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Research Article

COMPATIBILITY OF FUNGICIDES WITH *Trichoderma viridae* AGAINST FUSARIUM WILT CAUSED BY *Fusarium udum*

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Abstract- Five fungicides *viz.* Carbendazim Mancozeb Carboxin +Thiram Hexaconazole and Propiconazol were evaluated and found that carboxin + thiram, hexaconazol and propiconazol were completely inhibit the growth of *F. oxysporum* f. sp. *udum* followed by carbendazim 50 and 100 ppm respectively as compared to control. The growth reduction was less in mancozeb at all concentrations. *In vitro* sensitivity test conducted to evaluate the compatibility of *Trichoderma spp*. with fungicides at 100 ppm concentration and the result reveals that mancozeb and carboxin+thiram were compatible, 88.25 mm and 85.75 mm respectively. The fungal antagonist JN-S1 and TL-S1 isolate caused highly significant reduction in pigeonpea wilt fungus *F. oxysporum* f. sp. *udum* under *in vitro* conditions.

Keywords- compatibility, Fungicides, *Trichoderma, Fusarium udum*, Inhibition.

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Introduction

The pigeonpea (*Cajanus cajan* L. Millsp.) is an important pulse crop in India belonging to the family Fabaceae. Globally pigeonpea is cultivated about on 4.7 million ha area with 3.69 million tones annual production. India accounts 78% of the global output with current production of 2.78 million tones from 3.5 million ha. The average yield of pigeonpea in M.P. is 848 kg/ha which is much lesser than the potential yield of crop (1500-2000 kg/ha). Several biotic and abiotic factors are responsible for reducing the yield) [1]. It is widely used as a pulse, green vegetable, fodder and for a variety of other purposes. The seed protein content of pigeonpea (21%) compares well with that of other important grain legumes. High sensitivity of the crop to the attack of insect-pests and diseases appears to be the main reason for such low yields. The crop is attacked by more than 100 pathogens [2]. Including fungi, bacteria, viruses, phytoplasma like organisms and nematodes. However, only a few of them cause economic losses [3]. Fusarium wilt is the most important disease of pigeonpea in India resulting in yield losses upto 67 per cent at maturity.

Material and Method

Source of seed and other materials

The experiment was conducted at AICRP on Pigeonpea ZARS Khargone and laboratory facility was availed at Department of Plant Pathology, B. M. Collage of Agriculture, Khandwa (M.P.). The seeds of 76 pigeonpea genotypes were obtained from AICRP pigeonpea, ZARS, Khargone (M.P.). Infected samples were collected from selected experimental sites (ACRIP on pigeonpea at ZARS, Khargone). The pathogens were grown on potato dextrose agar (PDA) andstored at 4 ^{0}C until use. The pathogen was identified according to [4]. Colonies of *F. udum* were purified in PDA slants and stored at 4^{0}C.

Collection, isolation, purification, identification and storage of the pathogen

Infected roots samples were collected from diseased as well as healthy pigeon pea plants growing on selected experimental sites (ACRIP on pigeonpea at ZARS, Khargone). Roots were washed with tap water to remove the soil particles and cut into small pieces (1 cm) and surfaces were sterilized by dipping in 0.2% mercuric chloride solution for 1 minute and washed several times with distilled water. Pieces of roots were placed on filter paper to remove excess water; and then on potato dextrose agar (PDA), to isolate *F. udum.* The plates were incubated at 28+1^{o}C for seven days, and the pathogen was purified by hyphal tip culture technique. *F. udum* isolates were identified. Colonies of *F. udum* were purified in PDA slants and stored at 4^{0}C [4].

Collection of microorganism isolates

The *Trichoderma* isolates were obtained from Department of Plant Pathology B. M. College of Agriculture, Khandwa (M.P.)

Microorganism used and its maintenance

Strains of *Trichoderma* were used as a biocontrol agent on pigeonpea pathogen *F. oxyporum* f. sp. *udum* which was isolated from pigeonpea. *F. oxyporum* f. sp. *udum*can cause wilt disease in pigeonpea. In this study, biocontrol agent was cultivated on potato dextrose agar (PDA) at 25 ± 1 ^{0}C for 5-7 days and stored at 4 ^{0}C until use.

