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Research paper

Efficacy of different fungicides against *Ascochyta rabiei* and *Botrytis cinerea* associated with chickpea

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ABSTRACT

***Ascochyta rabiei* and *Botrytis cinerea* have been reported to cause blight and gray mold diseases in chickpea in different parts of the world and cause a significant economic loss to crop. In the present investigation, an *in vitro* evaluation of fungicides was conducted to find out the sensitivity and fungicide concentration in inhibiting the growth of *Ascochyta rabiei and Botrytis cinerea*. *In vitro* evaluation of fungicides revealed that, among five fungicides, bavistin (carbendazim 50 WP) a systemic fungicide was highly effective in inhibiting the mycelial growth with 100% inhibition at all the concentrations tested followed by baycor (bitertenol WP (25% w/w), with 98.88% inhibition at 500 ppm concentration and captan with 80% mycelia growth inhibition in *A.rabiei*. The fungicides such as chlorothalonil, copper oxychloride, and bitertanol at all concentrations were ineffective against *B. cinerea*. The fungicides that were effective under laboratory conditions could be further evaluated under field conditions to validate their efficacy and they could be incorporated as a component in integrated disease management in chickpea.**

Key words: *Ascochyta*, *Botrytis*, Efficacy, Evaluation, Fungicides, Sensitivity

INTRODUCTION

Ascochyta blight and botrytis gray mold diseases of chickpea (*Cicer arietinum*) caused by the fungus *Ascochyta rabiei* (Pass.) Labrousse, and *Botrytis cinerea* Pers. Ex. Fr. Is, respectively are the major yield-limiting constraints in chickpea worldwide. The blight occurs in major chickpea-growing regions of the world (Nene and Reddy, 1987; Khan *et al*., 1999; Kaiser *et al*., 2000; Chongo *et al*., 2003). The areas with intensive production of chickpea as a sole crop contributed to the build-up of inoculum for the severity of epidemics. The blight reduces seed yields and the quality of chickpea significantly, and in susceptible cultivars yield losses may reach up to 100 % (Reddy and Singh, 1990). Chickpea blight is the most devastating chickpea foliar disease in many countries (Pande *et al*., 2005). Disease severity increases with the increase in relative humidity (Trapero-Casas and Kaiser, 1992). Cloudiness and prolonged wet weather favor rapid development and spread of both diseases. Two to three foliar sprays with captan, mancozeb, or chlorothalonil at 2-3 g/l water can effectively manage *Ascochyta rabiei* (Gaur *et al.,* 2010). An adequate level of resistance is not available in the cultivated genotypes and under high disease pressure fungicides become useless. The seed and soil-borne nature of *Ascochyta rabiei* makes fungicidal seed treatments essential and useful (Maden *et al.,* 1975). Fungicides such as carbendazim, difenoconazole, and tebuconazole are being used on a relatively small scale in the Indian subcontinent (Gaur and Singh, 1996).

Botrytis gray mold (BGM) was responsible for 70–100 % yield losses in chickpea in Hissar and several parts of Punjab during 1979-82 (Grewal and Laha, 1982). BGM can devastate chickpea, resulting in complete yield loss in years of extensive winter rains and high humidity (Reddy *et al.,* 1993). Seed treatment with bavistin + thiram (1:1), indofil M-45, thiabendazole, ronilan, rovral, and bavistin at 0.3 % controls seed-borne inoculum of *B. cinerea* (Bakr *et al.,* 1993).

Although fungicide efficacy against *Ascochyta* spp. and *Botrytis cinerea* has been studied in other crops, such as field pea, lentil and straw berry, there is little information relating to fungicide efficacy against chickpea foliar pathogens. Among the various methods to test the efficacy of fungicides, the food poisoning technique (Rahman *et al*., 2019) was adopted to test fungicides (captan, chlorothalonil, copper oxychloride, bitertanol and carbendazim) against major foliar pathogens of chickpea (*Ascochyta rabiei* and *Botrytis cinerea*).

In the present study, fungicides which had effective in controlling foliar pathogens of chickpea were evaluated for their ability to reduce mycelial growth of the most common foliar pathogens of temperate region such as *Ascochyta rabiei and Botrytis cinerea* under *in vitro*. Then the efficacy of selected fungicides against artificially inoculated chickpea was carried out using potted chickpea plants. This would lead to finding an alternate use of the fungicides as potential seed treatment against these pathogens.

