The Pharma Innovation Journal 2020; 9(3): 321-324

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2020; 9(3): 321-324
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www.thepharmajournal.com
Received: 03-01-2020
Accepted: 06-02-2020

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Comparison among botanicals, bio-agents and chemical control against *fusarium oxysporum* f. sp. *ciceris in vitro*

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Abstract
Chickpea (*Cicer arietinum* L.) is one of the most important pulse crops, so far its acreage and production is concern. Chickpea has been well recognized for source of protein, nutritive value where majority of the population of India depend on the low priced food for meeting its dietary requirements. The disease is caused by *Fusarium oxysporum* f. sp *ciceris*. Fungicides used for disease management caused by pathogens are known to pollute the environment, soil and water resulting deleterious effects on human health and biosphere. In present investigation, extracts of five plant species namely, Neem (*Azadirachta indica*), Garlic (*Allium sativum*), Onion (*Allium cepa*), Zinger (*Zingiber officinale*), two bioagents *Trichoderma viride, T. harzianum* along with two fungicides Carbendazim and Thiram were evaluated for fungi toxicity against *Fusarium oxysporum* f. sp. *ciceris*. The results clearly showed that all fungicides were effective against pathogen to inhibit mycelial growth *in vitro* conditions. Plant extracts and bioagents also more or less inhibited mycelial growth of *Fusarium oxysporum* f. sp. *ciceris in vitro* conditions.

Keywords: Botanicals, bioagents, fungicides, *fusarium oxysporum* f. sp. *ciceris*

Introduction
Chickpea (*Cicer arietinum* L.) commonly known as Gram or Bengal gram belong to the family Fabaceae is an important *Rabi* season pulse crop in India. It is the world's third most important pulse crop, after dry beans (*Phaseolus vulgaris* L.) and dry peas i.e. *Pisum sativum* L. (Vishwadhar and Gurha, 1998) [10]. In India, total pulse had the area of 31.11 mha with production of 24.51 mt. and productivity 788 Kg /ha. In India total chickpea had the area of 10.76 mha with production of 11.16 mt. and productivity 1037 Kg /ha (Anonymous, 2018) [1, 2] However, six major states *viz*., Madhya Pradesh, Rajasthan, Maharashtra, Uttar Pradesh, Karnataka and Andhra Pradesh altogether contribute 91 per cent of the production and 90 per cent of the area. In Uttar Pradesh, chickpea is grown an area of 562 thousand ha with production of 626.00 thousand ton and productivity 1114 kg /ha. (Anonymous, 2018) [1, 2].
The total production and productivity per unit area of chickpea is very low. Among the various factors, different pathogens responsible for yield losses of chickpea. Wilt is one of the major diseases of chickpea at national level; the yield losses encountered was reported about 60 per cent (Singh *et al*., 2007) [9]. Wilt disease of chickpea caused by *Fusarium oxysporum* f. sp. *ciceris* is a complex and destructive in nature. The genus *Fusarium* had many soil borne species which were distributed worldwide and known as plant pathogens since a long time ago. In order to overcome to this disease, a laboratory experiment was conducted to know the efficacy of botanicals and bio-agents against *Fusarium oxysporum* f. sp. *ciceris*.

Materials and Methods
The efficacy of various plant extracts against *Fusarium* wilt of chickpea *viz*., Neem leaves extract (*Azadirachta indica)*, Garlic bulb extract (*Allium sativum)*, Tulsi leaves extract (*Ocimum sanctum*), Onion bulb extract (*Allium cepa*), Zinger rhizome extract (*Zingiber officinale*), two botanicals *Trichoderma viride, Trichoderma harzianum* and two fungicides Carbendazim and Thiram were used. Fresh leaves, rhizomes and bulb were collected and washed thoroughly in clean water. 100 gram of each washed plant material was grinded in pestle and mortar by adding equal amount (100 ml) of sterilized water (1 : 1 v/v) and heated at 80ºC for 10 minutes in hot water bath. The materials was filtered through double layered muslin cloth followed by filtering through sterilized Whatman No.1 filter paper and treated as standard plant extract (100%). To study the inhibitory effect of botanicals on mycelial growth