Laboratory bioassay of fungicides

Five fungicides given in [Table-1] were evaluated against the pathogen *F. udum* by poison food techniques [5]. The details of the fungicides used in the present investigation are summarized in [Table-1].

The different fungicides were screened for their efficacy against the pathogen by "Food Poison Techniques" described by [6] in which required quantity of each fungicide was thoroughly mixed with 60 ml well sterilized potato dextrose agar

medium contained in 100 ml flasks. Now 20 ml of this medium mixed with fungicides was poured in Petri-plates and allowed to solidify. Each treatment was replicated three times. One set of control was also kept in which the medium was not mixed with fungicides. Equal pieces of the fungal growth, cut by the cark borer were inoculated in each Petri-plate at the center. These inoculated Petri-dishes were incubated at 28±1^{0}C for 7days and after 7 days of the incubation, the fungal growth was recorded in each Petri-dishes.

Table-1 *Fungicide with their coined, trade and chemical name*

Fungicide	Trade name	Formulation	Chemical name
Carbendazim	Bavistin	50%WP	Methyl-1-2-benzimidazole carbamate (MBC)
Mancozeb	Dithane M-45	75%WP	[[1,2-thanediylbis[carbamodithioato]] (2-)]manganese mixture with [[1,2-ethanediylbis [carbamodithioato]] (2-)] zinc
Carboxin +Thiram	Vitavax power	37.5%WP+ 37.5%WP	5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilido + Tetramethyl thiurum disulphide
Hexaconazole	Trigger	5%WP	2-(2,4-dichlorophenyl)-1-(1H-1,2,4-trizol-1-yl)hexan-2-ol
Propiconazol	Bumper	25%WP	1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1,2,4-triazole

Compatibility of agro-chemical with *Trichoderma* strains

The agro-chemical was mixed in potato dextrose agar medium in different preparations. Fifteen ml media were poured into each of the sterilized 90 mm petri plate and 5 mm disc cut with the cork borer from the freshly growing culture of Trichoderma strains was placed in the center of each plate upside down. Plates were incubated at 28 ± 1^{0}C and growth of the colony was recorded after 7 days. Three replications were kept for each concentration and data were analyzed following complete randomized design.

Antagonism of Trichoderma strains against *F. oxyporum* f. sp. *Udum*

In vitro antifungal activity of Trichoderma stains against *F. oxyporum* f. sp. *Udum* was tested on dual culturing method. The 5 mm diameter Discs of pathogen/ strain of Trichoderma cut from edge of 7days old PDA culture are placed 5 cm from each other and incubated at 28 ± 1 ^{0}C. Inhibition of radial growth of fungi and encroachment over pathogens by Trichoderma were measured and compared with the control.

Statistical analysis

The data were subjected to statistical analysis after transformation. The data converted into percentage were transformed to angular values. The difference between the two means was subjected to further testing by computing critical difference at 5% probability level.

(1) Standard error for treatment mean:

$$S.\,Em.\pm = \sqrt{\frac{Em.s}{r}}$$

(2) Critical difference:

$$C.D. = S.\,Em. \times \sqrt{2} \times \bar{t}$$

Where,

Ems = Error means sum of square

r = Number of replication

t = 't' value at 5% probability levels

Results and Discussion

In vitro* evaluation of fungicides against *F. oxysporum* f. sp. *udum

In vitro evaluation of fungicides and their combination was carried out by poison food technique. Two concentrations (50 and 100 ppm) of each fungicide were assayed against *Fusarium oxysporum* f. sp. *udum*. Observations on radial growth of *Fusarium oxysporum* f. sp. *udum* were recorded after seven days of inoculation. Data presented in [Table-2] reveals the effect of fungicide sat 50 and 100 ppm concentrations on the radial growth of *F. udum*. Among the fungicides carboxin + thiram, hexaconazol and propiconazol completely inhibit the growth of *F. oxysporum* f. sp. *udum.* Followed by carbendazim (24.50 and 18.58 mm) respectively in 50 and 100 ppm concentrations. The growth reduction was less in and mancozeb (44.17 and 30.00 mm) respectively at 50 and 100 ppm.

