RESEARCH ARTICLE

Antifungal efficacy of floral extracts, biocontrol agents and fungicides against *Fusarium oxysporum* f. sp. *ciceri*

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ABSTRACT: The present investigation was conducted during 2011-2015 to evaluate the effectiveness of extracts of *Marigold* sp. (*Tagetes erecta* L.), *Gaillardia* sp. (*Gaillardia pulchella*), *Chrysanthemum* sp. (*Chrysanthemum indicum*) and *Calotropis* sp. (*Calotropis gigantea*) flowers, biocontrol agents and fungicides against *Fusarium oxysporum* f. sp. *ciceri*. Among the *Pseudomonas fluorescens*, *Bacillus subtilis* and *Trichoderma harzianum* tested by dual culture technique, *P. fluorescens* suppressed the colonisation of *F. oxysporum* f. sp. *ciceri* by 81.59%. The three fungicides viz., carbendazim (0.1%), metalaxyl (0.2%) and thiram (0.2%) were tested by poisoned food technique. Carbendazim @ 0.1% showed the highest effectiveness against *F. oxysporum* f. sp. *ciceri*. Seed treatment with *P. fluorescens* 10 g/kg seed + *Trichoderma harzianum* 4 g/kg seed + *Marigold* sp. (*Tagetes erecta* L.) floral water extract 4% proved highly effective in increasing seedling vigour index (3008.25) in chickpea in paper towel assay and was also effective to reduce incidence of Fusarium wilt (69.31%) under green house conditions.

Keywords: *Calotropis gigantea*, *Chrysanthemum indicum*, *Gaillardia pulchella*, *Tagetes erecta*

The antifungal compounds in plants play an important role in disease resistance (Mahadevan, 1982). Such compounds are selective of their toxicity, and play important role in plant disease management (Singh and Dwivedi, 1987). Several plant species also possess antifungal properties (Akinbode and Ikotun, 2008; Yasmin *et al.*, 2008). Medicinal plants viz., *Azadirachta indica*, *Ocimum basilicum*, *Datura stramonium*, *Tagetes erecta* and *Allium sativum* at 2, 4, 6 and 8% concentrations showed effectiveness against *Fusarium oxysporum* f. sp. *gladioli* (Chohan *et al.*, 2011).

Chickpea (*Cicer arietinum* L.) is one of the most important pulse crops of both tropical and temperate regions. Chickpea crop can be attacked by more than 50 pathogens (Nene *et al.*, 1981). Among the various diseases, wilt (*F. oxysporum* f. sp. *ciceri*) appear every year in Vidarbha region of Maharashtra and has become limiting factor for chickpea cultivation in many states of the country causing 10 per cent in yield annually (Dubey *et al.*, 2007). However, mortality losses due to this fungi vary from 10-100 percent (Rajput *et al.*, 2010). It is an important fungal pathogen in chickpea growing areas of India (Andrabi *et al.*, 2011), which is seed-borne (Pande *et al.*, 2007) as well as soil-borne (Jiménez - Fernández *et al.*, 2011).

Use of biocontrol agents in combination with reduced doses of chemical fungicide has recently been emphasized for sustainable agriculture (Andrabi *et al.*, 2011).

Cultural methods of disease management are not sufficiently effective against the pathogens like *Fusarium* spp. having prolonged saprophytic survival ability. The use of *Trichoderma* has attracted attention because of its efficacy against wide range of plant pathogens and for its growth promoting action (Harman *et al.*, 2004). The *Trichoderma* species have been reported quite effective against chickpea wilt under glasshouse and field conditions (Dubey *et al.*, 2007; Kaur and Mukhopadhyay, 1992). Selected isolates of *P. fluorescens* have also been found suppressive against chickpea wilt (Kapali *et al.*, 2016; Liu *et al.*, 2007).

In India, there are large number of temples where mainly, *Marigold* sp. (*Tagetes erecta* L.), *Gaillardia* sp. (*Gaillardia pulchella*), *Chrysanthemum* sp. (*Chrysanthemum indicum*) and *Calotropis* sp. (*Calotropis gigantea*) etc. are being used for garland, bouquets or in loose for worshipping gods and also as ornamentals in various functions and celebrations. Disposition of such used flowers poses a problem. Many of these flowers possess antimicrobial activity. The present investigation is an attempt to explore the possibilities for utilizing such huge biological waste along with biocontrol agents and fungicides for management of wilt of chickpea.

MATERIALS AND METHODS

An experiment was conducted to check the efficacy of different floral solvent extracts, biocontrol agents and fungicides against *F. oxysporum* f. sp. *ciceri* during years 2011-15. Flowers viz., *Marigold* sp., *Chrysanthemum* sp.

