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EFFICACY OF ORGANIC AMENDMENTS AND BIO-AGENTS AGAINST *FUSARIUM OXYSPORUM* F. SP. *CICERI IN VITRO*

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ABSTRACT

Chickpea (*Cicer arietinum* L.) is the second most important crop in the world after dry bean. It belongs to the family *Fabaceae*. A large number of diseases have been reported on chickpea among them wilt is caused by *Fusarium oxysporum* f. sp. *ciceri*. The six organic amendments *viz*., mustard cake, neem cake, groundnut cake, castor cake, cotton cake and poultry manure at 5 and 10 per cent concentration and four bio-agents *viz., Trichoderma viride, Trichoderma harzianum, Pseudomonas fluorescens* and *Bacillus subtillis* were evaluated *in vitro* condition. Among the organic amendments, highest mean growth inhibition of 72.97 per cent was recorded with neem cake followed by castor cake (63.66%) and mustard cake (49.07%) while among the bio-agents *Trichoderma viride* showed maximum growth inhibition (62.78%) which was statistically followed by *T. harzianum* (50.11%). In case of bacterial bio-agents *P. fluorescens* (28.89%) was potential antagonists followed by *B. subtillis* (23.85%).
***Keywords*:** Chickpea, wilt, organic amendments and bio-agents.

Introduction

Pulses are recognized as one of the most important sources of edible vegetable proteins, which are taken in the form of dal. Besides being a rich source of protein, they maintain soil fertility through biological nitrogen fixation in soil and thus play a vital role in furthering sustainable agriculture (Kannaiyan, 1999). Among the pulse crops, Chickpea (*Cicer arietinum* L.) is the second most important crop in the world after dry bean. It belongs to the family *Fabaceae*. Chickpea seed contains 17-24 per cent of protein, 61.2 per cent carbohydrates, 9.8 per cent moisture (Smartt, 1976) and essential amino acids like isoleucine, leusine, lysine, phenylalanine and valine (Karim and Fattah, 2006).

Global production of pulses is 60 MT. Globally India ranked first in pulses production. Area, production and productivity of chickpea in India are 31.03 million hectares, 27.69 MT and 892 kg ha^{-1} respectively. Among the states of India, Madhya Pradesh ranked first in area and production of chickpea followed by Maharashtra, Rajasthan and Gujarat (Anon., 2022a). In Gujarat, chickpea was grown in 11016 hectares, producing 21014 MT with an average productivity of 1908 kg ha^{-1}, which is high as compared to national average productivity (Anon., 2022b).

A large number of diseases have been reported on chickpea *viz.* fusarium wilt [*Fusarium oxysporum* Schlechtend.: Fr. f. sp. *ciceri* (Padwick) T. Matuo & K. Sato], black root rot [*Fusarium solani* (Mart.) Sacc.], dry root rot *[Macrophomina phaseolina* (Tassi) Goidanich], wet root rot [*Rhizoctonia solani* Kuhn] and collar rot [*Sclerotium rolfsii* Sacc.] are of considerable importance (Nene *et al*., 1981). Among them, chickpea wilt complex is considered the most important, devastating and challenging one, being responsible for seed rot, seedling blight, root rot and mature plant wilt, culminating in 60-70 per cent yield loss (Tewari and Mukhopadhyay, 2001) The disease complex observed first in the history caused by *Fusarium* and *Meloidogyne* species on cotton (Atkinson, 1892). Wilt

complex caused by several soil-borne pathogens is the major yield reducing malady.

Chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceri* was first reported from India by Butler (1918). The mystery of the chickpea wilt complex was solved by Nene *et al.* (1979) in India and also developed multiple disease resistance screening techniques and disease-resistant varieties. The wilting pathogens are seed-borne (Haware *et al*., 1978; Pande *et al*., 2007) as well as soil-borne (Jimenez-Fernndez *et al.,* 2011) in nature. The mycelium and chlamydospores can survive in seed and soil, and also infected crop residues, roots and stem tissue buried in the soil more than the six years in absence of host plant (Haware *et al*., 1986).

Organic amendments and biological agents are a sustainable and eco-friendly approach to managing *F. oxysporum* f. sp. *ciceri* and reducing reliance on chemical fungicides. Therefore, the present investigation was carried to evaluate efficacy of organic amendments and biological agents against *F. oxysporum* f. sp. *ciceri in vitro* condition.

