The Pharma Innovation Journal 2023; 12(6): 2063-2066

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(6): 2063-2066

www.thepharmajournal.com
Received: 08-03-2023
Accepted: 13-04-2023

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Inhibitory effect of fungicides (*in vitro* & *in vivo*) against the pathogen *Fusarium oxysporum* f. sp. *ciceri* causing wilt disease of chickpea

Jaish Raj Yadav, Ramesh Singh, Manish Kumar Maurya and Vikash Kumar Yadav

Abstract
Chickpea (*Cicer arietinum* L.) is one of the important pulse crops, in India. Among fungal diseases, wilt caused by *Fusarium oxysporum* f. sp. *ciceri* Butler has been observed to be predominant and most destructive in major chickpea growing areas. In the present investigation efficacy of ten fungicides were tested *in vitro* against *Fusarium oxysporum* f. sp. *ciceri*. Out of which five fungicides *viz.*, Benlate (88.15%), Companion (85.52%), Bavistin (76.97%), thiram (73.02%), Tilt (65.13%) and Chlorothalonil (59.21%) were found to check the growth of the fungus significantly. Among other fungicides, Topsin-M (49.08%), Vitavax (44.07%), Indofil M-45 (32.24%) and copper oxychloride (17.10%) check the growth of the fungus in descending order of superiority. All the fungicides proved better as compared to control. On the basis of above experiment, all the fungicides found effective *in vivo* test were used for seed treatment against wilt of chickpea in pots culture experiment in glass house compound. Percentage of seed germination was minimum in untreated control and maximum in case of Benlate (96%) treated seed followed by Companion (94%), Bavistin (92%), and Thiram (90%) which were statistically at par to each other. Thiram (90.00%), Topsin-M (90%), Chlorothalonil (90.00%), and Vitavax powder (88%) were statistically at par to each other. Least effective for seed germination was recorded treated with Indofil M-45 (86%), copper oxychloride (86%). On the basis of above experiment percentage of wilted plants minimum in case of Benlate (6.00%), followed by Tilt (12.00%), Companion (14.00%), thiram (16.00%) and Bavistin (18.00%) statistically at par to each other. Vitavax powder (20.00%), indofil M-45 (24.00%), Topsin-M (28.00%), Chlorothalonil (30.00%) and copper oxychloride (36.00%) at par to each other statistically.

Keywords: Chickpea, *Fusarium oxysporum* f. sp. *ciceri*, seed treatment, fungicide

Introduction
Chickpea (*Cicer arietinum* L.) commonly known as Gram or Bengal gram or Egyptian pea belongs to the subfamily, Papilionaceae (family- Leguminosae) with chromosome number, 2n = 2x = 16. It is an important *Rabi* season pulse crop in India. Pulses are basic ingredient in the diet of a vast majority of Indian population as they provide a perfect mixture of high biological value when supplemented with cereals. It has carbohydrates 27.42 gram, protein 8.86 gram and fat 2.59 gram per 100 gram of chickpea. Chickpea is high in protein, low in fat and sodium, cholesterol free and is excellent source of both soluble and insoluble fiber, as well as complex carbohydrates, vitamin and minerals especially calcium, phosphorus, iron and magnesium (Roy *et al*., 2010) [14].

Germinated seeds are recommended to cure scurvy disease in human being. Being a pulse crop, it is a good source of protein constituting about 99 per cent in grains on dry weight basis, which is very cheap and hence referred as “Poor man’s meat” (Muehlbauer and Rajesh, 2008) [10].

Chickpea is the third most important pulse crop, after dry bean and peas, produced in the world. It accounts for 20 per cent of the world pulses production. Major producers of chickpea include India, Pakistan and Mexico. India is the largest producer, with about 8 million tons, accounting of about 69 to 71 per cent of total world production.