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and *Gaillardia* sp. were collected from Lord Ganeshaji and Lord Datta temples whereas *Calotropis* sp. flowers were collected from Lord Shani temple from Akola city of Maharashtra. Biocontrol agent's viz., *T. harzianum*, *P. fluorescens* and *Bacillus subtilis* were obtained from Department of Plant Pathology, Dr. P.D.K.V., Akola. The chickpea genotype JG-62 was used as a susceptible host for *F. oxysporum* f. sp. *ciceri* throughout the experiment. Three fungicides viz., thiram 0.2%, metalaxyl 0.2% and carbendazim 0.1% were used for management studies.

Preparation of mass inoculum of *F. oxysporum* f. sp. *ciceri*

The sand sorghum medium (SSM) was used for the mass multiplication of *F. oxysporum* f. sp. *ciceri*. The sorghum grains were soaked in water for overnight; air-dried on blotter paper, calcium carbonate @ 25 g/kg was added to grains to avoid clumping. About 300 g moistened grains and 150 g dry sand were filled in a 1000 ml flask and autoclaved twice for 30 minutes at 1.04 kg/cm² pressure with a 24 hours gap. The grains were inoculated with the pure culture of *F. oxysporum* f. sp. *ciceri* under aseptic conditions; incubated at 28±2°C for two weeks; shaken on alternate days to break clumps and facilitate uniform fungus colonization on grains.

Pathogenicity of *F. oxysporum* f. sp. *ciceri*

Plastic pots (2 kg capacity) were filled with sterilized soil and inoculated with *F. oxysporum* f. sp. *ciceri* inoculum multiplied on sand sorghum medium (SSM) was added to each pot @ 100 g/kg soil; thoroughly mixed with upper 15 cm soil layer (Soil inoculation technique), for this experiment three replicates were maintained. Three pots with sterilized soil without inoculum were used as control. The pots were watered lightly (In each pot, daily 100 ml of sterilized distilled water was poured) and incubated for 4 days. Ten surface sterilized chickpea seeds of JG-62 were sown in each pot; pots kept open and seedling mortality was recorded. The wilting started after 10 days after seed sowing.

Preparation of floral solvent extracts

All floral parts of *Marigold* sp. (*Tagetes erecta* L.), *Gaillardia* sp. (*Gaillardia pulchella*), *Chrysanthemum* sp. (*Chrysanthemum indicum*) and *Calotropis* sp. (*Calotropis gigantea*) were thoroughly rinsed with tap water to remove impurities; dried separately under the shade with occasional up and down mixing for 4 weeks. The dried flowers were powdered with the grinder and stored in airtight container until further use (Thenmozhi *et al.*, 2011). Methanol, acetone, dichloromethane and sterilized distilled water were used as solvent for preparing flower extracts. Forty gram powder of each flower was soaked in 200 ml of each solvent in 500 ml conical flask cotton-plugged tightly and wrapped with paper; kept on the rotary shaker at 150 rpm and at 30°C temperature for four days and then allowed to stand for 5 hr to settle the flower debris. Supernatant from each flask was filtered

separately through Whatman No. 1 filter paper and evaporated at room temperature (30-35°C) up to complete removal of the solvent. Residual flowers were re-extracted thrice to harvest maximum metabolites. Concentrated air dried extracts were weighed separately and transferred into small vials and kept in the refrigerator at 5°C for further use. The resultant crude extracts were used at four different concentrations for growth inhibition assay against *F. oxysporum* f. sp. *ciceri*.

***In vitro* evaluation of floral extracts by liquid bioassay method**

The efficacy of methanol, acetone, dichloromethane and distilled water extracts of *Marigold* sp. (*Tagetes erecta* L.), *Gaillardia* sp. (*Gaillardia pulchella*), *Chrysanthemum* sp. (*Chrysanthemum indicum*) and *Calotropis* sp. (*Calotropis gigantea*) flowers were tested against *F. oxysporum* f. sp. *ciceri* under *in vitro* condition following liquid bioassay technique on potato dextrose broth (PDB) medium.

One gram residue of respective plant extracts was diluted with 10 ml DMSO and from each 250, 500, 750 and 1000 µl extract was mixed with 50 ml sterilized PDB in 250 ml conical flasks. For respective controls, only 250, 500, 750 and 1000 µl DMSO was used. Three replicates for each treatment were prepared. All conical flasks were inoculated individually with 5 mm diameter discs of the test fungal culture of *F. oxysporum* f. sp. *ciceri* and then incubated at 28±2°C for 7 days. Mycelial mat from each treatment was collected on; dried to a constant dry weight at 70°C for 6 hr and respective net dry mycelial weights recorded and used to determine the growth inhibition. The percent growth inhibition (I) of test fungus was calculated by using formula suggested by Vincent (1947).

