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Construing the Role of Plant Extracts, Fungicides and Bio-agents in Ameliorating Fusarium Wilt Management in Chickpea

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ABSTRACT

Background: Chickpea (*Cicer arietinum* L.) is the most important leguminous crop around the world. Fusarium wilt incited by *Fusarium oxysporum* f. sp. *ciceris* is a major biotic constraint in chickpea production. The present investigation was undertaken to evaluate the efficacy of plant extracts, fungicides and bio-agents against *Fusarium oxysporum* f. sp. *ciceris* under *in vitro* and field conditions.

Methods: Plant extracts and fungicides were evaluated using poison food technique while antagonistic activity of bio-agents was studied using dual culture technique under *in vitro* conditions. Field trials were conducted to evaluate the efficacy of different plant extracts, fungicides and bio-agents against Fusarium wilt at Experimental Area of Plant Pathology, CCS HAU, Hisar.

Result: Among thirteen plant extracts evaluated *in vitro*, neem leaves extract was found to be the most effective in inhibiting mycelial growth of *F. oxysporum* f. sp. *ciceris* followed by datura leaves' and garlic cloves' extract which were statistically at par. Out of six plant extracts tested against Fusarium wilt under field conditions, seed treatment with extracts of neem and datura leaves at 10% concentration were most effective and curtailed the wilt incidence by 39.02 and 34.14% along with 7.55 and 6.83% enhanced seed yield. Among fungicides, carbendazim 50 WP was the most toxic fungicide in restricting colony diameter of the pathogen with the least EC_{50} and EC_{90} values of 1.28 and 15.24 ppm a.i. followed by azoxystrobin 23 SC with corresponding values of 1.57 and 49.16 ppm a.i., respectively. Seed treatment with carbendazim 50 WP and azoxystrobin 23 SC were superior over other treatments and provided 88.41 and 85.98% reduction in disease incidence along with 12.85 and 10.99% higher seed yield over control. Among the bio-agents tested, *T. viride* and *T. harzianum* gave the best results in suppressing the pathogen growth *in vitro* and in minimizing the disease incidence coupled with improvement in seed yield under field conditions. The present study has provided chemical and non-chemical measures for integrated management of chickpea wilt.

Key words: Bio-agents, Chickpea, Fungicides, Fusarium wilt, Plant extracts.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the most important leguminous crop around the world which is extensively used in crop rotation with cereals to improve soil fertility due to its ability to form nodules and fix atmospheric nitrogen. Amongst various biotic constraints, wilt caused by *F. oxysporum* f. sp. *ciceris* is one of the most devastating diseases of chickpea in India inflicting an annual yield loss of 10-15% (Singh and Dahiya, 1973; Dubey *et al*., 2007) that reached 70-100% in years of severe outbreak (Halila and Strange, 1996). The widespread occurrence of disease has been reported in most chickpea growing areas in Africa, southern Europe, America and Asia including India (Jimenez-Diaz *et al*., 2015). Extracts of neem (*Azadirachta indica*) and garlic (*Allium sativum*) (Jamwal *et al*., 2017), datura (*Datura metel* var. *quinquencuspida*), congress grass (*Parthenium hysterophorus*), tulsi (*Ocimum sanctum*) at 40% concentrations (Jespers and Ward, 1993) and argel (*Solenostemma argel*), ginger (*Zingiber officinale*) and jatropha (*Jatropha curcus*) (Mohamed *et al*., 2016) have been found effective in inhibiting the mycelial growth of *F. moniliforme* f. sp. *ciceri.* Seed treatment with neem oil (Singh *et al*., 1980) and extracts of garlic bulbs (Chand and Singh, 2005) and garlic leaves (Singh *et al*., 1979) were effective in curtailing Fusarium wilt. Several fungicides *viz.* carbendazim, propiconazole, metalaxyl and benomyl (Jamwal *et al*., 2017), tebuconazole, copper oxychloride (Golakiya *et al*., 2018) are known to suppress the growth of the pathogen. Seed treatment with carbendazim, carbendazim + mancozeb (Golakiya *et al*., 2018) and soil drenching with carbendazim at 200 and 500 ppm (Mahmood *et al*., 2015) provided maximum reduction in disease incidence in pot and glass house experiments while seed treatment with thiram and carbendazim (Singh and Jha, 2003) proved highly effective in minimizing the wilt incidence along with enhanced seed yield under field conditions. In addition, the bio-control agents namely, *Trichoderma* sp., *Aspergillus niger*, *A. flavus* and *Bacillus* sp. (Mane and

