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Antifungal activity of zinc sulphate on *Macrophomina phaseolina* (Tassi.) Goid and it's synergistic action with *Trichoderma viride* Pers.

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ABSTRACT: **Antifungal activity of zinc sulphate against *Macrophomina phaseolina*, causing root rot disease in black gram was investigated under *in vitro* condition. The result of experiment revealed that among the nine nutrients tested, zinc sulphate at 500 and 750 ppm completely inhibited the mycelial growth of *M. phaseolina*. Synergistic action of zinc sulphate with *T. viride* showed increased mycelial growth (66.25mm, 207x10⁶ spore/sq. cm), mycelial dry weight (565.22 mg) and sporulation (81.80 x 10⁶ spores/ ml) produced at 500 ppm and hence *T. viride* significantly reduced the mycelial growth of *M. phaseolina* in zinc sulphate amended medium.**

KEY WORDS: *Macrophomina phaseolina*, nutrients, *Trichoderma viride*, synergistic action, zinc sulphate

INTODUCTION

Black gram (*Vigna mungo* L.) root-rot caused by *Macrophomina phaseolina* (Tassi.) Goid in an important disease, responsible for low productivity in the crop. The pathogen is seed and soil borne having a wide host range. It colonizes the roots and stem of young plants, producing many sclerotia in the collapsed plants. Growers currently rely on frequent application of fungicides to protect the crop. However, repeated fungicide use causes environmental problems. Moreover, the pathogen may develop resistance, thereby reducing the efficacy of chemicals. Hence control of root-rot with mineral nutrients has received considerable attention. Disease reduction is most often attributed to improved nutrition that boosts host defenses or to direct inhibition of fungal growth and activity. Sivasithamparam (1996) stated that nutrients affect severity of disease not only by influencing root physiology and host resistance but also affect the interaction between host, pathogen and antagonist each of which also be affected independently by the availability of nutrients. The present study was taken up to find the effectiveness of nutrients against *M. phaseolina* and it¢s mycotonic effect with *T. viride* was also evaluated.

MATERIALS AND METHODS

Effect of nutrients on *M. phaseolina*

Potato Dextrose Agar (PDA) medium was

amended with nine nutrients *viz.*, calcium chloride, calcium nitrate, calcium sulphate, ammonium sulphate, ammonium nitrate, potassium chloride, borax, zinc sulphate, ferrous 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). Control plates were maintained without nutrients. Three replications were maintained. The colony growth in mm was recorded 72 hours after inoculation.

Effect of nutrients on biomass production of *M. phaseolina*

To study the effect of nutrients on biomass production of *M. phaseolina*, 100 ml of potato dextrose broth (PDB) was taken in a sterile conical flask and mixed with 0.025, 0.05 and 0.075 g of the above nutrients to obtain a 250, 500 and 750 ppm concentration and the pH of the medium was adjusted to 7. An eight mm mycelial disc of *M. phaseolina* was inoculated to each conical flask and incubated at room temperature. After seven days, the mycelial mat was filtered through filter paper (Whatman No. 42) and oven dried at 60°C for 48 hours (Singh and Malhotra, 1994). The dry weight of the mycelial mat was recorded.

Tolerance of *T. viride* to zinc sulphate

Mycelial growth of *T. viride* was tested in PDA medium containing zinc sulphate at different concentrations. Mycelial disc taken from the margin of the fungal growth was then placed in the middle of the Petri-plates containing poisoned medium and incubated at room temperature. Radial growth (% inhibition) was measured at regular intervals. The tests were conducted with three replicates.

The zinc sulphate amended plates were kept for further observation until growth fully stopped. The sporulation of *T. viride* was determined by counting the number of spores in one square cm area using a heamocytometer. From each plate, the numbers of conidia were counted in three places (Angappan, 1992).

Effect of zinc sulphate on the biomass production and sporulation by *T. viride*

Effect of zinc sulphate on mycelial dry weight of *T. viride* at different concentration *viz.*, 250, 500 and 750 ppm was estimated by the procedure given by Singh and Malhotra (1994). Spore production was recorded with heamocytometer at 10^{-6} dilution and the number of spores/ml was recorded (Jayaraj and Ramabadran, 1998).

Efficacy of *T. viride* on the inhibition of *M. phaseolina* on zinc sulphate amended medium

The antagonism of *T. viride* against *M. phaseolina* was tested in zinc sulphate amended media by dual culture technique (Dennis and Webster, 1971). PDA medium was amended with zinc sulphate at 250, 500 and 750 ppm concentration. Twenty ml sterilized and cooled medium was poured in Petri - dish. An eight mm mycelial disc of *M. phaseolina* was placed on one end of Petri-dish and at the opposite end similar size disc of *T. viride* was placed. Three replications were maintained for each treatment. The plates were incubated at room temperature (28 ± 2°C) and the radial growth of each fungus was measured at 3 days after incubation.

