Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(3): 2908-2911
Received: 23-03-2018
Accepted: 28-04-2018

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Screening of fungicides *in vitro* and seed treatment against fusarium wilt of chickpea caused by *Fusarium oxysporum* F. sp. ciceris

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Abstract
Chickpea (*Cicer arietinum* L.) is an important rabi season pulse crop in India belonging to leguminous family. It is good source of protein and possesses nitrogen fixing ability. Various diseases affect the chickpea viz. Ascochyta blight, Phytophthora root rot, Botrytis grey mold, Damping off, Pythium rot/seed rot; Rust and Fusarium wilt in which Fusarium causes severe yield loss of about 60 percent. Among the ten fungicides tested in laboratory, Benlate, Companion, Bavistin, Thiram and Chlorothalonil completely (100%) inhibited the growth of the fungus while seven fungicides were used for seed treatment, where all of them were found significantly superior over control and Bavistin gave best result for seed treatment.

Keywords: fungicide, fusarium, seed treatment, wilt

Introduction
Chickpea (*Cicer arietinum* L.) belonging to leguminosae, 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 with the largest area (135.40 lakh ha.) and highest production (131.02 lakh tones) in the world (Anonymous 2016) [3]. Six countries including India, Australia, Turkey, Myanmar, Pakistan and Ethiopia account for about 90 per cent of world chickpea production. India is the largest consumer of chickpea in the world. As a result, the country occupied second place in the world despite contributing for about 70 per cent of world's total production (Anonymous, 2015) [2]. In India, it is grown in an area of 96 lakh ha with the production of 88.32 lakh tones with the productivity of 968 kg /ha (Anonymous, 2016) [3]. Besides the good source of protein (22%), it also contains calcium (280mg/100gm), carbohydrate (61-62%), Iron (12.3 mg/100gm). It fixes about 30-50 kg /ha of nitrogen (Singh, 1998). The region between the South of the Caucasus and in the North of Persia are the place of possible origin of chickpea as this was also supported by Van der Maesen (1972) [16].

Chickpea is affected by various diseases viz. Ascochyta blight, Phytophthora root rot, Botrytis grey mold, Damping off, Pythium rot and seed rot, Rust and Fusarium wilt in which fusarium causes severe yield loss about 60 percent (Singh and Gupta, 2007) [14]. Fusarium wilt is a seed and soil born fungal disease. As elimination of the pathogen is one of the principles of disease management, contaminated seeds are treated chemically to kill the surface contaminants of those that are not deep seated (H.C. Dube, 2016) [16]. Specific fungicides can control the disease or suppress the fungal growth in very short period of time. Bavistin and thiram are reported effective for the control of wilt disease of the chickpea (Verma *and* Vyas, (1977) [17], Shukla *et al.,* (1981) [12]. Agallol and brassicol are also reported as best fungicides (Das, 1987) [5]. Some fungicides used with the combination of bioagent viz. Trichoderma with vitavax and thiram, (Mukhopadhyay *et al.,* 1992) [10], bacillus subtilis with carboxin gives significant control over *Fusarium oxysporum f. sp*. ciceri and Carboxin also reported to reduce wilt incidence and increased seed yield (De *et al.,* 1996) [6].

Maheshwari *et al.,* (2008) [9] reported Carbendazim to be the most effective fungicide for checking the mycelial growth of *fusarium oxysporum f. sp.* lentis (5.6 mm) followed by Capton (9.9 mm), hexaconazole (12.5 mm) and diniconazole (16.44 mm).

Seed treatment is done by various methods. It is may divided into three categories depending on the nature and purpose of the treatment i.e. seed disinfection, seed disinfestation and seed protection.

Generally systemic fungicides are used for seed treatment. Seed treatment with Bavistin against chickpea wilt was found effective and it used as best chemical for seed treatment (Verma *et al.,* 1977) [17].

Other studies also showed that Bavistin and Thiram are effective in controlling the wilt of chickpea (Shukla *et al.*, 1981) [12].

Materials and Methods

The present investigations based on laboratory and glasshouse experiments were undertaken at the Department of Plant Pathology, C. S. Azad University of Agriculture & Technology, Kanpur, during the year 2014-15, 2015-16.

Fungus isolation and Suspension

Fungus have been isolated from infected plant from Legume Research Farm of Nawabganj, isolation of fungus was done in under lab conditions. The pathogenicity test of the isolated fungus was done in artificial conditions of inoculation. It was found those typical wilt symptoms on roots and other part of the chickpea plants in pots (Tripathi *et al.*, 2007) [15] and the culture of *fusarium oxysporum f. sp.* cerci grown in potato dextrose agar (PDA) medium and incubated at 28+1 °C for seven days. After that, this culture was inoculated into 250 ml of culture flask containing 100 ml of potato dextrose broth (PDB) medium. The cultures were shaken on a shaker at 60 rpm for 14 days. The fungal mycelia were removed using sterile nylon cloth to obtain stock of fungal conidia. The density of conidial suspension was counted using haemocytometer (Gregory, 1983) [8] and the desirable densities was obtained by diluting the stock using sterile water.