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Yadav, 2015); *T. viride* and *T. harzianum* (Abhiram and Masih, 2018); *T. viride*, *Bacillus subtilis* and *Pseudomonas fluorescens* alone and in combinations showed the highest inhibition of mycelial growth of the pathogen *in vitro* (Keote *et al.*, 2019) while *T. polysporum*, *G. virens*, *P. variotii*, *T. pseudokoningii* and *T. harzianum* (Maitlo *et al.*, 2019) provided 89-94% protection against chickpea wilt under field conditions coupled with enhanced seed germination, plant growth and seed yield of chickpea.

Identification of new effective plant extracts, fungicides and bio-control agents is pre-requisite to develop a sustainable management strategy against Fusarium wilt. Moreover, most of the earlier studies pertaining to efficacy of plant extracts and bio-control agents are confined to pot and glasshouse experiments. Keeping in view the importance of chickpea wilt and need for alternative methods of its management, an attempt was made to evaluate the efficacy of certain plant extracts, fungicides and bio-control agents against *F. oxysporum* f. sp. *ciceris* under *in vitro* and field conditions.

MATERIALS AND METHODS

In vitro evaluation of plant extracts, bio-agents and fungicides against *F. oxysporum* f. sp. *ciceris*

Evaluation of plant extracts

Fresh and healthy parts of thirteen plants were collected from the surroundings of CCS HAU, Hisar. Aqueous extracts of different parts of plants *viz.* leaves of neem (*Azadirachta indica*), henna (*Lawsonia alba*), lantana (*Lantana camara*), congress grass (*Parthenium hysterophorus*), bougainvillea (*Bougainvillea glabra*), guava (*Psidium guajava*), eucalyptus (*Eucalyptus teretecornis*), aak (*Calotropis gigantea*), datura (*Datura stramonium*), chrysanthemum (*Chrysanthemum indicum*), turmeric (*Curcuma longa*) powder, garlic (*Allium sativum*) cloves and ginger (*Zingiber officinale*) rhizomes were evaluated for their efficacy against *F. oxysporum* f. sp. *ciceris*. The plant extracts were prepared by crushing surface washed plant parts with equal amount of water in a grinder. Macerated extract was centrifuged to remove the plant debris. The supernatant was then passed through bacterial proof filter paper lining over Buchner's funnel. Equal amount of mixture of plant extract and water on weight basis was considered as 100% (stock solution) from which further dilution was made.

For *in vitro* evaluation, various concentrations of 20, 30, 40 and 50% were prepared by adding required quantity of sterile distilled water to aqueous stock solution of each plant extract. The solutions of these concentrations were added in equivalent volume of double strength potato dextrose agar (PDA) medium in Petri plates to get the final concentration of 10, 15, 20 and 25%. PDA plates without plant extracts served as control. Hyphal plugs of 5 mm diameter were cut from the periphery of 7 days old active culture and transferred aseptically to plates containing PDA medium amended with plant extracts. Four replications were kept for each treatment. The plates were sealed with parafilm and incubated at 27±1°C. The colony diameter was measured in different treatments when radial growth in the control plates reached the periphery. The antifungal activity of plant extracts was expressed as percentage of mycelial growth inhibition (MGI) as described below.

$$\text{MGI (\%)} = \frac{\text{Growth in un-amended plate - Growth in plant extract amended plate}}{\text{Growth in un-amended plate}} \times 100$$

