemic diseases, the root-knot caused by *Meloidogyne javanica* and *Meloidogyne incognita* are associated with okra. Existing practice of chemical control is too expensive, particularly, for poor farmers of Bangladesh as well as a difficult task to determine the precise dose of the chemical for its application to the field. In addition, their harmful effect is responsible for air, soil and water pollution (Alam, 1987). For these reasons, the extent of use of chemicals in controlling plant pathogens including nematodes has been discouraged all over the world. Biological control of pathogens offers environmentally safe, durable and cost effective alternatives to chemicals (Papavizas and Lumsden, 1980). Biological agents are now being used in many developed countries for combating diseases with the aim of increasing food production. Bio-control management with beneficial microbes manipulates the environment for better plant health and vigour (Kowk et al., 1987). In biological control, *Trichoderma* spp. stimulates the growth of the plant against plant pathogenic nematodes (Inber et al., 1994).

Various plant extracts and plant parts have recently been reported to have anti-nematode properties (Haseeb et al., 1982; Mahmood et al., 1982; Hussain et al., 1984; Sartaj et al., 1985; Arya and Saxena, 1988; Chhabra et al., 1988; Ahmad et al., 1990; Ganai et al., 1992; Mukhter et al., 1994; Sellami and Mouffarrah, 1994). Plant grown in the cultivated land, road side may be used to prepare the solution or paste for application against root-knot disease of vegetables (Irshad et al., 1982; Stephan et al., 1989). Hence, this experiment has been carried out with BAU-Biofungicide, standard leaf extract of Bermuda grass (*Cynodon dactylon*), Water spinach (*Ipomoea aquatica*) and Ivy leaf morninglory (*Ipomoea hederocea*) to evaluate the efficacy and superiority of BAU-Biofungicide and plant extract against root-knot of okra caused by *Meloidogyne javanica*.

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2. Materials and methods

The pot experiments were conducted from October 2009 to March 2010 in the Department of Plant Pathology, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh. Two sets of pot experiments namely; seed treatment and soil drenching with BAU-Biofungicide and plant extracts on growth of 60, 90 and 120 days old okra plant inoculated at 30 days of growth stage with *M. javanica* before 7 days of drenching. BAU-Biofungicide, standard aqueous leaf extracts of Bermuda grass (*Cynodon dactylon*), Water spinach (*Ipomoea aquatica*) and Ivy leaf morninglory (*Ipomoea hederacea*) were used in the following treatments:T_0 = Control (without extract/ BAU-Biofungicide) T_1 = BAU-Biofungicide solution (2%), T_2 = Bermuda grass (*Cynodon dactylon*) extract (S), T_3 = Water spinach (*Ipomoea aquatica*) leaf extract (S), T_4 = Ivy leaf morninglory (*Ipomoea hederacea*) leaf extract (S) arranged in completely randomized design.

Sandy loam soil, sand and well decomposed cowdung were mixed properly at the ratio of 2:2:1 followed by sterilization with formalin at the rate of 3% ft^{-3} soil. After 72 hours, the sterilized soil was exposed to air-drying for 48 hours. Earthen pots (30 cm diameters) were washed with water and sterilized with formalin, then filled with 5 kg sterilized dried soil. Disease free okra seeds cv. BARI-1 were surface sterilized with low concentration of mercuric chloride solution (0.001%) for 1 minute followed by washing thrice with water. Petridish was taken and surface sterilized with methylated spirit before placing seeds for treating with BAU-Biofungicide @ 1:40 w w^{-1} (BAU-Biofungicide: seed) collected from Disease Resistance Laboratory, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh. The seeds were thoroughly mixed with the BAU-Biofungicides by shaking the Petridish and placed for shade drying, followed by sowing in the pot on the same day in the afternoon. Leaf (20 g) of Bermuda grass was collected and macerated in an electric blender and soaked separately in l00 ml distilled water in several conical flasks. The filtrate collected was considered as standard concentration of Bermuda grass extract. The same procedure was followed for the preparation of leaf extracts of Water spinach and Ivy leaf morning glory. The seeds were thoroughly soaked in the standard Bermuda grass extract (30 ml) for 30 minutes @ 1:10 w w^{-1} (extract : seed). Seed treatments and sowing were followed as before. Soil drenching was done following normal procedure. Egg masses were collected from the brinjal roots which was inoculated with a single egg mass of *M. javanica* before the experiment. Ten reddish brown mature egg masses pot^{-1} were placed around the both sides of plant root at 2.5 cm deep, five egg masses on each side of the plant. Inoculation was done on the 30 days old seedlings in pots.

After 30, 60 and 90 days of inoculation, data on the length of shoot (cm), length of root (cm), weight of shoot (g), weight of root (g), number of galls g^{-1} of root, number of fruits $plant^{-1}$, weight of fruits $plant^{-1}$. The recorded parameters were analyzed by F-test for the treatment means and replication means using Duncan's New Multiple Range Test at 0.05 level.

