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Fusarium wilt in chickpea (*Cicer Arietinum* L.) caused by *Fusarium oxysporum* f. sp. *ciceris* management through combination of Essential oils and bioagents

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Abstract

Chickpea is one of the most important pulse crops contributes as largest producer in India with 75% of world acreage and production. Chickpea is grown in *Rabi* season. Chickpea is known to be infested by 172 pathogens (67 fungi, 3 bacteria, 80 nematodes and mycoplasma) from all over the world. Wilt of Chickpea caused by *Fusarium oxysporum* f.sp. *ciceri* is the most important disease, causing maximum economic damage to the crop. In the December month of 2018 to check the effect of seed treatment with bio-agents and essential oils against Chick pea wilt diseases to minimize the disease intensity. The treatments were Control (water irrigation), Neem oil 5%, 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 oil 1.25% seed treatment was done. Among all the treatment in managing the wilt disease, Neem oil + *Trichoderma viride* + *Pseudomonas* sp. showed better results.

Keywords: Bio-agents, essential oil, chickpea, *Trichoderma viride*, *Pseudomonas* sp, Neem oil, Eucalyptus oil and wilt disease

Introduction

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. Pulses provide significant nutritional and health benefits, and are known to reduce several non-communicable diseases such as colon cancer and cardio-vascular diseases. (Laxmipathi *et al.*, 2013) [7].

The centre of origin of chickpea is considered to be lies in South Western Asia from where it has spread to different countries including India. Despite chickpea being a member of the “founder crop package”, with potential nutritional/medicinal qualities, it has not received due attention for research like other founder crops (e.g. wheat or barley). Chickpea has been and is being consumed by humans since ancient times owing to its good nutritional properties. 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.) (Vishwadhar and Gurha, 1998) [12]. In India, it is one of the most important pulse crops commonly known as ‘chana’, contributes as largest producer with 75% of world acreage and production of gram. Chickpea was grown on 9.92 million hectare with the production of 9.80 million tones and productivity 945 kg/in India. In India, Madhya Pradesh stood first as far as acreage and production were concerned. The chickpea was grown on 5.75 lakh hectares with the production of 5.54 lack tones during 2016-17 in Uttar Pradesh.

Pulses plant bearing symbiotic bacteria contain in the nodules on their roots take so little and give so much to our soil by fixing the atmospheric Nitrogen that their significance in restoring and maintaining the soil cannot be ignored.

Chickpea is grown in *Rabi* season. Chickpea is known to be infested by 172 pathogens (67 fungi, 3 bacteria, 80 nematodes and mycoplasma) from all over the world (Nene *et al.*, 1996) [9]. Wilt of Chickpea caused by *Fusarium oxysporum* f.sp. *ciceri* is the most important disease, causing maximum economic damage to the crop and at national level the yield losses encountered was reported to the tune of 60 per cent. The disease can appear at any stage of plant growth, symptoms in a highly susceptible cultivar can develop any time between 25 days after sowing till as late as podding stage (Nene and Reddy, 1987) [8]. The phyto-toxin produced by the pathogen causes wilting and leaf burning. In India it causes annually a loss of 10–15%,

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which in years of severe epidemics may rise to 60–70% depending on varietal susceptibility and agro-climatic conditions. When disease occurs at seedling stage, seedlings collapse and lie flat on soil surface and cause losses up to 46.6% whereas in adult plants, brown to black discoloration of xylem vessels occurs and severe yield loss extends up to 100%. The purpose of this study is to find out the best suitable biological management of the disease.

Materials and Method

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.

Climate

Prayagraj is situated at 25.27° North latitude 80.50° 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 °C in summer and drops down to 1.5°C in winters. Isolation have been made from rhizosphere or infected stem and pure culture has been procured.

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

Pure culture of *F. oxysporum* f. sp. *ciceri* 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 °C to 180 °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) [1].

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 started 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 °C temperature. (Aneja, 2004) [1] 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 HgCl₂ 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 grams 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. *ciceri* was inoculated under aseptic condition in those flask containing grains and incubated at 28± 2 °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) [1]

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

Application of Treatment

Field trial

Field trial consisted of seven treatments, with control and three replications with total number of 24 plots. Treatments were Neem Oil, Eucalyptus oil, *Trichoderma viride*, *Pseudomonas* sp. and Three combinations Neem oil + *T. viride*, Neem oil + *T. viride*, + *Pseudomona* and Neem oil + *T. viride* + Eucalyptus oil which were replicated three times. Observations on disease incidence and plant growth parameters were taken at 30, 45 and 60 days after sowing (DAS).

