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Comparative Studies of Fusarium Wilt in Chickpea on Effect of Bio-agents and Essential Oils in Aspects of Cost Benefit Ratio

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A B S T R A C T

Pulses are major sources of proteins among the vegetarians in India, and complement the staple cereals in the diets with proteins, essential amino acids, vitamins and minerals. They contain 22-24% protein, which is almost twice the protein in wheat and thrice that of rice. Chickpea (*Cicer arietinum* L.) is the world's third most important pulse crop, after dry beans (*Phaseolus vulgaris* L.) and dry peas (*Pisum sativum* L.). *Fusarium oxysporum* f.sp. *ciceris* is a wilt fungus causing severe damage wherever this crop is grown **.** It causes complete loss in grain yield if the disease occurs in the vegetative and reproductive stages of the crop. The treatments were Control (water irrigation), Neem oil5%, Eucalyptus oil 5%,*Trichoderma viride* 5%, *Pseudomonas sp.* 5%, Neem oil 2.5% + *Trichoderma viride* 2.5%, Neem oil 1.25% + *Trichoderma viride* 1.25% +*Pseudomonas sp.*1.25%, Neem oil 1.25% + *Trichoderma viride* 1.25% +Eucalyptus oil1.25%seed treatment was done. Among all the treatment in managing the wilt disease, Neem oil + *Trichoderma viride* +*Pseudomonas sp.* showed best cost benefit ratio of 1:2.89.

Introduction

Chickpea (*Cicer arietinum L.*) is a bushy annual plant of the pea family, with short, hairy pods containing usually two seeds. Chickpea is the world's third most important pulse crop, after dry beans (*Phaseolus vulgaris L.*) and dry peas (*Pisum sativum L.*) (Vishwadhar and Gurha, 1998). Chickpea is valued for its nutritive seeds with high protein content (25.3-28.9%) after dehulling (Hulse, 1991). India, accounts for 75% of world's chickpea production on 13.98 million ha area with production 137.3 lakh tonnes and productivity 982 kg/ha (Haware, *et al.*, 2016). *Fusarium* wilt is a serious disease of chickpea in India, Iran, Pakistan, Nepal, Burma, Spain, Tunisia and Mexico. It causes complete loss in grain yield if the disease occurs in the vegetative and reproductive stages of the crop (Haware and Nene, 1980; Haware *et al.*, 1990; Halila and strange, 1996; Navas *et al.*, 2000).This fungus, *Fusarium oxysporium*f. sp. *ciceris*is primarily a soil borne pathogen; however, few reports indicated that it can be transmitted through seeds (Haware *et al.*, 1978).

The disease manifests as mortality of young seedlings (within 25 to 30 days after sowing) to wilt or death of adult plants (Haware *et al.*, 1978). Seedlings that die due to wilt disease can be confused with other diseases of wilt complex, if not examined carefully. *Fusarium* wilt infected seedlings collapse and lie flat on the ground retaining their dull green color. Adult plants show typical wilt symptoms of drooping of petioles, rachis and leaflets.

The roots of the wilting plants do not show any external rotting but when split open vertically, dark brown discoloration of internal xylem is seen (Nene *et al.*, 1991). Pods from the wilted plants look normal but seeds are generally smaller, wrinkled and discolored. Though such seeds can be detected visually, a normal looking seed harvested from wilted plants may also harbor the wilt pathogen. Seeds from wilted plants when mixed with the seeds from healthy plants may be responsible in introducing wilt in new areas. Management of *Fusarium* wilt of chickpea is difficult to achieve and no single control measure is fully effective.

The most effective and practical method of control worldwide is to use fungicides (Gupta *et al.*, 1988) or resistant cultivars. However, the effectiveness of host resistances is curtailed by the occurrence of pathogenic races in *Fusarium oxysporium* f. sp. *ciceris* (Haware and Nene, 1982; Jimenez-Diaz *et al.*, 1989; Jimenez-Gasco *et al.*, 2004).