of *Fusarium oxysporum* f. sp. *ciceris*, 5.0 per cent concentration were used by applying food poison technique (*in vitro*). The bio-agents *Trichoderma viride* and *Trichoderma harzianum* were used 0.6 per cent concentration of each bioagents incorporated in 100 ml PDA. Two fungicides Carbendazim and Thiram were used at one concentration of each (0.3 per cent) then incorporated in 100 ml PDA and mixed thoroughly. The flasks were taken to get uniform mix of the plant extract, bioagents and fungicide and poured twenty ml medium into each Petri plate. After solidify and these plates were centrally inoculated with the 5 mm diameter disc of pathogen (cut by sterilized cork borer), taken from the margin of actively growing 7 days old culture. Control was used without adding extract, bioagents and fungicide in the medium. Three replications of each were kept and incubated at 28±2 °C. The efficacy of fungicides was observed by measuring the radial growth of the fungal colony at 4 to 7 days after incubation, by using formula.

Per cent growth inhibition was calculated by using formula.

$$I = \frac{C - T}{C} \times 100$$

Where,
I = Per cent inhibition of fungal growth
C = Radial growth of control Petri plate
T = Radial growth of treated Petri plate

Results and Discussions

The ten treatments (6 botanicals, 2 bioagents, 2 fungicides and 1control) were evaluated during present investigation for their efficacy (*in vitro)* against *Fusarium oxysporum* f. sp. *ciceris* using food poison technique and observation were recorded after 4 and 7 days of incubation at 5 and 10 per cent concentration. After 4 days of incubation, the data presented in Table-1showed that the minimum radial growth was obtained at 10 per cent concentration in garlic bulb extract (4.51mm) followed by Neem leaf extract (7.72mm), Zinger rhizome extract (8.28mm), Onion bulb extract (10.63mm) and Tulsi leaf extract (11.84mm). The maximum radial growth was recorded in control (25mm). The radial growth significantly differed in different treatment with other except Neem and Zinger, Onion and Tulsi which were at par to each other at 10% concentration. The maximum per cent inhibition was recorded in garlic bulb extract (80.61%) followed by Neem leaf extract (68.62%), Zinger rhizome extract (66.54%), Onion bulb extract (56.83%) and Tulsi leaf extract (52.26%) at 10% concentration (Table 1). The same trend was found after 7 days, minimum radial growth was recorded at 10% concentration of Garlic bulb extract (5.17mm) followed by Neem leaf extract (8.44mm), Zinger rhizome extract (16.60mm), Onion bulb extract (19.04mm) and Tulsi leaf extract (18.57mm). The maximum radial growth was recorded in control (45mm). The radial growth significantly differed in different treatment with other except Onion and Tulsi which were at par to each other. Similarly there was no significant difference observed in radial growth of Onion and Tulsi at 10% concentration. The maximum per cent inhibition was recorded in garlic bulb extract (88.66%) followed by Neem leaf extract (81.23%), Zinger rhizome extract (65.20%) Onion bulb extract (58.72%) and Tulsi leaf extract (57.68%) at 10% concentration (Table 2). Efficacy of bio-agents and fungicides against *Fusarium oxysporum* f. sp. *ciceris* (*in vitro*). After 4 and 7 days incubation. The data indicates that the similar trend was found in 4 and 7 days incubation so all the treatments were significantly superior over control. It may be seen from the data (Table 3) that the radial growth was recorded minimum in Carbendazim (4.40 mm) followed by Thiram (6.44 mm), *Trichoderma viride* (10.34 mm) and *Trichoderma harzianum* (17.27 mm). The radial growth in control was 45 mm. The radial growth significantly differed with each other in all the treatment after 7 days of incubation. The maximum per cent inhibition was recorded in Carbendazim (90.72%) followed by Thiram (88.52%), *Trichoderma viride* (78.50%), and *Trichoderma harzianum* (67.40%) at 7 days incubation, respectively which significantly differed each other (Table 3). Present studies are in conformity with the reports of Ogechi *et al*., (2006) [7] gave the effect of crude extracts of Neem leaf (*Azadirachta indica*) and Garlic bulb (*Allium sativum*). All the extracts inhibited mycelial growth at various levels. Mukhtar (2007) [6] worked antifungal effect of aqueous extracts of four plant species *viz.*, *Azadirachta indica*, A. Juss., *Datura metel* L. var. *quinquecuspida* Torr. *Ocimum sanctum* L. and *Parthenium hysterophorus* L against *Fusarium oxysporum* f. sp. *ciceris (in vitro)*. They further reported that plants extracts, *A. indica* and *D. metel* inhibited fungal growth by 80%. Even at 10% concentration. Dubey *et al*., (2006) [3] evaluated *Trichoderma viride* and *Trichoderma harzianum* against *Fusarium oxysporum* f. sp. *ciceris* commonly prevalent in India. *T. viride* inhibited maximum mycelial growth of the pathogen followed by *T. harzianum*. It decreased wilt incidence under greenhouse condition. Ramezani (2010) [8] to test efficacy of two fungal bio agents *viz.*, *Trichoderma harzianum*, *Trichoderma viride*, were evaluated against the chickpea vascular wilt pathogen and he report that *T. viride* produced the maximum inhibition zone of 17.00 mm compared to the minimum of 7.00 mm by *T. harzianum*. Mahmood *et al*., (2015) tested side by side both (*in vitro*) and in glass house against chickpea wilt pathogen. *In vitro* study showed that Carbendazim proved best by checking the mycelial growth with mean of 83.7% inhibition over the control. Jayalakshmi *et al.,* (2009) [4] tested the seed treatment with chemical, namely bavistin (1.0 g/kg seed) + thiram (1.25 g/kg seed), bavistin (2.0 g/kg seed), thiram (2.5 g/kg seed), funginil (4.0 g/kg seed) were superior in increasing seed germination and reducing plant mortality. Seed treatment with thiram + bavistin and bavistin alone was most effective in increasing seed germination and in reducing plant mortality under field conditions.