Material and Methods

Poison food technique for organic amendments

All the amendments were crushed to make fine powder. Fifty grams powder of each amendment was taken into 250 ml flask and then 150 ml sterilized water added to the flask. All these flasks were plugged with cotton and allowed for decomposing the material for 15 days. After 15 days, the material was strained with muslin cloth to obtain the extract. The strained liquid was autoclaved at 1.045 kg cm^{-2} pressure for 20 minutes and considered as cent per cent concentration (standard solution). The measured quantity of standard solution of the organic amendments were incorporated separately in melted PDA medium in conical flasks aseptically at the time of pouring the medium to obtain desired concentration. The medium was shaken well to give uniform dispersal and then poured about 20 ml in each sterilized Petri plate. After solidification of the medium, the Petri plates were inoculated in the centre by placing seven days old mycelial discs and then incubated at 28 ± 2°C temperature. Control was also maintained by growing the pathogen on organic amendment free medium.

Table 1 : Details of experiment:

Sr. No.	Content	Descriptions
1	Location	Dept. of Plant Pathology, CPCA, S. D. Agricultural University, Sardarkrushinagar
2	Experimental design	FCRD
3	Treatment	Seven (7)
4	Repetitions	Four (4)
5	Technique	Poison food technique

Table 2 : List of organic amendments ware used against *F. oxysporum* f. sp. *ciceri in vitro*

Sr.no.	Amendments	Concentration (%)	
1.	Mustard cake	5	10
2.	Neem cake	5	10
3.	Ground cake	5	10
4.	Castor cake	5	10
5.	Cotton cake	5	10
6.	Poultry manure	5	10
7.	Control	-	-

Observations recorded:

When the control plate filled with fungal growth, observation of radial growth of the fungus in all treatment was recorded. The per cent growth inhibition was calculated by using the formula of Vincent (1947).

$$PGI = \frac{C - T}{C} \times 100$$

Where:

PGI= Per cent growth inhibition

C = Colony diameter in control (mm)

T= Colony diameter in treatment (mm)

Dual culture technique for bio-agents

Different antagonist bio-agents were evaluated for their antagonistic activity against *F. oxysporum in vitro* by dual culture technique (Dennis and Webster, 1971). The tested bio agents and *F. oxysporum* were grown separately on PDA medium. The mycelial disc (5 mm diameter) of pathogen and fungal antagonists were placed on the same plate 60 mm away from each other.

To test for antagonistic bacteria, a 5 mm of the mycelial disc of pathogen culture was placed on one side of a Petri plate containing PDA medium, and loopful of bacteria was streaked 30 mm away from the disc of *F. oxysporum* on the same plate. Paired cultures were incubated at 27±2 °C. The plates inoculated only with test pathogen served as control. Four repetitions were maintained for each antagonist. The antagonistic fungal culture was maintained on PDA culture media and bacterial cultures were maintained on nutrient agar media. The assay for antagonism was performed on PDA media on Petri plates by the dual culture method.

Table 3 : Detail of experiment

Sr. no.	Content	Descriptions
1	Location	Dept. of Plant Pathology, CPCA, S. D. Agricultural University, Sardarkrushinagar
2	Experimental design	CRD
3	Treatment	Five (5)
4	Repetitions	Four (4)
5	Technique	Dual culture technique

Table 4: List of bio-agents were used against *F. oxysporum* f. sp. *ciceri in vitro*

Treatments	Bio-agent	Source
1	*Trichoderma viride*	Department of Plant Pathology
2	*Trichoderma harzianum*	Department of Plant Pathology
3	*Pseudomonas fluorescens*	Department of Microbiology
4	*Bacillus subtillis*	Department of Microbiology
5	Control	-

Observations recorded

When the control plate filled with fungal growth, observation of radial growth of the fungus in all treatment was recorded. The per cent growth inhibition was calculated by using the formula of Vincent (1947).

$$PGI = \frac{C - T}{C} \times 100$$

Where:

PGI= Per cent growth inhibition

C = Colony diameter in control (mm)

T= Colony diameter in treatment (mm)

Result and Discussion

Many organic amendments are known to have an inhibitory effect on the growth and reproduction of various fungi.

In the present investigation total six organic amendments *viz.,* mustard cake, neem cake, groundnut cake, castor cake, cotton cake, poultry manure with control were evaluated at 5 and 10 per cent concentrations, respectively by poisoned food technique *in vitro* to know their inhibitory effects on the growth of *F. oxysporum* f. sp. *ciceri.*

The results (Table 5) revealed that all the organic amendments were showed inhibitory effect on growth of *F. oxysporum* f. sp. *ciceri in vitro*. The highest mean growth inhibition of 72.97 per cent was recorded with neem cake followed by castor cake (63.66%) and mustard cake (49.07%) whereas, lowest in poultry manure (28.50%) followed by groundnut cake (40.59%).

