EFFECT OF MINERAL COMPONENTS ON BIO-CONTROL AGENT AGAINST BLACK GRAM ROOT ROT CAUSED BY *MACROPHOMINA PHASEOLINA*

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Abstract: Macrophomina phaseolina (Tassi). Goid is an important plant pathogen distributed worldwide and causes disease on more than 500 hosts. M. phaseolina is classified as a Deuteromycete, which shows two asexual sub -phases**.** Root rot caused by *Macrophomina phaseolina* is responsible for the considerable loss (4-20%) in black gram. Under *in vitro* conditions six nutrients *viz*., zinc sulphate, boron, ammonium sulphate, ferrous sulphate, calcium sulphate and magnesium sulphate were screened among them two effective nutrients (zinc sulphate and boron) were culled out and the compatibility of *Trichoderma viride* was checked and developed commercial formulation of *Trichoderma viride* enriched zinc sulphate and boron which inhibits the mycelial growth and dry weight of *Macrophomina phaseolina.* Under glass house conditions the soil application of zinc sulphate and boron enriched *T. viride* (10 g / 5 kg of pot soil) talc formulation reduced the root rot incidence (12.50) and increased the zinc (7.45), boron (4.54) uptake and ultimately reflected in an increased yield (16.38[a]).

Keywords: *Macrophomina phaseolina, Trichoderma viride,* soil application, nutrient uptake.

Introduction: Root rot disease caused by the soil-borne fungus *M. phaseolina* (Tassi). Goid is a major limiting factor in the mung bean production causing considerable losses (Raguchander *et al*., 1993) [9]. Although only a single species has been recognized in the genus *Macrophomina,* high levels of variation in pathogenicity have been found. Mayek-Perez *et al.,* 2001 [8], Su *et al*., 2001 [12], Sundaravadana, 2002) [13]. Crous *et al.* (2006) [3] demonstrated that although the teleomorph is unknown, *M. phaseolina* is a member of the family *Botryosphaeriaceae* which comes under the phylum Ascomycota and order Botryosphaeriales. In India, root rot commonly occurs in the states of Madhya Pradesh, Maharashtra, Rajasthan and Delhi. A variety of symptoms were caused by *M. phaseolina* which ranged from root rot, leaf spotting, leaf blighting, blighting of petiole, browning of leaf veins, twisting of leaf lamina, blighting of stem and rotting of root. The most striking symptom is the sudden wilting and drying of the whole plant, most of the leaves remaining green. Latha *et al.* (2000) [7] reported that the earliest incidence of root rot was observed at 15 days after sowing. The fungus is reported to be soil, seed and stubble-borne. Among the seed pathogens, *M. phaseolina* is the most important since it causes seed and seedling rot. Hiromath and Shambulingappa (1981) [5] reported that 4.1 to 52.2 per cent of number of pods/plant and 3.5 to 11.5 per cent of 100 grain weight was reduced by *M. phaseolina* on black gram. The effect of mineral nutrients on plant disease control has received considerable attention over the years but little of this attention has been directed towards the trace elements *viz*., zinc, silicon, boron, calicium etc. Macro and micro nutrients play an important role in inhibiting the growth of the pathogen. *Trichoderma* species are among the most frequently isolated soil fungi and present in plant root ecosystems. The fungi are opportunistic, avirulent plant symbionts and function as parasites and antagonists of many phytopathogenic fungi, thus protecting plants from diseases. So far *Trichoderma* spp. are among the most studied fungal biocontrol agents and commercially marketed as a potent biopesticides, biofertilizer and also used in soil amendments.

Materials and Methods: Survey and sampling were done randomly from individual plants of black gram that were naturally infected by *M. phaseolina*. Samples were collected in different places in the Tamil Nadu state, India and transported in plastic bags to the laboratory and then either immediately used for the pathogen isolation or stored in a refrigerator for subsequent usage. Root bits of *Macrphomina phaseolina* were sterilized (0.1% mercuric chloride) and washed with distilled water three times, then transferred into a 90 mm Petri plate with PDA medium. The isolates were incubated at 25°C to 30°C, which allowed them to grow, further they were transferred into PDA slants.

