Plant Archives Vol. 20 Supplement 1, 2020 pp. 1547-1550 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

BIO –EFFICACY OF CULTURE FILTRATE OF *BACILLUS CEREUS* AGAINST ON THE GROWTH OF *MACROPHOMINA PHASEOLINA* CAUSING ROOT ROT OF GROUNDNUT AND DIFFERENT ORGANIC AMENDMENTS ON THE SURVIVABILITY OF *BACILLUS CEREUS*

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Abstract

Root rot disease of groundnut caused by *Macrophomina phaseolina* is considered as one of the major constraints to groundnut production. To overcome these problems the present study, we investigated the *in vitro* biological control of *M. phaseolina* by using biocontrol agent like *Bacillus cereus* and different organic amendments on the survivability of *B. cereus.* The culture filtrate of *B. cereus* at 40 and 50 % conc. completely inhibited the mycelial growth and minimum mycelial dry weight of *M. phaseolina.* The survival of *B. cereus* was assessed through periodical sampling. The final population of *B. cereus* was the highest in FYM amended soil (82.17) followed by neem cake (78.00) at 60th day which were at par with each other. After 60th day the population gradually decreased in all the treatments.

Key words: Groundnut, *Macrophomina phaseolina*, *Bacillus cereus*, Survivability, *Culture* filtrate.

Introduction

Groundnut (*Arachis hypogaea*) the king of oilseeds is popularly called as wonder nut and poor men's cashew nut. While being a valuable source of all the nutrients, it is a low priced commodity and is one of the most important food and cash crops of our country. The demand for groundnut is increasing due to increase in population. But, the problems posed by pests and disease are enormous and among these, the root rot disease caused by *Macrophomina phaseolina* (Tassi.) Goid is considered as the major constraint in increasing productivity of groundnut in India and is the most widespread and serious disease of groundnut crop in the world (Kata, 2000).

M. phaseolina causes complex disease syndromes like root rot, seedling blight, charcoal rot, ashy stem blight, wilt, collar rot, dry rot, pod rot and seed rot in several crops (Raut and Ingle, 1989). The fungus can attack all parts of groundnut plants and the disease may appear at any stage of crop development. Water soaked necrotic spots appear on the stem and then wilting follows. The dead tissues are covered by abundant black sclerotia giving the appearance of light covering of soot. Pychidia are also found in some cases. Roots are commonly attacked which turns black and later become rotten and shredded. Infection was also noted in capsule inner wall, septum and placenta of the seed. Stem near the soil level showed discolouration and shredding of the bark. Premature opening of pods and discolouration of seeds were the other symptoms (Suriachandraselvan *et al.,* 2005).

The biological management of plant pathogens using antagonistic microorganisms fits well within the frame work of the integrated disease management and has now become a distinct possibility for the successful management of soil borne plant diseases. However, soil has enormous untapped potential antagonistic microbes, which are helpful in reducing pathogen inoculum through different modes of action. Hence, it was thought that the heterotrophic rhizobacteria *viz., Bacillus cereus* could be tested against *M. phaseolina* causing root rot disease of groundnut.

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Materials and methods

Preparation of the culture filtrates of *Bacillus*

cereus

The *B. cereus* isolate was grown for 10 days at room temperature (28 ± 2°C) in Erlenmeyer flasks containing 50 ml of sterilized potato dextrose broth. The cultures were filtered under vacuum through bacteriological filter to remove the cells and the spores and the filtrate thus obtained was used for the studies.

Poisoned food technique (Solid media)

The culture filtrates of *B. cereus* isolate were incorporated into sterilized PDA medium at 5, 10, 20 and 40 percent conc. by adding the calculated quantity of culture filtrate to the medium by means of a sterile pipette. The PDA medium without the culture filtrate served as control. The flasks were inoculated with nine mm mycelial disc of *M. phaseolina* collected from the periphery of seven days old culture and incubated at room temperature (28 ± 2°C). Three replications were maintained for each treatment. The diameter of the mycelial growth (in mm) of *M. phaseolina* was measured when the mycelial growth fully covered the control plates.

Poisoned food technique (Liquid media)

A similar experiment as described above was conducted with incorporation of the culture filtrate in the liquid media. The flasks were incubated for seven days at room temperature (28 ± 2°C) and filtered through Whatman No. 42 filter paper under vacuum. Then the bio mass was dried until attaining constant weight in an oven and the mycelial dry weight was recorded in mm.