3. Results and Discussion

3.1. Effect of seed treatment

Significantly, the highest 36.88, 80.67 and 103.60 cm shoot length were recorded with T_1 but T_0 resulted the lowest 29.17, 57.43 and 83.17 cm shoot length at 60, 90 and 120 days, respectively (Table 1). T_1 was found with the maximum root length 28.40, 43.40 and 49.50 cm while the minimum 20.25, 30.57 and 32.72 cm were in T_0, respectively. The highest 39.54, 172.70 and 174.50 g shoot weight were recorded with T_1 and the lowest 27.56, 109.50 and 111.30 g was in T_0. T_1 carried out higher root weight (10.06, 35.10 and 71.25 g) than T_0 (5.59, 25.08 and 58.86 g). The maximum 15.67, 21.00 and 29.33 galls was found in T_0 but T_1 resulted the lowest 7.33, 13.67 and 16.33 galls g^{-1} of fresh root, respectively. T_1 gave the highest 3.00, 12.00 and 16.00 number of fruit while the lowest 1.33, 6.33 and 9.33 number of fruit was in T_0, respectively. The highest 52.67, 246.80 and 400.90 g weight of fruit were found in T_1 but the lowest 24.60, 146.70 and 208.70 g were in T_0, respectively. From the present investigation, it was revealed that extract of neem and *Trichoderma* were effective in controlling plant parasitic nematode. These results are in close proximity with Alam (1990) and Khan et al. (2001) where they reported that Neem (*Azadirachta indica*) had good properties in controlling plant parasitic nematodes. Bettiol (1996) reported that *Trichoderma* spp. hinders the establishment of nematode. Similar observations were also noted by El-Moity et al. (1998) and Khan et al. (2001). *Trichoderma* based BAU- Biofungicide may have some kinds of antibiotics which suppressed the activity of nematode. *T. harzianum* improved the growth of root of *Meloidogyne* infested plants and decreased the galling index, number of eggs which enhanced plant height, shoot weight, root length, root weight and reduced nematode populations (Rao et al., 1998; Yang-Xiu Juan et. al. 2000; Manuzca 2001; Devi et al., 2002; Hemlata et al., 2001). Sharma (1999) also showed that *T. harzianum* caused the highest (69.6%) mortalities of *M. incognita.*

3.2. Effect of soil drenching

Significantly, the highest shoot length was recorded with T_1 having 31.67, 73.58 and 90.50 cm while T_0 showed the lowest 25.85, 53.75 and 75.17 cm shoot length at 60, 90 and 120 days, respectively (Table 2). The maximum root length 24.51, 42.75 and 39.00 cm was recorded with T_1 but the minimum 17.51, 32.18 and 27.62 cm root length was in T_0, respectively.

Table 1: Effect of seed treatment with BAU-Biofungicide and plant extracts on growth of 60, 90 and 120 days old okra plant inoculated at 30 days of growth stage with *M. javanica*

Days	Treatments	Length of shoot (cm)	Length of root (cm)	Weight of shoot (g)	Weight of root (g)	Number of galls g^{-1} root	Number of fruit $plant^{-1}$	Weight of fruit (g) $plant^{-1}$
60	T_0	29.17^{c}	20.25^{d}	27.56^{d}	5.59^{e}	15.67^{a}	1.33^{d}	24.60 c
	T_1	36.88^{a}	28.40^{a}	39.54^{a}	10.06^{a}	7.33^{e}	3.00^{a}	52.67^{a}
	T_2	36.50^{a}	28.27^{a}	37.99^{b}	7.79^{b}	8.67^{d}	2.00^{b}	40.67^{b}
	T_3	35.33^{b}	22.70^{b}	31.89^{c}	7.12^{c}	11.67^{c}	2.00^{b}	37.31^{b}
	T_4	30.25^{c}	21.70^{c}	31.14^{c}	6.49^{d}	12.67^{b}	1.67^{c}	29.13^{c}
90	T_0	57.43^{d}	30.57^{e}	109.50^{e}	25.08^{d}	21.00^{a}	6.33^{e}	146.70^{e}
	T_1	80.67^{a}	43.40^{a}	172.70^{a}	35.10^{a}	13.67^{d}	12.00^{a}	246.80^{a}
	T_2	74.83^{b}	40.65^{b}	163.60^{b}	34.08^{a}	14.67^{bc}	10.33^{b}	205.80^{b}
	T_3	73.74^{b}	36.26^{c}	151.40^{c}	32.75^{b}	14.33^{cd}	8.33^{c}	180.80^{c}
	T_4	61.09^{c}	33.58^{d}	111.00^{d}	27.75^{c}	15.33^{b}	7.67^{d}	160.70^{d}
120	T_0	83.17^{d}	32.72^{e}	111.30^{e}	58.86^{e}	29.33^{a}	9.33^{e}	208.70^{e}
	T_1	103.60^{a}	49.50^{a}	174.50^{a}	71.25^{a}	16.33^{d}	16.00^{a}	400.90^{a}
	T_2	94.92^{b}	45.50^{b}	152.40^{b}	67.53^{b}	21.33^{c}	14.33^{b}	331.30^{b}
	T_3	94.10^{b}	36.75^{c}	130.80^{c}	64.53^{c}	23.67^{b}	13.33^{c}	293.20^{c}
	T_4	86.67^{c}	34.58^{d}	114.40^{d}	60.49^{d}	24.33^{b}	11.67^{d}	269.50^{d}

In a column, values having same letter(s) do not differ significantly at p=0.05 level.