Table 1: Effect of plant extract against *Fusarium oxysporum* f. sp. *ciceris* on radial growth and growth inhibition at 5 and 10 per cent concentration *in vitro* after 4 days incubation.

Treatments	Concentration (5%)		Concentration (10%)	
	Radial growth (mm)	Growth inhibition (%)	Radial growth (mm)	Growth inhibition (%)
Neem	11.28	56.57 (48.75)	7.72	68.62 (55.91)
Onion	14.35	41.46 (40.06)	10.63	56.83 (48.90)
Garlic	6.56	77.20 (61.46)	4.51	80.61 (63.85)
Zinger	11.65	52.44 (46.38)	8.28	66.54 (54.64)
Tulasi	14.54	39.75 (39.06)	11.84	52.26 (46.27)
Control	25.00	0.00 (0.00)	25.00	0.00 (0.00)
CD at 5%	1.62	4.88	1.92	6.22
CV	6.55	5.69	9.52	6.32

* Figure given in the parenthesis are transformed value

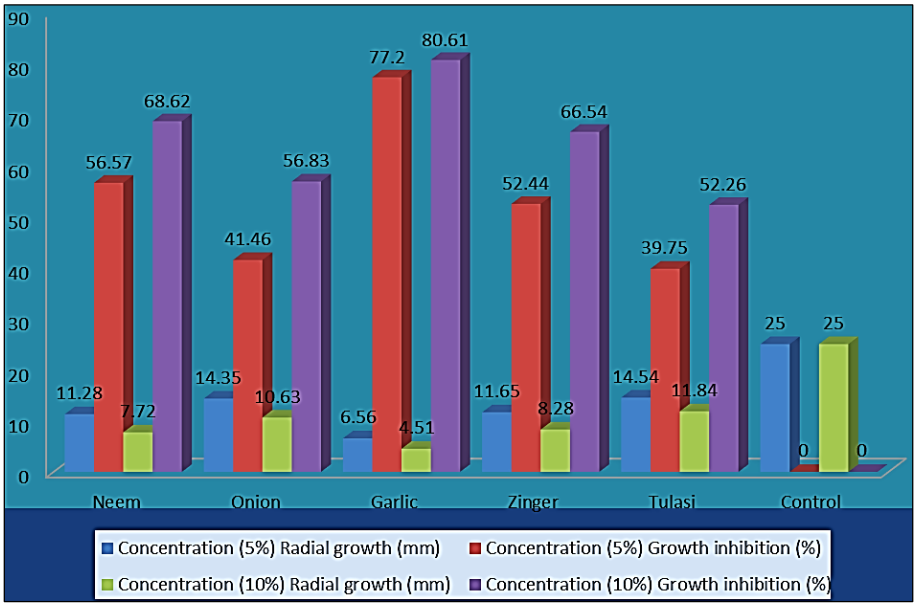

Fig 1: Effect of plant extract against *Fusarium oxysporum* f. sp. *ciceris* on radial growth and growth inhibition at 5 and 10 per cent concentration *in vitro* after 4 days cubation

Table 2: Effect of plant extract against *Fusarium oxysporum* f. sp. *ciceris* on radial growth and growth inhibition at 5 and 10 per cent concentration *in vitro* after 7 days incubation.

Treatments	Concentration (5%)		Concentration (10%)	
	Radial growth (mm)	Growth inhibition (%)	Radial growth (mm)	Growth inhibition (%)
Neem	12.57	70.86 (57.31)	8.44	81.23 (64.31)
Onion	20.61	55.01 (47.85)	18.57	58.72 (50.00)
Garlic	9.17	80.06 (63.46)	5.17	88.66 (70.34)
Zinger	15.98	64.87 (53.63)	16.60	65.20 (53.83)
Tulasi	22.46	50.42 (45.22)	19.04	57.68 (49.39)
Control	45.00	0.00 (0.00)	45.00	0.00 (0.00)
CD at 5%	2.12	5.45	1.62	6.07
CV	5.68	5.60	5.83	5.79

