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EFFICACY OF SOME FUNGAL ANTAGONIST AGAINST CHICKPEA WILT PATHOGEN *FUSARIUM OXYSPORUM* f. sp. *ciceri*

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Abstract: *Chickpea is a well-known rainfed crop of high value. Wilt caused by Fusarium oxysporum f.sp. ciceri (FOC) is the major seed, soil borne disease which results in excessive damage to the crop. The present study was aimed to determine the potentiality of locally isolated bioagents (Trichoderma Harzianum, Trichoderma viride and Aspergillus niger) against seven isolates of Fusarium oxysporum f.sp.ciceri causing chickpea wilt. Under in vitro conditions all the tested antagonist species inhibited the radial growth of the pathogen. Among all the bioagents the inhibition of the pathogen was least with A. niger and maximum with T. harzianum. Under pot experiments all the treatments were able to significantly control the wilt incidence. The bioagents at different concentrations viz. (2%, 4%, 6%, 8% w/w) were tested against the susceptible variety viz. JG62. T. harzianum and T. viride at 8% and A. niger at 10% concentration (w/w), inhibited the wilt incidence upto 100%.*

Keywords: *Bioagents, Chickpea, Wilt.*

Introduction

Chickpea is amongst the predominant grain legume crop grown in India. It's pivotal role in maintaining soil fertility particularly in dry areas has assigned it a special significance in the development of sustainable agriculture of the arid and semi-arid tropical regions. Amongst the major biotic constraints limiting its yield in the Indian subcontinent and the Mediterranean Basin[1], *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *ciceri* holds a cardinal place causing annual losses ranging from to 10% to 100% under conditions favourable for the disease[2- 3].

The disease is primarily managed by resistance breeding programme. The high incidence of pathogenic variability and mutability limits the effectiveness of any naturally selected resistance against the pathogenesis[4]. Disease management with fungicides is uneconomical because of the soil and seed borne nature of the pathogen besides it being hazardous to the environment[5]. Fungicides not only contribute to ground water pollution but also cause loss of non-target beneficial flora and evolving of fungicidal resistance variant of the pathogen[6].

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Management of plant disease through biological control has been considered as a viable alternative method as against the use of chemical pesticide and cultural practices [7-8]. Different mode of action of bio control active micro-organism in controlling fungal plant disease include hyper-parasitism, predation, antibiosis, cross protection, competition for site and nutrient and induced resistance.[9]

Aspergillus species are well known for producing various kinds of active compounds including antifungal and antibacterial[10-11]. Effectiveness of Aspergillus species against tomato wilt, brinjal wilt and foot rot of black pepper causing pathogens , *Fusarium oxysporum* f. sp. *lycopersici* *Fusarium solani* and *Phytopthora capsici* respectively have been reported.[12-13] *Trichoderma* species have become popular biological agents to protect crop against plant pathogen all over the world[14] . They can parasitize fungal pathogen and produce antibiotics [15]. *Trichoderma* species were found as effective biological inducers of plant's own defence mechanism in coconut [16], cucumber [17], and tomato [18-20]

The present study was undertaken to estimate the effectiveness of *T. harzianum, T. viride* and *A. niger* species against chickpea wilt pathogen *Fusarium oxysporum* f. sp. *Ciceri.*

Material and methods

A. Sample Collection

All the 7 isolates of *Fusarium oxysporum* f. sp. *ciceri* (FOC) used in the present study were isolated from roots of wilt infected chickpea plants collected from across six farm fields of Kanpur and Unnao district. The antagonists *viz T. harzianum T. viride* and *A. niger* were isolated from the rhizosphere soil of healthy chickpea plants.

B. Isolation Purification and maintenance of the pathogen

The root of each collected plant sample was washed thoroughly in running tap water and then surface sterilized in 0.01% mercuric chloride solution for one minute. The sterilized root pieces were then kept in potato dextrose agar (PDA) medium, incubated for 5 days at 25ºC. As soon as the growth of causal fungus was obtained it was transferred to PDA slants.

