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Evaluation of different fungicides and their compatibility with potential *Trichoderma* spp. for the *m*anagement of *Aspergillus niger*, Incitant of collar rot of groundnut

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Abstract

Among the 14 isolates of *Trichoderma* spp. TAG-2, TAG-10, TAG-13 were characterized as very fast growing, while TAG-14, TAG-11 and TAG-7 are fast growing and remaining were moderate growing. In dual culture technique, the isolates TAG-2, TAG-13 and TAG-10 have shown maximum inhibition percentage of 81.36, 78.51 and 75.97 against the test pathogen *Aspergillus niger* while, the least inhibition percentage of 63.24 was observed in the isolate, TAG-9. *In vitro* efficacy of four systemic fungicides viz., carbendazim, propiconazole, tebuconazole and hexaconazole and two non-systemic fungicides viz., mancozeb and captan, were evaluated against *A. niger* at 250, 500, 1000 and 1500ppm concentrations. All systemic fungicides were found highly effective and completely inhibited the mycelial growth of the pathogen even at 250ppm followed by mancozeb. Out of four systemic fungicides and two non systemic fungicides tested under *in vitro* for compatibility with potential bioagent, mancozeb was found highly compatible with TAG-2. An integrated management strategy was developed for collar rot of groundnut under glass house conditions. Out of 12 treatment combinations, maximum disease reduction was achieved by integrated use of seed treatment with mancozeb @ 2g/kg + soil application with effective fungal antagonist TAG-2 @ 8g/kg (T_{10}) as this treatment recorded PDI of 7.16 percent. This treatment not only reduced the disease incidence to a maximum extent, but also recorded maximum plant height (37.64cm), root length (28.50cm) and maximum dry weight of shoot (6.84gm) and root (0.71gm) of groundnut when compared to all other treatments.

INTRODUCTION

Among soil-borne diseases, collar rot of groundnut caused by *Aspergillus niger* is one of the important soil-borne as well as seed-borne disease of groundnut affecting the crop at three stages of growth viz., germination, emergence and maturity. Pre-emergence rotting occurs when seeds are affected immediately after sowing. Post-emergence seedling blight occurs when germinated seeds are invaded. Sometimes mature plants may also be attacked causing rapid dying of entire plant. It is one of the important disease reported from different parts of country being more severe in *Kharif* than in *Rabi* [1]. The pathogen was reported to cause damage to an extent 26% in different groundnut genotypes [2]. About 20 per cent reduction in plant population was reported in the crops grown in sandy loam soils in Chittoor district of the state due to collar rot. Integration of biological and chemical control methods has the potential to control plant pathogens with minimal interference in the natural biological equilibrium. Biological approaches can be successful if the biocontrol agents are compatible with fungicides and biopesticides [3]. Information on efficacy of different fungicides and their compatibility with bioagents was meager. Hence, the present studies were taken up with a view to understand the effect of different fungicides and their compatibility with fungal antagonists and integrated management of disease under glasshouse conditions.

MATERIALS AND METHODS

A rowing survey was conducted for the occurrence of collar rot incidence in groundnut and collection of disease affected plants and soil samples for isolation of pathogen and native *Trichoderma* spp. in major groundnut growing villages in and around Tirupati. The test pathogen, *Aspergillus niger* was isolated from the stems of infected groundnut plants by tissue segment method [4] using potato dextrose agar (PDA) medium. Composite soil samples were collected from rhizosphere of healthy plants in collar rot infected groundnut field and shade dried. Serial dilution technique [5,6] was used to isolate *Trichoderma* spp. from rhizosphere soil of groundnut. Fourteen isolates of *Trichoderma* spp. were isolated and tested for their efficacy against *A. niger*. *In vitro* efficacy of fungicides and their compatibility with potential native antagonistic fungus was evaluated using poisoned food technique [7]. Four systemic fungicides (Carbendazim, Propiconazole, Tebuconazole, Hexaconazole) and two non-systemic fungicides (Mancozeb, Captan) were used. A 7 mm mycelial disc of 5 day old antagonistic fungus was inoculated at the centre of fungicide amended PDA medium. The compatibility of each fungicide was tested at four concentrations (250, 500, 1000 and 1500 ppm). A control plate without fungicide was maintained. Each treatment was replicated thrice. The plates were incubated at 28 ± 2 °C until full growth was observed in control. Per cent inhibition of growth of the antagonistic fungus was calculated using the formula I = (C-T)/C X 100. Where, I = Inhibition percentage, C = Growth of fungus in control T = Growth of fungus in treatment. The fungicide having inhibitory effect on *A. niger* and compatible with potential antagonist was used in management of the disease in pot culture. The treatments imposed were T1: Seed treatment with fungicide @ 2g/kg, T2: Seed treatment with effective fungal antagonist TAG-2 @ 4g/kg, T3: Seed treatment with fungicide @ 4g/kg, T4: Seed treatment with effective fungal antagonist @ 8 g/kg, T5: Soil application with effective fungicide @ 2 g/kg, T6: Soil application with effective antagonist @ 4 g/kg, T7: Soil application with effective fungicide @ 4 g/kg, T8: Soil application with effective antagonist (T_8) @ 8 g/kg, T9: Seed treatment with fungicide @ 2g/kg + Seed treatment with effective