***In vitro* assay of responses to bio-control agent:**

T. viride isolates were isolated from the soil using a *Trichoderma* selective medium (TSM) developed by Elad and Chet (1983) [4] following the dilution plate technique. The inhibitory effect of *T. viride* isolates against *Macrphomina phaseolina* was determined by the dual culture technique. The fungal antagonists and the pathogen were cultured on PDA medium. The test antagonist (8 mm) were then cut from the periphery of the colony and placed at one end of the sterilized Petri dish containing 15 ml of PDA medium. A similar disc of the pathogen was placed at the

opposite end approximately 75 mm from the first. The linear growth of the antagonist and the pathogen was measured at regular intervals after inoculation.

***In vitro* assay of responses to nutrients:** The efficacy of the following nutrients *viz.,* zinc sulphate, boron, ammonium sulphate, ferrous sulphate, calcium sulphate and magnesium sulphate at 3 levels *viz.,* 250, 500 and 750 ppm (w/v) were tested on mycelial growth of *M. phaseolina* by poisoned food technique (Schmitz, 1930) [10]. The desired amounts of the above combination of nutrients were incorporated in the molten PDA. To prepare nutrient amended PDA, 100 ml of PDA was taken in a sterile conical flask and mixed with 0.025 g, 0.050 g, 0.075 g of different combination of each nutrient to obtain 250, 500 and 750 ppm concentrations of nutrients, pH was adjusted to 7. An 8 mm mycelial disc of *M. phaseolina* was kept in the center of the each nutrient amended medium and incubated at room temperature (28 + 2°C). Control plates were maintained without nutrients. Three replications containing nutrient amended PDA broth and incubated at room temperature (28 + 2°C). After seven days, the mycelial mat was filtered through (Whatman No.42) filter paper and oven dried at 60°C for 48 hours (Singh and Malhotra, 1994) [11]. The dry weight of the mycelial mat was recorded.

Estimation of Metal tolerant: Growth of fungi was evaluated by the biomass yield in the nutrient amended liquid broth. The dry weight of the mycelial mat was recorded. These results were expressed in terms of a Tolerance Index (TI) as per the Colpaert *et al.* (2000) [2].

$$TI_{DW} = \frac{\text{DW of treated mycelium}}{\text{DW of control mycelium}} \times 100$$

Where, TI - Tolerance index, DW - Dry weight**.** Values are mean of three replications.

Efficacy of bio-control formulations *Trichoderma viride formulation:* Talc-based formulation of *Trichoderma* was prepared according to the method described by Jeyarajan *et al.* (1994) [6]. Eight mm disc of 5 days old culture grown on PDA was inoculated in 250 ml of conical flasks containing 100 ml of yeast molasses medium. Eight days after incubation, the contents of the flask including the mycelial mat and metabolite were homogenized. Later it was mixed with talc powder at the ratio of 1:2 (v/w) and shade dried for two days. About 10 g of Carboxy Methyl Cellulose (CMC) was added as sticking material per 1 kg of talc powder.

Effect of nutrient and nutrient enriched talc formulation against *Macrophomina phaseolina* under pot culture condition: The virulent isolate of *M. phaseolina* was mass multiplied in the sand-maize medium and was mixed with the potting mixture. were maintained. The colony growth in mm was recorded 72 hours after inoculation. A difference in colony diameter between the poisoned medium and the control was used to calculate the per cent inhibition as follows:

$$Mi = \frac{Mc - Mt}{Mc} \times 100$$

Where: Mi- inhibition of mycelial growth, Mc- colony diameter of the control set, Mt - colony diameter of fungi on the poisoned medium

Effect on biomass production: To study the effect of nutrients on biomass production of *M. phaseolina* 100 ml of potato dextrose broth (PDB) was taken in three separate sterile conical flasks and mixed with 0.025, 0.050 and 0.075 g of nutrients in each to obtain a 250, 500 and 750 ppm concentration of the above nutrient**s** and the pH of the medium was adjusted to 7. An 8 mm mycelial disc of *Macrophomina phaseolina* was inoculated to each conical flask

Surface sterilized black gram seeds (Co-6) were sown in pots containing potting mixture @ 3 seeds per pot. The treatments of pot culture experiments were as follows. Soil application of *T. viride* alone (20 g / 5 kg of pot soil) – talc formulation followed by Soil application of zinc sulphate (12.5 mg / 5 kg of pot soil), Soil application of boron (5.0 mg / 5 kg of pot soil), Soil application of zinc suphate enriched *T. viride* (15 g / 5 kg of pot soil) talc formulation, Soil application of boron enriched *T. viride* (20g / 5kg of pot soil) - talc formulation, Soil application of zinc sulphate and boron enriched *T. viride* (mixture) (10g / 5kg of pot soil) - talc formulation, Inoculated Control and Un-inoculated Control. The experiment was conducted in completely randomized block design replicated three times. The observations on wilt incidence were done at 15, 30, 45 and 60 DAS. The per cent disease incidence (PDI) was assessed using the following formula.