Table-2 *Radial growth of Fusarium oxysporum f. sp. udum in fungicide amended medium at seven days after inoculation*

Treatment	Radial growth of *Fusarium oxysporum* f. sp. *Udum* (mm)*		
	50 ppm	100 ppm	Mean A
Carbendazim	24.50	18.58	21.54
Mancozeb	44.17	30.00	37.08
Carboxin +Thiram	0.00	0.00	0.00
Hexaconazole	0.00	0.00	0.00
Propiconazol	0.00	0.00	0.00
Control	60.17	60.83	60.50
Mean B	21.47	18.24	
Factors	**S.E(m)**	**C.D.**	
Factor(A)	0.92	2.65	
Factor(B)	0.53	1.53	
Factor(A X B)	0.75	2.16	

*Mean of four replications

Compatibility of fungicides with *Trichoderma spp.*

Fungicides were evaluated for the compatibility reaction with *Trichoderma spp*. at 100 ppm concentration *In vitro and the data revels that* only two fungicides mancozeb and carboxin+thiram were compatible with 88.25 mm and 85.75 mm mean redial growth after seven days of inoculation respectively while all the other fungicides were toxic. Among the isolates JN-S1 gave the highest mycelial growth (89.67 mm) with mancozeb followed by Carboxin +Thiram (86.00 mm) whereas TL-S1 showed maximum 86.83mm with mancozeb followed by Carboxin +Thiram (85.50mm). The fungicides Carbendazim, Hexaconazole and Propiconazol completely check the growth of *Trichoderma viridi.*

Table-3 *Growth of Trichoderma strains on fungicide amended PDA at 100 ppm after two and seven days of inoculation*

Treatment	Radial growth of *Trichoderma viridi* at 2 DAI (mm)*			Radial growth of *Trichoderma viridi* 7DAI (mm)*		
	JN-S1	TL-S1	Mean A	JN-S1	TL-S1	Mean A
Carbendazim	0.00	0.00	0.00	0.00	0.00	0.00
Mancozeb	19.83	14.17	17.00	89.67	86.83	88.25
Carboxin +Thiram	14.00	14.67	14.33	86.00	85.50	85.75
Hexaconazole	0.00	0.00	0.00	0.00	0.00	0.00
Propiconazol	0.00	0.00	0.00	0.00	0.00	0.00
Control	43.50	40.50	42.00	90.00	90.00	90.00
Mean B	12.89	11.56		44.28	43.72	
Factors		**S.E(m)**	**C. D.**		**S.E(m)**	**C. D.**
Factor(A)		0.11	0.32		0.31	0.89
Factor(B)		0.06	0.18		0.18	0.51
Factor(A X B)		0.09	0.26		0.25	0.72

*Mean of four replications

Efficacy of *Trichoderma* spp. against *Fusarium oxysporum* f. Sp. *udum*

As a bio-control agent, *Trichoderma strain* JN-S1 could successfully inhibit the growth of *Fusarium udum* with inhibition zone of 63.14%. Whereas *Trichoderma* strain TL-S1could successfully inhibit *Fusarium udum* with inhibition zone of 57.89 %. Average mean of growth inhibition of *Fusarium udum* was noticed 57.25 % at five days after inoculation and 63.78 % at seven days after inoculation. The inhibition zone indicates that the both *Trichoderma strains* (JN-S1 and TL-S1) significantly inhibit the growth of *Fusarium udum*.

Table-4 *Mean of growth inhibition of Fusarium udum by Trichoderma strains*

S.No.	Trichoderma	Inhibition zone (%)*		
		5 DAI	7 DAI	Mean A
1	JN-S1	61.20	65.07	63.14
2	TL-S1	53.29	62.49	57.89
	Mean B	57.25	63.78	

* Mean of four replications.