Evaluation of fungicides

Six fungicides *viz.* carbendazim 50% WP (Bavistin), mancozeb 75% WP (Indofil M 45), azoxystrobin 23% SC (Amistar), hexaconazole 5% EC (Contaf), tebuconazole 25% EC (Folicur) and captan 50% WP (Captan) were evaluated for their toxicity to *F. oxysporum* f. sp. *ciceris* under *in vitro* conditions at different concentrations using poisoned food technique (Grover and Moore, 1962). Stock solutions of the fungicides were prepared by dissolving their required quantity in small amount of acetone (5 ml) and diluting further with sterile distilled water. The additional dilutions from stock solution of each fungicide were prepared to have a series of concentrations 10-times higher than the test concentrations. Flasks holding 90 ml of sterilized PDA with ingredients of 100 ml were incorporated with 10 ml of fungicide solution of concentration 10-times higher than the required concentration in the amended PDA. For control, flasks of 90 ml PDA were supplemented with 10 ml sterilized distilled water. The supplemented PDA from each flask was poured in four sterilized Petri plates (25 ml/plate). After solidification of the medium, the plates were inoculated with 5 mm mycelial disc taken from active culture of *F. oxysporum* f. sp. *ciceris* on PDA and incubated at 27±1°C. The colony diameter was measured in different treatments and the mycelial growth inhibition was calculated as stated above. Dose response curve was drawn by plotting per cent growth inhibition against fungicide concentrations on a log probit scale (Bliss, 1967). The fungicide concentration triggering 50 and 90% growth inhibition (EC_{50} and EC_{90}) were worked out from the dose response curve (Horsfall, 1956).

Evaluation of bio-agents

Four bio-agents *viz. Pseudomonas fluorescens, Trichoderma viride, T. harzianum* and *Gliocladium virens* were evaluated for their inhibitory potential against *F. oxysporum* f. sp. *ciceris* under *in vitro* conditions following dual culture technique. The PDA medium was poured aseptically in Petri plates and inoculated with the test pathogen as well as antagonist. Four replications were maintained for each bio-agent. The Petri plates were incubated at 27 ± 1°C and split growth of pathogen was observed when the control plates were completely filled with it. The per cent growth inhibition was calculated following the formula given by Vincent (1947).

Mass culturing of *F. oxysporum* f. sp. *ciceris* and bio-agents

Fusarium oxysporum f. sp. *ciceris* was mass cultured on sorghum seeds in polypropylene bags. The bags were plugged with non-absorbent cotton and autoclaved for 20 minutes at 15 psi. The bags were inoculated with seven days old culture of the pathogen under sterilized conditions. The inoculated bags were incubated at 27±1°C for 10 days and the culture was used for artificial inoculation in field studies.

The cultures of *Trichoderma viride*, *T. harzianum, Gliocladium virens* and *Pseudomonas fluorescens* were obtained from the local habitat, CCS HAU, Hisar. The fungal bio-agents namely, *T. viride*, *T. harzianum* and *G. virens* were mass cultured on sterilized sorghum grains in 500 ml Erlenmeyer flasks while the bacterial bio-agent, *P. fluorescens* was multiplied on nutrient broth. The inoculated flasks were incubated at 27±1°C for 10 days and shaken thoroughly for proper multiplication of bio-agents.

Evaluation of plant extracts, fungicides and bio-agents under field conditions

Separate field trials were conducted to evaluate the efficacy of different plant extracts, fungicides and bio-agents against Fusarium wilt at Experimental Area of Plant Pathology, CCS HAU, Hisar during *Rabi* 2018-19 and 2019-20 using a susceptible chickpea variety JG 62. The seeds were sown in 3×2 m^2 plots with a row to row and plant to plant spacing of 30 cm and 10 cm, respectively. The crop was raised following recommended Package of Practices of CCS HAU, Hisar.

Evaluation of plant extracts

Based on *in vitro* efficacy of botanicals, six best plant extracts were selected for evaluation under field conditions. The seeds of wilt susceptible chickpea variety JG 62 were soaked in 10% concentration of different plant extracts for one hour, dried under shade and sown in plots in a randomized block design (RBD) with three replications for each treatment along with untreated control. The observations on disease incidence were recorded by counting the infected and healthy plants in different treatments. The seed yield was recorded on plot basis and expressed as kg/ha.

Evaluation of fungicides

Field experiments were conducted to study the efficacy of different fungicides against Fusarium wilt of chickpea in RBD with three replications during 2018-19 and 2019-20 under artificial inoculation conditions. The plot size, row to row and plant to plant distance was the same as indicated above. The seeds of susceptible chickpea variety JG 62 were treated with fungicides before sowing (Table 1). The observations on disease incidence and seed yield were recorded as stated earlier.

Evaluation of bio-agents

Field experiments were conducted in RBD with three replications for each treatment during 2018-19 and 2019-20. The seeds of chickpea variety JG 62 were treated separately with *Pseudomonas fluorescens, T. viride, T. harzianum* and *G. virens* at 0.4% before sowing. The plot size, row to row and plant to plant distance was the same as indicated above. The observations on disease incidence and seed yield were recorded similarly as described under evaluation of plant extracts.

