Effect of seed treatment with biological agents on germination and vigour of bottle gourd seedlings following tray method

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Abstract: An experiment was conducted in the greenhouse of the Seed Pathology Centre, Bangladesh Agricultural University, Mymensingh, Bangladesh in April 2012 to determine the effect of seed treatment with different biological agents including BAU-Biofungicide on germination of bottle gourd seeds under greenhouse condition. Two factors factorial experiment with two varieties and seven treatments was conducted in Randomized Complete Block Design (RCBD) and each treatment was replicated four times. The Two factors were Treatments (T_0 = Control (water), T_1 = BAU-Biofungicide, T_2 = Mustard oil cake, T_3 = Poultry litter, T_4 = Neem leaf powder, T_5=Garlic clove extract and T_6 = Paradox 5 G (Chemical nematicide) and Varieties (V_1= Hi-green and V_2= Martina). All the treatments showed increased germination over untreated control where maximum germination was recorded by seed treatment with BAU-Biofungicide followed by garlic clove extract and neem leaf powder.
Key words: Biological agents, germination, vigour, seedlings.

Introduction

Root-knot nematodes are serious and economically most important pest of many cultivated crops around the world (Trifonova *et al.,* 2009). They are particularly damaging vegetables in tropical and sub-tropical countries (Sikora and Fernandez, 2005) and cause losses up to 80% in heavily infested fields (Kaskavalci, 2007). Nahar *et al.* (1996) estimated yield loss in bottle gourd due to*Meloidogyne incognita* as 26.44 percent. Removal of key nematicides from the market in the last few decades due to concerns about the environment safety as well as human health (Rich *et al.,* 2004) and development of resistance in pathogen against these chemicals, which create problems to control them, presently researchers have diverted their attention to manage plant nematode through use of organic amendments (Bari *et al,*. 2004) and biological measures like *Trichoderma* spp., *Pacecilo myceslilacinus, Pasturia penetrans* and *Pseudomonas aeruginosa* (Siddiqui *et al.,* 1999). Biological control has been proposed as a replacement for chemical control of plant diseases (Harman, 2000). Amongst various organic amendments, the nematicidal potential of the *Azadirachta indica* (neem), castor, mustard, and *Citrullus* have been reported by various researchers (Khan *et al.,* 2011). Use of BAU-Biofungicide as nematode killing agent is a new approach to eco-friendly measures. As bio-control agents' micro-organisms like *Trichiderma* spp., *Pasturia penetrans* act either as antagonists, parasites or predators (Siddiqui *et al.,* 1999). *Trichoderma* spp. as bio-fungicidal agents has been found to be effective against soil borne plant pathogenic nematodes like *Meloidogyne* spp. (Sheron *et al.,* 2001). Concerns about the health, safety and environmental effects of agricultural chemicals in our water, soil and food, the use of biological control need to be emphasized strongly.Under the above circumstances, the present research programme was undertaken to investigate the effect of seed treatment with biological agents on germination and vigour of bottle gourd seedlings using tray method.

