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Research Article

Screening of *Bacillus* isolates against *Aspergillus niger* causing collar rot of groundnut

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ABSTRACT

The investigation was carried out on the biocontrol of common collar rot of groundnut caused by *Aspergilus niger* by using isolates of *Bacillus*. The four isolates of *Bacillus* were isolated from the groundnut rhizosphere soil. Each isolate was characterized and identified and designated as B1 to B4. *A. niger* was isolated from rhizosphere soil of groundnut. The B1. isolate showed medium inhibitory activity (45.73%) on radial growth of *A. niger* on 96 hours incubation in dual culture method. Maximum per cent inhibition ef radial growth of fungi was observed with isolates of B-1 (48.98%) in volatile method. Groundnut seed inoculated with bioisalate showed highest percentage of seed germination and B_2, B_3 and B_4 isolates produced 66.66 per cent, 57.98 per cent and 50.15 per cent mortality, respectively.

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INTRODUCTION

Plant diseases need to be controlled to maintain the quality and abundance of food, feed, and fiber produced by growers around the world. Different approaches may be used to Control plant diseases, Such as chemical fertilizers and pesticides. The environmental pollution caused by excessive use and misuse of agrochemicals some pest management have focused their efforts on developing alternative inputs to synthetic chemicals for controlling pests and diseases. Among these, alternatives are those referred to as biological control. Members of the U.S. National Research Council took into account modern biotechnological developments and referred to biological control as the use of natural or modified organisms, genes, or gene products, to reduce the effects of undesirable organisms and to favor desirable organisms such as crops, beneficial insects, and microorganisms", but this definition spurred much subsequent debate and it was frequently considered too broad by many' scientists who worked in the field (US Congress, 1995). The use of a gram-positive *Bacillus* species as a biocontrol agent is relatively rare, and has received less intensive study than the use of gram-negative bacteria. The antagonists studied have been mainly *Bacillus* subtilis and occasionally B. megaterium, B. cereus, B. pumilus, and B. polymyxa (Utkhede, 1984). As *Bacillus* spp. have the characteristics of, being widely distributed in nearly all agricultural soils and in other environments, having high thermal tolerance, showing rapid growth in liquid culture, and readily form resistant spores. Moreover, they are considered safe biological agents, and their potential as Bio-control agents is considered to be high. However, the evaluation of bacteria has focused primarily on disease suppression (Siala and Gray, 1974).

Bacillus spp. can be used as biological control agent for bacteria and fungal diseases like gray mold, powdery mildews, early and late blight, bacterial spot and walnut blight through production of antimicrobial proteins namely bacteriocin, chitinase, glucanase etc and antibiotics as well as antifungal synthesized by secondary metabolism pathways.

MATERIALS AND METHODS

Isolation of *Bacillus* isolates:

Rhizosphere soil samples obtained from agriculture fields cultivated with groundnut from several location of Dharmapuri district and brought to laboratory in polythene bag. For isolation of *Bacillus* species, each gram of soil sample was suspended in 99m1 of sterile distilled water and shaken

vigorously for 2 min The samples were heated at 60°C for 60 min in a water bath. Then the soil suspensions were serially diluted in sterile distilled water, and the dilution from 10-1 and 10-5 were placed on nutrient agar medium. The plates were incubated at 28-37°C for 24-48 hrs (Watanabe and Hayano, 1993; Chilcott and Wigley. 1993). Colonies were isolated on the basis of their different visual characteristics. After isolation, all colonies were purified by single colony isolation after re-streaking on nutrient agar medium.

Identification of *Bacillus* spp.:

The bacterial isolates were characterized morphologically and biochemically by Gram's staining, Spore staining, Colony Morphology, Cell Shape and Motility test

Isolation of *Aspergillus niger*:

A.niger was isolated from rhizosphere soil of groundnut which was collected from Dharmapuri District. The dilution was prepared by adding 10 g of sample to 90 ml sterilized distilled water and homogenized by mechanical shaking for 2 min. From this diluted sample, 0.1 ml was pipette onto the surface of petri plates containing the Potato dextrose agar and spread using sterilized L rod. The petri plates were incubated at 28^0C for 5 to 7 days. A. niger was isolated based on colony morphology.