The inhibition of fungal growth was increased with increase in concentration in all the tested organic amendments. The interaction effect of organic amendments and concentration was also found significant and noted that the maximum per cent growth inhibition (80.05%) in neem cake at 10 per cent concentration which was followed by castor cake (72.27%) at 10 per cent whereas, lowest growth inhibition was recorded with poultry manure (31.00%) at 10 per cent.

Table 5: Efficacy of organic amendments against *F. oxysporum* f. sp. *ciceri in vitro*

Tr. No.	Treatment	Growth inhibition (%)		
		Concentration (%)		Mean
		5.0	10.0	
T_1	Mustard cake	37.12 (36.42)	51.78 (61.72)	44.45 (49.07)
T_2	Neem cake	54.26 (65.88)	63.47 (80.05)	58.87 (72.97)

T_3	Groundnut cake	32.97 (29.61)	45.90 (51.57)	39.43 (40.59)
T_4	Castor cake	47.89 (55.04)	58.23 (72.27)	53.06 (63.66)
T_5	Cotton cake	33.69 (30.78)	50.55 (59.63)	42.12 (45.20)
T_6	Poultry manure	30.65 (25.99)	33.83 (31.00)	32.24 (28.50)
T_9	Control	4.05 (0.50)	4.05 (0.50)	4.05 (0.50)
Mean		34.38 (34.89)	43.97 (50.96)	-
		Treatment	**Concentration**	**Treatment × Concentration**
S. Em. ±		0.21	0.12	0.29
C. D. at 5%		0.60	0.35	0.85
C. V. %		1.31		

Figures in parenthesis are retransformed values of arcsine transformed values.

These findings were close enough with the results of Shrivastava (2021) reported that neem cake showing excellent inhibitory effect of 70.87 per cent followed by mustard cake (65.36 %), linseed cake (62.99%), groundnut cake (53.36%) against *F. oxysporum* f. sp. *ciceri.* These findings were also close enough with the results of Ghante *et. al*. (2019b) reported that maximum reduction of *F. oxysporum* f. sp. *udum* over control was recorded in neem seed cake (73.33 %) followed by castor seed cake (66.66 %). Same findings were also reported by Kumari *et al.* (2020) and Maurya *et al.* (2023).

Further the investigation was carried out to evaluate the *in vitro* efficacy of two fungal bio-agents as well as two bacterial bio-agents against *F. oxysporum* isolates of chickpea by dual culture method.

The results presented in Table 6 revealed the significant difference in the growth inhibition of all the antagonist. Among the tested fungal and bacterial antagonist, the fungal antagonist found superior over bacterial antagonist. It was recorded that *Trichoderma viride* showed maximum growth inhibition (62.78%) which was statistically followed by *T. harzianum* (50.11%). In case of bacterial bio- agents *P. fluorescens* (28.89%) was potential antagonists followed by *B. subtillis* (23.85%).

Table 6: Efficacy of bio-agents against *F. oxysporum* f. sp. *ciceri*

Tr. No.	Treatments	Growth inhibition (%)
T1	*Trichoderma viride*	52.40 (62.78)
T2	*Trichoderma harzianum*	45.64 (50.11)
T3	*Pseudomonas fluorescens*	32.51 (28.89)
T4	*Bacillus subtillis*	29.23 (23.85)
T5	Control	04.05 (0.50)
Mean		32.38 (32.75)
S. Em. ±		0.39
C. D. at 5%		1.18
C. V. %		2.38

Figures in parenthesis are retransformed values of arcsine transformed values.

This finding was close enough with the results of Thaware *et al.* (2016) tested six fungal and two bacterial bioagents in *in vitro*, exhibited significant mycelial growth inhibition of *F. oxysporum* f. sp. *ciceri,* however, *T. viride* recorded significantly highest mycelial growth inhibition (75.55%), followed by *T. harzianum* (73.77%) *T. koningii* (71.88%) and *P. fluorescens* (43.77%). The same results were obtained by Penchala *et al.* (2008), Andrabi *et al.* (2011), Rehman *et al.* (2013), Patil *et al*. (2015), Ghante *et al*. (2019a) and Charpota *et al.* (2023) in different crops.

Conclusion

Among the *in vitro* study of organic amendments, neem cake showed highest inhibition percentage and among the bio-agents *T. viride* showed highest inhibition percentage.

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