Statistical analysis

Data were analysed statistically after angular transformation using appropriate design (Panse and Sukhatame, 1978) and the treatments were compared at p = 0.05.

RESULTS AND DISCUSSION

Evaluation of plant extracts, fungicides and bio-agents against *Fusarium oxysporum* f. sp. *ciceris* under *in vitro* and field conditions

Evaluation of plant extracts

Among aqueous extracts of 13 plants evaluated at different concentrations, neem leaves' extract was found to be the most effective in suppressing the mean mycelial growth of *F. oxysporum* f. sp. *ciceris* by 73.26% followed by datura leaves' and garlic cloves' extract (statistically at par). However, the least per cent growth inhibition was recorded in extracts of congress grass, guava and lantana leaves. There was a significant increase in mycelial growth inhibition of the pathogen with increasing concentrations of plant extracts. Maximum mean mycelial growth inhibition of 53.16% was observed at 25% concentration of different plant extracts. The interaction between treatments and concentrations was found to be significant. All the plant extracts inhibited the mycelial growth at 10% concentration, however, the extracts varied with respect to the concentrations up to which the growth inhibition occurred. In leaves extract of chrysanthemum, the growth inhibition was observed up to a concentration of 20% while in remaining plant extracts increase in inhibition continued up to 25%. The per cent growth inhibition was statistically on par at 10 and 15% concentrations in aqueous extract of ginger rhizomes, henna, parthenium and datura leaves and at 15 and 20% concentrations in guava leaves (Table 2).

Under field conditions, seed treatment with extract of neem and datura leaves (statistically at par) were the most effective in curtailing the wilt incidence by 39.02 and 34.14% along with 7.55 and 6.83% enhanced seed yield of chickpea variety JG 62, respectively. Other plant extracts had no significant effect on disease incidence (Table 3). Various antifungal substances produced from plant extracts have been reported to inhibit different pathogens. The results of present study are in conformity with those of Singh and Chand (2004), Irum (2007) and Jamwal *et al.* (2017) who reported aqueous extract of neem to be effective against *F. oxysporum* f. sp. *ciceris*. The effectiveness of garlic leaf extract and neem oil against *F. oxysporum* f. sp. *ciceris* has also been established by Singh *et al.* (1979) and Singh *et al.* (1980), respectively. Jespers and Ward (1993) reported that aqueous extract of *Datura metel* var.

Table 1: Efficacy of fungicides against *Fusarium oxysporum* f. sp. *ciceris* under *in vitro* conditions.

Fungicides	Trade name	Test concentrations (ppm a.i.)*	EC_{50} (ppm a.i.)	EC_{90} (ppm a.i.)
Hexaconazole 5 EC	Contaf	0-50	4.79	87.71
Azoxystrobin 23 SC	Amistar	0-75	1.57	49.16
Tebuconazole 25 EC	Folicur	0-50	4.60	52.19
Captan 50 WP	Captan	0-1000	4.68	147.42
Mancozeb 75 WP	Indofil M 45	0-1250	3.80	4148.77
Carbendazim 50 WP	Bavistin	0-20	1.28	15.24

*The test concentrations of hexaconazole 5 EC, tebuconazole 25 EC and mancozeb 75 WP were enhanced to 100, 100 and 4500 ppm a.i. to obtain their EC_{90} values.

Table 2: Effect of plant extracts on Fusarium wilt incidence in chickpea variety JG 62 under field conditions.

Plant extracts**	Disease incidence (%)		Mean	Disease control (%)	Seed yield (kg/ha)		Mean	Increase in seed yield (%)
	2018-19	2019-20			2018-19	2019-20		
Neem leaves	44.17 (41.61)*	39.17 (38.71)	41.67 (40.16)	39.02	1635.98	1691.31	1663.64	7.55
Turmeric powder	57.50 (49.30)	60.00 (50.83)	58.75 (50.07)	14.02	1542.45	1606.96	1574.71	1.80
Garlic cloves	54.17 (47.38)	50.83 (45.46)	52.50 (46.42)	23.17	1576.70	1664.77	1620.73	4.78
Bougainvillea leaves	63.33 (52.72)	65.83 (54.35)	64.58 (53.54)	5.48	1521.98	1604.03	1563.00	1.04
Eucalyptus leaves	66.67 (54.73)	63.33 (52.76)	65.00 (53.75)	4.87	1538.18	1577.35	1557.77	0.70
Datura leaves	48.33 (44.02)	41.67 (40.18)	45.00 (42.10)	34.14	1616.34	1688.78	1652.56	6.83
Control	71.67 (58.04)	65.00 (53.77)	68.33 (55.91)		1518.76	1574.97	1546.87	
C.D. (p= 0.05)	(6.55)	(7.77)			81.15	80.37		
C.V. (%)	7.32	9.00			3.88	3.80		