Antagonistic activity of *Bacillus* isolates against *A. niger*:

Dual culture method (Montealegre *et al.*, 2003), Bioefficacy for volatile antagonistic activity of *Bacillus* isolates against A. niger (Dennis and Webster, 1971), Pot Culture Trial (Padmodaya and Reddy, 1998), Preparation of *A. niger* infested soil: Preparation of inoculum of antagonists: Seed treatment:

Experimental details:

Crop	:	Groundnut
Variety	:	TMV-7
No. of treatments	:	6
No. of replications	:	3
Date of sowing	:	03.1. 2010

Treatments		
Sr. No.	Name of the treatments	
1.	Control	T_1
2.	Seeds + *A.niger*	T_2
3.	Seeds + *A.niger* + B1	T_3
4.	Seeds + *A.niger* + B2	T_4
5.	Seeds + *A.niger* + B3	T_5
6.	Seeds + *A.niger*+B4	T_6

RESULTS AND DISCUSSION

The findings of the present study have been presented in the following sub heads :

Characterization of *A. niger* isolate:

The colony morphology of A. niger was found as when immature they are covered with white fluffy aerial mycelia and when mature they covered with black spores. They produce septate hyphae and globose shaped conidial head with large black to brownish black. The conidiophore was a hyaline and the conidia were globose and echinulate with 4- 5 µm in diameter.

Isolation and identification of *Bacillus* isolates:

Four isolates of *Bacillus* species were isolated from the rhizosphere soil of groundnut in Dharmapuri district, of Tamil Nadu. The soil samples for isolating the *Bacillus* species were collected from the field whose pH range from 6.5 to 7.4 with red soil type. All isolates were aerobic with straight rods, motile, endospore forming, gram positive, strongly catalase positive and indicative of *Bacillus* species, namely *Bacillus* - 1, *Bacillus*-2, *Bacillus*-3 and *Bacillus*-4 which was designated as B_1 to B_4.

Antagonistic activity of *Bacillus* isolates against:
A. niger by Dual culture method:

On Potato Dextrose Agar (PDA) Medium, the antagonistic *Bacillus* isolates was found to restrict the growth of collar rot causing *A. niger* (Table 1). The antagonistic activities of *Bacillus* spp. isolates were excellent but they were showed different antagonism towards the mycelial radial growth of *A. niger*. The B_1 isolate showed maximum inhibitory activity (45.73%) on radial growth of *A. niger* on 96 hours incubation. The remaining isolates like B_2, B_3 and B_4 showed less inhibitory action against *A. niger* which radial growth was restricted in percentage of 21.34, 21.95 and 37.00, respectively.

Bioefficacy for volatile antagonistic activity of *Bacillus* isolates against *A. niger*:

All the isolates of *Bacillus* inhibited the fungi by production of a volatile inhibitory factor. Maximum percent inhibition of radial growth of fungi was observed with isolates of B_1-1 (48.98%). The least volatile antagonistic activity of *Bacillus isolate* – B_4 was shown by 2.04 per cent only. The remaining isolates B_2 and B_3 were inhibited the radial growth of *A.niger* in the range of 26.53 per cent and 25.50 per cent, respectively.

Pot culture trial:

Pot culture trail was conducted to evaluate the efficiency of *Bacillus* isolate on control of collar rot causing *A.niger* in inivo condition and presented in (Table 3 and Fig. 1).

