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Efficacy of fungicides and endophytic bacteria against Fusarium wilt of chickpea caused by *Fusarium oxysporum* f. sp. *ciceris* under *in vitro* conditions

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ABSTRACT

Chickpea (*Cicer arietinum* L.) is an important leguminous crop originated from Southwestern Asia region and Fusarium wilt disease caused by *Fusarium oxysporum* f. sp. *ciceris* (Foc) is limiting factor in chickpea cultivation. Efficacy of fungicides and endophytic beneficial bacteria were tested *in vitro* against chickpea wilt pathogen. Four different fungicides namely, carbendazim 50% WP (bavistin), hexaconazole 5% EC (contaf plus), propiconazole 25% EC (Tilt) and thiophanate methyl 70% WP (Roko) at 0.10, 0.15 and 0.20% were evaluated *in vitro* against Fusarium wilt of chickpea. Carbendazim and propiconazole proved the most effective exhibiting mean mycelial growth inhibition of 100% at all concentrations followed by hexaconazole and thiophanate methyl inhibit mycelial growth of 78.35 and 77.25% at 0.20% respectively. Four endophytic bacterial strains of *Bacillus* designated as ECP1 (*Bacillus cereus*), ECP5 (*Bacillus subtilis*), ECP8 (*Bacillus amyloliquefaciens*) and ECP10 (*Bacillus cereus*) were also evaluated against the Foc. The endophytic *Bacillus* strains revealed that ECP1 is the most efficacious resulted in 69.62 % mean inhibition of mycelial growth followed by ECP5 with 67.03%. ECP8 (*Bacillus amyloliquefaciens*) and ECP10 (*Bacillus cereus*) showed 65.18% growth reduction over control respectively.

Keywords: *Bacillus*, Chickpea, Endophytes, Fungicides, *Fusarium oxysporum* f. sp. *ciceris*

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a leguminous crop with chromosomal number of 2n=16 and was originated from Southwestern Asia. It is a major Rabi pulse crop in tropical and sub-tropical regions. It is a rich source of proteins, especially lysine and low in sulfur containing amino acids, making it a popular nutritious snack as well as rich in mineral content. India leads the globe in chickpea production (9.94 million tonnes), followed by Turkey (0.63 million tonnes), Russia (0.51 million tonnes), Myanmar (0.50 million tonnes), Pakistan (0.45 million tonnes) and Ethiopia (0.44 million tonnes). India produces 70% of the world's chickpeas (FAO, 2019). Chickpea is grown in Meghalaya, with an area (1856 ha), production (2187 metric tonnes) and productivity (1178 kg per ha) respectively (Anonymous, 2016). Though chickpea is attacked by many diseases but, only a handful are responsible for large production losses, such as wilt (*Fusarium oxysporum* f.sp. *ciceris*), dry root rot (*Rhizoctonia bataticola*), collar rot (*Sclerotium rolfsii*), wet root rot (*Rhizoctonia solani*), Ascochyta blight (*Ascochyta rabiae*) and Botrytis grey mould (Nene *et al.,* 1981). Wilt disease caused by *F. oxysporum* f.sp. *ciceris* (Foc) is a major constraint for chickpea production in various countries, including India, Iran, Pakistan, Nepal, Burma, Spain, Mexico, Peru, Syria and the United States. It is one of India's major limiting factors for chickpea production. This disease causes crop losses of 10-15 % per year of the total seed grain production and in severe condition it may go up to 100 % losses under suitable climatic conditions (Navas *et al.,* 2000). Chickpea wilt is a vascular pathogen that perpetuates both in seed as well as soil and can also survive up to 3- 6 years (Ayyub *et al.,* 2003). This pathogen can cause infection at all the stages of plant growth with more severe at flowering and podding stage. Under field conditions, the typical wilting can be appeared within 3-4 weeks after sowing, if the variety is susceptible (Haware, 1990). Thus, the disease can be effectively managed through different strategies such as use of resistant varieties, cultural practices, use of fungicides and through endophytic bacteria. Although each of these methods of disease management practices has their own importance, but none of the method give completely success when applied alone for disease control (Chandel and Deepika, 2010). Despite many attempts to control chickpea wilt pathogen Foc, the problem is still important throughout the world. The use of fungicide is most effective and reliable method of controlling disease. The application of fungicides in the farmers field can only be recommended against the *F. oxysporum* f.sp. *ciceris* after a successful laboratory evaluation. Therefore, the present study is carried out to evaluate different fungicides and endophytic bacteria against the *F. oxysporum* f.sp. *ciceris* under *in vitro* conditions.