*Figures in the parentheses are angular transformed values; **Plant extracts were applied at 10% concentration.

Table 3: Efficacy of fungicides against Fusarium wilt incidence in chickpea variety JG 62 under field conditions.

Fungicides	Dosage (per kg seed)	Disease incidence (%)		Mean	Disease control (%)	Seed yield (kg/ha)		Mean	Increase in seed yield (%)
		2018-19	2019-20			2018-19	2019-20		
Carbendazim 50 WP	2 g	8.33 (16.73)*	7.50 (15.74)	7.92 (16.24)	88.41	1707.25	1825.55	1766.40	12.85
Mancozeb 75 WP	2.5 g	25.83 (30.50)	22.50 (28.28)	24.17 (29.39)	64.63	1644.27	1742.96	1693.61	8.20
Azoxystrobin 23 SC	1 ml	10.83 (19.09)	8.33 (16.59)	9.58 (17.84)	85.98	1678.79	1795.93	1737.36	10.99
Hexaconazole 5 EC	1 ml	16.67 (24.07)	15.00 (22.73)	15.83 (23.40)	76.83	1627.32	1766.88	1697.10	8.42
Tebuconazole 25 EC	1 ml	14.17 (22.04)	15.83 (23.38)	15.00 (22.71)	78.05	1635.11	1739.41	1687.26	7.79
Captan 50 WP	2 g	21.67 (27.72)	19.17 (25.92)	20.42 (26.82)	70.12	1597.45	1719.76	1658.61	5.96
Control		72.50 (58.47)	64.17 (53.24)	68.33 (55.86)		1503.16	1627.38	1565.27	
C.D. (p=0.05)		(3.80)	(3.580			85.49	100.53		
C.V. (%)		7.45	7.50			3.92	4.20		

*Figures in the parentheses are angular transformed values.

quinquencuspida at 40% concentration was effective in suppressing the mycelial growth of *F. moniliforme* f. sp. *ciceri*. However, in present study the aqueous leaves extract of *Datura stramonium* suppressed the growth of wilt pathogen by more than 60% at a concentration of 20%. Previous reports have also indicated the toxic effect of extract of garlic bulbs (Chand and Singh, 2005; Jamwal *et al.,* 2017), argel, ginger and jatropha (Mohamed *et al.,* 2016) to *F. oxysporum* f. sp. *ciceris*. Reduction in disease incidence in aqueous extracts of different plants might be due to their direct toxic effect on mycelial growth of the pathogen.

Evaluation of fungicides

The fungicides namely, hexaconazole 5 EC, azoxystrobin 23 SC, tebuconazole 25 EC, captan 50 WP, mancozeb 75 WP and carbendazim 50 WP differed considerably in their toxicity to mycelial growth of *F. oxysporum* f. sp. *ciceris* under *in vitro* conditions. The EC_{50} values of fungicides ranged from 1.28 to 4.79 ppm a.i., while the EC_{90} varied between 15.24 to 4148.77 ppm a.i. Amongst various fungicides, carbendazim 50 WP proved to be the most toxic fungicide in inhibiting mycelial growth of the fungus with the least EC_{50} and EC_{90} values of 1.28 and 15.24 ppm a.i. followed by

azoxystrobin 23 SC with EC_{50} and EC_{90} values 1.57 and 49.16 ppm a.i., respectively (Table 4). These observations were in agreement with the findings of Mahmood *et al.* (2015) and Jamwal *et al.* (2017) who reported that carbendazim 50 WP was highly inhibitory to fungal growth of *F. oxysporum* f. sp. *ciceris*. However, Mohamed *et al.* (2016) recorded 95% inhibition of mycelial growth of *F. oxysporum* f. sp. *ciceris* by thiram followed by bayfedan.