Pure cultures of the pathogenic fungus was obtained by adopting dilution method of Keitt[21]. Small piece of PDA culture along with the pathogen was transferred to a tube containing 10 ml sterile distilled water, shaken vigorously till a homogenous suspension was obtained, 10 fold serial dilution was made up to 10^{-4}. Single loop containing a single spore was then transferred to PDA plate. The colonies so obtained were then transferred to PDA slants and kept at 4ºC for further use.

C. Isolation of the Bioagents

Isolation of the bioagents *viz. (T. harzianum, T viride* and *A. niger*) was done from the rhizosphere soil of healthy chickpea plants by serial dilution method of V. N Pathak [22].One ml of soil suspension from dilution of 10^{-5} and 10^{-6} was aseptically added to sterile plate containing 15-20 ml of PDA . After incubation individual colonies were picked up with sterile loop and transferred to PDA slants and kept at 4ºC for further use.

D. Identification of the Fungal strains (Pathogen and Bioagents)

Based on the microscopic studies all the 7 isolates of pathogenic *F. oxysporum* f. sp. *ciceri* were identified on the basis of size and

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shape of the micro and macroconidia [23]. All the bioagents isolated *viz T. harzianum, T. viride* and *A. niger* were identified on the basis of morphological and cultural characteristics [24-27].

E. In-vitro Evaluation of antagonistic behaviour of fungal antagonist against of isolates *F. oxysporum* f. sp ciceri

All the bioagents were inoculated according to dual culture technique of Johnson and Curl [28] on PDA petridishes and the inhibition of the radial growth of the test pathogen in treated and control plates were recorded after one week of incubation. Percent inhibition of mycelial growth of the pathogen was calculated using formula [29]:

$$I(\%) = (C-T)/C*100$$

Where I = percent inhibition, C= colony diameter in in control, and T= Colony diameter in treatment

F. Study of the interaction pattern of the pathogen and antagonist *in -vitro*

The interaction pattern among the pathogen and bioagents was studied according to the key of Johnson and Curl [28] where:

A is mutual intermingling of the two organism

B is mutual inhibition on contact

C is mutual inhibition at a distance

D is Inhibition on contact, the antagonist continues to grow, at an unchanged or reduced rate through the colony of the inhibited organism

E is inhibition at a distance, the antagonist continues to grow resulting in a clear zone at an unchanged or reduced rate.

G. Efficacy of the antagonist in pot experiments

Multiplication of the inoculum of the7 test fungal isolates as well as the bioagents (*T. harzianum T. viride* and *A. niger*) were done as per the method of Miller[30], comprising of 190 gm of field soil sieved through 2 mm sieving mesh , 10gm of finely grounded maize meal and 70 ml of distilled water. The 200 gm of this soil maize meal medium was sterilized in 500 ml of Erlenmeyer flask. Later these flask were inoculated with the test fungal isolates and the antagonist and incubated at 25 ±2ºC for 20 days to obtain the respective inoculums.

H. Preparation of pots infested with F. oxysporum f. sp. ciceri.

For all the pot experiments 15 cm pots were taken, surface sterilized in 5 percent Lysol and then rinsed thoroughly. The pots were then filled with sterilized soil maize meal medium (190:10) and 5 % (w/w) wilt fungus inoculum multiplied on soil maize meal medium. The antagonist *T. harzianum, T. viride* and *A. niger* were mixed at different concentrations *viz.*2, 4, 6, 8 and 10% (w/w) in infested soil sand mixture in 15 cm plastic pots. The seeds @ 5 seeds per pot of susceptible variety JG62, were surface sterilized and sown for a total of 10 pots. The pots were lightly irrigated as and when required.

I. Statistical analysis

All values were expressed as mean ± SD, n = 3 and the results on the percent reduction of colony growth of the FOC isolates *in-vitro* were analysed by analysis of variance (ANOVA). $P \leq 0.05$ was considered statistically significant. Statistical evaluation was carried out using SAS system and the mean values were compared using the Least Significant Difference (LSD) at $P<0.05$.