The area, production and productivity of chickpea among the major pulses in India is estimated to be 9.93 mha; 9.53 mt. and 960 kg/ha respectively. It is grown in Madhya Pradesh, Rajasthan, Uttar Pradesh, Jharkhand, Maharashtra, Bihar, Punjab, Haryana, Andhra Pradesh and Chhattisgarh. 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.

The area, production and productivity of chickpea in U.P. is estimated to be 6.11 Lakh ha; 6.84 Lakh tons and 1119.48 kg/ha (Anonymous, 2018) [1].

Inspite of the excluding efforts made by different agencies to boost up its production, the total production and productivity per unit area is very less. Among the various factors responsible for yield, one of the major factors is the wilt of chickpea caused by different pathogens. The pathogen of the disease is soil borne and can survive in the soil for at least six years in the absence of host. It is one of the major disease of chickpea at national level; the yield losses encountered was reported about 60 per cent (Singh and Gupta, 2007) [15].

F. oxysporum f. sp. *ciceri* infects chickpea at seedling stage as well as at flowering and pod forming stage with more incidence at flowering and podding stage if the crop is subjected to sudden temperature rise and water stress (Choudhary *et al*., 2007) [3]. Losses of chickpea from Fusarium wilt have been reported to vary from 10 to 15 per cent (Jalali and Chand, 1991) [6] but losses of up to 70 per cent have been reported in some years in Northern India and Pakistan.

Four races (1 to 4) of Fusarium wilt have been identified from India (Haware and Nene, 1982) [5]. Therefore, in view of the seriousness of the disease and importance of the crop, present studies were undertaken with the following objectives:- Inhibitory effect of fungicides (*in vitro* & *in vivo*) against the pathogen *Fusarium oxysporum* f. sp. *ciceri* causing wilt disease of chickpea.

Methods and Material

Screening of fungicides against the pathogen (*in vitro)*

The following ten fungicides were evaluated against the pathogen under laboratory conditions to screen out the best fungicides depending upon their inhibitory effect on the growth of the fungus (*Fusarium oxysporum* f. sp. *ciceri*).

S. No.	Fungicides	Active ingredients	Dose (%)
1.	Benlate	Benomyl (50 per cent W.P.)	0.2
2.	Companion	Carbendazim (12 per cent) + Mancozeb (64 per cent) W.P.	0.2
3.	Topsin-M	Thiophanate methyl (70 per cent W.P.)	0.2
4.	Bavistin	Carbendazim (50 per cent W.P.)	0.2
5.	Thiram	Thiram (75 per cent W.P.)	0.2
6.	Tilt	Propiconazole (25 per cent EC)	0.2
7	Chlorothalonil	Chlorothalonil 82.5 W.P.	0.2
8.	Vitavax powder	Carboxin (37.5 per cent) + Thiram (37.5 per cent) W.P.	0.2
9.	Indofil M-45	Mancozeb (75 per cent W.P.)	0.2
10.	Copper oxychloride	Copper oxychloride (50 per cent W.P.)	0.2
11.	Control		

The different fungicides were screened for their efficacy against the pathogen by “Food poison techniques” in which required quantity of each fungicide was thoroughly mixed with 100 ml well sterilized Potato dextrose agar medium contained in 150 ml flasks.

Now this medium mixed with fungicides was poured in Petri-plates and allowed to solidify. Each treatment was replicated three times. One set of control was also kept in which the medium was not mixed with fungicides. Equal pieces of the fungal growth, cut by the sterilized corn borer were inoculated in each Petri-dish at the centre. These inoculated Petri-dishes were incubated at 28±1 °C and after 7 days of the incubation, the fungal growth was recorded in the each Petri-dish.

Evaluation of fungicides against the disease (*in vivo)*

The effectiveness of fungicides on seed treatment have been made in earthen pot culture experiment during the *rabi* crop season of 2018-19 in glass house compound, Department of Plant Pathology, CSA University of Agriculture & Technology, Kanpur.