Effect of organic amendments on the survivability of biocontrol agents

Two hundred g of garden land soil was filled in earthen pots (15 cm dia.). The organic amendments *viz.,* farm yard manure, press mud, poultry manure, neem cake and vermicompost were incorporated in soil at 1% level (w/w) (Ayyappan, 2005). The conidial suspensions of the antagonists were prepared with adequate CFU and added to soil @ two ml/100g of soil and mixed thoroughly. The pots were maintained inside the glasshouse with judicious, uniform and regular watering. Samples were drawn periodically at 0, 30, 60 and 90 days after incubation and the population of *B. cereus* was assessed using serial dilution technique and appropriate selective medium.

Efficacy of organic amendments on plant growth promotion and root rot incidence of groundnut

A separate pot culture experiment was conducted by incorporating organic amendments @ one per cent level as per the treatment schedule to the sick soil to assess their efficacy on the management of groundnut root rot pathogen and plant growth promotion. Earthen pots (30 cm dia) were filled with soil at the rate of five kg pot^{-1}. Each treatment was replicated thrice and a suitable control was also maintained. Required quantity of organic amendments were amended in these pots before 2 weeks of sowing and maintained with sufficient moisture for their decomposition. All the pots were inoculated with *M. phaseolina* multiplied on sand-maize medium @ 5 per cent level soil before one week of sowing except one set of control. Pots of respective experiments were sown with seeds of groundnut TMV-13 @ five seeds pot^{-1}. The pots were maintained in a glass house with need based irrigation and all the agronomic procedures were followed as per the standard protocols. The observations on per cent plant stand; plant growth and disease intensity were recorded at the time of harvest.

Results and Discussion

Effect of culture filtrate of *B. cereus* on the mycelial growth and mycelial dry weight of *M. phaseolina*

In vitro studies were conducted to find out the effect of culture filtrate of *B. cereus* at different concentrations on the mycelial growth and mycelial dry weight of *M. phaseolina* as described under materials and methods and the results are summarized in Table 1 & 2. The results revealed that, in general increasing trend in per cent inhibition was observed while increasing the concentration of culture filtrates. Among the various conc. of the culture filtrate tested the culture filtrate of *B. cereus* at 40 percent conc. and above completely, inhibited the mycelial growth of *M. phaseolina*. The untreated control completely covered the entire Petri plates.

The results revealed that the flasks inoculated with *M. phaseolina* alone (control) recorded the maximum dry weight whereas, the flask inoculated which *M. phaseolina* plus different concentration of culture filtrate of *B. cereus* recorded significant reduction in the mycelial dry weight. The culture filtrate of *B. cereus* at 40 percent and above completely inhibited the mycelial growth of *M. phaseolina.* Among all concentrations, 10 per cent concentration of the *B. cereus* was found to be the least effective. In general, increasing trend in per cent inhibition was observed while increasing the concentration of culture filtrate from 10 to 50 percent.

The antagonistic activity of *Bacillus* spp. has been reported by many workers. The effectiveness of *B. cereus* against *Fusarinm roseum* var. *sambucinum*, the casual agent of potato dry rot was reported (Sadfi *et al.,* 2001). *Bacillus* strains *viz., B. cous, B. circulans, B. lentus* and two strains of *Bacillus* sp. have suppressed the incidence of bacterial blight caused by *Xanthomonas oryzae* Pv. *oryzae* by more than 50 percent in *indica* rice cultivars (Preetivasudevan *et al.,* 2003). In the present investigation the culture filtrate of *B. cereus* was found to be highly inhibitory to the *M. phaseolina* radial growth in solid and liquid media. The quantity and quality of inhibiting substances present in the culture filtrate of *B. cereus* and different mechanism of actions might be attributed as the reason for the enhanced inhibitory action against the test pathogen.

Table 1: Effect of culture filtrate of *B. cereus* against *M. phaseolina* (Solid media).

S. No.	Concentration of culture filtrate	Mycelial growth of pathogen (mm)	% Decrease over control
1.	10	86.00	4.44
2.	20	45.00	50.00
3.	30	38.00	57.77
4.	40	00.00	100.00
5.	50	00.00	100.00
6.	Control	90.00	-
	SED	2.761	-
	CD (p=0.05)	5.560	-

Table 2: Effect of culture filtrate of *B. cereus* against *M. phaseolina* (Poisoned food technique - liquid media).

S. No.	Concentration of culture filtrate	Mycelial dry weight of pathogen	% Inhibition over control
1.	10	167.36	42.75
2.	20	100.70	65.78
3.	30	42.29	85.62
4.	40	1.20	99.59
5.	50	1.05	99.64
6.	Control	294.28	-
	SED	0.87	-
	CD (p=0.05)	1.95	-

Table 3: Effect of certain organic amendments on the survivability of *B. cereus* (Pot culture).