***In vitro* evaluation of floral water extracts by filter paper disc method**

The efficacy of distilled water extracts of crushed flowers of *Marigold* sp. (*Tagetes erecta* L.), *Gaillardia* sp. (*Gaillardia pulchella*), *Chrysanthemum* sp. (*Chrysanthemum indicum*) and *Calotropis* sp. (*Calotropis gigantea*) flowers at concentration 25, 50, 75 and 100 mg crude extract/ml of DMSO were tested against *F. oxysporum* f. sp. *ciceri* under *in vitro* condition by filter paper disc method (Zape *et al.*, 2009). Culture suspension of each test fungus was prepared separately by adding 10 ml distilled sterilized water in pure culture plates of respective test fungus, the diameter of colony of fungus was 9 cm. One ml culture suspension of test fungus was uniformly spread on solidified PDA in petriplates. The filter paper disc (5 mm dia.; Whatman No. 1) impregnated with the floral water extracts at 25, 50, 75 and 100 mg/ml concentrations were placed on the petriplates colonized by fungal pathogen. The plates were incubated at 28±2°C and the inhibition zones formed due to respective extracts were observed every 24 hours interval up to 72 hours.

***In vitro* evaluation of bioagents by dual culture assay**

Antagonistic activity of *P. fluorescens*, *B. subtilis* and *T. harzianum* on the growth of *F. oxysporum* f. sp. *ciceri* was studied by dual culture technique on PDA in petriplates. The PDA discs of 5 mm diameter were taken from 3 days old culture of *F. oxysporum* f. sp. *ciceri*. The discs were placed at center of respective PDA plates. Then bacterial antagonists were streaked parallel on both sides of fungal pathogens leaving a gap of 3 cm distance between them. In case of the fungal antagonist, each plate was inoculated with 5 mm mycelial disc of fungal pathogens and *T. harzianum* were placed at side by side on medium in each plate approximately at a distance of 4 cm away from each other. Similarly, one set of each fungus without any biocontrol agent culture served as control.

The inoculated plates were incubated at $28 \pm 2^\circ\text{C}$ for 7 days. Colony diameter of test fungus in each plate was measured and mean diameter and percent inhibition was calculated by using formula suggested by Vincent (1947).

***In vitro* evaluation of fungicides by liquid bioassay technique on PDB medium**

The efficacy of three fungicides viz., thiram (0.2%), carbendazim (0.1%) and metalaxyl (0.2%) were evaluated *in vitro* against *F. oxysporum* f. sp. *ciceri* by liquid bioassay technique on PDB medium. Required quantity of respective fungicidal formulations as per their concentrations were added in 250 ml conical flasks containing 50 ml sterilized potato dextrose broth (PDB) separately to make a desired (0.2, 0.1 and 0.2% respectively) concentration of each fungicide. The efficacies of fungicides were evaluated by following the same procedure as described for floral extracts by Vincent (1947).

***In vitro* evaluation of fungicides, bioagents and floral extracts by paper towel method**

This method was used to know the effect of seed treatments with *Marigold* sp. water extract, *P. fluorescens*, *T. harzianum* and fungicides viz., carbendazim, thiram and metalaxyl on growth promoting activity in chickpea. Randomly selected 100 seeds treated with *Marigold* sp. flower extract, bioagents and fungicides were placed on two layers of moist germination paper and rolled carefully to avoid any excess pressure on seeds. These towels were incubated in seed germinator at $20 \pm 2^\circ\text{C}$ for 10 days. Similarly, control set was maintained by using non-treated seeds of chickpea. Three replications were maintained for each treatment separately. The first count was taken on 10th day. All morphologically normal seedlings were counted and germination was expressed in percentage. The seedling vigour, was recorded by taking ten normal seedlings randomly from the germination test. The root length was recorded at collar region up to tip of the primary root. The mean root length was recorded in cm. The same seedlings were used for the measurement of shoot length. The measurement of shoot length was taken

from collar region to the point of junction of cotyledons. The mean shoot length was recorded in cm. Vigour index was calculated by the following formula, given by Abdul Baki and Anderson (1973).

Greenhouse experiment

An experiment was conducted to determine the individual and combined effect of *P. fluorescens*, *T. harzianum* and distilled water extract of marigold flower on *Fusarium* wilt of chickpea during the November 2013 to March 2014. The talc-based formulations of *T. harzianum* and *P. fluorescens* were prepared by following the method described by Papavizas *et al.* (1984) and Vidhyasekaran and Muthamilan (1995). Seeds of JG 62 were surface disinfected in 2% sodium hypochlorite for 30 seconds washed with distilled water and dried for overnight. Ten seeds were planted per pot filled with sterilized potting soil (1.5 kg) (Latha *et al.*, 2009). The inoculum of fungal pathogens multiplied on sand: sorghum medium was incorporated into the separate pots at 1:20 (w/w) ratio of pathogen and soil. In every treatment, the talc-based formulations of *T. harzianum* and *P. fluorescens* were applied as a seed treatment at 4 and 10 g/kg of seed, respectively. For *Marigold* sp. (*Tagetes erecta* L.) floral water extract treatment, seeds were soaked in 2, 3 and 4% solutions separately for 3 hr and air dried overnight before sowing. The fungicides metalaxyl (0.2%), carbendazim (0.1%) and thiram (0.2%) were also used for comparison. The control sets with and without the pathogen were also maintained. Three replications were maintained for each treatment in a completely randomized design in a glass house. The incidence of wilt in chickpea was recorded at 30 and 60 days after sowing.