MATERIALS AND METHODS

In vitro evaluation of different fungicides against the *F. oxysporum* f.sp. *ciceris*

In vitro efficacy of 4 fungicides namely carbendazim, hexaconazole, propiconazole and thiophanate methyl at 0.10, 0.15 and 0.20% were tested against the pathogen by poisoned food technique as given by Sharvelle (1961). Requisite quantities of the fungicides were added separately into 250 ml conical flasks containing 100 ml sterilized molten and warm PDA medium and thoroughly mixed in order to dissolve the fungicides properly. 20 ml of the poisoned medium from each specific concentration were poured into sterilized Petri plates

separately and allowed to solidify. Mycelial discs of 5 mm size from three day old actively growing cultures of the pathogen were cut with the help of sterilized cork borer and one such disc was centrally placed into each of the Petri plate. Three replications were maintained for each treatment and also for the control plates without the fungicides. The seeded plates were incubated at 27 ± 1 °C and mean colony diameter of each treatment was recorded after the mycelia in the control plates attained full growth. The per cent inhibition of mycelial growth was calculated by using the formula given by Vincent (1947).

Per cent mycelial growth inhibition = $C - T/C$ x 100

Where, C = radial growth of pathogen in control (cm) T = radial growth of pathogen in treatment (cm)

In vitro evaluation of antagonistic endophytic bacteria against the *F. oxysporum* f.sp. *ciceris*

The pure culture of for antagonistic endophytic bacteria i.e., *Bacillus cereus* (ECP1), *Bacillus subtilis* (ECP5), *Bacillus amyloliquefaciens* (ECP8) and *Bacillus cereus* (ECP10) were isolated from roots with root nodules of chickpea plants collected from different localities of Ri Bhoi district of Meghalaya. All the four isolates were tested for their inhibitory/antagonistic activity against *F. oxysporum* f.sp. *ciceris* by dual culture technique (Barhate *et al.,* 2012). *Foc* was cultured on Petri plate for 3 days. With a sterilized cork borer, 5 mm disc were cut from the periphery of culture and was transferred aseptically to a new plate containing PDA. Bacterial endophytes (24 h old) were streaked parallel to each other 1 cm away from the disc on both sides in the same plate. The plate with only fungal disc served as control. All the treatments were replicated 3 times. The plates were incubated at 28 ± 1°C till the control plates attained full growth. Inhibition percentage were calculated using the formula described by Vincent (1947).

Inhibition (%) = $C - T/C$ x 100

Where, C = radial growth of pathogen in control (cm) T = radial growth of pathogen in treatment (cm)

RESULTS AND DISCUSSION

In vitro evaluation of different fungicides on mycelial growth inhibition of *F. oxysporum* f. sp. *ciceris*

The four fungicides, carbendazim, hexaconazole, propiconazole and thiophanate methyl at three different concentrations @ 0.10, 0.15 and 0.20% was found effective against *F. oxysporum* f.sp. *ciceris*. Carbendazim and propiconazole gave 100% inhibition of growth of the pathogen at all the concentrations (0.10, 0.15 and 0.20%) tested and was found significantly different from hexaconazole and thiophanate methyl which gave inhibition per cent of 78.35 and 77.25% at 0.20% respectively (**Fig. 1; Table 1**).

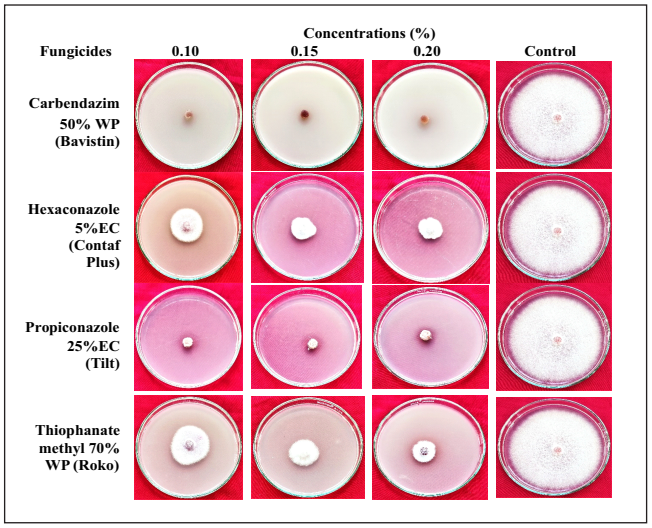

Fig. 1: Effect of different fungicides against the radial mycelial growth of *F. oxysporum* f. sp. *ciceris*