Result

Comparative effect on plant height against bio-agents and botanical: Plant height of chickpea was observed at 30, 45 and 60 days after sowing (cm).

Observation recorded on 30 DAS pertaining to mean plant height was highest in Treatment (T₆) *T. viride* + Neem oil + *Pseudomonas* sp. (8.75). Observation recorded on 45 DAS pertaining to mean plant height was highest in treatment (T₆) *T. viride* + Neem oil + *Pseudomonas* (13.24). Observation recorded on 60 DAS pertaining to mean plant height was highest in treatment (T₆) *T. viride* + Neem oil + *Pseudomonas* sp. (20.39)

Comparative effect on number of branches against bio-agents and botanical: Number of branches of chickpea was observed at 30, 45 and 60 days after sowing.

Observation recorded on 30 DAS pertaining to mean number of branches was highest in treatment (T₆) *T. viride* + Neem oil + *Pseudomonas* sp. (4.20). Observation recorded on 45 DAS pertaining to mean number of branches was highest in treatment (T₆) *T. viride* + Neem oil + *Pseudomonas* (4.867).

Observation recorded on 60 DAS pertaining to mean number of branches was highest in treatment (T₆) *T. viride* + Neem oil + *Pseudomonas sp.* (5.6).

Comparative effect of bio-agents and essential oils on wilt incidence: *Per cent* disease incidence of chickpea was observed at 30, 45 and 60 days after sowing.

Observation recorded on 30 DAS pertaining to mean percent of disease incidence reveal that it was lowest in treatment (T₆) *T. viride* + Neem oil + *Pseudomonas sp.* (1.13). Observation

recorded on 45 DAS pertaining to mean percent of disease incidence reveal that it was lowest in treatment (T₆) *T. viride* + Neem oil + *Pseudomonas sp.* (3.57). Observation recorded on 60 DAS pertaining to mean percent of disease incidence reveal that it was lowest in treatment (T₆) *T. viride* + Neem oil + *Pseudomonas sp.* (5.77).

Comparative effect of bio-agents and essential oils on yield of chickpea (Q/ha)

Table 1: Data was observed in the mean of maximum yield qt./h was recorded in treatment (T₆) *T. viride* + Neem oil + *Pseudomonas* (28.75).

S.No.	Treatment	Plant height (cm)			Number of branches			Disease incidence (%)			Yield (Q ha ⁻¹)
		30 DAS*	45 DAS	60 DAS	30 DAS*	45 DAS	60 DAS	30 DAS*	45 DAS	60 DAS	
T ₀	Control	6.51	12.00	17.25	2.467	3.400	4.07	12.60	21.93	41.00	20.04
T ₁	Neem oil	7.33	12.43	18.96	3.200	3.867	4.667	9.93	15.48	20.93	17.62
T ₂	Eucalyptus oil	6.97	12.01	18.53	2.600	3.533	4.333	11.30	18.40	29.03	21.79
T ₃	<i>Trichoderma viride</i>	7.72	12.55	19.20	3.400	4.000	4.800	7.90	13.98	17.90	18.54
T ₄	<i>Pseudomonas</i>	7.22	12.23	18.72	2.800	3.667	4.467	10.63	16.53	22.27	22.75
T ₅	Neem oil + <i>T. viride</i>	8.24	12.85	19.10	3.600	4.267	5.067	3.40	7.66	11.60	28.75
T ₆	<i>T. viride</i> + Neem oil + <i>Pseudomonas</i>	8.75	13.24	20.39	4.200	4.867	5.600	1.13	3.57	5.77	24.25
T ₇	Eucalyptus oil + <i>T. viride</i> + +Neem oil	8.44	13.07	19.76	3.867	4.600	5.333	2.00	5.23	9.57	15.58