Three fungicides used under *in vitro* were tested alone for comparing results. This experiment included the following seventeen treatments: *P. fluorescens* alone (10 g/kg seeds of chickpea), *T. harzianum* alone (4 g/kg seeds of chickpea), *Marigold* sp. water extract alone @ 2%, *Marigold* sp. water extract alone @ 3%, *Marigold* sp. water extract alone @ 4%, *P. fluorescens* (10 g/kg seeds of chickpea) + *T. harzianum* (4 g/kg seeds of chickpea), *P. fluorescens* (10 g/kg seeds of chickpea) + *Marigold* sp. water extract 2%, *P. fluorescens* (10 g/kg seeds of chickpea) + *Marigold* sp. water extract 3%, *P. fluorescens* (10 g/kg seeds of chickpea) + *Marigold* sp. water extract 4%, *T. harzianum* (4 g/kg seeds of chickpea) + *Marigold* sp. water extract 2%, *T. harzianum* (4 g/kg seeds of chickpea) + *Marigold* sp. water extract 3%, *T. harzianum* (4 g/kg seeds of chickpea) + *Marigold* sp. water extract 4%, *P. fluorescens* (10 g/kg seeds of chickpea) + *T. harzianum* (4 g/kg seeds of chickpea) + *Marigold* sp. water extract 4%, metalaxyl (0.2%), thiram (0.2%), carbendazim (0.1%) and control (*F. oxysporum* f. sp. *ciceri* inoculated).

Experimental design and statistical analysis

An experiment of *in vitro* effect of floral extracts on test pathogen was carried out by using completely

randomized block design (FRBD) with three factors having four levels in each factor. Greenhouse experiment was carried out by using completely randomized design and each treatment had three replications. 'F' test of significance was used to identify significant treatments. The standard error (SE) and critical difference (CD) at 1% level of probability were calculated.

RESULTS AND DISCUSSION

The pathogenicity of *F. oxysporum* f. sp. *ciceri* was tested on susceptible variety JG-62 of chickpea by soil inoculation technique. The initiation of symptoms was observed after 10 days of inoculation of *F. oxysporum* f. sp. *ciceri*. The infected leaves of plants were brown in colour. Dropping of leaves and seedling collapsing were noticed after 10 days of inoculation. Internal tissues became dark and discoloured. When the plant was uprooted and cut across the collar region, the central tissues were found to be discoloured, brown to dark

brown due to vascular infection of the pathogen. Merkuz and Getachew (2012) observed symptoms in chickpea cultivar JG-62. Leaves of wilted plants were mildly chlorotic and later turned brown (Woltz and Jones, 1981).

In vitro evaluation of floral extracts against *F. oxysporum* f. sp. *ciceri*

In vitro evaluation of floral extracts revealed that all tested treatments of floral extracts had the considerable inhibitory effect on the biomass production of *F. oxysporum* f. sp. *ciceri*. Data shown in Table 1 have reveals that at the highest concentration (1000 µl), complete inhibition of dry mycelial weight of test fungus was recorded in S4F1 and S1F1. Lowest inhibition 42.43% was reported in interaction S2F2. Such results indicate that these extracts may have fungicidal properties that can be used as seed treatments for controlling *F. oxysporum* f. sp. *ciceri*. Gopalakrishnan *et al.* (2010) reported that organic fractions of parthenium

Table 1. Effect of interaction mean of solvents x flowers x concentrations (S x F x C) on dry mycelial weight of *Fusarium oxysporum* f. sp. *ciceri*