For seed treatment the required amount of fungicides were added to 100gm of seed in 250 ml conical flask and mixed thoroughly to achieve uniform coating of the fungicide and the seed coat. Thus 10 treated seed with each fungicide were sown in earthen pots (30 cm in diameter) containing inoculums of the pathogen mixed with sterilized soil and untreated seeds were sown in earthen pots containing with inoculums, mixed with sterilized soil. Each treatment was repeated five times. Observations were recorded as, percentage of seeds germination & diseased plants (wilted plants). Ten fungicides were used for this purpose.

Experimental Findings

Screening of fungicides against the pathogen *in vitro* and *in vivo*

A. Laboratory screening of fungicides against the pathogen

Ten fungicides were tested against the pathogen under laboratory conditions. The screening of the best and effective fungicides was done on the basis of the inhibitory effect of the fungicides on the growth of the fungus by the Agar plate method after 7 days of incubation at 28±1°C temperature. The average diameter of the fungal colonies was noted in the poured plates containing different fungicides as mentioned in Table-7.

Table 1: Inhibitory effect of fungicides on the growth of *Fusarium oxysporum* f. sp. *ciceri in vitro* incubated at 28±1 °C after 7 days

S. No.	Fungicides	Dose %	Average diameter of fugal growth (cm)	% inhibition over control
1.	Benlate	0.2	0.90	88.15
2.	Companion	0.2	1.10	85.52
3.	Bavistin	0.2	1.75	76.97
4.	Thiram	0.2	2.05	73.02
5.	Tilt	0.2	2.65	65.13
6.	Chlorothalonil	0.2	3.10	59.21
7	Topsin- M	0.2	3.87	49.08
8.	Vitavax powder	0.2	4.25	44.07
9.	Indofil M-45	0.2	5.15	32.24
10.	Copper oxychloride	0.2	6.30	17.10
11.	Control	0.0	7.60	-
	SEM ±	-	0.32	-
	C.D. at 5%	-	0.94	-

It is evident from the results of Table 7 and its corresponding histogram that out of 10 fungicides tested in laboratory, Benlate (88.15%), Companion (85.52%), Bavistin (76.97%), thiram (73.02%), Tilt (65.13%), Chlorothalonil (59.21%), inhibited the growth of the fungus completely. Other fungicides which were also found effective to check the growth of fungus were Topsin- M (49.08%), Vitavax powder (44.07%), Indofil M-45 (32.24%) and Copper oxychloride (17.10%). All the fungicides were effective to check the growth of the fungus when compared with the control.

B. Effect of seed treatment with fungicides against the disease in pot culture experiment

All the fungicides found effective under laboratory conditions were further tested for their effectiveness in pot culture experiments according to the technique described under "Materials and Methods". Results are presented in Table 8.-

Table 2: Effect of fungicides in seed treatment under pots culture experiment (*in vivo*) (a) Percentage of seed germination

S. No.	Fungicides	Dose %	Number of seed sown	Number of seed germination	Germination (%)
1.	Benlate	0.20	50	48	96.00
2.	Companion	0.20	50	47	94.00
3.	Bavistin	0.20	50	46	92.00
4.	Thiram	0.20	50	45	90.00
5.	Tilt	0.20	50	44	88.00
6.	Chlorothalonil	0.20	50	45	90.00
7	Topsin –M	0.20	50	45	90.00
8.	Vitavax powder	0.20	50	44	88.00
9.	Indofil M-45	0.20	50	43	86.00
10.	Copper oxychloride	0.20	50	43	86.00
11.	Control	-	50	41	82.00
	SEM±			0.676	1.480
	C.D. at 5%			1.995	4.369

It is evident from the Table 8(a) that seed treatment of chickpea variety 'C-104' (susceptible) with ten fungicides were found significantly effective in raising seed germination under pot culture experiments. Percentage seed germination was minimum in untreated control and maximum in case of Benlate (96%) treated seed followed by Companion (94%), Bavistin (92%), thiram (90%), Chlorothalonil (90%), Topsin-M (90%), and Tilt (88%) Vitavax powder (88%) which were statistically at par to each other. Least seed germination was recorded with Indofil M-45 (86) and Copper oxychloride (86%) treated seeds.