Among the fungicides carboxin + thiram, hexaconazol and propiconazol were completely inhibit the growth of *F. oxysporum* f. sp. *Udum* followed by carbendazim 50 and 100 ppm respectively as compared to control. The growth reduction was less in mancozeb at all concentrations. The fungicides and stated that the propeconazol and hexaconazol significantly inhibited the *Fusarium udum in-vitro* conditions [7]. In support of present investigation reported that *F. udum* was completely inhibited by bavistin (0.1%), topsin-M-70 (0.1%), thiram (0.1%), captan (0.15%) and dithane-Z-78 (0.37) in *in-vitro* [8]. The effect of carbendazim, captan, Dithane –Z-78, thiophanatemethyl and thiram against *Fusarium udum* under *in vitro* [9]. Among which, carbendazim was found to be effective at 100, 250 and 500 ppm concentrations. [10] reported that mancozeb showed maximum inhibition of *F. udum* as compared to carbendazim, mancozeb, sulphur and companion (mancozeb 63% + carbendazim 12%) fungicides. There are reports on the compatibility of fungicides with *Trichoderma* spp., which are effective in managing disease like root rot, damping off and wilt caused by soil borne pathogen. However, biological control alone will not be feasible to combat the diseases in case of severe incidence. Biological control, in integration with fungicidal treatment was found to be a more reliable approach to manage such soil borne plant pathogens. The present study was aimed to study the compatibility of *Trichoderma* spp. with common fungicides and chemicals at 100 ppm. *Trichoderma* isolate JNKVV-S1 was highly compatible with the fungicides followed by TNAU-S1. Among the fungicides mancozeb at 100 ppm was significantly least toxic to both the isolates of *Trichoderma* followed by carboxin+thiram as compared to other fungicides. Interaction of fungicide and *Trichoderma* isolates was also significant. In mancozeb treated medium significantly maximum mycelial growth was recorded in JNKVV-S1. While in carboxin+thiram treated medium maximum mycelia growth was found in strain TNAU-S1 which was at par with JNKVV-S1 at two days after inoculation however after five and seven day after inoculation JNKVV-S1 show maximum mycelia growth in carboxin+thiram treated medium which was at par with TNAU-S1. Carbendazim, hexaconazol and propiconazol at100 ppm showed inhibitory effect to both the isolates during two, five and seven days after inoculation. It observed that vitavax was compatible with *G.virens* and *T. harzianum* [11]. It evaluated the *in vitro* efficacy of different fungicides against *T. harzianum* @ 1500 ppm and found that the mycelial growth of *T. harzianum* was completely inhibited by Carbendizam and Hexaconazole @ 1500 ppm and the inhibition with Copper oxychloride and mancozeb was 90 and 41 per cent respectively [12]. Also observed Carbendazim was incompatible with *Trichoderma viride* TV2 isolate whereas carboxin was compatible for integrated treatment [13]. The *T. harzianum were tested for in vitro* compatibility with mancozeb, carbendazim and copper oxychloride and found that carbendazim at 0.1 per cent completely inhibited the mycelial growth whereas mancozeb and copper oxychloride showed compatibility with the antagonist at 0.2 and 0.1 per cent respectively [14].

Fungal species belonging to the genus *Trichoderma* are worldwide in occurrence and easily isolated from the soil. The potential of *Trichoderma* species as bioconrol agents against various plant diseases has been reported by several workers [15,16]. In the present investigation, fungal antagonist JNKVV-S1 and TNAU-S1 isolate caused highly significant reduction in pigeonpea wilt fungus *F. oxysporum* f. sp. *udum* under *in vitro* conditions. Also reported the demands for *in vitro* effectiveness of *Trichoderma* against species of *Fusarium* [17]. The antagonist *Trichoderma harzianum*, *T. coningi* and *T. viride* were reported to be equally antagonistic to *F. udum* under *in vitro* [18]. The efficacy of 30 different *Trichoderma* isolates against *F. udum* and reported that two isolates showed highest colony growth reduction of 65.5 to 67.2 per cent, seven between 50 to 60 per cent and 21 isolates between 41-50 per cent [19]. They further observed that eleven isolates reduced conidia production by >90 per cent. The culture filtrates of *Trichoderma* spp. also reduced > 90 per cent colony growth of *F. udum.* It reported that *T. viride* and *Pseudomonas fluorescens* significantly reduced the growth of *F. udum* [20]. Also reported that *Trichoderma* spp. successfully controlled *Fusarium* spp. on cotton, wheat and muskmelon [21].

Conclusions

The fungicides carboxin + thiram, hexaconazol and propiconazol completely inhibit the growth of *F. oxysporum* f. sp. *udum.* The fungicides mancozeb and carboxin+thiram were react compatibly with Trichoderma. The inhibition zone indicates that the both *Trichoderma strains* (JN-S1 and TL-S1) significantly inhibit the growth of *Fusarium udum.*

Application of research: carboxin and mancozeb fungicides are suitable to use with the Trichoderma in the management Fusarium wilt of pigeonpea

Research Category: Plant Pathology

Abbreviations:
JN-S1= (JNKVV -S1)
TL-S1= (TNAU-S1)

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