S x F x C Solvent x Flower x Conc.)	% inhibition over control			
	C1	C2	C3	C4
S 1 F 1	19.83 (26.43)*	35.21 (36.40)	58.84 (50.09)	100.00 (90.00)
S 1 F 2	10.91 (19.21)	31.32 (34.03)	64.91 (53.68)	73.53 (59.05)
S 1 F 3	14.85 (22.65)	28.78 (32.43)	64.51 (53.45)	73.66 (59.13)
S 1 F 4	18.14 (25.19)	38.90 (38.58)	52.57 (46.48)	74.11 (59.44)
S 2 F 1	15.80 (23.39)	33.66 (35.45)	53.46 (46.99)	75.24 (60.23)
S 2 F 2	1.30 (6.42)	19.75 (26.36)	26.57 (31.00)	42.43 (40.43)
S 2 F 3	7.97 (16.30)	31.02 (33.83)	49.37 (44.64)	69.32 (56.38)
S 2 F 4	8.57 (16.85)	27.53 (31.61)	47.85 (43.77)	54.79 (47.76)
S 3 F 1	26.80 (31.15)	53.32 (46.90)	65.39 (53.98)	82.51 (65.34)
S 3 F 2	2.44 (8.32)	18.70 (25.60)	39.89 (39.15)	51.89 (46.08)
S 3 F 3	2.34 (8.80)	19.45 (26.01)	46.71 (43.11)	61.72 (51.80)
S 3 F 4	27.87 (30.80)	34.56 (36.00)	52.61 (46.50)	60.60 (51.13)
S 4 F 1	30.29 (33.36)	57.71 (49.46)	94.45 (76.41)	100.00 (90.00)
S 4 F 2	14.65 (22.21)	40.85 (39.72)	65.57 (54.08)	81.77 (64.75)
S 4 F 3	23.42 (28.92)	50.32 (45.19)	60.55 (51.10)	74.56 (59.79)
S 4 F 4	26.46 (30.92)	47.08 (43.32)	71.52 (57.78)	86.70 (68.71)
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Source	S.E (M)±			C.D. at (p=0.01)
Solvent (S)	0.39			1.45
Flowers (F)	0.39			1.45
Concentrations (C)	0.39			1.45
Solvent x Flowers (S x F)	0.79			2.91
Solvent x Concentrations (S x C)	0.79			2.91
Flowers x Concentrations (F x C)	0.79			2.91
Solvent x Flowers x Concentrations (S x F x C)	1.57			5.82

*Figures in parenthesis are arc sin transformed values
Average of three replications

Solvents (S)	Flowers (F)	Concentrations (C)
S1- Methanol	F1- Marigold flower extract	C1- 250 µl
S2- Acetone	F2- <i>Gaillardia</i> sp. flower extract	C2- 500 µl
S3- Dichloromethane	F3- <i>Chrysanthemum</i> sp. flower extract	C3- 750 µl
S4- Distilled water	F4- <i>Calotropis</i> sp. flower extract	C4- 1000 µl

at 0.5% concentration inhibited the biomass production of *F. oxysporum* by 91% compared to control. Satish *et al.* (2009) evaluated 46 plants including *Calotropis gigantea* for their antifungal activity against eight *Fusarium* spp. and observed that 12 plants had significant antifungal activity.

In vitro evaluation of the effect of floral water extracts against *F. oxysporum* f. sp. *ciceri*

Among the water extract treatments a maximum of 18.3 mm inhibition zone was observed with *Marigold* sp. (*Tagetes erecta* L.) flower extract at 100 mg/ml, followed by 16.0 mm in *Marigold* sp. (*Tagetes erecta* L.) flower extract at 75 mg/ml (Table 2). Lowest 2.5 mm inhibition zone was recorded due to *Gaillardia* sp. (*Gaillardia pulchella*) flower extract. Similar results were observed by Zape *et al.* (2009) in which they tested alcoholic extract of different medicinal plant parts by disc diffusion method against *F. oxysporum* f. sp. *ciceri* at 250, 500 and 1000 µg/ml concentration and observed maximum fungitoxicity in bawchi, ashwagandha and kali haldi, while vatrak, jangli haldi, shatavari, vanjeera and kali musli did not show fungitoxic effect.

Evaluation of biocontrol agents against *F. oxysporum* f. sp. *ciceri* by dual culture technique

All the biocontrol agents gave the significant reduction of the pathogen *in vitro* compared to the control (Table 3). *P. fluorescens* recorded the highest (81.59%) reduction of the radial growth of *F. oxysporum* f. sp. *ciceri*,

Table 2. Effect of different floral extracts using water as solvent on growth of *F. oxysporum* f. sp. *ciceri* by filter paper disc method

Extract	Concentrations (mg/ ml)	Zone of inhibition (mm) <i>F. oxysporum</i> f. sp. <i>ciceri</i>
Marigold flower extract	25*	5.3
	50	8.7
	75	16.0
	100	18.3
<i>Gaillardia</i> flower extract	25	2.5
	50	6.5
	75	10.8
	100	13.3
<i>Chrysanthemum</i> flower extract	25	3.7
	50	7.7
	75	9.5
	100	11.7
<i>Calotropis</i> flower extract	25	4.3
	50	7.7
	75	11.7
	100	14.0
S.E (M)±		0.70
C.D. at (p=0.01)		2.74

*Average of three replications

Table 3. Efficacy of bioagents on mycelial growth of *Fusarium oxysporum* f. sp. *ciceri*

Tr. No.	Treatment	Radial mycelial growth (mm) <i>F. o. ciceri</i>	Per cent inhibition <i>F. o. ciceri</i>
1	<i>T. harzianum</i>	20.43	77.30 (61.58)
2	<i>P. fluorescens</i>	16.57	81.59 (64.68)
3	<i>B. subtilis</i>	22.86	74.60 (59.80)
4	Control	90.00	0.00(0.00)
S.E (M)±		0.72	0.57
C.D. at (p=0.01)		2.83	2.21

*Figures in parenthesis are arc sin transformed values
Average of five replications

whereas, the lowest inhibition was observed due to *Bacillus subtilis* (74.60%). Hesamedin (2009) evaluated different bioagents against *F. oxysporum* f. sp. *ciceri* and reported that all the bioagents were effective in reducing mycelial growth of the fungus but maximum inhibition was done by in *T. harzianum*. Khan *et al.* (2012) found maximum inhibition (83.33) of *F. oxysporum* f. sp. *ciceri* by *T. harzianum* and 76.66% inhibition by *B. subtilis*.