Antifungal effect of fungicides against pathogen *in vitro*

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 cork-borer were inoculated in each Petri-dish at the center. These inoculated Petri-dishes were incubated at 28±1°C and after 10 days of the incubation, the fungal growth was recorded in the each Petri-dish. Mechanism of interaction was observed and the data were expressed as percent inhibited by following formula (Bliss, 1934) [4].

$$\text{Per cent inhibition (P.I.)} = \frac{\text{Growth in Control} - \text{Growth in treated plates}}{\text{Growth in control}} \times 100$$

Seed treatment

Wilt susceptible variety "C-104" was used for the experiment. The experiment was carried out in 9 inch earthen pots (30 cm in diameter). For seed treatment, the required amount of fungicides were added to 100 gm 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. Observation were recorded, percentage of seeds germination & diseased plants (wilted plants). Seven fungicides were used for this purpose.

The both experiment were conducted in complete randomized deign (CRD) in three replications.

Table 1: List of fungicides which are used against the pathogen *in vitro*

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

Table 2: List fungicides which are used for Seed Treatment

S. No.	Fungicides	Active ingredients	Dose percent
1.	Benlate	Benomyl (50percent W.P.)	0.2
2.	Companion	Carbendazim (12percent) + Mancozeb (64percent) W.P.	0.2
3.	Topsin-M	Thiophanate methyl (70percent W.P.)	0.2
4.	Bavistin	Carbendazim (50percent W.P.)	0.2
5.	Thiram	Thiram (75percent W.P.)	0.2
6.	Indofil M-45	Mancozeb (75percent W.P.)	0.2
7.	Copper oxychloride	Copper oxychloride (50percent W.P.)	0.2

Result and Discussion

Out of 10 fungicides tested in laboratory, Benlate, Companion, Bavistin, thiram and Chlorothalonil, completely (100%) inhibited the growth of the fungus. Other fungicides which were also found effective to check the growth of fungus were Topsin- M (80.95%), Vitavax power (60.31%), Indofil M-45 (46.91%) and Copper oxychloride (40.56%). All the fungicides were effective to check the growth of the fungus when compared with the control.

On the basis of above experiment, seven fungicides found effective *in vivo* test were used for seed treatment against wilt of chickpea in pots culture experiment in glass house compound. The wilted plants minimum in case of Bavistin (6.00%) followed by thiram (10.00%) and Benlate (12.00%) statistically at par to each other. Companion (14.00%), indofil M-45 (28.00%), copper oxychloride (30.00%) and Topsin-M (32.00%) at par to each other statistically. Similar results have also been reported by verma and Vyas, (1977) [17], Shukla *et al.*, (1981) [12], das (1987) [5], Mayur *et al.*, (2001) and Maheswari *et al.*, (2008) [9].

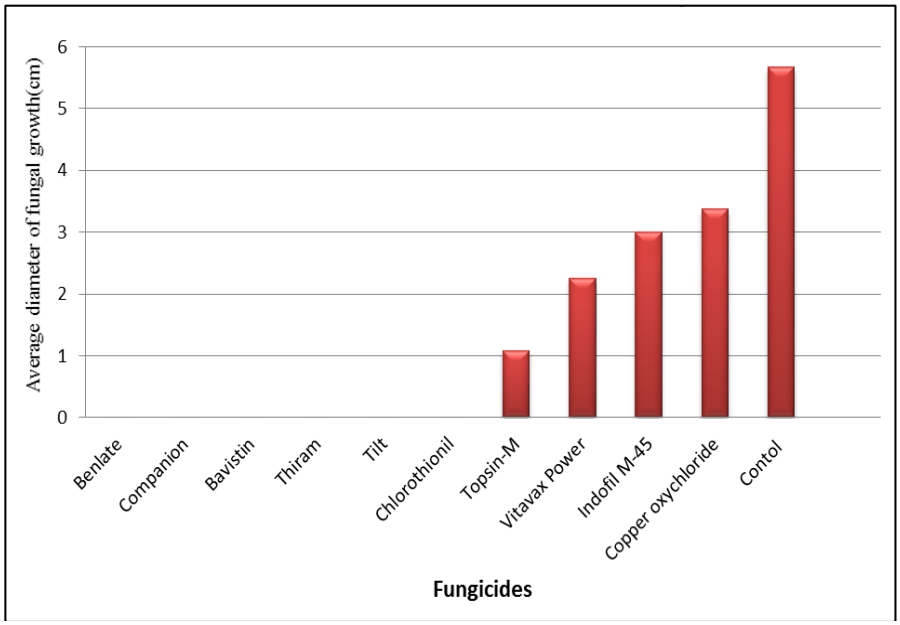
Benlate, Dompanion, Bavistin, thiram, Tilt and Chlorothalonil completely inhibited the growth of F. oxysporum, the cause of wilt, whereas bavistin proved best for seed germination as well as control of the disease under field treatment with best chemical is can prevent the crop from fusarium wilt (Mukhopadhyay *et al.*, 1992) [10].

