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Evaluation of genetically mutated *Trichoderma* spp. for the management of *Macrophomina phaseolina*, incitant of charcoal rot of Sunflower

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ABSTRACT

A glasshouse experiment was conducted to evaluate the efficacy of genetically mutated *Trichoderma* spp. for the management of *Macrophomina phaseolina,* charcoal rot of sunflower. Out of 10 treatment combinations, maximum disease reduction was achieved by a combination of seed treatment + soil application of mutated strain TM_{17} as this treatment recorded zero percent disease incidence. The same treatment also recorded maximum plant height (43.62 cm), shoot length (40.00 cm), root length (3.62 cm), maximum fresh weight of shoot (11.90 gm), root (3.40 gm) and maximum dry weight of shoot (0.98 g) and root (0.56 gm) of sunflower when compared to other treatments.

KEY WORDS: Charcoal rot, genetic enhancement, integrated disease management, Sunflower

INTRODUCTION

Sunflower (*Helianthus annus* L.) is valued for its nutritive value as vegetative oil in the world. The pathogen *Macrophomina phaseolina* incites charcoal rot disease, which is of major concern for crop growers. Of late, the disease is observed in severe proportion in the sunflower growing areas of Andhra Pradesh, Bihar, Karnataka and Maharashtra. The pathogen is both seed borne and soil borne (Dhingra and Sinclair, 1994; Singh and Singh 1983) and attacks the crop during later stages of growth. The pathogen survives in the form of sclerotia either in soil or remains embedded in diseased plant tissues. Since the pathogen has broad host range, management is quite difficult through application of fungicides. Hence the present study has been taken up to evaluate the efficacy of potential wild and mutant biocontrol strains of *Trichoderma* keeping a chemical fungicide as check against the pathogen under glasshouse conditions.

MATERIALS AND METHODS

The studies were carried out at Department of Pathology, S. V. Agricultural College, Tirupati during 2008-09 under glass house conditions. Sunflower cv. NDSH I was raised in pots in the green house to which the treatments were imposed. The treatments comprised of seed treatment and soil application with antagonistic wild and mutant *Trichoderma* sp. The treatments that were imposed for the study are given below. Four replications were maintained for each treatment.

Treatment No.	Treatment
T_1	Seed treatment with effective wild antagonist *Trichoderma* @ 4gm/kg.
T_2	Seed treatment with mutant strains of *Trichoderma* @ 4gm/kg.
T_3	Soil application with wild antagonist *Trichoderma*
T_4	Soil application with mutant strain of *Trichoderma*
T_5	$T_1 + T_3$
T_6	$T_2 + T_4$
T_7	Seed treatment with carbendazim @ 1g/kg of seed
T_8	Soil application with carbendazim @ 1000 ppm
T_9	Un inoculated control
T_{10}	Inoculated control

The pathogen *M phaseolina* was mass multiplied on sterilized sorghum grains. For this, 100 g of sorghum seeds were washed thoroughly in tap water and soaked in water for overnight in 250 ml conical flask with addition of 20 ml of 4% dextrose. The flasks were then autoclaved for 20 min at 15 psi. After cooling the flasks at room temperature, they were shaken well to separate the sterilized grains and were inoculated with 2-3 discs of 4 days old cultures of *M. phaseolina* and incubated at 28 ± 2°C for 7 days. After seven days, the inoculum was mixed with sterilized soil in pots @100 g/kg (Rajeswari *et al.,* 1999).

Mass multiplication of the potential wild **and mutant *Trichoderma***

Preparation of powder formulation was carried by using stationary culture method. The biomass from 15 days culture of *Trichoderma* in flasks was used for preparation of formulation. The biomass along with medium in conical flasks was mixed with a carrier in the ratio 1:2. The mixture is air dried for 3 to 4 days and blended to have a flour powder to which 5 g of Carboxy methyl cellulose was added (Jeyarajan *et al.,* 1994). Sorghum-grain flour (50 g) was taken in each 250 ml conical flask and 10 ml tap water was added for adjusting moisture to 50 per cent (W/V).

Mass multiplication of *Trichoderma* spp. was carried out by inoculating 2 to 3 discs of seven days old culture of potential *Trichoderma* spp. onto substrate separately into conical flasks and incubated at room temperature for 28 ± 2°C.

Seed treatment with antagonistic wild and mutant *Trichoderma* spp

Sunflower seeds were treated with potential wild and mutated *Trichoderma* spp. @ 4g/kg of seed and Carbendazim@1gm/kg of seeds. The treated seeds were sown in the pathogen inoculated pots @ 6 seeds/pot (Suriachardraselvan and Seetharaman, 2003).