In vitro evaluation of fungicides against *F. oxysporum* f. sp. *ciceri* by liquid bioassay method

Carbendazim (0.2%) and thiram (0.2%) showed efficiency to inhibit radial growth of the *F. oxysporum* f. sp. *ciceri* compared to the control (Table 4). However, carbendazim (0.2%) was most effective fungicides, to give cent percent inhibition in radial growth of the fungus. Gupta *et al.* (1997) screened six fungicides against *F. oxysporum* f. sp. *ciceri* and reported carbendazim at 100 mg/ml as most effective to inhibit the growth of fungus *in vitro*.

In vitro evaluation of *Marigold* sp. (*Tagetes erecta* L.) water extracts, *P. fluorescence* and *T. harzianum* alone and in combination on seedling growth parameters of chickpea seedling (JG-62 cultivar) by paper towel method

Effect of seed treatment with *Marigold* sp. (*Tagetes erecta* L.) water extract and bioagents alone and in combination

Table 4. Efficacy of fungicides on dry mycelial weight of *F. oxysporum* f. sp. *ciceri*

Tr. No.	Treatment	Dry mycelial weight (mg) <i>F.o. ciceri</i>	Per cent inhibition <i>F.o. ciceri</i>
1	Carbendazim (0.1%)	0.00	100.00 (90.00)
2	Metalaxyl (0.2%)	590.86	12.61 (20.79)
3	Thiram (0.2%)	134.14	80.16 (63.55)
4	Control	676.14	0.00 (0.00)
S.E (M)±		1.39	0.16
C.D. at (p=0.01)		5.42	0.62

*Figures in parenthesis are arc sin transformed values
Average of five replications

on growth parameters of chickpea variety JG-62 was evaluated by paper towel method. Three seed dressing fungicides were also tested for comparing results of Marigold water extract and bioagents.

Observations on percent germination, mean shoot length, mean root length and seedling vigour index were recorded and presented in table 5. In case of variety JG-62, highest 93.5% germination, maximum 9.9 cm mean shoot length, maximum 21.6 cm mean root length and maximum seedling vigour index (3008.25) were recorded in T13 (*P. fluorescens* 10 g/kg seeds of chickpea + *T. harzianum* 4 g/kg seeds of chickpea + Marigold water extract 4%). Rajput *et al.* (2010) recorded an increase in seedling vigour index of chickpea seedlings with the application of *Trichoderma* sp. and *P. fluorescens*. Karimi *et al.* (2012) also reported that bacterial antagonists were significantly effective in increasing plant growth compared to control. The growth promotion effect of *P. fluorescens* may be due to the activity of solubilization of phosphorus present in the non-soluble form in soil (Dube and Yeole, 1997) or production of auxins, gibberellins etc. (Glick, 1995). *T. Harzianum* solubilizes phosphorus (Sharma, 2003) and stimulates plant growth even in the absence of the pathogen (Lifshitz *et al.*, 1986).

The current findings suggest that combined application of *P. fluorescens*, *T. harzianum* and Marigold water extract can be used as seed treatments for the control of *F. oxysporum* f. sp. *ciceri* in chickpea seeds and for improving chickpea seedling growth and are safe and eco-friendly on chickpea compared to synthetic chemicals. Results of the present study are in

confirmation with the results of Mavi (2014) who reported that Hydropriming and compared control seeds with organic priming (*Tagetes patula* and *Tagetes erecta*) for seed vigor index. The application of organic priming (*T. patula* and *T. erecta*) recorded the difference in germination (82 and 80%), and also in emergence (73 and 76%) when compared control with Hydropriming.

This is the first report which results showed that marigold species can be used as a potential organic priming agent. Increase in root and shoot lengths of chickpea was also reported by Nagamani *et al.* (2011) with the application of carbendazim along with *T. viride*.

Evaluation of water extract of *Marigold* sp. (*Tagetes erecta* L.) flowers, bioagents and fungicides against *F. oxysporum* f. sp. *ciceri* under greenhouse conditions

Effect of different treatments on percent seed germination: Results in Table 6 revealed that treatment T13 (*P. fluorescens* 10 g/kg seeds of chickpea + *T. harzianum* 4 g/kg seed + Marigold sp. (*Tagetes erecta* L.) water extract 4%) showed maximum (94.3%) seed germination and was significantly superior over all the other treatments. Lowest percent germination (68.6%) was reported in T17 (control).