In vitro evaluation of antagonistic endophytic bacteria against *F. oxysporum* f. sp. *ciceris*

In dual culture test of four potent endophytic bacteria with *Foc* showed that *Bacillus cereus* (ECP1) had more mycelial growth inhibition of pathogen (Foc) with 69.62 % inhibition over control. *Bacillus subtilis* (ECP5) was proved to be second best followed by *Bacillus amyloliquefaciens* (ECP8) and *Bacillus cereus* (ECP10) with 67.03, 65.18 and 65.18% growth reduction over control respectively (**Fig. 2; Table 2**).

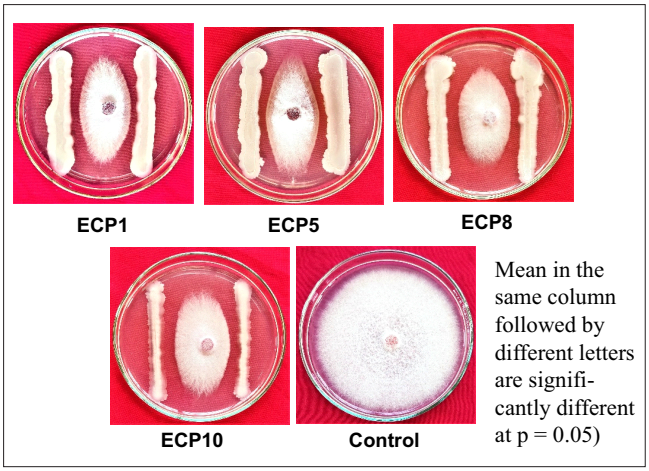

Fig. 2: *In vitro* evaluation of bacterial endophytes against *F. oxysporum* f. sp. *ciceris*

Chemical control based on the use of the fungicides in spite of its all health hazards has proved to be the management strategy. Four chemical fungicides with three different concentrations (0.10, 0.15 and 0.20%) were screened against *F. oxysporum* f.sp. *ciceris* under in vitro conditions. Out of the for fungicides carbendazim and propiconazole were found as the most effective against the *F. oxysporum* f.sp. *ciceris*. The

Table 1. Effect of different fungicides against the mycelial growth of *F. oxysporum* f. sp. *ciceris*

Fungicides	Growth (cm) at conc. (%)			Inhibition (%) over control at concentrations (%)			Control (cm)
	0.10	0.15	0.20	0.10	0.15	0.20	
Carbendazim 50% WP (Bavistin)	0±0 (0.00)	0±0 (0.00)	0±0 (0.00)	100±0^{d} (90.00)	100±0^{d} (90.00)	100±0^{d} (90.00)	9.00±0 (3.00)
Hexaconazole 5%EC (Contaf Plus)	2.35±0.057 (1.53)	2.15±0.05 (1.45)	1.95±0.10 (1.39)	73.88±0.640^{c} (59.27)	76.38±0.555^{c} (60.92)	78.35±1.11^{c} (62.27)	9.00±0 (3.00)
Propiconazole 25% EC (Tilt)	00±0 (0.00)	0.00±0 (0.00)	0.00±0 (0.00)	100±0^{d} (90.00)	100±0^{d} (90.00)	100±0^{d} (90.00)	9.00±0 (3.00)
Thiophanate methyl 70% WP (Roko)	3.25±0.43 (1.80)	2.55±0.10 (1.59)	2.05±0.57 (1.43)	63.88±4.84^{b} (53.06)	71.66±1.11^{b} (57.84)	77.25±6.38^{b} (61.51)	9.00±0 (3.00)
CD (0.05)	1.33	0.34	1.76	0.29	0.07	0.39	0.05
SEm(±)	0.44	0.11	058	4.88	1.24	6.48	0.02