Mass multiplication of wild and mutant *Trichoderma* on FYM

It is less expensive for multiplication of inoculum in large scale. It takes 10-15 days for the colonization of the FYM substrates.(FYM:10 kg; Sorghum grain multiplied with *Trichoderma*: 200gms)

Spread thick layer of FYM on ground under shade, and above it add a layer of *Trichoderma* multiplied sorghum grains and above it spread one layer of FYM. In the same manner, made a heap with alternate layers of FYM and sorghum grains. Cover the heap with gunny bag and sprinkle water

daily to maintain required moisture content (Kousalya and Jeyarajan, 1988).

In this manner both wild and mutant potential *Trichoderma* were mass multiplied on FYM separately and used for the study.

RESULTS AND DISCUSSION

In the present investigation, combination of wild and mutant *Trichoderma*, carbendazim were taken up to study their effect on *Macrophomina phaseolina*, a soil-borne pathogen. Wherein, an attempts was made to observe whether the treatments imposed have any effect on disease incidence and also any stimulatory or inhibitory effect on the seed germination and growth parameters like root and shoot length, fresh and dry weight of the sunflower cultivar NDSH-1.

Per cent disease incidence (PDI)

It is evident that of 10 treatments tested against *M. phasealina*, treatment (T_6) seed treatment with mutant TM-17 @ 4g/kg of seed + soil application with TM-17 recorded no disease incidence and treatment T_9 also recorded no PDI over control followed by treatment T_8, recorded 10.68 per cent.

Seed treatment + soil application mixtures of bio -agents might have given double protection to the germinating seeds and later onto the sunflower seedling by colonizing the seed and root surface by producing a variety of metabolites which might have resulted a synergetic biochemical protection against the invading fungal pathogen in the infection court.

Further, Haggag and Mohamed (2002) reported that *Trichoderma* mutants significantly reduced the onion white rot disease caused by *Sclerotium cepivorum*. Hunjan *et al*. (2004) reported that seed + soil application of mutant strain TV 34-M-5 gave highest (72.8%) disease control of black scurf of potato. During the present investigation the results of the experiment are in agreement with Jayaraj and Radha Krishnan (2003) who reported that soil application of mutant of *T. harzianum* resulted in better plant stand, less damping-off disease caused by *Rhizoctonia solani*. Bharati *et al.* (2002) also reported that soil application of *T. viride* reduced the soil borne disease in sunflower.

While the results of treatment T_5 (seed treatment with mutant TM-17 @ 4g/kg of seed + soil application with wild *Trichoderma*) are in agreement with Rettinasababathi and Ramdoss (2000) who reported that seed + soil application of *T. viride* recorded reduced percentage of disease incidence (Charcoal rot) in sunflower.

The results obtained in the treatment T_8 (soil application with carbendazim @ 20 ml/kg of soil) with 10.68 percent disease incidence are in agreement with the findings of Raghuchander *et al.* (1997). Soil drenching with bavistin proved excellent control of root rot disease in Mung bean caused by *M.phaseolina*.

The result obtained in the treatment T_2 (seed treatment with mutant TM-17 @ 4g/kg of seed) with 11.50 per cent disease incidence was in agreement with Okigho and Ikediugwv (2001) who reported that tuber treatment with mutant *T. viride* showed a drastic reduction of *Rhizoctonia*

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spp on tuber surface of potato during their five months storage.

Mutants of *Trichoderma* significantly improved the yield of onion in addition to controlling *sclerotium cepivorum* which cause white rot disease in onion (Huggag and Mohammed, 2002).

The results obtained in the treatment T_1 (seed treatment with mutant TM-17 @ 4g/kg of seed) are in agreement with Surichandraselvan *et al*. (2004) who reported that seed treatment with talc based formulation of *T. harzianum* (4 g/kg) significantly reduced the charcoal rot incidence (22-27%) compared to untreated control (67%). Ramakrishnan *et al*. (1994) developed talc based formulation of *T. viride* for biological control of *M. phaseolina*.

The results obtained in the treatment with (T_8) carbendazim with 20.56 per cent are in agreement with Abrahm Mathew and Gupta (1996) who reported that seed treatment with bavistin (2 g/kg seed) reduced the severity of web blight of French bean.

Plant height and root length

Among the ten treatments tested against *M. phaseolina*, it is obvious from the Table 1 that the treatment T6 (seed treatment with mutant TM-17 @ 4g/kg of seed + soil application of TM-17) recorded a highest shoot length 40.00 cm followed by the treatments T_8 (Soil application with carbendazim) with 36.00 cm and T_9 (un-inoculated control) with 35.00 cm respectively.