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3. Results

The perusal of data in Table 1 reveals that the growth of all the isolates of Fusarium oxysporum f. sp. ciceri was inhibited considerably by the bioagents, *T. harzianum*, *T. viride* and *A. niger (*P<0.05)

A. Inhibition of colony growth of FOC isolates by the bioagents and their respective interaction pattern.

The interaction pattern between the isolates of wilt pathogen and *T. harzianum* was either of D or E type. It was D type (with isolate 1, 2, 3, 5 and7) meaning growth of antagonist *i.e T. harzianum* continuing after coming in contact with *F. oxysporum* f. sp. *ciceri*. Whereas with the other isolates it was E type, meaning inhibition at a distance and the antagonist continued its growth resulting in a clear zone either at an unchanged or reduced rate. The inhibition was significant (P<0.05) ranged from 20.11% (isolate7) to 65.78% (isolate 6) while it was 36.15, 56.64, 61.76, 52.39, 55.65, with isolate 1, 2, 3, 4, and 5 respectively.

The percentage reduction in the colony growth of the FOC isolates with *T. viride* was significant(P<0.05) ranging from 36.16% (isolate 4) to 63.30 %(isolate 5). It recorded an inhibition of 56.26, 59.76, 53.40, 58.79, 48.09 % with isolate 1, 2, 3, 6 and 7 respectively while the interaction pattern was of C, D, and E type meaning (i) mutual inhibition at a distance (ii) the antagonist continues to grow after coming in contact with other organism (iii) inhibition at a distance and antagonist continued to grow resulting in a clear zone at an unchanged or reduced rate respectively. It was E type with isolate 2 and D type with isolate 1, 3, 4 and 7 while C type with isolate 6 and 5.

The interaction pattern between the wilt pathogen *F. oxysporum* f. sp. *ciceri* isolates and *A. niger* revealed minimum inhibition of the former if compared with species of *T. harzianum* and *T. viride.* The interaction pattern was of B, C and D type. Isolates 2, 3, 6, 7 showed B type of interaction where mutual inhibition of fungal colonies took place when they came in contact with each other. They exhibited 23.33, 17.95, 25.55, and 14.16 % inhibition respectively while isolate 1 and 5 exhibited C type of interaction pattern and inhibition was 47.22 and 40.28 % respectively. Isolate 4 exhibited D type of interaction with 21.29 % inhibition.

B. Efficacy of antagonist under pot conditions

The results in Table 2 clearly indicates that all the three bioagents viz. *T. harzianum T. viride* and *A. niger* were effective at all levels of inoculum percentage against the susceptible variety JG62 chickpea seeds. *T. harzianum* at 2. 4. 6 and 8% controlled the wilt as its incidence was reduced to 45.8, 13.1, 16.3, 6.25 and 0%.

T. viride when tested in infested pot with the same sequence of concentrations revealed 48.97, 38.29, 17.39 and 0 % wilting of seeds (JG62). *A. niger* was tested in the same manner. The results showed 45.8, 19.1, 16.3, 6.25 and 0% wilting at 2, 4, 6, 8 and10 % inoculum respectively. It can be inferred that efficacy of *A. niger* was lesser when compared to the other two bioagents, as it required 10% inoculum of *A. niger* (w/w) to check the wilt incidence completely.

Though *T. harzianum* was the best antagonist but *T. viride* and *A. niger* or a combination of the above species may be utilized to check the wilt menace effectively.

4. Discussion

In recent years growing concern against the use of chemical pesticides has forced the

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scientific community to look for various alternative measures to manage plant diseases. The use of biocontrol agents is gaining momentum as it is environment friendly and also compatible with other models of agriculture: organic, biological and integrated pest/pathogen management [31]. Biological control of soil borne plant pathogen is a potential alternative to the use of chemical pesticides which have been proven harmful to the environment [32]. The fungal antagonist may compete for ecological niche by consuming available nutrients and by secreting a spectrum of biochemical. These biochemical may include cell wall degrading enzymes, siderophores, chelating iron, and a wide variety of volatile and non-volatile antibiotics [33] .