Therefore, integrated management strategies are the only solution to maintain plant health, including minimum use of chemicals for checking the pathogen population, encouragement of beneficial biological agents to reduce pathogen inoculum (Bendre and Barhate, 1998). The purpose of this study is to find out the best cost benefit ratio for suitable antagonists to manage the disease.

Materials and Methods

The field experiment was conducted during *Rabi* season at Central Research Farm, Department of Plant Pathology, Allahabad School of Agriculture, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, U.P., entitled "Biological approaches against Wilt caused by *Fusarium oxysporum* f. sp. *ceri*of Chickpea (*Cicer arietinum* L.)".The detail of the methods employed and materials used during the course of investigations are as follows-

Climate

Prayagraj is situated at 25.27^{o} North latitude 80.50^{o} East longitude and at an altitude of 98 meter above sea level. The climate is typically semi arid and sub tropical.

The maximum temperature reaches up to 47^{o} C in summer and drops down to 1.5^{o}C in winters. Isolation will be made from rhizosphere or infected stem and pure culture will be procured.

Mass multiplication of *F. oxysporum* f. sp. *ciceri*

Pure culture of *F. oxysporum* f. sp. *ceri*was procured from Department of Plant Pathology, SHUATS, Prayagraj.

In laboratory all glass wares used were thoroughly cleaned with detergent, washed dried and sterilized at 150°C for 4 h and Potato Dextrose Agar (PDA) was used for isolation of fungus as method described by The petri dishes and pipettes were wrapped in clean paper and sterilized in hot air oven at a temperature of 150^{0} C to 180^{0} C for two to four hours.

For isolating and growing of pathogen *F. oxysporum* f. sp. *ciceri*, Potato Dextrose Agar

(PDA) medium was used. The procedure for the preparation of medium was adopted as mentioned by Aneja, (2004).

Material Required

Agar 20 g
Dextrose 20 g
Peeled potatoes 200 g
Distilled water 1000 ml
pH 5.5-6.5

Procedure

Weighed 200g of peeled Potato gently with the help of weighing machine. It was boiled in 500 ml of distilled water till potatoes were mashed easily by the finger.

Potato extract was extracted with the help of muslin cloth. Measured the amount of sieved extract on measuring cylinder and made it 1000 ml by the addition of distilled water.

Again stared boiling of extract and added bit by bit Dextrose powder and Agar powder respectively. The PDA was transfer in the conical flask (250 ml) for sterilization. It was kept for 30 min at 15lbs and 120±2^{0}C temperature (Aneja, 2004). For further analysis and mass multiplication of *Fusarium* sp. slants was prepared.

Isolation and identification of the pathogen

Sample of infected root was randomly collected from experimental plot. Root was washed with tap water to removed soil particles and warp gently with the bloating sheet.

One present concentration of $HgCl_2$ was used for surface sterilization of root. Small pieces of roots were cut with sharp blade under aseptic condition and plot on petri dishes which contend PDA. Inoculated petri dishes were incubated in B.O.D. After recommended period of incubation mycelium growth of pathogen was observed on the petri dishes. Single spore technique was applied for purification of *Fusarium sp*. and pure culture of *Fusarium sp*. was transferred in several slants for further analysis.

Procedure for mass multiplication of *F. oxysporum* f. sp. *ciceri*

The chickpea seeds were soaked partially for overnight and then spread on the clean blotting paper for air drying. About 250 gm. of seeds of chickpea were filled in each 1000 ml flask and autoclaved for 30 minutes at 15 lbs. psi pressure.

The mycelium bit of pure culture of *F. oxysporum* f. sp. *Cicero* was inoculated under aseptic condition in those flask containing grains and incubated at 28±2^{0}C for 10 days.

Meanwhile flasks were shaken to avoid clumping of grains and to facilitate early growth of fungus. These mass inoculums were spread in the experimental sick plot before two week of sowing (Aneja, 2004).

Sowing chick pea seeds into inoculated plot

The experiment was conducted in Randomized block design (RBD), using 2x2 m plot size with three replications. Sowing date was 18 December, 2018. The sowing was done row to row and plant to plant spacing with of 30×10 cm.

Post planting operation

Irrigation, weeding, earthing, thinning, etc. were carried out routinely for the proper growth of the crop.