Materials and Methods

The experiment was conducted in the greenhouse of the Seed Pathology Centre, Bangladesh Agricultural University, Mymensingh, Bangladeshin April 2012.Two factors factorial experiment with two varieties and seven treatments was conducted in Randomized Complete Block Design (RCBD). Each treatment was replicated four times. All the trays were arranged randomly.The Two factors wereTreatments (T_0 = Control (water),T_1 = BAU-Biofungicide, T_2 = Mustard oil cake,T_3 = Poultry litter,T_4 = Neem leaf powder, T_5 = Garlic clove extract andT_6 = Paradox 5 G (Chemical nematicide) and Varieties (V_1= Hi-green and V_2 = Martina). The varieties were screened out from by a pot experiment that were highly susceptible to *Meloidogyne incognita.* The collected sand was sterilized with formalin (40%) at the rate of 5ml formalin diluted with 20 ml of water for 4kg sand (Dashgupta, 1988). The formalin treated sand was covered with polythene sheet for 48 h and then exposed for 48 h for aeration before setting of the experiment. Thus the sand was ready for sowing the seeds. The plastic trays (12”×8”) were filled with the sand and moistened with water. The seeds were thoroughly mixed with BAU-Biofungicide @ 1:40 w/w (BAU-Biofungicide: Seed) following the method of Hossain (2003) by shaking the plastic potsthat were surface sterilized with methylated spirit before placing seeds. In case of mustard oil cake,poultry litter,neem leaf powder,garlic clove extract and distilled water, seeds were soaked and thoroughly mixed with the composition and stirred with the help of a glass rod for 2-3 minutes (Yadav *et al.,* 2005). Water soaked two hundred seeds of selected two varieties were taken in two flasks separately with Paradox 5 G (2 g a.i/kg seed) and thoroughly mixed with Paradox 5 G by shaking (Fazal *et al.,* 2001). The coated, soaked and seeds treated were then placed in a cool and dry place under shade for drying and the seeds were ready for sowing in the tray on the same day.Treated seeds were sown in plastic trays (50 seeds per tray) maintaining equal distances among the seeds. Four trays were allocated per treatment and each tray was considered as one replication. Fourteen days after sowing, the sand of the tray was watered to make it moist for easy uprooting of the bottle gourd seedlings. Randomly selected 10 seedlings were uprooted carefully from each tray and washed thoroughly with running tap water. The modified root portion of each seedling was separated from the shoot by a sharp knife and mean shoot and root length and mean shoot and root weight were measured. Data were recorded three times from each treatment at 14 days after sowing (DAS) following the International Rules for Seed Testing

Association (ISTA, 2001). Data about germination (%), abnormal seedlings (%), normal seedlings (%), shoot length (cm), root length (cm), shoot weight (g), root weight (g) parameters and the vigour Index (VI) was computed using the formula of Baki and Anderson (1973).

Results and Discussion

In case of germination (%), the treatments were found to differ significantly over control where germination of seeds ranged from 77.75 to 98.25 %. Seed treatment with BAU-Biofungicide gave 98.25% germination which was 26.36% higher than the untreated control, while garlic clove extract and neem leaf powder treated seeds showed 90.50% and 88.25% germination and 16.40% and 13.50% higher values, respectively over the untreated control. Similar results were also observed by Rahman, (2006) that BAU-Biofungicide significantly increased the seed germination in different crops. Bhuiyan *et al.,* (2006) used BAU-Biofungicide for seed treatment of different winter vegetables and found that the germination of BAU-Biofungicide treated resulted up to 22% higher germination over the untreated control.

The formation of normal seedlings varied significantly among the different treatments and ranged from 89.25 to 97.75 %. The highest normal seedlings were observed when the seeds were treated with BAU-Biofungicide (97.75%)and the lowest normal seedlings were in untreated control (89.25%). No significant differences were found among the treatments BAU-Biofungicide, garlic clove extract and poultry litter. Again, no significant differences were observed among the treatments poultry litter, neem leaf powder and garlic clove extract. Maximum abnormal seedlings were recorded in untreated control followed by Paradox 5 G and mustard oil cake. No significant difference was found between these two treatments. Again, no significant differences were found among the treatments poultry litter, neem leaf powder and garlic clove extract. The lowest percentage of abnormal seedlings was found with BAU-Biofungicide. The highest vigour index was recorded in BAU-Biofungicide (35.65) followed by poultry litter (28.41), garlic clove extract (27.24) and neem leaf powder (27.15) treated seeds having 75.87%, 40.15%, 34.38% and 33.94% higher vigour indexes, respectively were recorded at 14 DAS over the untreated control (Table 1).