* Figure given in the parenthesis are transformed value

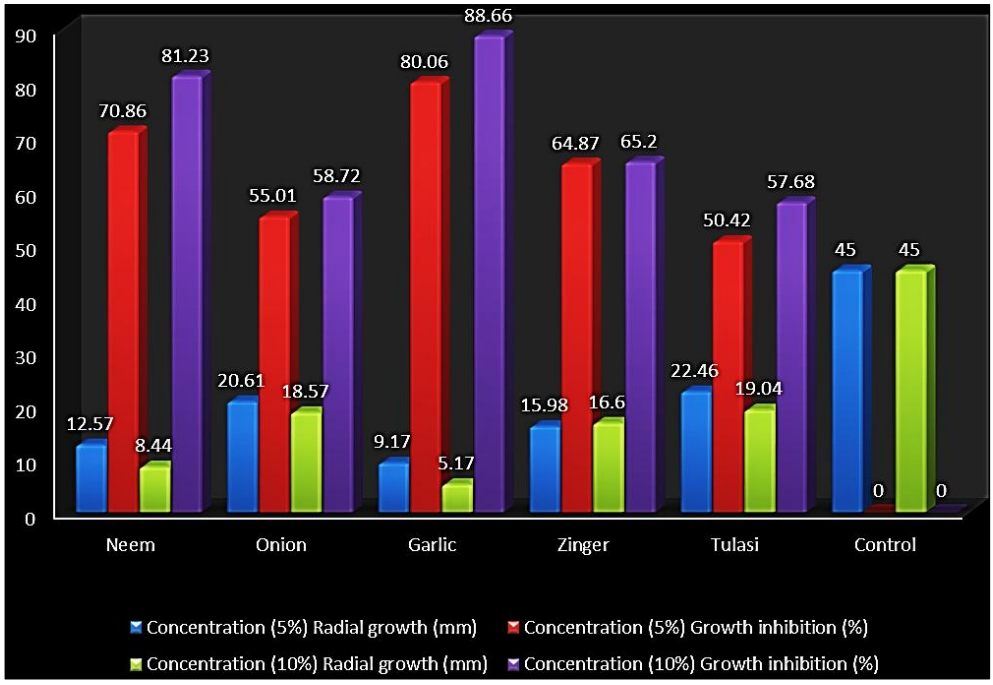

Fig 2: Effect of plant extract against Fusarium oxysporum f. sp. ciceris on radial growth and growth inhibition at 5 and 10 per cent concentration in vitro after 7 days incubation

Table 3: Effect of bioagents (0.6%) and fungicides (0.3%) against *Fusarium oxysporum* f. sp. *ciceris* on radial growth and growth inhibition *in vitro* after 4 and 7 days.

Treatments	4 Days		7 Days	
	Radial growth (mm)	Growth inhibition (%)	Radial growth (mm)	Growth inhibition (%)
T. viride	8.11	67.61 (55.29)	10.34	78.50 (62.35)
T. harzianum	10.87	56.33 (48.62)	17.27	67.40 (55.16)
Carbendazim	2.92	88.29 (69.98)	4.40	90.72 (72.24)
Thirum	3.74	84.31 (66.64)	6.44	88.52 (70.18)
Control	25.0	0.00 (0.00)	45.00	0.00 (0.00)
CD at 5%	1.12	6.25	1.91	7.05
CV	6.08	5.52	6.29	5.76

* Figure given in the parenthesis are transformed value

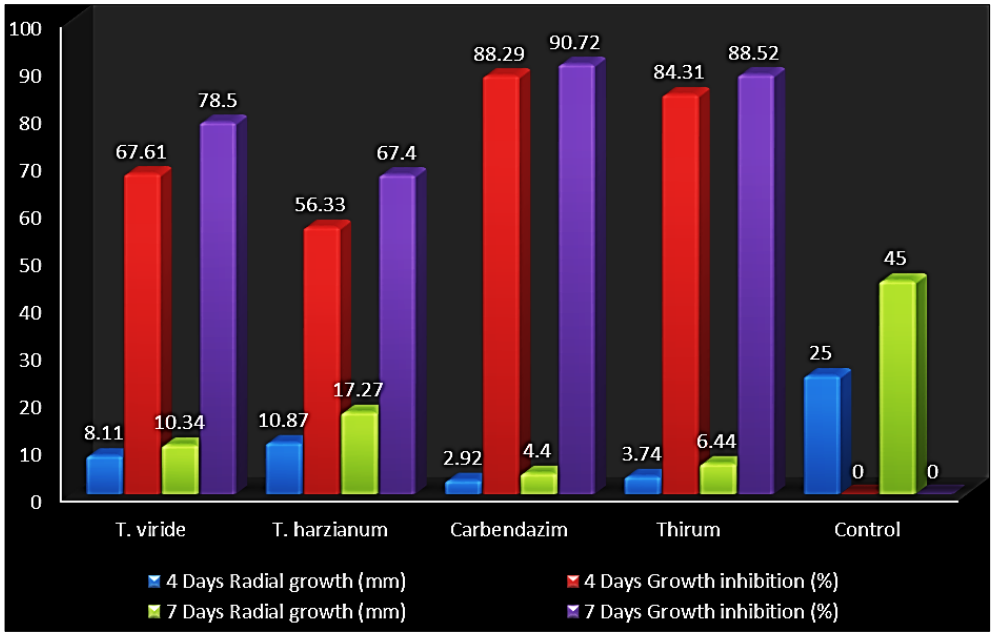

Fig 3: Effect of bioagents (0.6%) and fungicides (0.3%) against Fusarium oxysporum f. sp. ciceris on radial growth and growth inhibition in vitro after 4 and 7 days

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