The difference in colony diameter between poisoned medium and control was used to calculate the per cent inhibition.

RESULTS AND DISCUSSION

Influence of nutrients on *M. phaseolina*

The antifungal activity of nutrients was tested against *M. phaseolina* causing black gram root- rot. Among the nine nutrients, the mean minimum colony diameter and mycelial dry weight of *M. phaseolina* was 3.2 mm and 199.66 mg in zinc sulphate amended medium as against the control, which recorded 89.5 mm mycelial growth and mycelial dry weight 452.67 mg, respectively. Zinc sulphate at 250 ppm recorded 9.7 mm mycelial growth whereas the other two concentrations *viz.*, 500 and 750 ppm completely (100 %) inhibited the

growth of the pathogen, proving its effectiveness over the other nutrients (Table 1). Zinc sulphate at 250 and 500 ppm recorded a mycelial dry weight of 441.26 and 153.73 mg whereas at 750 ppm it completely inhibited the growth of the pathogen proving its effectiveness (Table 2). As the concentration of zinc sulphate increased, the mycelial growth and mycelial dry weight was reduced. The treatments *i.e.*, ferrous sulphate, calcium chloride, calcium nitrate, calcium sulphate, ammonium sulphate and ammonium nitrate were ineffective. Similarly Lakpale *et al.* (1997) reported that zinc sulphate at 500 and 750 ppm concentration retarded the mycelial growth and dry weight of *Rhizoctonia solani* causing sheath blight of paddy. Zinc sulphate inhibited the growth of *M. phaseolina* (Gupta, 1999) and reduced the root - rot incidence in pulses (Latha *et al.*, 1997).

Effect of zinc sulphate on growth and sporulation of *Trichoderma viride*

The synergistic action of zinc sulphate with fungal antagonist *T. viride* has been explored under *in vitro* conditions (Table 3). The mean colony diameter of *T. viride* in the zinc sulphate amended solid medium at 250, 500 and 750 ppm was 64.25, 66.25 and 47.16 mm, respectively. As the concentration increased from 500 to 750 ppm the colony diameter decreased. The colony diameter recorded after 72 h of inoculation was 88.00 mm and 88.50 mm at 250 and 500 ppm, respectively and they were on par with each other and with the control (89.50 mm). Hence, *T. viride* was compatible with zinc sulphate at 250 and 500 ppm.

In addition to the mycelial growth of *T. viride* the extent of sporulation in one square cm in the zinc sulphate amended medium was counted. The sporulation of *T. viride* at 250 ppm was 212.65 x 10^8 spores/ sq.cm at 168 h after inoculation as against the control, which recorded 149.05x10^8 spores/ sq.cm. The sporulation at 500 ppm was significantly higher than the control, which recorded 258.12 x 10^8 spores/ sq.cm at 168 h after inoculation (HA1).

Table 1. Efficacy of nutrients on mycelial growth of *M. phaseolina*

Nutrient	Colony diam 72 h after inoculation (mm)			
	250 ppm	500 ppm	750 ppm	Mean
Calcium chloride	87.7	89.0	84.3	87.0
Calcium nitrate	89.3	89.0	87.0	88.4
Calcium sulphate	89.0	87.3	85.0	87.1
Ammonium sulphate	88.0	85.0	82.3	85.1
Ammonium nitrate	89.3	86.0	82.3	85.9
Potassium chloride	83.7	51.7	40.0	58.4
Borax	70.0	4.2	3.0	25.7
Ferrous sulphate	62.3	11.7	8.3	27.4
Zinc sulphate	9.7	0.0	0.0	3.2
Mean	75.8	59.3	56.2	63.8
CD (P=0.05)	Treatments = 1.62, Concentration = 0.39, Treatments x Concentration = 2.81			

Table 2. Efficacy of nutrients on mycelial dry weight of *M. phaseolina*

Nutrient	Colony diam 72 h after inoculation (mm)			
	250 ppm	500 ppm	750 ppm	Mean
Calcium chloride	435.50	435.23	412.2	427.66
Calcium nitrate	441.00	412.00	483.33	445.44
Calcium sulphate	459.46	483.13	432.26	458.28
Ammonium sulphate	410.07	453.63	408.10	423.93
Ammonium nitrate	444.10	413.26	418.30	425.22
Potassium chloride	414.86	313.06	309.13	345.69
Borax	429.10	281.73	148.40	289.41
Ferrous sulphate	445.96	182.70	74.11	234.12
Zinc sulphate	441.26	153.73	0.0	199.66
Mean	437.34	358.73	268.58	354.88
CD (P=0.05): Treatments = 8.65, Concentration = 4.64, Treatments x Concentration = 14.67				