fungal antagonist @ 8 g/kg , T10: Seed treatment with fungicide @ 2g/kg + Soil application with effective antagonist (T_8) @ 8 g/kg , T11: Un−inoculated control, T12: Inoculated control. *To inoculate soil with test pathogen, A. niger* was mass multiplied on sterilized sand-corn meal medium . For this, 100 g of partially broken maize grains, 100 g of sand and 20 ml of distilled water were taken into 250 ml conical flask. Then, the flasks were autoclaved for 20 min at 15 psi. After cooling the flasks at room temperature, they were shaken well and inoculated with 2-3 discs of 4 days old culture of *A. niger* and incubated at 28 ± 2^{0}C for 7 d. After seven days, the inoculum was mixed with sterilized soil in pots @ 100 g kg^{-1} one week before sowing the seeds. The antagonistic fungus was mass multiplied on modified wheat bran saw dust (WBSD). 30 g of wheat bran and 10 g of saw dust were added to 35 ml of water in 250 ml conical flask and mixed thoroughly with the help of glass rod and sterilized in autoclaved at 15 psi for 20 min for two successive days. The flasks were inoculated with young growing mycelial discs of fungal antagonist and then incubated for 2 weeks at 28 ± 2 C. Then the inoculum was added to the soil @ 10 g kg^{-1} of soil [8].

For treating the seeds with antagonistic fungus, Two to three discs of 8 days old culture of potential antagonistic fungus was inoculated into 250 ml conical flask containing 70 ml yeast-molasses broth. After 7 days of inoculation a thick mycelia mat was formed and it was mixed by using blender. Further, it was mixed with talc powder at 1:2 (v/w) ratio and shade dried for 2 days. Carboxy methyl cellulose (10g) was added as sticky material for 1 kg of talc powder. The talc based formulation was used for treating the seeds according to the doses included in the treatments. The seeds were mixed with effective fungicide with desired concentration according to the treatments and it was shaken in closed vessel for 5-15 min to facilitate even spreading of fungicide over the surface of all seeds. In case of combined treatments, seeds were initially treated with fungicide and later treated with antagonist just before sowing. For soil application of antagonist, antagonist fungus was mass multiplied on sterilized wheat bran (100 g/250 ml flask). Two discs of 6 mm of 7 day old fungus were added to the flask and incubated at 28 ± 2° C for 15 days. Then the inoculum was added to the soil according to the doses included in the treatments at the time of sowing. For soil application of fungicide, the fungicide was mixed in water with desired concentration according to treatments and applied to the soil surface. In case of combined application, initially soil was treated with fungicide and later with antagonist. The

Table 1: In vitro screening of Trichoderma isolates against A. niger (AN2) by dual culture technique.

S.No.	Isolates	Radial growth of Aspergillus niger (mm)*	Per cent inhibition over control
1.	TAG-1	21.53	71.67 (57.84)
2.	TAG-2	14.16	81.36 (64.42)
3.	TAG-3	27.43	63.90 (53.07)
4.	TAG-4	26.20	65.53 (54.05)
5.	TAG-5	24.20	68.16 (55.65)
6.	TAG-6	26.10	65.66 (54.13)
7.	TAG-7	22.00	71.05 (57.45)
8.	TAG-8	23.73	68.77 (56.02)
9.	TAG-9	27.93	63.24 (52.68)
10.	TAG-10	18.26	75.97 (60.64)
11.	TAG-11	25.30	66.71 (54.76)
12.	TAG-12	23.30	69.34 (56.38)
13.	TAG-13	16.33	78.51 (62.38)
14.	TAG-14	20.46	73.07 (58.74)
15.	Control	76.00	00.00 (00.00)
	S.Ed.	0.2010	
	S.Em (±)	0.1421	
	CD (0.05)	0.4104	
	CV (%)	0.461	

* Mean of three replications
** Figures in parenthesis are angular transformed values
* TAG Trichoderma against A.niger

observations recorded were percentage of disease incidence (PDI) and other parameters like plant height, shoot height, root weight and root length. The PDI was calculated by using the formula PDI = (Number of diseased plants/ Total no. of plants) X 100.