$$(PDI) = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Results and Discussion: The survey was conducted in black gram extensive growing areas of Tamil Nadu. The survey revealed that root rot caused by *Macrophomina phaseolina* was prevalent and the infection ranged from 5 to 75% (Table1). Among the six nutrients tested, zinc sulphate (750 ppm) effectively reduced the mycelial growth and mycelial dry weight of *M. phaseolina* (32.00 mm and 406.75 mg) (Table 2). As the concentration increased from 250 to 750 ppm the effectiveness of the nutrient against the target pathogen also increased. This was followed by boron which

inhibited the mycelial growth both in the solid and liquid medium. The compatibility of zinc sulphate in the zinc sulphate amended solid medium after 72 hours of inoculation was individually significant and recorded 74.45, 80.87 and 87.00 mm colony diameter at 250, 500 and 750 ppm respectively. As the concentration increased from 250 to 750 ppm the colony diameter also increased. The same trend has followed in the liquid medium. The mycelial dry weight recorded after 72 hours of inoculation was individually significant and recorded a mycelial dry weight of 443.12, 496.20 and 608.00 mg at 250, 500 and 750 ppm, respectively (Table 3).

The *T. viride* enriched zinc sulphate and boron had recorded minimum incidence of root rot under pot culture studies. The *T. viride* enriched zinc sulphate and boron had reduced the root rot (12.50%) incidence of black gram and increased the shoot length (27.00 cm), root length (22.80 cm), DMP (0.0883 g /3 seedlings), Zinc (7.45 mg/pot) and boron uptake (4.54 mg/pot), vigour index (2920) and ultimately an increased yield (16.38 g/pot) (Table 4). The nutrient enriched *T. viride* was able towards the fungal antagonist *T. viride* has been explored under *in vitro* conditions. The colony diameter of *T. viride* to reduce the soil-borne disease significantly and increased the nutrient uptake.

Akladious, S.A and Abbas, S.M (2012) [1] revealed that the *Trichoderma* treatments were highly significant in increasing the growth of maize plants comparing to the control, this may be attributed to that *Trichoderma* treated plants were able to enhance nutrient uptake resulting in increasing the root and shoot growth and improving the plant vigour to grow more rapidly and to enhance plant greenness which might be resulted in higher photosynthetic rates. The basal application of *Trichoderma*, zinc sulphate and boron individually reduced the soil-borne diseases of pulses. The study revealed that the nutrient enriched *T. viride* (zinc sulphate and boron mixture) has resulted in reducing the soilborne diseases and the enrichment had an additive effect on the yield and nutritional status of the crop.

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Table 1. Effect of inoculum levels of *Macrophomina phaseolina* incitant of root rot of black gram			
Inoculum levels (g / kg of pot soil)	**Root rot (%)**		
	15 DAS	**30 DAS**	**45 DAS**
5	2.38^{d} (8.87)	14.8^{d} (22.7)	32.0^{d} (34.4)
10	5.89^{c} (14.04)	17.0^{c} (24.4)	52.5^{c} (46.4)
15	12.0^{b} (20.3)	23.0^{b} (28.7)	64.9^{b} (53.7)
20	16.2^{a} (23.7)	27.8^{a} (31.8)	75.0^{a} (60.0)
Un inoculated Control	0.00^{e}	0.00^{e}	0.00^{e}

Mean of four replications. Means in a column followed by same superscript letters are not significantly different according to Duncan's multiple range test at P = 0.05.