Among the 10 treatments tested against *M. phaseolina*, it is obvious from the Table 11 that the treatments T_6 (seed treatment with mutant TM-17 @ 4g/kg of seed + soil application TM-17 with 100gm FYM/ kg of soil) recorded a highest root length 3.62 cm followed by the treatment T_8 (soil drench with carbendazim) with 3.39 cm and T_9 (uninoculated control) with 3.21 cm root length, respectively.

The results obtained in the treatment T_6 are in agreement with Jayaraj and Radha Krishnan (2003) who also reported that seed treatment with carbendazim followed by soil application of carbendazim resistant mutants of *T. harzianum* resulted in increased plant biomass apart from reducing the infection by *R. solani*. Mutants of *Trichoderma* significantly improved the yield of onion in addition to controlling *Sclerotium cepivorum* which causes white rot disease in onion (Haggag and Mohammad, 2002).

Sankar and Sharma (2001) reported that maize seed treatment with *Trichoderma viride* @ 12 g/kg gave maximum shoot length (180.8 cm), dry matter (24.5 g/plant) and grain weights of 10 cobs (910.5g) in management of collar rot.

Fresh weight of shoot and root

Among 10 treatments tested against *M. phaseolina* it is evident from the Table 1 that the treatment T_6 (seed treatment + soil application with TM-17) recorded highest shoot and root fresh weights 11.90, 3.40 gm respectively followed by treatment T_9 recorded with 11.01, 2.90 gm fresh weights of shoot and root respectively

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Dry weight of shoot and root

Among ten treatments tested against *M. phaseolina* it is evident from the Table 1 that the treatment T_6 (seed treatment + soil application of TM-17) recorded highest shoot and root dry weights 0.98, 0.56 gm respectively followed by treatment T_9 recorded with 0.83, 0.38 gm weights of shoot and root.

Trichoderma spp. are known to promote growth in crop plants by stimulating host plant hormone mechanism (Cook and Baker, 1988, Chet 1987, Hornby, 1990). However, the results obtained with the mutants of *Trichoderma* spp in sunflower crop in present study also proved the same.

CONCLUSION

From the present study, T_6 treatment (comprising of seed treatment + soil application with potential mutant TM_{17}) was found to be the best in increasing germination of sunflower seedlings, significantly reducing the incidence of charcoal rot disease as compared to the control.

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Table 1 : Effect of different treatments on percentage of disease incidence and on growth parameters of sunflower (cv. NDSH-1)

Treatments	Per cent disease incidence	Shoot length (cm)	Root length (cm)	Total plant height (cm)	Shoot fresh weight (gm)	Root fresh weight (gm)	Shoot Dry weight (gm)	Root dry weight (gm)
T_1 : Seed treatment with potential native antagonist *Trichoderma* @ 4gm/kg.	12.22 (20.45)**	22.40	2.19	24.59	6.01	1.06	0.35	0.19
T_2 : Seed treatment with mutant strains of *Trichoderma* @ 4gm/kg.	11.50 (19.82)	29.40	3.11	32.51	8.99	2.82	0.39	0.36
T_3 : Soil application with native antagonist *Trichoderma*	17.68 (24.86)	21.06	1.92	22.98	5.30	0.98	0.32	0.12
T_4 : Soil application with mutant strain of *Trichoderma*	18.82 (25.71)	26.80	2.90	29.70	7.86	2.01	0.56	0.29
T_5 : $T_1 + T_3$	10.68 (19.02)	33.40	3.21	36.61	9.82	2.90	0.78	0.33
T_6 : $T_2 + T_4$	**00.00 (0.00)**	**40.00**	**3.62**	**43.62**	**11.90**	**3.40**	**0.98**	**0.56**
T_7 : Seed treatment with carbendazim @ 1g/kg of seed	12.35 (20.56)	25.60	2.70	28.30	7.28	1.89	0.48	0.26
T_8 : Soil application with carbendazim @ 1000 ppm	10.68 (19.06)	36.00	3.39	39.39	10.80	2.98	0.88	0.44
T_9 : Un-inoculated control	00.00 (0.00)	35.00	3.21	38.21	11.01	3.20	0.83	0.38
T_{10} : Inoculated control	86.20 (69.20)	18.20	1.21	21.52	4.60	0.90	0.10	0.05
SEM	1.68639	1.5958	0.18537	1.7654	0.47852	0.112382	0.0337638	1.6329
CD	4.9749	4.7079	0.5469	5.1197	1.4117	0.3315	0.0996	0.048
Mean	18.01	28.79	2.96	31.74	8.36	2.21	0.60	0.30

*Mean of three replications ** Figures in parenthesis are angular transformed values

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