RESULTS

A survey was carried out on the occurrence of collar rot disease in four major groundnut growing villages of Tirupati and its surrounding areas viz., Srikalahasti, Renigunta, Ramachandrapuram and Chandragiri and the average percentage of disease incidence recorded as 11.21, 9.04, 7.39 and 6.47 per cent respectively. The highest average per cent disease incidence was recorded in Srikalahasthi mandal (11.21%) and least average per cent disease incidence was noticed in Chandragiri mandal (6.47%).

A total of 146 rhizosphere soil samples were collected from healthy plants of collar rot infected groundnut fields. Serial dilution technique was used to isolate antagonistic mycoflora from rhizosphere soil. Colonies of *Trichoderma* spp. were observed on *Trichoderma* selective medium (TSM) on 7 days after inoculation. The antagonistic *Trichoderma* spp. were identified based on mycological keys described [9]. The antagonistic *Trichoderma* spp. were screened against the highly virulent isolate AN_2 of *A .niger*. All 14 isolates of *Trichoderma* spp. were screened for their efficacy against test pathogen. All the fourteen isolates of *Trichoderma* inhibited the growth of *A. niger* in dual culture. The isolate TAG-2 showed maximum percentage of inhibition (81.36) followed by TAG-13 (78.51%) and TAG-10 (75.97%)(Table 1). However, both were found to be on a par with each other in inhibiting the pathogen. The inhibition percentage of other isolates were TAG-14 (73.07%), TAG-1 (71.67%), TAG-7 (71.05%), TAG-12 (69.34%), TAG-8 (68.77%), TAG-5 (68.16%), TAG-11 (66.71%), TAG-6 (65.67%), TAG-4 (65.53%), TAG-3 (63.90%) and TAG-9 (63.24%). Least

Table 2: Efficacy of antagonist and fungicide on per cent incidence of collar rot of groundnut in pot culture

Treatment No.	Treatment	Per cent disease incidence	Plant height (cm)	Root length (cm)	Dry weight (mg)	
					Shoot	Root
T_1	Seed treatment with Mancozeb @ 2 g/kg	45.66 (42.51)	30.41	20.50	4.03	0.41
T_2	Seed treatment with effective fungal antagonist TAG-2 @ 4 g/kg	40.12 (39.30)	30.59	21.07	4.22	0.48
T_3	ST with Mancozeb @ 4g/kg	42.41 (40.64)	31.04	21.13	4.06	0.47
T_4	ST with effective fungal antagonist TAG-2 @ 8g/kg	36.32 (37.06)	32.89	22.65	4.25	0.52
T_5	Soil application with Mancozeb @ 2 g/kg	37.51 (37.77)	31.68	22.02	4.17	0.49
T_6	SA with effective fungal antagonist TAG-2 @ 4 g/kg	26.44 (30.95)	33.46	24.53	5.30	0.55
T_7	SA with Mancozeb @ 4g/kg	28.51 (32.28)	32.37	23.38	4.83	0.53
T_8	SA with effective fungal antagonist TAG-2 @ 8 g/kg	22.50 (28.31)	33.48	25.50	6.06	0.61
T_9	Seed treatment with Mancozeb @ 2 g/kg + ST with effective fungal antagonist TAG-2 @ 8g/kg	10.68 (19.06)	35.91	27.03	6.46	0.66
T_{10}	Seed treatment with Mancozeb @ 2 g/kg + SA with effective fungal antagonist TAG-2 @ 8 g/kg	**7.16 (15.48)**	**37.64**	**28.50**	**6.84**	**0.71**
T_{11}	Un-inoculated control	0.00 (0.00)	29.42	20.11	3.92	0.38
T_{12}	Inoculated control	83.10 (65.74)	24.69	12.14	2.18	0.29
	S.Em (±)	0.366	0.214	0.043	0.032	0.009
	C.D (0.05)	1.058	0.619	0.126	0.094	0.026
	C.V (%)	2.006	1.151	0.330	1.170	3.164

percentage inhibition was observed with the isolate TAG-9 (63.24%). The isolate TAG-2 was found to be more effective due to its more percentage inhibition and it was used for further studies. The results were in conformation with [10] who reported *T.harzianum* inhibited 63% and 58% of two virulent isolates of *A. niger* respectively in dual culture method by producing branches inside the conidiophore results in the rupture of conidiophore.