Effect of different treatments on wilt (%) incidence at 30 and 60 DAS: Lower wilt incidence (10.6%) at 30 DAS was recorded with T16 (Carbendazim @ 0.1%), followed by 14.3% in T13 (*P. fluorescens* 10 g/kg seeds of chickpea + *T. harzianum* 4 g/kg seeds of chickpea + Marigold sp. (*Tagetes erecta* L.) water extract (4%).

Table 5. Effect of Marigold water extract, *P. fluorescens* and *T. harzianum* alone and in combination on seedling growth parameters of chickpea Variety JG-62 by paper towel method

Tr. No.	Treatment	JG 62			
		% Germination	Mean shoot length (cm)	Mean root length (cm)	SVI
T1	<i>P. fluorescens</i> alone (10 g/kg)	84.50	7.00	10.30	1461.85
T2	<i>T. harzianum</i> alone (4 g/kg)	87.00	7.30	12.10	1687.80
T3	Marigold water extract alone @ 2%	74.50	5.30	8.60	1035.55
T4	Marigold water extract alone @ 3%	77.33	5.60	9.80	1190.88
T5	Marigold water extract alone @ 4%	81.67	6.70	11.10	1453.73
T6	<i>P. fluorescens</i> (10 g/kg) + <i>T. harzianum</i> (4 g/kg)	91.50	7.70	12.20	1820.85
T7	<i>P. fluorescens</i> (10 g/kg) + Marigold water extract 2%	86.00	8.00	10.00	1548.00
T8	<i>P. fluorescens</i> (10 g/kg) + Marigold water extract 3%	88.50	8.30	10.40	1654.95
T9	<i>P. fluorescens</i> (10 g/kg) + Marigold water extract 4%	89.67	9.00	12.20	1901.00
T10	<i>T. harzianum</i> (4 g/kg) + Marigold water extract 2%	90.00	8.40	13.70	1989.00
T11	<i>T. harzianum</i> (4 g/kg) + Marigold water extract 3%	91.50	8.90	17.30	2397.30
T12	<i>T. harzianum</i> (4 g/kg) + Marigold water extract 4%	94.00	9.20	18.10	2566.20
T13	<i>P. fluorescens</i> (10 g/kg) + <i>T. harzianum</i> (4 g/kg) + Marigold water extract 4%	95.50	9.90	21.60	3008.25
T14	Metalaxyl (0.2%)	74.50	7.00	9.00	1192.00
T15	Thiram (0.2%)	85.33	5.10	13.20	1561.54
T16	Carbendazim (0.1%)	79.33	6.80	15.40	1761.13
T17	Control	69.33	3.10	4.70	540.77

(SVI= Seedling vigour index)

Maximum wilt incidence (59.6%) at 30 DAS was observed in control treatment, followed by 46.3% in T14 (Metalaxyl @ 0.2%) (Table 6). Treatment T16 (Carbendazim @ 0.1%) exhibited minimum wilt incidence (24.3%) at 60 DAS, followed by 26.0 and 28.3% wilt in T15 (Thiram @ 0.2%) and T13 (*P. fluorescens* 10 g/kg seeds of chickpea + *T. harzianum* 4 g/kg seeds of chickpea + *Marigold* sp. (*Tagetes erecta* L.) water extract 4%), respectively (Table 6). Maximum wilt incidence (92.33%) was recorded in T17 (control), followed by 71.3% in T14 (Metalaxyl @ 0.2%). Khan *et al.* (2004) evaluated efficacy of bioagents and fungicides against *F. oxysporum* f. sp. *ciceri* under field condition and observed 12.5% wilt incidence in combined seed treatment of *T. harzianum* and *P. fluorescens*, 24.9% in *T. harzianum*, 27.1% in *P. fluorescens* and 36.4 % in carbendazim as compared to 61.8% in control.

Effect of different treatments on disease reduction (%) i.e. effect on wilt (%): Maximum reduction (73.6%) in chickpea wilt caused by *F. oxysporum* f. sp. *ciceri* was observed in T16 (Carbendazim @ 0.1%). De *et al.* (1996) Coated chickpea seeds with carbendazim @ 0.2% and found effective in reducing wilt, followed by 71.8 and 69.3% inhibition in T15 (Thiram @ 0.2%) and T13 (*P. fluorescens* 10 g/kg seeds of chickpea + *T. harzianum* 4 g/kg seeds of chickpea + *Marigold* sp. (*Tagetes erecta* L.) water extract 4%), respectively. Lowest reduction in disease incidence (22.7%) was reported in T14 (Metalaxyl @ 0.2%).

The present study showed that the tested plant extracts suppressed the growth of *F. oxysporum* f. sp. *ciceri* at varying levels. The distilled water extracts inhibited the growth of the pathogen more than the other solvents. The *Marigold* sp. (*Tagetes erecta* L.) floral extracts was found effective in reducing the radial growth of the pathogen as against other extracts. Fungitoxic effects have been recorded earlier by the higher plants against mycelial growth of different phytopathogenic fungi (Obboh *et al.*, 2007). *T. harzianum* was earlier also proved as a potential biocontrol agent of soil-borne plant pathogens (Dubey, 2003). However, *P. fluorescens* and *T. harzianum* alone or in combination with *Marigold* sp. (*Tagetes erecta* L.) flower water extract was found highly effective in seed treatment against the wilt disease in chickpea.