Table 3: Inhibitory effect of fungicides on the growth of *Fusarium oxysporum f. sp.* cerci *in vitro* incubated at 28±1 °C after 10 days

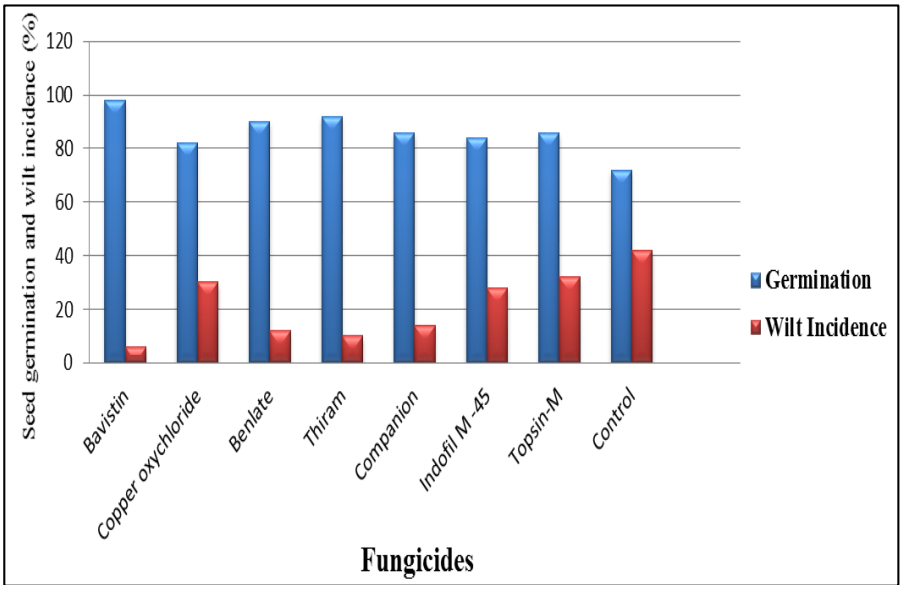
S. No.	Fungicides	Dose %	Average diameter of fugal growth (cm)	Per cent inhibition
1.	Benlate	0.2	00	100
2.	Companion	0.2	00	100
3.	Bavistin	0.2	00	100
4.	Thiram	0.2	00	100
5.	Tilt	0.2	00	100
6.	Chlorothionil	0.2	00	100
7	Topsin- M	0.2	1.08	80.95
8.	Vitavax power	0.2	2.25	60.31
9.	Indofil M-45	0.2	3.01	46.91
10.	Copper oxychloride	0.2	3.37	40.56
11.	Control	0.0	5.67	
	C.D. at 5%			0.1340

Table 4: Effect of fungicides in seed treatment under pots culture experiment (*in vivo*)

S. No.	Fungicides	Dose (%)	Number of seed sown	Number of wilted plants	Wilted plants (%)
1.	Bavistin	0.20	50	3	6.00
2.	Copper oxychloride	0.20	50	15	30.00
3.	Benlate	0.20	50	6	12.00
4.	Thiram	0.20	50	5	10.00
5.	Companion	0.20	50	7	14.00
6.	Indofil M-45	0.20	50	14	28.00
7	Topsin -M	0.20	50	16	32.00
8.	Control	-	50	21	42.00
	C.D. at 5%				0.6120



Graph 1: Inhibitory effect of fungicides on the growth of *Fusarium* o*xysporum f. sp.* cerci *in vitro* incubated at 28±1°C after 10 days



Graph 2: Effect of fungicides of seed treatment on germination and incidence under pots culture experiment (*in vivo*)

Conclusion

The total yield loss due to plant diseases is about 14.1 per cent in the world. (Agrios, 2005) [1]. In India, a total crop yield loss due to plant diseases is about 26 per cent. To reduce this yield loss, we need to develop new management strategies. In next 15-20 years, there will be huge demand of crop production for our population to meet their food demand, these management strategies will help to crotrol yield loss due to plant diseases.

Benlate, Companion, Bavistin, thiram, Tilt and Chlorothalonil completely inhibited the growth of *F. oxysporum*, the cause of chickpea wilt, whereas bavistin proved best for seed treatment as well as control of the disease under field conditions. Fungicidal seed treatment provides robust disease control and has been associated with more rapid and increased emergence of seedlings. The result is healthier plants right out of the ground.

Present study can serve as a handy recommendation for the farmers who can use Bavistin @ 0.2 % for seed treatment against chickpea wilt. This recommendation can also be incorporated as an essential component in Integrated Disease Management chickpea wilt along with other eco-friendly methods.

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