The present study was undertaken to assess the effectiveness of *T. harzianum T. viride* and *A. niger* against the chickpea wilt pathogen *F. oxysporum* f.sp. *ciceri*. The *in-vitro* assay revealed that all the three bioagents rapidly colonized the medium and were effective in checking the radial growth of the pathogen. *T. harzianum* was the most effective and was able to reduce the pathogen growth (isolate6) upto 65.78%, while *T. viride* followed closely behind with 63.30% inhibition (isolate 5).

The results are in agreement with various investigations stating the use of *T. harzianum* and *T. viride* as biocontrol agents. *Trichoderma* species when added to the soil or applied as seed treatments have been found to grow readily along with the developing root system of the treated plant. [34-35].

However *A. niger* showed a maximum of 47.22 % reduction in colony diameter of FOC whereas under pot conditions it was 100 percent successful in controlling the wilt at 10% inoculum. The inhibitory effects of *Aspergillus spp*. against several plant pathogens have been reported [36-37] .The positive response of bean plants on the addition of *A. niger* have been reported due to the fungistatic activity or the plant growth promoting activities in soil.[38-39],[29] Alwathnani et al.[32] found that under pot conditions, *T. harzianum* and *A. niger* boosted plant growth significantly and reduced the wilt incidence to (44.4%) and (35.6%), respectively as compared to FOL inoculated plant. The antagonistic potential of the bioagents has been attributed to fungistatic effect [40] or might be due to the secretion of antibiotics by the fungi or other inhibitory substances produced by the antagonists. [41-43]

5. Conclusion

The present study indicates the success of the bioagents *T. harzianum T. viride* and *A. niger* against FOC. *T. harzianum* was found to be most effective under *in vitro* and pot experiments followed by *T. viride* and *A. niger* to control chickpea wilt. The above bioagents could be used as eco-friendly cost effective alternative for the biological control which may help to obtain higher yield and promote sustainable agriculture.

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TABLES

Table 1: Inhibition of radial growth of different isolates of *F. oxysporum f. sp.ciceri* due to *T. harzianum T. viride* and *A. niger* and their respective interaction pattern

S. No	Isolate /Antagonist	Radial growth of isolates on 7th Day in mm(in presence of the antagonist)	Percent inhibition	Type of interaction
1	Isolate 1(Control)	57.16±0.62	**0.00**	
	Isolate 1+*T.harzianum*	37.16±1.31	36.15±0.87	D
	Isolate 1+*T.viride*	25.0±0.81	56.26±1.74	D
	Isolate 1+*A.niger*	30.16±0.84	47.22±1.82	C
2	Isolate 2(Control)	57.83±0.62	**0.00**	
	Isolate 2+*T.harzianum*	25.07±0.49	56.64±1.03	D
	Isolate 2+*T.viride*	23.27±0.60	59.76±1.27	E
	Isolate 2+*A. niger*	44.33±0.57	23.33±1.21	B
3	Isolate 3(Control)	61.26±0.91	**0.00**	

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	Isolate 3+*T.harzianum*	23.42±0.46	61.76±0.91	D
	Isolate 3+*T.viride*	28.55±0.48	53.40±0.96	D
	Isolate 3+ *A. niger*	50.26±0.52	17.95±1.04	B
4	Isolate 4(Control)	49.0±0.81	**0.00**	
	Isolate 4+*T.harzianum*	22.62±0.55	52.39±1.37	E
	Isolate 4+*T.viride*	31.25±0.65	36.16±1.64	D
	Isolate 4+*A niger*	38.56±0.49	21.29±1.23	D
5	Isolate 5(Control)	59.5±1.08	**0.00**	
	Isolate 5+*T.harzianum*	26.38±0.46	55.65±0.90	D
	Isolate 5+*T.viride*	21.83±0.62	63.30±1.28	C
	Isolate 5+*A. niger*	35.53±0.55	40.28±1.13	C
6	Isolate 6(Control)	54.48±0.40	**0.00**	
	Isolate 6+*T.harzianum*	18.64±0.26	65.78±0.61	E
	Isolate 6+*T.viride*	22.45±0.72	58.79±1.63	C
	Isolate 6+*A. niger*	40.55±0.60	25.55±1.37	B
7	Isolate 7(Control)	64.5±0.41	**0.00**	
	Isolate 7+*T.harzianum*	51.52±0.46	20.11±0.87	D
	Isolate 7+*T.viride*	34.48±0.61	48.09±1.17	D
	Isolate 7+*A. niger*	55.36±0.76	14.16±1.44	B