(Data in the table are mean values of 4 replicates
Values in parantheses are square root transformed for mean growth and arc sine transformed for per cent inhibition)

other fungicides like hexaconazole and thiophanate methyl were moderately effective against *F. oxysporum* f.sp. *ciceris*. Generally, a positive correlation was observed between the different concentrations of the tested fungicides and inhibition of *F. oxysporum* f.sp. *ciceris*. Higher doses of fungicides were found to be more effective than their lower doses. There were several reports from elsewhere regarding *in vitro* evaluation of chemical fungicides *F. oxysporum*. Our results are in conformity with those reported by Poddar *et al.* (2004), Rajput *et al.* (2006), Mukhtar (2007), Sultana and Ghaffar (2010), Khan *et al.* (2012), Patra and Biswas (2016) and Bashir *et al.* (2017). Generally, all the treatments check the activities of the inoculated fungus (*Foc*) and hence promote the growth of chickpea plant. Although the higher concentrations of the few fungicides completely inhibited the pathogen. Similarly, biological control of the plant diseases can be defined as management of the plant diseases by reducing the inoculum of the pathogen with the help of the beneficial micro-organisms (Campbell, 1994). In the present study *Bacillus cereus* was proved most effective in *in vitro* and these findings were completely in agreement with many workers who found many isolates/strains of *Bacillus*, isolated from the roots with root nodules of host crop plants were found effective to manage the plant pathogens (Burr *et al.,* 1998; Prasad *et al.,* 2002; Postma *et al.,* 2003; Saikia *et al.,* 2003). The results of the current investigation were supported by the findings of Arfaoui *et al.* (2006) who reported 14 endophytic bacterial isolates of chickpea gave more than 30% inhibition against *F. oxysporum* f.sp. *ciceris*. The results of Sallam *et al.* (2013) also supported the present investigation who reported that twenty-seven bacteria were isolated from rhizosphere region of cantaloupe plants. They tested *in vitro* against the growth of *F. solani* and found that 9 isolates showed more than 70% inhibition.

Table 2: *In vitro* evaluation of bacterial endophytes against Foc

Bacterial isolates	Per cent Inhibition over control
ECP1	69.62±1.28 (56.55)
ECP5	67.03±0.64 (54.95)
ECP8	65.18±1.28 (53.83)
ECP10	65.18±1.28 (53.83)
SEm(±)	0.371
CD (0.05)	1.128
C.V.	1.389

(Data in the table are mean values of 4 replications
Values in parentheses are square root transformed)

From the present study, it can be concluded that fungicide, carbendazim and endophytic bacteria, *Bacillus* sp. can manage Fusarium wilt of chickpea caused by *F. oxysporum* f.sp. *ciceris* effectively and could be included as a part of integrated disease management in eco-friendly manner for increasing production and productivity of chickpea. The present study was directed to identify and characterize of these endophytic *Bacillus* sp. on molecular basis, which has a key role in managing the wilt disease in chickpea.

REFERENCES

Anonymous. 2016. *Area and Production of Pulses. Directorate of Agriculture, Government of Meghalaya*. www.megagriculture.gov.in/PUBLIC/crops_pulses.aspx. Accessed on 28 January 2022.

Arfaoui, A., Sifi, B., Boudabous, A., Hadrami, I.E. *et al.* 2006. Identification of *Rhizobium* isolates possessing antagonistic activity against *Fusarium oxysporum* f. sp. *ciceris*, the causal agent of Fusarium wilt of chickpea. *J. Plant Pathol.* 67-75.

Ayyub, M.A. Khan, S.M. Ahmad, R. and Iftikhar, K. 2003. Screening of chickpea germplasm for the sources of resistance against chickpea wilt (*Fusarium oxysporum* f. sp. *ciceris*). *Pak. J. Phytopathol*. **15(1-2):** 25-27.

Barhate, G. Bidbag, M.B. Ilhe, B.M and Shivagaje, A.J. 2012. *In vitro* evaluation of fungicides and bioagents against *Colletotrichum capsici* causing die-back of capsicum. *J. Plant Dis. Sci.* **7:** 64-66.