Application of Treatment

Symbols	Treatments	Method of Application	References
T_0	Control	-	-
T_1	Neem oil	Seed treatment	**Singh *et al.*, (2016)**
T_2	Eucalyptus oil	Seed treatment	**Dawar *et al.*, (2007)**
T_3	*Trichoderma viride*	Seed treatment	**Chand *et al.*, (2009)**
T_4	*Pseudomonas sp.*	Seed treatment	**Shrivastava *et al.*, (201**
T_5	Neem oil + *T. Viride*	Seed treatment	**Chakraborty *et al.*, (20**
T_6	*T. viride* + Neem oil + *Pseudomonas sp.*	Seed treatment	-
T_7	*T. viride* +Neem oil+ Eucalyptus oil	Seed treatment	-

Results and Discussion

The data presented in Table revealed that maximum yield of 28.75q/ha with cost benefit ratio 1:2.89 was obtained with Neem oil+ *T. viride* + *P. fluorescens* as seed treatment. Next effective treatment was Neemoil+ Eucalyptus Oil + *T. viride* seed treatment with effective CB (1**:**2.31), followed by Neem oil+ *T. viride* seed treatment with CB (1**:**2.30), *T. viride* seed treatment with CB (1:2.25), neem oil seed treatment with CB (1:2.02), *Pseudomonas fluorescens* seed treatment with CB (1:1.90) and eucalyptus oil seed treatment with CB (1:1.64) over the control.

Table.1 Economics of treatments

S.No	Treatment	Rate Seed treatment	Cost of Chemical	Cost of Treatment
01	**T_1** Neem oil	50ml/kg seed	250Rs/lt	1250/-
02	**T_2** Eucalyptus oil	50ml/kg seed	1000Rs/lt	5000/-
03	**T_3** *T. viride*	50ml/kg seed	70Rs/lt	350/-
04	**T_4** *P. fluorescens*	50ml/kg seed	100Rs/Kg	500/-
05	**T_5** Neem oil+ *T. viride*	50ml of oil + 5gm/kg seed	320Rs./Kg	1280/-
06	**T_6** Neem oil+ *T. viride* + *P. fluorescens*	50ml of oil + 5gm/kg seed	420 Rs/Kg	1510/-
07	**T_7** Neemoil+Eucalyptus Oil + *T. viride*	50ml of oil + 5gm/kg seed	1320 Rs./ Kg.	4030/-
08	**T_0** Control	----------	----------	----------

Table.2 Comparative effect of Bio-agents and essentiall oils on Cost Benefit Ratio

S.No.	Treatment	Yield of q/ha	Cost of yield	Total cost of yield (Rs.)	Common Cost (Rs.)	Treatment cost (Rs.)	Total cost	C:B ratio
01	**T_1** Neem oil	20.04	4500 Rs/q	90180/-	43200/-	1250/-	44450/-	1:2.02
02	**T_2** Eucalyptus oil	17.62	4500 Rs/q	79290/-	43200/	5000/-	48200/-	1:1.64
03	**T_3** *T. viride*	21.79	4500 Rs/q	98055/-	43200/	350/-	43550/-	1:2.25
04	**T_4** *P. fluorescens*	18.54	4500 Rs/q	83430/-	43200/	500/-	43700/-	1:1.90
05	**T_5** Neem oil+ *T. Viride*	22.75	4500 Rs/q	102375/-	43200/	1280/-	44480/-	1:2.30
06	**T_6 Neem oil+ *T. viride* + *P. Fluorescens***	**28.75**	**4500 Rs/q**	**129375/-**	**43200/**	**1510/-**	**44710/-**	**1:2.89**
07	T_7 Neemoil+Eucalyptus Oil + *T. Viride*	24.25	4500 Rs/q	109125/-	43200/	4030/-	47230/-	1:2.31
08	T_0 Control	15.58	4500 Rs/q	70110/-	43200/	---------	43200/-	1:1.62

The present investigation indicates that application of Neem oil+ *T. viride* + *P. fluorescens* with seed treatment can be used as an effective treatment of wilt disease.

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