Values shown are the mean ± SD of 3 replicates, significant at $p \leq 0.05$

Table 2: Effect of different concentrations of Trichoderma spp. and *A. niger* on incidence of wilt of chickpea caused by *Fusarium oxysporum* f. sp. Ciceri (isolate 3) at 10 %(w/w) inoculum in pot cultures

S.no	**Different concentrations of the antagonists with 10 % mixed inoculum of *F. oxysporum* f.sp.*ciceri***		**No. of seeds sown @5seeds per pot**	**No. of seedlings emerged**	**No. of plants wilted**	**Percent incidence of wilt**
1	*T. harzianum* 2%	JG62	50	50	20	44
2	4%	JG62	50	48	9	18.6
3	6%	JG62	50	49	3	6.52
4	8%	JG62	50	50	0	0
5	Control	JG62	50	50	50	100
6	*T. viride* 2%	JG62	50	49	24	48.97
7	4%	JG62	50	47	18	38.29
8	6%	JG62	50	46	8	17.39
9	8%	JG62	50	50	0	0

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10	Control		JG62	50	49	49	100
11	*A. niger*	2%	JG62	50	48	22	45.8
12		4%	JG62	50	47	9	19.1
13		6%	JG62	50	49	8	16.3
14		8%	JG62	50	48	3	6.25
15		10%	JG62	50	48	0	0
16	Control		JG62	50	49	49	100

FIGURE

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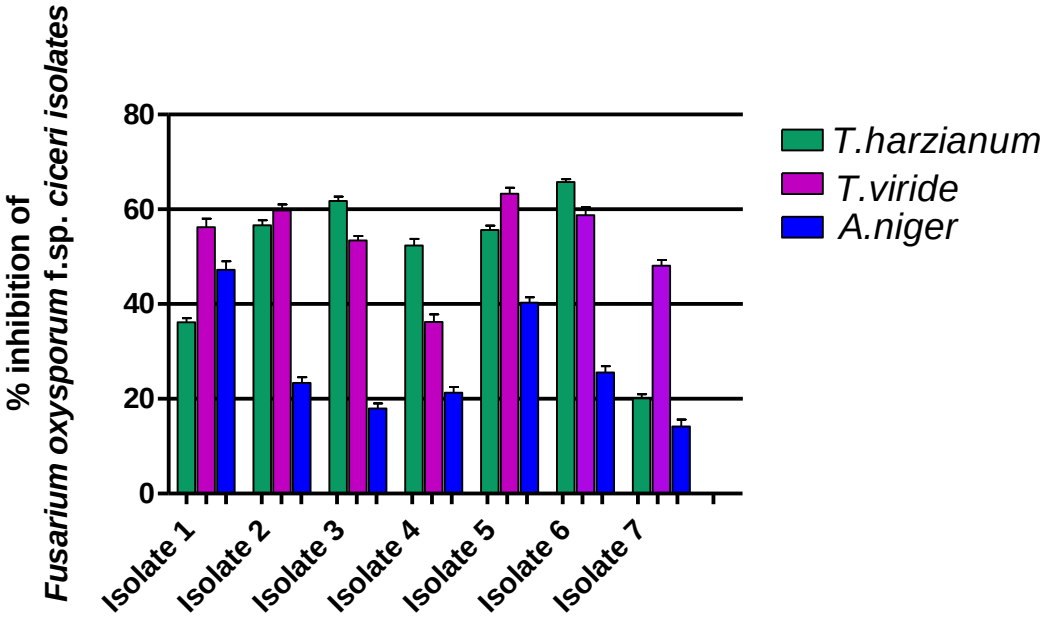

Figure1: Percent inhibition of *F. oxysporum f.sp. ciceri* isolates with the bioagents: All tested bioagents showed significant reduction in the colony growth of FOC isolates. T. harzianum was the most effective among the tested bioagents.