Bashir, S., Mughal, M.N., Dar, S. A., Nissa, S. *et al.* 2017. *In vitro* efficacy of fungicides and biocontrol agents against wilt of chickpea caused by *Fusarium oxysporum* f.sp. *ciceris* (Foc). *Int. J. Curr. Microbiol. App. Sci.* **6(11):** 1392-1399.

Burr, A., Ortuno, A. and Armero, T. 1998. Phosphate solubilizing effect of *Aspergillus niger* and *Pseudomonas*. *Microbiol.* **30:** 113

Campbell, R. 1994. Biological control of soil born disease: some present problems and different approach. *Crop Prot.* **13:** 4-13.

Chandel, S. and Deepika, R. 2010. Recent advances in management and control of Fusarium yellows in *Gladiolus* species. *J. Fruit and Ornamental Plant Res.* **18(2):** 361-380.

Haware, M.P. 1990. Fusarium wilt and other important diseases of chickpea in the Mediterriananean area. *Options Méditerra néennes-Série Séminaires* **9:** 61-64.

Khan, H.S., Saifulla, I.M., Nawaz, A.S., Somashekharappa, N.P.R. *et al.* 2012. Efficacy of fungicides and biocontrol agents against *Fusarium oxysporum* f. sp. *ciceris* causing wilt of chickpea. *J. Enviro. Ecol.* **3:** 570-572.

Mukhtar, I. 2007. Comparison of phytochemical and chemical control of *Fusarium oxysporum* f. sp. *ciceris*. *Mycopath*. **5(2):** 107-110.

Navas, C.J.A., Hau, B and Jiménez, D.R.M. 2000. Yield loss in chickpeas in relation to development of Fusarium wilt epidemics. *Phytopathology* **90(11):** 1269-1278.

Nene, Y.L., Haware, M.P and Reddy, M.V. 1981. Chickpea disease resistance screening technique. *ICRISAT Information Bulletin* **28:** 28-29.

Patra, S. and Biswas, M.K. 2016. Efficacy of fungicides for the management of chickpea wilt disease caused by *Fusarium oxysporum* f. sp. *ciceri*. *J. Adv. Res.* **4(10):** 1457-1461.

Poddar, R.K., Singh, D.V. and Dubey, S.C. 2004. Integrated application of *Trichoderma harzianum* mutants and carbendazim to manage chickpea wilt (*Fusarium oxysporum* f. sp. *ciceri*). *Ind. J. Agric. Sci.* **74:** 346-348.

Postma, J., Montanari, M. and Van den Boogert, J.F. 2003. Microbial enrichment to enhance disease suppressive activity of compost. *Eur. J. Soil Biol.* **39:** 157-163.

Prasad, R.D., Rangeshwaran, R., Anuroop, C.P and Rashmi, H.J. 2002. Biological control of wilt and root rot of chickpea under field conditions. *Ann. Plant Prot. Sci.* **10:** 72-75.

Rajput, A.Q., Arain, M.H., Pathan, M.A., Jiskani, M.M. *et al.* 2006. Efficacy of different fungicides against Fusarium wilt of cotton caused by *Fusarium oxysporum* f. sp. *vasinfectum*. *Pak. J. Bot.* **38(3):** 875-880.

Saikia, R., Singh, T., Kumar, R., Srivastava, J., Srivastava, A.K. *et al.* 2003. Role of salicylic acid in systemic resistance induced by *Pseudomonas fluorescens* against *Fusarium oxysporum* f. sp. *ciceris* in chickpea. *Microbiol. Res.* **158:** 203-213.

Sallam, N.A., Riad, S.N., Mohamed, M.S. and El-eslam, A.S. 2013. Formulations of *Bacillus* spp. and *Pseudomonas fluorescens* for biocontrol of cantaloupe root rot caused by *Fusarium solani*. *J. Plant Prot. Res.* **53(3):** 210-218.

Sharvelle, E.G. 1961. *The Nature and Uses of Modern Fungicides*. Burgers Publication Company Minneapolis, Minnesota, U.S.A., p. 308.

Sultana, N. and Ghaffar, A. 2010. Effect of fungicides, microbial antagonist and oilcakes in the control of *Fusarium solani*, the cause of seed rot, seedling and root infection of bottle gourd, bitter gourd and cucumber. *Pak. J. Bot.* **42(4):** 2921-2934.

Vincent, J.M. 1947. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature* **159:** 850.