Table 2: Effect of soil drenching with BAU-Biofungicide and plant extracts on growth of 60, 90 and 120 days old okra plant inoculated at 30 days of growth stage with *M. javanica*

Days	Treatments	Length of shoot (cm)	Length of root (cm)	Weight of shoot (g)	Weight of root (g)	Number of galls g^{-1} root	Number of fruit $plant^{-1}$	Weight of fruit (g) $plant^{-1}$
60	T_0	25.85^{c}	17.51^{c}	14.75^{c}	4.06e	11.33^{a}	1.00^{c}	17.02^{c}
	T_1	31.67^{a}	24.51^{a}	22.48^{a}	5.47^{a}	6.33^{d}	2.00^{a}	27.09^{a}
	T_2	31.50^{a}	24.20^{a}	22.33^{a}	5.18^{c}	8.67^{c}	1.33^{b}	19.41^{bc}
	T_3	31.08^{a}	23.58^{a}	22.25^{a}	5.30^{b}	9.00^{c}	1.33^{b}	21.20^{b}
	T_4	28.42^{b}	20.24^{b}	17.22^{b}	5.08^{d}	9.67^{b}	1.00^{c}	16.27^{c}
90	T_0	53.75^{e}	32.18^{d}	135.90^{d}	16.57^{d}	18.67^{a}	4.67^{d}	107.70^{d}
	T_1	73.58^{a}	42.75^{a}	157.40^{a}	24.60^{a}	12.33^{e}	8.33^{a}	203.10^{a}
	T_2	72.00^{b}	41.58^{a}	153.40^{b}	22.74^{b}	13.33^{d}	7.00^{b}	168.70^{b}
	T_3	63.75c	35.92^{b}	149.90^{c}	21.07^{c}	17.00^{c}	6.67^{b}	157.20^{b}
	T_4	59.42^{d}	33.92^{c}	148.50^{c}	16.96^{d}	17.00^{b}	5.67^{c}	131.70^{c}
120	T_0	75.17^{d}	27.62^{d}	107.20^{e}	32.20^{e}	26.67^{a}	8.67^{d}	211.20^{e}
	T_1	90.50^{a}	39.00^{a}	146.30^{a}	43.68^{a}	14.33^{e}	12.00^{a}	310.80^{a}
	T_2	89.33^{a}	38.33^{a}	138.60^{b}	41.19^{b}	16.67^{d}	11.33^{b}	289.80^{b}
	T_3	84.23^{b}	36.22^{b}	127.60^{c}	37.52^{c}	20.67^{c}	11.00^{b}	259.90^{c}
	T_4	78.65^{c}	32.16^{c}	108.70^{d}	33.59^{d}	24.33^{b}	9.67^{c}	238.90^{d}

In a column, values having same letter(s) do not differ significantly at p=0.05 level.

T_1 (22.48, 157.40 and 146.30 g) performed better shoot weight than T_0 (14.75, 135.90 and 107.20 g). The maximum 5.47, 24.60 and 43.68 g root weight was in T_1 but T_0 showed the minimum 4.06, 16.57 and 32.20 g, respectively. In respect of galling, T_0 gave the highest 11.33, 18.67 and 26.67 number of galls g^{-1} although the lowest galling was in T_1 having 6.33, 12.33 and 14.33 galls, respectively. T_1 achieved superior fruit number (2 .00, 8.33 and 12.00) to T_0 (1.00, 4.67 and 8.67). T_1 resulted the highest fruit weight (27.09, 203.10 and 310.80) compared to T_0 (16.27, 107.70 and 211.20 g). T_1 (BAU-Biofungicide) was effective in increasing plant growth, yield and reduced galling incidence compared to other treatments.

These findings corroborate with the reports of a good number of researchers (Sharon et al., 2001; Manuzca, 2001; Devi and Hassan, 2002). They also reported the effectiveness of *Trichoderma* spp. to reduce gall formation on okra caused by *M. incognita* and *M. javanica.* It is observed that *Trichoderma* isolates reduced nematode infestation and increased plant growth over control and was most effective antagonistic against *Meloidogyne* spp. (Devila et al., 1999; Faruk et al., 1999; Siddiqui et al., 1999; Devi and Hassan 2002).

4. Conclusion

Seed treatment and soil drenching of BAU-biofungicide solution (2%) as well as Bermuda grass leaf extract recorded the highest shoot and root length as well as weight, number of galls g^{-1}, number of fruit $plant^{-1}$ and weight of fruit (g) $plant^{-1}$. Therefore, BAU-biofungicide solution (2%) and Bermuda grass leaf extract may be recommended for the control of root knot of Okra.

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