Table 3: (b) Percentage of wilted plants

S. No.	Fungicides	Dose %	Number of seed sown	Number of wilted plants	Wilted plants (%)	% decrease in wilted plant over control
1.	Benlate	0.20	50	3	6.00	88.46
2.	Companion	0.20	50	7	14.00	73.08
3.	Bavistin	0.20	50	9	18.00	65.38
4.	Thiram	0.20	50	8	16.00	69.23
5.	Tilt	0.20	50	6	12.00	76.92
6.	Chlorothalonil	0.20	50	15	30.00	42.30
7	Topsin –M	0.20	50	14	28.00	46.15
8.	Vitavax powder	0.20	50	10	20.00	61.53
9.	Indofil M-45	0.20	50	12	24.00	53.85
10.	Copper oxychloride	0.20	50	18	36.00	30.77
11.	Control			26	52.00	
	SEm±			0.212	0.471	
	C.D. at 5%			0.626	1.390	

It is evident from the above Table 8(b) that all the fungicides found significantly superior over control. Minimum percentage of wilted p1ants was with Benlate (6.00%) followed by Tilt (12.00%), Companion (14.00%), Thiram (16.00%), Bavistin (18.00%). Statistically they were at par to each other. Higher percentage of wilted plants was with Vitavax powder (20.00%), Indofil M-45 (24.00%), Topsin-M (28.00%), Chlorothalonil (30.00%) and Copper oxychloride (36.00%) which were at par to each other approximately.

Discussion

In the present investigation efficacy of ten fungicides were tested *in vitro* against *Fusarium oxysporum* f. sp. *ciceri*. Out of which five fungicides *viz.*, Benlate (88.15%), Companion (85.52%), Bavistin (76.97%), and thiram (73.02%), Tilt (65.13%) and Chlorothalonil (59.21%) were found to check the growth of the fungus significantly. Among other fungicides, Topsin-M (49.08%), Vitavax (44.07%), Indofil M-45 (32.24%) and copper oxychloride (17.10%) check the growth of the fungus in descending order of superiority. All the fungicides proved better as compared to control.

On the basis of above experiment, all the fungicides found effective *in vivo* test were used for seed treatment against wilt of chickpea in pots culture experiment in glass house compound. Percentage of seed germination was minimum in untreated control and maximum in case of Benlate (96%) treated seed followed by Companion (94%), Bavistin (92%), and Thiram (90%) which were statistically at par to each other. Thiram (90.00%), Topsin-M (90%), Chlorothalonil (90.00%), and Vitavax powder (88%) were statistically at par to each other. Least effective for seed germination was recorded treated with Indofil M-45 (86%), copper oxychloride (86%). On the basis of above experiment percentage of wilted plants minimum in case of Benlate (6.00%), followed by Tilt (12.00%), Companion (14.00%), thiram (16.00%) and Bavistin (18.00%) statistically at par to each other. Vitavax powder (20.00%), indofil M-45 (24.00%), Topsin-M (28.00%), Chlorothalonil (30.00%) and copper oxychloride (36.00%) at par to each other statistically. Similar results have also been reported by Nikam *et al.* (2007) [11], Korde (2011)

[7], Subhani *et al.* (2011) [16], Taskeen *et al.* (2011) [17], Kumari *et al.* (2014) [8], Brahate *et al.* (2015) [2], Mane *et al.* (2015) [9], Ravichandran and Hegde (2015) [13], Patra and Biswas (2016) [12], Thaware *et al.* (2016) [18].

Conclusion

It is concluded that Benlate, Companion, Bavistin, thiram, Tilt and Chlorothalonil significantly inhibited the growth of *F. oxysporum* f. sp. *ciceri*, the cause of wilt, whereas Benlate proved best for seed germination as well as control of the disease under field conditions.

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