Effect of nutrients on mycelial growth of *Macrophomina phaseolina* incitant of root rot of black gram on solid and liquid medium							
Nutrients	**Mycelial growth (mm)**			**Mycelial dry weight (mg)**			**Metal tolerance Index @ 750 ppm (%)**
	250 ppm	**500 ppm**	**750 ppm**	**250 ppm**	**500 ppm**	**750 ppm**	
Zinc sulphate	76.5^{fg}	62.4^{c}	32.7^{a}	675.5^{d}	577.2^{c}	406.2^{a}	40.7
Boron	81.7^{hi}	69.2^{d}	42.4^{b}	720.8^{e}	724.9^{e}	524.5^{b}	52.5
Ammonium sulphate	86.9^{j}	76.5^{g}	71.3d^{e}	786.9^{ghi}	767.1^{fgj}	741.3^{ef}	74.2
Ferrous sulphate	87.5^{j}	80.2^{ghi}	73.9^{ef}	855.1^{kl}	830.3^{k}	784.0^{h}	78.5
Calcium sulphate	88.0^{j}	82.7^{gi}	79.0^{ghi}	871.5^{l}	867.6^{jkl}	827.9^{ijk}	82.9
Magnesium sulphate	87.3^{j}	78.6^{h}	76.7^{g}	885.7^{l}	826.7^{ik}	808.9^{hij}	81.0
Control	90.0^{j}	90.0^{j}	90.0^{j}	998.6^{m}	998.7^{m}	999.0^{m}	-

Mean of three replications. Means in a column followed by same superscript letters are not significantly different according to Duncan's multiple range test at P = 0.05.

Table 3. Compatibility of the zinc sulphate with fungal antagonist *Trichoderma viride* (solid and liquid media)

Zinc sulphate (ppm)	Colony diameter (mm)				Liquid media		Zinc content (mg/pot)	Metal tolerance Index @ 750 ppm (%)
	24 hrs	48 hrs	72 hrs	Sporulation of *T. viride* 1x10^6 spores/sq. cm	Mycelial dry weight (mg)	Sporulation of *T. viride* 1x10^6 spores/sq. cm		
250	24.5^a	67.0^a	74.4^a	102.4^b	443.1^a	100.9^a	27.3^c (31.5)	80.5
500	39.3^b	76.0^b	80.8^b	123.2^c	496.2^b	109.0^b	29.8^b (33.1)	90.1
750	44.5^c	82.0^c	87.0^c	142.0^d	608.0^d	126.0^d	32.1^a (34.5)	110.4
Control	52.4^d	88.6^d	89.5^d	94.0^a	550.3^c	117.1^c	1.9^d (7.96)	-

Mean of five replications. Means in a column followed by same superscript letters are not significantly different according to Duncan's multiple range test at P = 0.05.Figures in parentheses are arcsine transformed values.

Table 4. Effect of nutrients and nutrient enriched *Trichoderma viride* (talc formulation) on the root rot incidence of black gram

Treatments Soil application	*Germination (%)	*Root rot (%)	*Shoot length	*Root length	*DMP (g 3	*Zinc uptake	*Boron uptake	*Vigour index	*Yield (gm/pot)
T. viride (20 g / 5 kg of pot soil) – talc	94 (75.8)	28.6^e (97.1)	18.8^d	11.5^d	0.063^d	1.88^f	1.02^f	2230.4^d	8.79^d
$Znso_4$ (12.5 mg / 5 kg of pot soil)	92 (73.6)	31.6^f (102.7)	18.3^d	11.3^d	0.064^d	3.32^d	1.38^e	2254.4^d	8.58^d
Boron (5.0 mg / 5 kg of pot soil)	91 (72.5)	36.4^g (111.4)	16.6^e	08.2^e	0.053^e	2.30^e	1.76^d	1832.8^e	7.52^e
T. viride + $ZnSo_4$ (15g / 5kg of pot	95 (77.1)	15.5^c (69.6)	24.5^b	18.1^b	0.077^b	6.72^b	3.99^b	2629.6^b	13.47^b
T. viride + Boron (20g / 5kg of pot	92 (73.6)	21.3^d (82.5)	21.0^c	14.5^c	0.068^c	4.85^c	3.70^c	2479.0^c	11.25^c
T. viride + $ZnSo_4$ + Boron	95 (77.1)	12.5^b (62.1)	27.0^a	22.8^a	0.088^a	7.45^a	4.54^a	2920.0^a	16.38^a
T_7 -Inoculated control	91 (72.5)	73.5^h (177.2)	11.3^g	05.1^g	0.031^g	0.64^h	0.49^h	1389.2^g	4.21^g
T_8 -Un inoculated control	100 (90.0)	0.0^a (0.86)	15.0^f	10.8^f	0.043^f	1.73^g	1.00^g	1600.0^f	6.98^f

Mean of three replications. Means in a column followed by same superscript letters are not significantly different according to Duncan's multiple range test at P = 0.05. Figures in parentheses are arcsine transformed values.

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