Under field conditions, the disease incidence was significantly reduced by seed treatment with the fungicides during both the years. On mean basis, seed treatment with carbendazim 50 WP and azoxystrobin 23 SC (statistically at par) were superior over other treatments and provided 88.41 and 85.98% reduction in wilt incidence. Mancozeb 75 WP and captan 50 WP were observed to be less effective. The highest seed yield of 1766.40 and 1737.36 kg/ha, respectively was obtained in carbendazim 50 WP and azoxystrobin 23 SC treated plots, which was 12.85 and 10.99% higher than the control (Table 1). Effective management of Fusarium wilt with carbendazim 50 WP has also been documented by several researchers (Haware *et al.*, 1978; De *et al.,* 1996; Singh and Jha, 2003; Andrabi *et al.,* 2011; Mahmood *et al.,* 2015).

Table 4: Evaluation of bio-agents against *Fusarium oxysporum* f. sp. *ciceris* under *in vitro* conditions using dual culture technique.

Bio-agents	Mycelial growth inhibition (%)
Trichoderma viride	75.27 (60.18)
Trichoderma harzianum	68.05 (55.57)
Gliocladium virens	44.44 (41.78)
Pseudomonas fluorescens	46.66 (43.05)
C.D.(p=0.05)	(4.41)

*Figures in the parentheses are angular transformed values.

Evaluation of bio-agents

Out of four bio-agents *viz. T. viride, T. harzianum, G. virens* and *P. fluorescens*, maximum reduction (75.27%) in mycelial growth of the pathogen was observed with *T. viride* followed by *T. harzianum* (68.05%) while *P. fluorescens* and *G. virens* (statistically at par) were found less effective in inhibiting the fungal growth (Table 5). *Trichoderma* spp. (*T. harzianum* and *T. viride*) are well documented for being effective bio-agents against soil-borne fungi namely, *Fusarium*, *Pythium* and *Rhizoctonia* (Papavizas *et al.,* 1984; Mohiddin *et al.,* 2010). In present study, *G. virens* inhibited mycelial growth of test pathogen by 44.44%, corroborating the results of Maitlo *et al.* (2019).

In field tests, all the bio-agents significantly reduced the wilt incidence during both the years. Based on mean of two years' data, seed treatment with *T. viride* and *T. harzianum* (statistically at par) gave the best results in reducing the disease incidence followed by *P. fluorescens* while seed treatment with *G. virens* proved to be less effective. *T. viride* and *T. harzianum* suppressed the Fusarium wilt by 48.35 and 42.25% along with 6.91 and 6.81% increase in seed yield of chickpea, respectively (Table 6). Soil application of *T. harzianum* and *T. viride* has been found effective in managing wilt diseases (Prasad *et al.,* 2002; Dubey *et al.,* 2012). In the present investigation, bio-priming seeds of chickpea variety JG 62 with *T. viride* or *T. harzianum* provided better disease control along with enhanced seed yield, indicating their possible use in managing chickpea wilt in low-input sustainable agriculture, supporting the findings of Mane and Yadav (2015) and Abhiram and Masih (2018). *Trichoderma* species have been highlighted as potential bio-agent in controlling Fusarium wilt of chickpea (Wani *et al.*, 2007; Verma *et al.*, 2014) and

Table 5: Evaluation of plant extracts against *Fusarium oxysporum* f. sp. *ciceris* under *in vitro* conditions.