S. No.	Treatments	Sampling period			
		0	30	60	90
1.	FYM	62.05	69.24	82.17	64.30
2.	Pressmud	52.28	62.50	69.60	54.12
3.	Neem cake	60.78	64.75	78.00	60.15
4.	Vermicompost	59.78	61.69	70.38	54.35
5.	Poultry manure	60.65	68.70	72.30	57.05
6.	Control	52.35	58.38	65.45	40.59
	SED	0.532	1.736	4.562	1.552
	CD (p=0.05)	1.069	3.490	9.169	3.119

Table 4: Effect of organic amendments on the incidence of root rot and biometrics of groundnut (pot culture).

S. No.	Treatments	Germination (%)	Shoot length (cm)	Root length (cm)	Root rot incidence	Percent decrease
1.	FYM	84.96	12.70	12.20	38.75	35.65
2.	Pressmud	80.00	10.00	10.21	42.15	30.00
3.	Neem cake	84.25	12.60	12.24	40.00	33.57
4.	Vermicompost	82.60	11.78	11.87	41.25	31.50
5.	Poultry manure	81.21	9.97	11.05	44.25	26.51
6.	Control	79.46	8.86	6.72	60.22	—
	SED	0.304	0.030	0.010	1.015	—
	CD (p=0.05)	0.611	0.060	0.020	2.130	—

Some strains of *B. cereus* produce antibiotics able to suppress fungal diseases of the rhizopshere (Milner *et al.,* 1996). *B. subtilis* was known to produce extra cellular antibiotics that were inhibitory to some plant pathogens (Podile *et al.,* 1988). *Bacillus* sp. produce also a range of other metabolites including chitinases and other cell wall-degrading enzymes (Sadfi *et al.,* 2001), volatiles (Fiddman and Rossal, 1994) and compounds which elicit plant resistances, mechanisms (Kehlenbeck *et al.,* 1994). Thus, our study demonstrated that the mycelial inhibition occurred due to production of appropriate, deleterious antifungal metabolites by *B. cereus* which caused growth inhibition of *M. phaseolina*.

Effect of different organic amendments on the survivability of *B. cereus* (pot culture)

The survival of *B. cereus* was assessed through periodical sampling. The final population of *B. cereus* was the highest in FYM amended soil (82.17) followed by neem cake (78.00) at 60th day which were at par with each other. After 60th day the population gradually decreased in all the treatments. The lowest population of *B. cereus* was observed in pressmud (69.60) amended soil. The population showed decreasing trend upon increase in sampling period (Table 3). Alagarsamy *et al.* (1987) observed a stimulated level of antagonist population in soils amended with organic matter. Krishnamoorthy and Bhaskaran (1990) reported about the beneficial effect of FYM, neem cake, saw dust and tamarind compost to *T. harzianum* when applied to soil. A similar increase in population in soil applied with soybean residue was also observed (Myriam and Fernandez, 1992). Lewis and Papavizas (1984) clarified that the antagonists applied to soil along with a food base survived more efficiently than devoid of food base. These earlier reports lend support to the present findings.

Effect of organic amendments on the plant growth parameters and root rot incidence of groundnut TMV 13 (pot culture)

The data on the effect of various treatments with organic amendments on the growth parameters and root rot incidence were recorded in Table 4. All the treatments had significantly increased the growth parameters when compared to control. Among the treatments, the maximum germination percent (84.96), shoot length (12.70 cm), root length (12.20 cm), minimum root rot incidence (38.75) were observed in FYM amended soil followed by neem cake and vermicompost in the decreasing order of merit. The maximum disease incidence (60.22) and the minimum growth parameters were recorded in control.

Ebenezar *et al.,* (1995) reported that application of decomposed neem leaves and pungam leaves reduced the root rot incidence of greengram. Soil application of neem cake and FYM reduced the incidence of charcoal rot disease with increased yield in cowpea and soybean (Raguchander *et al.,* 1998) and sunflower Suriachandraselvan, 1997). Muthusamy (1989) stated that application of neem cake, farmyard manure, neem leaves and *Pongamia glabra* leaves reduced the number of propagates of *M. phaseolina* in soil besides enhancing the soil antagonistic population. These earlier reports are in line and lend support to the present findings. The presence of higher beneficial microflora and higher organic content might be the reason for the enhanced plant growth and suppression of root rot disease incidence.

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