The results revealed that groundnut seed together with

Table 1 : Comparative study on antagonism of *Bacillus* isolates against *A. niger* by Dual culture method on PDA

Sr. No.	Isolates	PDA medium with different incubation period							
		24 hours		48 hours		72 hours		96 hours	
		Radial growth of fungi (mm)	% inhibition of radial growth	Radial growth of fungi (mm)	% inhibition of radial growth	Radial growth of fungi (mm)	% inhibition of radial growth	Radial growth of fungi (mm)	% inhibition of radial growth
1.	Control	31.00	-	52.00	-	65.00	-	82.00	-
2.	B_1	28.00	9.68	36.50	29.81	39.50	39.23	44.50	45.73
3.	B_2	31.00	0.00	50.00	3.85	53.50	17.69	64.50	21.34
4.	B_3	30.00	3.22	49.50	4.81	53.50	17.69	64.00	21.95
5.	B_4	29.50	4.83	47.00	9.61	48.66	25.13	51.66	37.00

Table 2 : Comparative study on antagonism of Bacllus isolates against *A. niger* by volatlle method on PDA

Sr. No.	Isolates	PDA medium with different incubation period							
		24 hours		48 hours		72 hours		96 hours	
		Radial growth of fungi (mm)	% inhibition of radial growth	Radial growth of fungi (mm)	% inhibition of radial growth	Radial growth of fungi (mm)	% inhibition of radial growth	Radial growth of fungi (mm)	% inhibition of radial growth
1.	Control	22.00	-	32.00	-	40.00	-	49.00	-
2.	B_1	16.00	27.27	17.50	46.87	21.00	47.50	25.00	48.98
3.	B_2	18.50	15.90	26.50	17.19	31.00	22.50	36.00	26.53
4.	B_3	18.00	18.18	26.00	18.75	30.50	23.75	36.50	25.50
5.	B_4	21.50	2.27	30.50	4.69	38.00	5.00	48.00	2.04

different isolates of *Bacillus* significantly increased the germination of seeds. The maximum germination was observed in T_1 treatment and least germination percentage was in T_2. Groundnut seed inoculated with B1 isolate showed highest percentage of seed germination and B_2, B_3 and B_4 isolates produced 66.66 per cent, 57.98 per cent and 50.15 per cent mortality, respectively.

(Table 4) exhibited the influence of plant growth by

Table 3 : Effect of *A. niger* and *Bacillus* isolates on germination of groundnut seeds on 7 DAS

Sr. No	Name of the treatments		No. of seed germinated	Germination percentage	Mortality percentage
1.	Control	T_1	10	100	-
2.	Seeds + *A niger*	T_2	5.33	12.38	87.62
3.	Seeds + *A niger* + B_1	T_3	7.66	69.45	30.55
4.	Seeds + *A niger* + B_2	T_4	6	33.34	66.66
5.	Seeds + *A niger* + B_3	T_5	6.33	42.02	57.98
6.	Seeds + *A niger* + B_4	T_6	6.66	49.85	50.15

Table 4 : Effect of *A. niger* and *Bacillus* isolates on height of groundnut seedling on different DAS

Sr. No	Name of the treatments		7 DAS	14 DAS	21 DAS	28 DAS
1.	Control	T_1	7.27	8.23	8.97	9.5
2.	Seeds + *A niger*	T_2	3.77	4.03	5.4	6.1
3.	Seeds + *A niger* + B_1	T_3	8.1	9.07	10.13	10.87
4.	Seeds + *A niger* + B_2	T_4	6.63	7.63	9.1	10.1
5.	Seeds + *A niger* + B_3	T_5	6.26	7.13	8.96	9.33
6.	Seeds + *A niger* + B_4	T_6	6.03	7.1	8.56	9.22

Table 5 : Effect of *A. niger* and *Bacillus* isolates on survival of groundnut seedling on 28 DAS

Sr. No	Name of the treatments		No. of survival	Survival percentage
1.	Control	T_1	100	100
2.	Seeds + *A niger*	T_2	2.33	24.97
3.	Seeds + *A niger* + B_1	T_3	7.66	82.1
4.	Seeds + *A niger* + B_2	T_4	5.66	60.66
5.	Seeds + *A niger* + B_3	T_5	6.33	67.85
6.	Seeds + *A niger* + B_4	T_6	6.33	67.85