Plant extracts	Mycelial growth inhibition (%) at				Mean
	10%	15%	20%	25%	
Neem leaves (*Azadirachta indica*)	61.11 (51.40)*	68.61 (55.91)	75.56 (60.37)	87.78 (69.57)	73.26 (59.31)
Turmeric powder (*Curcuma longa*)	48.89 (44.35)	50.56 (45.30)	53.89 (47.21)	64.44 (54.94)	54.44 (47.95)
Garlic cloves (*Allium sativum*)	49.44 (44.66)	54.17 (47.37)	59.72 (50.59)	67.50 (55.23)	57.71 (49.46)
Ginger rhizome (*Zingiber officinale*)	49.44 (44.66)	49.72 (44.82)	53.61 (47.05)	60.00 (50.75)	53.19 (46.82)
Henna leaves (*Lawsonia alba*)	40.00 (39.21)	41.11 (39.86)	43.33 (41.15)	51.11 (45.62)	43.89 (41.46)
Lantana leaves (*Lantana camara*)	6.11 (14.24)	13.61 (21.62)	16.39 (23.87)	18.61 (25.54)	13.68 (21.32)
Congress grass leaves (*Parthenium hysterophorus*)	9.44 (17.86)	10.28 (18.63)	11.67 (19.87)	13.33 (21.40)	11.18 (19.44)
Bougainvillea leaves (*Bougainvillea glabra*)	42.22 (40.51)	45.56 (42.43)	58.89 (50.10)	68.61 (55.91)	53.82 (47.24)
Guava leaves (*Psidium guajava*)	10.00 (18.41)	12.78 (20.90)	13.61 (21.62)	18.06 (25.13)	13.61 (21.52)
Eucalyptus leaves (*Eucalyptus teretecornis*)	43.06 (40.99)	45.83 (42.59)	51.67 (45.94)	68.06 (55.57)	52.15 (46.27)
Aak leaves (*Calotropis gigantea*)	25.00 (29.98)	39.44 (38.89)	47.78 (43.71)	64.17 (53.21)	44.10 (41.45)
Datura leaves (*Datura stramonium*)	56.94 (48.98)	58.06 (49.62)	61.39 (51.56)	65.28 (53.88)	60.42 (51.01)
Chrysanthemum leaves (*Chrysanthemum indicum*)	31.94 (34.40)	35.83 (36.75)	43.06 (40.99)	44.17 (41.63)	38.75 (38.44)
Mean	36.43 (36.13)	40.43 (38.82)	45.43 (41.85)	53.16 (46.80)	
C.D. (p=0.05)		Treatment		(1.65)	
		Concentration		(0.91)	
		Treatment × Concentration		(3.29)	

*Figures in the parentheses are angular transformed values.

Table 6: Effect of bio-agents on the incidence of Fusarium wilt in chickpea variety JG 62 under field conditions.

Bio-agent	Disease incidence (%)		Mean	Disease control (%)	Seed yield (kg/ha)		Mean	Increase in seed yield (%)
	2018-19	2019-20			2018-19	2019-20		
Trichoderma viride	39.38 (38.84)	29.38 (32.77)	34.38(35.81)	48.35	1574.07	1634.88	1604.47	6.91
Trichoderma harzianum	41.88 (40.30)	35.00 (36.22)	38.44(38.26)	42.25	1581.13	1624.87	1603.00	6.81
Pseudomonas fluorescens	48.13 (43.91)	43.75 (41.37)	45.94(42.64)	30.99	1541.25	1584.30	1562.77	4.13
Gliocladium virens	58.75 (50.05)	51.88 (46.07)	55.31(48.06)	16.90	1488.61	1558.25	1523.43	1.51
Control	68.75 (56.05)	64.38 (53.57)	66.56(54.81)		1464.68	1536.85	1500.77	
C.D. p=0.05)	(4.47)	(7.11)			79.48	42.42		
C.V. (%)	6.27	10.87			3.34	1.72		

*Figures in the parentheses are angular transformed values.

were superior to *B. subtilis* and a commercial bio-pesticide Kalisena™ (Dubey *et al.*, 2007). Moradi *et al.* (2012) reported that seed and liquid inoculation of *B. subtilis* and *T. harzianum*, singly or in combination reduced disease severity substantially up to 40% while Subhani *et al.* (2013) reported *T. harzianum* followed by *P. fluorescens* as an effective bio-agent in curtailing disease incidence by 67.93% under screen house conditions.

CONCLUSION

The results of the present study revealed that seed treatment with extract of neem and datura leaves at 10% concentration curtailed the wilt incidence by 39.02 and 34.14% along with 7.55 and 6.83% enhanced seed yield, respectively. Seed treatment with carbendazim 50 WP and azoxystrobin 23 SC provided 88.41 and 85.98% reduction in disease incidence along with 12.85 and 10.99% higher seed yield over control. *Trichoderma viride* and *T. harzianum* suppressed the pathogen growth *in vitro* and minimized the disease incidence under field conditions. The judicious use of chemical and non-chemical measures for integrated management of chickpea wilt will be beneficial for increasing the chickpea productivity.

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Conflict of interest

All authors declared that there is no conflict of interest.

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