The fungitoxic effect in flowers could be due to the antifungal compounds present in fresh marigold tissues (Baker, 1981). Similar findings correlate with the findings of Martínez *et al.* (2014) who reported that marigold is effective against the *F. oxysporum*, because it restricts the disease symptoms 88.5% caused by the pathogen. The compounds *viz.*, sesquiterpenes, saponins and flavonoids have certain anti-fungal properties, which is cent percent in marigold. Martinez (2012) reported that flavonoids are possessing antifungal activity against pathogens such as *Penicillium* sp. and *Rhizopus* sp. Phenolic compounds are active against the pathogen cell membranes, resulting in leakage of cytoplasmic

Table 6. Effect of *Pseudomonas fluorescens*, *Trichoderma harzianum* and Marigold water extract alone and in combination on wilt of chickpea caused by *F. oxysporum* f. sp. *ciceri*

Tr. No.	Treatment	Germination (%)	Wilt (%)		% Disease control
			30 DAS	60 DAS	
T1	<i>P. fluorescens</i> alone (10 g/kg)	83.33 (65.91)*	24.00 (33.42)*	40.00 (43.66)*	56.70
T2	<i>T. harzianum</i> alone (4 g/kg)	86.67 (68.58)	30.33 (29.33)	47.67 (39.23)	48.38
T3	Marigold water extract alone @ 2%	73.67 (59.13)	41.33 (40.01)	58.00 (49.60)	37.18
T4	Marigold water extract alone @3%	76.33 (60.89)	39.33 (38.84)	56.33 (48.64)	38.99
T5	Marigold water extract alone @4%	81.00 (64.16)	35.67 (36.67)	53.00 (46.72)	42.60
T6	<i>P. fluorescens</i> (10 g/kg) + <i>T. harzianum</i> (4 g/kg)	90.67 (72.21)	22.00 (27.97)	32.00 (34.45)	65.33
T7	<i>P. fluorescens</i> (10 g/kg) + Marigold water extract 2%	84.33 (66.68)	24.33 (33.21)	36.33 (41.17)	60.65
T8	<i>P. fluorescens</i> (10 g/kg) + Marigold water extract 3%	90.00 (71.57)	23.00 (32.16)	33.67 (40.40)	63.54
T9	<i>P. fluorescens</i> (10 g/kg) + Marigold water extract 4%	89.67 (71.25)	22.00 (31.09)	32.33 (38.25)	64.98
T10	<i>T. harzianum</i> (4 g/kg) + Marigold water extract 2%	88.00 (69.73)	30.00 (29.56)	43.33 (37.07)	53.07
T11	<i>T. harzianum</i> (4 g/kg) + Marigold water extract 3%	90.33 (71.89)	28.33 (28.66)	42.00 (35.47)	54.51
T12	<i>T. harzianum</i> (4 g/kg) + Marigold water extract 4%	92.33 (73.93)	26.67 (27.97)	38.33 (34.65)	58.48
T13	<i>P. fluorescens</i> (10 g/kg) + <i>T. harzianum</i> (4 g/kg) + Marigold water extract 4%	94.33 (76.23)	14.33 (22.25)	28.33 (32.16)	69.31
T14	Metalaxyl (0.2%)	73.67 (59.13)	46.33 (42.90)	71.33 (57.63)	22.74
T15	Thiram (0.2%)	83.67 (66.16)	12.00 (20.27)	26.00 (30.66)	71.86
T16	Carbendazim (0.1%)	78.67 (62.49)	10.67 (19.06)	24.33 (29.56)	73.66
T17	Control (pathogen inoculated)	68.67 (55.96)	59.67 (50.57)	92.33 (73.93)	0.00
S.E (M)±		1.41	0.72	1.06	-
C.D. at (p=0.01)		5.22	2.65	3.90	-

*Figures in parenthesis are arc sin transformed values
Average of three replications

contents. sesquiterpenes contain more than 5000 compounds found primarily in Compositae family (Wedge *et al.*, 1999). Sesquiterpenes play an important role in the defense mechanisms of the plant. It was also found that sesquiterpene lactone inhibits growth of *Colletotrichum*, *Phomopsis*, *Botrytis*, but was found ineffective against *F. oxysporum* isolated from *Leucadendron* sp. Antifungal activity of all the selected floral species was confirmed and the results revealed *Marigold* sp. (*Tagetes erecta* L.) floral extract is the most effective inhibitor for the mycelia growth of the tested pathogen. This could be an important step to use floral parts as biopesticides against *F. oxysporum* f. sp. *ciceri*. However, the study further needed to determine the chemical identity of the compounds which is having antifungal activity.

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