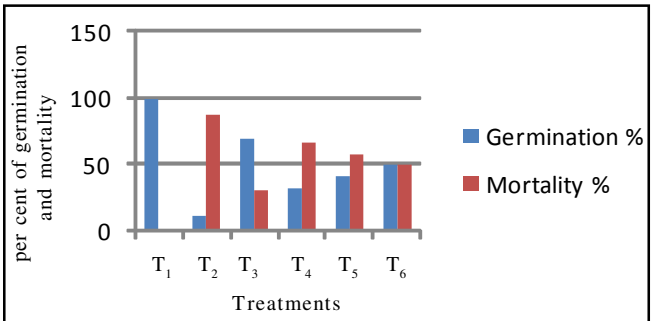

Fig. 1 : Effect of *A. niger* and *Bacillus* isolates on germination of groundnut seeds on 7 DAS

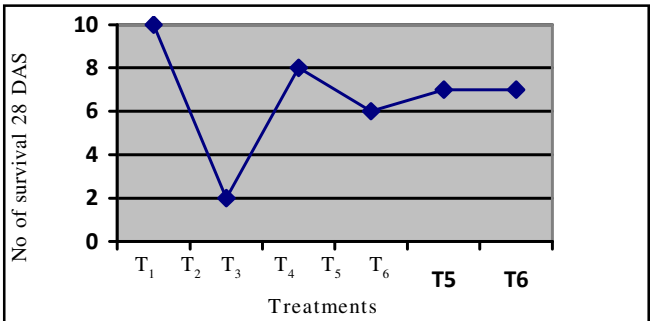

Fig. 2 : Effect of *A. niger* and *Bacillus* isolates on germination of groundnut seeds on 28 DAS

Bacillus isolates. T_1 and T_2 treatments produced 9.50cm and 6.10cm height of the growth, respectively. The remaining all isolates influenced uniform growth of plants. B_3 treatment showed maximum seedling growth (10.87cm) that was higher than control. The treatments B_4, B_5 and B_6 exhibited 10.10 cm, 9.33 cm and 9.22 cm growth of plants.

The effects of *A.niger* and *Bacillus* isolates on survival of groundnut seedling on 28 DAS were presented in Table 5 and Fig. 2. The control treatment was produced 100 per cent survival of groundnut seedling on 28DAS but *A.niger* allowed withstanding of seedling only 24.97 per cent. The seed with B_1 produced 82.10 per cent of survival but (B_2 and B_3 retained 60.66 and 67.85 per cent of seedling in the pots. B_4 isolates in treatment T_6 allowed to growth in the range of 67.85 that was on par with T_5 of B_3.

The control treatment was produced 100 per cent survival of groundnut seedling on 28DAS but *A.niger* allowed withstanding of seedling only 24.97 per cent. The seed with B_1 isolate produced 82.10 per cent of survival but B_2 and B_3 isolates retained 60.66 and 67.85 per cent of seedling in the pots. B_4 isolate in treatment T_6 allowed to growth in the range of 67.85 that was at par with T_5 of B_3. The present result confirmed the statement of Luz (2000) who reported that the best isolates of *B. megatherium* (Embr.9790) and *B. subtilis* (Embr.9786) significantly diminished the disease incidence and severity Fusarium graminearum on wheat up to 50 per cent and 67 per cent, respectively.

From all these results it may be concluded that the biocontrol effect of antagonistic bacteria isolated from soils (*Bacillus* spp.) against *A.niger* are adequate for their use at the field level. Within the mechanisms used by these bacteria are the secretions of volatile and diffusible metabolites but not of fungal cell wall hydrolytic enzymes. Therefore, these bacteria could be used at the field level to biocontrol agent against the *A.niger* disease in groundnut.

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