Discussion

Similar finding has been reported by Dhanya *et al.* (2016) [4] also observed that application of *Trichoderma viride* + *pseudomonas* controlled soil borne disease wilt in effective manner. Jaiswal *et al.* (2015) [5] conducted *Fusarium oxysporum*. Sp. *Lycopersici* (FOL) is a highly destructive pathogen in both green house and field conditions. There are options for the management of the disease viz., use of botanical, bioagents, chemicals or genetic resistance. The present investigation was under taken to evaluate chemical, bioagents and botanicals viz. Bavistin (1g l-1 of water seedling dip), *Trichoderma viride* (25 kg ha-1), Neem oil cake (250 kg ha-1), NSKE (5%), combined treatment of *T. viride* + Neem oil, Bavistin + Neem oil cake and *T. viride* + FYM 1kg 100kg-1 FYM) for the management of wilt. Nikam *et al.* (2007) [11] evaluated *in-vitro* and *in-vivo* trial on the management of chickpea wilt caused by *F. oxysporum* f.sp. *ciceri*. Soil amendment with groundnut cake is proved to be effective against *F. oxysporium* f. sp. *ciceri* followed by neem cake. Thus, chickpea wilt incited by *F. oxysporium* f. sp. *Ciceri* being soil borne disease could be managed by the integration of various practices like using resistant varieties, seed treatment with chemicals, seed and soil application of Bio-agents and amendment of soils with oilseeds cakes. Dawar *et al.* (2007) [3] used of aqueous extract of leaves, stem, bark and fruit Eucalyptus sp., in the control of root rot fungi viz., *Fusarium* sp., *Rhizoctonia solani* and *Macrophomina phaseolina* by paper disc and well methods were examined. Eucalyptus sp., was more effective @ 5% w/v against *M. phaseolina*, *R. solani* and *Fusarium* sp. Soil amendment with leaves, stem, bark and fruit of Eucalyptus sp., @ 5% w/w showed significant increase in germination, shoot length, shoot weight, root length and root weight of chick-pea and mung bean plants. Zote *et al.* (2007) [13] studied on the management of wilt (*F. oxysporum* f. sp. *ciceri* [*F. oxysporum* f. sp. *ciceri*]) of chickpea (*Cicer arietinum*) was attempted by integrating a biological control agent *T. viride*, three fungicides (Thiram, Carbendazim and Captan) and four oil cakes (groundnut, cotton, neem and castor bean). soil application of oil cakes neem and castor bean, which recorded 86.60 and 85.40% seed germination and 38.09 and 47.60% wilt incidence and 61.91 and 52.40% wilt reduction,

respectively followed by fungicidal seed treatment with Thiram and Carbendazim. Chakraborty *et al.* (2009) [2] tested several fungal antagonists and botanicals for antimicrobial activity against the pathogen under *in vitro* and *in vivo* conditions. Soil solarization alone or with low dosages of a fungicide, biocide and bio-agent resulted in complete reduction of the pathogen. Soil solarization integrated with applications of *T. harzianum*, bavistin and neem was the most effective treatment. Kumar *et al.* (2011) [6] revealed in *in vitro* studies, neem extract at 20% concentration provided 55.6% inhibition. *Trichoderma harzianum* showed the highest mycelial growth inhibition (58.9%) against *R. bataticola*. Integrated methods showed higher disease reduction compared with single method. Bavistin (2 g kg-1 seed) + neem extract (20%) was the most effective treatment with 67.3% reduction followed by *T. harzianum* (15 g kg-1 seed) + Bavistin (54.2%) and neem extract + *T. harzianum* (44.0%). Animisha *et al.* (2012) conducted to diminish wilt of chickpea by use of integrated disease management. Neem cake at concentrations 7% followed by 5 and 3% in that order which give maximum inhibition of test pathogen under field condition. Lowest percentage of incidence of wilt (19.0%) was found with *T. Viride* (T2 followed by carbendazim (21.0%), neem cake (42.6%), carbendazim + neem cake (45.2%), carbendazim+ *T. viride* (47.2%), neem cake+ *T. viride* (48.2%). Gakuubi *et al.* (2017) evaluated the antifungal activity of essential oil (EO) of *Eucalyptus camaldulensis* Dehnh. Against five *Fusarium* sp. commonly associated with maize. The most abundant compounds identified in the EO were 1, 8-cineole (16.2%), α -pinene (15.6%), α -phellandrene (10.0%), and p-cymene (8.1%). The EO produced complete mycelial growth inhibition in all the test pathogens at a concentration of 7-8 μ L/ml after five days of incubation.

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