Table 1. Effects of seed treatment with biological agents and Paradox 5 G on germination, formation of normal and abnormal seedlings, shoot and root lengths, shoot and root weights and vigour index of bottle gourd at 14 days after sowing

Treatment	%Germination	% Normal seedlings	% Abnormal seedlings	Shoot length (cm)	Root length (cm)	Shoot wt. (g)	Root wt. (g)	Vigour index
Control (untreated)	77.75 f	89.25 d	10.75 a	18.33 d	7.75 d	1.67 e	0.17 d	20.27 f
BAU Bio fungicide	98.25 a (+26.36)	97.75 a (+9.52)	2.25 d (-79.07)	23.46 a (27.99)	12.82 a (65.41)	2.62 a (56.88)	0.30 a (72.98)	35.65 a (+75.87)
Mustard oil cake	86.75 d (+11.57)	93.00 c (+4.20)	7.00 b (-34.88)	20.04 c (9.32)	9.71 c (25.29)	1.81 d (8.38)	0.20 c (17.81)	25.81 d (+27.33)
Poultry litter	86.75 d (+11.57)	96.75 ab (+8.40)	3.25 cd (-69.77)	21.94 b (19.69)	10.81 b (39.48)	2.18 b (30.53)	0.22 b (30.45)	28.41 b (+40.15)
Neem leaf powder	88.25 c (+13.50)	96.50 b (+8.12)	3.50 c (-67.44)	19.96 c (8.89)	10.80 b (39.35)	2.14 bc (28.14)	0.19 c (11.49)	27.15 c (+33.94)
Garlic clove extract	90.50 b (+16.40)	97.25 ab (+8.96)	2.75 cd (-74.41)	19.21cd (4.80)	10.90 b (40.64)	2.10 c (25.74)	0.19 c (13.21)	27.24 c (+34.38)
Paradox 5 G	79.75 e (+2.57)	92.75 c (+3.92)	7.25 b (-32.56)	18.76 d (2.35)	7.98 d (2.96)	1.86 d (11.37)	0.17 d (2.87)	21.33 e (+5.23)
Significant level	**	**	**	**	**	**	**	**

Data represent the means of four replications, similar letter(s) in a column are not significantly different at 5% level by DMRT, ** = Significant at 1% level of probability, Data in parentheses indicate % increased (+) and decreased (-) over control

It was observed that shoot length among the treatments varied significantly and maximum shoot length 23.46 cm was found in case of BAU-Biofungicide treated seedlings which resulted 27.99% increase in length over the untreated control. Minimum shoot length 18.33 cm was found with control treatment. Maximum root length was found when the seeds were treated with BAU-Biofungicide which resulted 65.41% increased root length over untreated control. Minimum root length was observed in untreated control as well as in seeds treated with Paradox 5 G. In case of shoot weight, maximum shoot weight 2.62 g was found with BAU-Biofungicide treated seedlings which resulted 56.88 % increase over untreated control (1.86 g) at 14 DAS. Maximum root weight was found when seeds were treated with BAU-Biofungicide which resulted 72.98 % increased root weight over the untreated control (Table 1).

From the results, it was revealed that germination, formation of normal and abnormal seedlings and vigour index were 87.07 %, 95.00 %, 5.00% and 26.61 in variety Hi-green and 86.64%, 94.50%, 5.50% and 26.49 in variety Martina, respectively. The difference in shoot length of two varieties was not significant. Similarly, root length between the two varieties was not significant. Fresh weight of shoot was significantly higher in Martina compared to Hi-green, while fresh weight of root was significantly higher in Hi-green compared to Martina (Table 2).

Interaction effects of the treatments and varieties on the germination, formation of normal and abnormal seedlings,

vigour index, shoot and root lengths were found to be insignificant. Maximum germination (98.50%) was recorded with the treatment BAU-Biofungicide interacting with variety Martina. On the other hand, the highest number of normal seedlings (98.00%), the lowest number of abnormal seedlings (2.00%) were observed with the treatment BAU-Biofungicide interacting with variety Hi-green. The highest vigour index (35.85) was recorded with the treatment BAU-Biofungicide interacting with variety Martina (Table 3).