Table 3. Effect of zinc sulphate on mycelial growth and sporulation of *T. viride*

$ZnSO_4$ (ppm)	Colony diam (mm)				Reduction over control (%)	Sporulation of *T. viride* (1×10^8 spores/sq. cm)		
	24 h	48 h	72 h	Mean		120h	168h	Mean
250	40.75	64.0	88.00	64.25	10.30	127.87 (10.11)	212.65 (10.33)	170.25 (10.23)
500	42.75	67.5	88.50	66.25	7.54	156.85 (10.20)	258.12 (10.41)	207.48 (10.32)
750	22.25	43.00	73.25	47.16	34.18	75.17 (9.88)	112.95 (10.05)	94.06 (9.97)
Control	44.25	81.25	89.50	71.66	-	123.35 (10.09)	149.05 (10.17)	136.21 (10.13)
Mean	38.25	63.93	84.83	62.33	-	120.81 (10.08)	183.19 (10.26)	152.0 (10.18)
CD (P=0.05)	3.60	4.53	4.34	-	-	0.058	0.054	-

Note: Figures in parentheses are Logarithmic transformed value.

Effect of zinc sulphate on sporulation and dry weight of *T. viride* (Liquid medium)

The effect of zinc sulphate on sporulation and mycelial dry weight of *T. viride* at the three concentrations *viz.*, 250, 500 and 750 ppm same as that of solid medium was observed. As the concentration of zinc sulphate increased from 500 to 750 ppm the sporulation decreased. At 500 ppm of $ZnSO_4$ the sporulation was 81.80 x 10^8 spores/ml as against the control, which recorded 63.71 x 10^8 spores/ ml. At 750 ppm of $ZnSO_4$, the sporulation was reduced to 48.57 x 10^8 spores/ml. The mycelial dry weight at 250 and 500 ppm of zinc sulphate was 565.22 and 536.33 mg, respectively as against the control, which was 570.08 mg (Table 4).

Table 4. Effect of zinc sulphate on mycelial dry weight and sporulation of *T. viride*

$ZnSO_4$ (ppm)	Sporulation (x 10^8 spores/ml)	Mycelial dry weight (mg)
250	67.12 (9.83)	565.22
500	81.80 (9.91)	556.33
750	48.57 (9.69)	445.55
Control	63.71 (9.80)	570.08
Mean	65.30 (9.81)	534.31
CD (P=0.05)	(0.071)	10.48

Note: Figures in parentheses are Log transformed values.

Effect of *T. viride on M. phaseolina* cultures in zinc sulphate amended medium

The effect of the fungal bioagent *T. viride* was tested against the growth of *M. phaseolina* in the zinc sulphate amended medium by dual culture technique. Growth of *M. phaseolina* in 250, 500 and 750 ppm amended medium was 0.82 mm, 0.64 mm and 0.96 mm, respectively and they were on par with each other. The growth of *M. phaseolina* in the unamended medium was 39.60 mm. *Trichoderma viride* placed in the above three concentrations of $ZnSO_4$ inhibited the mycelial growth of *M. phaseolina* and recorded an inhibition of 97.9, 98.4 and 97.8 per cent, respectively over the control (Table 5). It supported the finding of Duffey and Defago (1999) who reported that zinc sulphate improved the biocontrol activity of *Pseudomonas fluorescens* against crown and root- rot of tomato. *T. hazianum* with Hi-gro combination reduced the root rot incidence by 72 per cent (Zaki and Gaffar, 1996).

Table 5. Effect of *T. viride* against *M. phaseolina* on zinc sulphate amended medium - dual culture

$ZnSO_4$ (ppm)	*M. phaseolina* colony diameter (mm)	Reduction over control(%)
250	0.82 (4.56)	97.9
500	0.64 (4.54)	98.4
750	0.96 (4.58)	97.8
Control	39.60 (7.70)	-
Mean	10.51 (5.35)	-
CD (P=0.05)	0.90	-

Note: Figures in parentheses are square root transformed values.

It is therefore inferred that zinc sulphate (500 ppm) not only direct inhibition over the root- rot pathogen but also enhance the mycoparasitic ability of biocontrol agents. This proved that activity of zinc sulphate could be effectively used as viable control strategy for agriculturally important soil-borne diseases. This study would also pave a way to amend nutrients along with commercial product of biocontrol agents for curtailing plant diseases.

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