Based on the results obtained, the isolates which showed inhibition percentage, TAG-2 (81.36%), TAG-13 (78.51%) and TAG-10 (75.97%) were categorised under highly effective group and the isolates TAG-14 (73.07%), TAG-1 (71.67%) and TAG-7 (71.05%) were placed in moderately effective group whereas the isolates TAG-12 (69.34%), TAG-8 (68.77%), TAG-5 (68.16%), TAG-11 (66.71%), TAG-6 (65.67%), TAG-4 (65.53%), TAG-3 (63.90%) and TAG-9 (63.24%) were pooled in the less effective group. These grouping of the isolates based on the efficacy in dual culture assay were in coincidence with the results of [10]. The systemic fungicides used to test their efficacy against *A. niger* were carbendazim, propiconazole, tebuconazole and hexaconazole and the non-systemic fungicides used were mancozeb and captan at four different concentrations viz., 250, 500, 1000 and 1500 ppm. Results revealed that all the four systemic fungicides inhibited the mycelial growth completely in all the concentrations even at 250 ppm. By using systemic fungicides similar findings were observed by [11] reported complete inhibition of *A. niger* in poisoned food technique. Of the non-systemic fungicides tested, 100 per cent inhibition of mycelial growth was observed at 1000 ppm with mancozeb and at 1500 ppm with captan. By using non-systemic fungicide, mancozeb similar results were obtained in poisoned food technique by [12]. In the present investigation, all the systemic fungicides were found to be completely inhibitory to TAG-2 even at 250 ppm concentration and hence these were incompatible with potential *Trichoderma* isolate, TAG-2. Similar findings were observed by [13] who reported complete inhibition of growth of different *Trichoderma* spp. in poisoned food technique when systemic fungicides were tested for compatibility with bioagent. Among the non-systemic fungicides tested, overall per cent reduction in TAG-2 colony diameter was maximum with captan (90.77) and minimum with mancozeb (55.55%). Among all the fungicides that were tested mancozeb was found to be highly compatible with TAG-2. The results were in accordance with [14] as mancozeb was highly compatible with *Trichoderma* sp.

DISCUSSION

Even through systemic fungicides carbendazim, hexaconazole, tebuconazole and propiconazole found effective against *A. niger* under *in vitro* conditions, owing to its toxicity to TAG-2 its use in integrated control was not considered and the next best fungicide i.e. mancozeb having high per cent inhibition of *A. niger* and also having high compatibility with TAG-2 was selected for integrated disease management. In the present investigation, maximum disease control was observed in the treatment T_{10}, that comprises seed treatment with mancozeb @ 2g/kg + soil application with effective fungal antagonist TAG-2 @ 8g/kg, which recorded less per cent disease incidence of 7.16 per cent when compared to inoculated control (83.10%) (Table 2). Integrated seed treatment with fungicides and antagonists protected pea from *Fusarium solani* f.sp. *pisi* was reported [15]. The fungicides provided initial protection to germinating seeds and after that the seedlings were protected by colonized growth of antagonists may be due to suppressed growth of pathogen through antibiosis and mycoparasitism. In the present investigation, maximum plant height (37.64 cm) and root length (28.50 cm) and also maximum shoot and root dry weight was recorded in integrated treatment T_{10}. This treatment was found stimulatory on the plant height and root length when compared to un-inoculated control and least plant height and root length was recorded in inoculated control. Seed + soil treatment with *Trichoderma* spp. which improved the growth parameters of groundnut was reported [16]. These results indicate that mancozeb and antagonist have stimulatory effect on plant height and root length.

CONCLUSION

A major challenge in plant disease management is to introduce or develop new disease management strategies, as the more traditional control method become obsolete and to do so without greater use of chemicals. Although it is well established that certain diseases can be controlled either completely or partially by the use of biocontrol agents, it is understood that the best method to control disease through integrated disease management where in a biological control component would be significant. It is attributed to that mancozeb could arrest the pathogen and antagonist could parasitize the pathogen and promote growth by secreting growth promoting metabolites. Two mechanisms have been advanced most frequently to explain the increased growth response induced by certain microflora. The first hypothesis was that enhanced growth might be due to biological control of plant pathogens in the soil. The other hypothesis so far not demonstrated clearly for any biological system was that a microbial agent produces growth regulatory metabolites. The present investigation shows that, the integrated treatment with biocontrol agent increased plant height, dry weight of shoot and root of groundnut.

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