Table 2. Response of two varieties of bottle gourd on germination, formation of normal and abnormal seedlings, shoot and root lengths, shoot and root weights and vigour index of bottle gourd seedlings at 14 days after sowing

Variety	% Germination	% Normal seedlings	% Abnormal seedlings	Shoot length (cm)	Root length (cm)	Shoot weight (g)	Root weight (g)	Vigour index
Hi-green	87.07	95.00	5.00	20.24	10.10	2.02 b	0.21 a	26.61
Martina	86.64	94.50	5.50	20.23	10.11	2.09 a	0.20 b	26.49
Significant level	NS	NS	NS	NS	NS	**	*	NS

Data represent the means of four replications, similar letter(s) or without letter in a column are not significantly different at 5% level by DMRT, ** = Significant at 1% level of probability, *= Significant at 5% level of probability and NS= Not significant

Table 3. Interaction effects of treatments and varieties on germination, formation of normal and abnormal seedlings and vigour index of bottle gourd at 14 days after sowing

Treatments	% Germination		% Normal seedlings		% Abnormal seedlings		Vigour index	
	Hi-green	Martina	Hi-green	Martina	Hi-green	Martina	Hi-green	Martina
Control (untreated)	78.00	77.50	90.00	88.50	10.00	11.50	20.16	20.38
BAU-Biofungicide	98.00	98.50	98.00	97.50	2.00	2.50	35.84	35.85
Mustard oil cake	87.00	86.50	93.00	93.00	7.00	7.00	26.35	25.26
Poultry litter	87.00	86.50	97.00	96.50	3.00	3.50	28.41	28.41
Neem leaf powder	88.50	88.00	96.50	96.50	3.50	3.50	27.11	27.19
Garlic clove extract	90.50	90.50	97.50	97.00	2.50	3.00	26.96	27.53
Paradox 5 G	80.50	79.00	93.00	92.50	7.00	7.50	21.46	21.19
Significant level	NS		NS		NS		NS	

Each value is an average of four replications, Values in the column having common letter(s) or without letter do not differ significantly at 5% level by DMRT, NS= Not significant

Table 4. Interaction effects of treatments and varieties on shoot and root length, shoot and root weight of bottle gourd seedlings at 14 days after sowing

Treatments	Shoot length (cm)		Root length (cm)		Shoot weight (g)		Root weight (g)	
	Hi-green	Martina	Hi-green	Martina	Hi-green	Martina	Hi-green	Martina
Control (untreated)	18.05	18.60	7.80	7.70	1.64 f	1.71 f	0.16 f	0.18 ef
BAU-Biofungicide	23.77	23.15	12.80	12.85	2.62 a	2.63 a	0.29 a	0.30 a
Mustard oil cake	20.50	19.57	9.80	9.62	1.66 f	1.95 d	0.21 c	0.19 de
Poultry litter	21.90	21.97	10.75	10.87	2.19 b	2.17 b	0.24 b	0.21 c
Neem leaf powder	19.92	20.00	10.70	10.90	2.17 b	2.12 b	0.19 de	0.19 de
Garlic clove extract	18.92	19.50	10.87	10.92	2.04 c	2.16 b	0.20 cd	0.19 de
Paradox 5 G	18.65	18.87	8.02	7.95	1.81 e	1.92 d	0.19 de	0.16 f
Significant level	NS		NS		**		*	

Each value is an average of four replications, Values in the column having common letter(s) or without letter do not differ significantly at 5% level by DMRT, ** = Significant at 1% level of probability, *= Significant at 5% level of probability, NS= Not significant

The highest shoot length (23.77 cm) was found with the treatment BAU-Biofungicide interacting with variety Hi-green and the highest root length (12.85 cm) was found with the treatment BAU-Biofungicide interacting with variety Martina. Interaction effects of shoot and root weights were found to be significant. The highest 2.62 g shoot weights were found with treatment BAU-Biofungicide interacting with variety Hi-green and same was true for this treatment interacting with the variety Martina having maximum 2.63 g shoots weight.Like that of shoot weight, the highest 0.29 g root weight was noted with the treatment BAU-Biofungicide interacting with the variety Hi-green and same was true for this treatment interacting with the variety Martina having maximum 0.30 g root weight. The interaction effects of the treatments and varieties revealed that the treatment BAU-Biofungicide interacting with the varieties Hi-green and Martina gave significantly the highest fresh weight of

shoot and root, while the least responses were found with control treatment interacting with both the varieties (Table 4).

Recommendations: Considering the above results it can be concluded that all the treatments showed increased germination over untreated control where maximum germination was recorded by seed treatment with BAU-Biofungicide followed by garlic clove extract and neem leaf powder.

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