MATERIALS AND METHODS

Fungal isolates

Fungal isolate, *Ascochyta rabiei* of Kanpur, Uttar Pradesh, and *Botrytis cinerea* of Pantnagar was isolated from the cultivars 'Bharath gram' and 'JG 62' of chickpea, respectively. The isolated fungal cultures were characterized based on morphological and molecular techniques and these cultures were stored as mycelial plugs in 20% glycerol stocks at -40 ^{0}C in the Division of Crop Protection, ICAR-Indian Institute of Pulses Research, Kanpur. The isolates were cultured on potato dextrose agar at 20±1^{0}C alternating with 12 hrs light and dark period (Bais *et al.,* 2019).

Pathogenicity tests

For testing the pathogenicity (Koch's postulate) of the fungus *A. rabiei* and *B. cinerea*, the experiment was conducted in the controlled environmental facility (CEF) at 20 ± 1 ^{0}C temperature alternating with 12 hrs light and dark with 90% relative humidity maintained till the completion of the experiment. The *Ascochyta rabiei* susceptible (L-550) and the *Botrytis cinerea* susceptible (JG-62) genotypes were planted in 10 cm diameter pots containing one kg of sand: soil: vermiculite (4:4:1 v/v) sterilized with four percent formaldehyde. The five seeds were planted at 2.5 cm deep with three replicates. For inoculation of plants, spore suspension of fungus (AR and BC) isolates grown on autoclaved Kabuli seeds prepared by mixing the 10-day-old cultures with sterilized distilled water. The spore suspension was filtered through sterilized muslin cloth and adjusted to 1×10^6 spore/ml (AR) (Jamil *et al*., 2000) and 1×10^5 spore/ml (BC) with the aid of a haemocytometer. Tween 20 (5 µl/100 ml) was added to the spore suspension as a wetting agent, and then the two-week-old chickpea seedlings were inoculated with the spore suspension until run-off by using the 500 ml hand sprayer. Three pots for each were sprayed in the same manner with sterilized distilled water and kept as a control and these pots were kept under the CEF facility until the appearance of the respective disease symptoms. The appearance date of color and size of symptoms on the leaves and stems were determined for each cultivar 15 days after inoculation.

Effect of fungicides on mycelial growth of fungi

Six fungicides belonging to different chemical groups (Table 1) were evaluated in vitro against *A. rabiei and B. cinerea* to determine their efficacy for mycelia growth inhibition using modified poisoned food technique (Grover and Moore, 1962). The fungicides tested were suspended in sterile distilled water and the amount to achieve each concentration (100 ppm, 200 ppm and 500 ppm, selected based on the reported activity range of each product) was added to chickpea seed meal agar (CSMA) at 60 °C and mixed immediately then poured 15 ml from each into 90 mm × 10 mm Petri plates. The Petri plates containing 15 ml of poisoned solidified media were inoculated in the center of the poisoned plates with five mm diameter of respective fungal mycelial discs of 10-day-old *A. rabiei* and seven-day-old *B. cinerea* cultures that were grown on CSMA medium. Each fungicide was tested at three different concentrations and control plates consisting of only CSMA (Without any fungicide). Three replications for each combination of fungicide concentration were set up by using a complete randomized design (CRD). Negative control was maintained without the addition of the fungicides to the media. The observations were taken and results were calculated two weeks after incubation (Fonseka *et al.,* 2023),

The treated plates were incubated at 20 ± 1^{0}C. Measurement of radial growth (mm) of mycelium was taken using a scale and percent inhibition of fungal mycelia growth was calculated using the following formula (Vincent 1947):

Percent growth inhibition (%) = ***A–B/A*** × 100

Where, A = Colony growth of the *Ascochyta rabiei/Botrytis cinerea* in the control plate

B= Colony growth of the *Ascochyta rabiei/ Botrytis cinerea* in fungicide treated plate

Statistical analysis

The experimental data were statistically analyzed and interpreted with ANOVA as per the completely randomized design (CRD) method for laboratory studies suggested by Panse and Sukathme (1985).

RESULTS AND DISCUSSION

The results of the findings in terms of percent mycelia inhibition of *A. rabiei* and *B. cinerea* were determined using the different concentrations of contact and systemic fungicides. Once the control plates showed maximum growth, data on the radial growth of the fungus was recorded.

Among five fungicides, bavistin (carbendazim 50 WP) a systemic fungicide was found significantly superior with 100% mycelial growth inhibition at all the concentrations followed by Baylor [bitertenol WP (25% w/w)] 98.88% and captan with 80% inhibition of *Ascochyta rabiei* at 500 ppm fungicide concentration. Chlorothalonil was found to be least effective than carbendazim, and Baycor but possessed more inhibition capacity than captan against *A. rabiei* at 500 ppm concentration. While the inhibitory effect of chlorothalonil and captan increased with an increase in the concentrations (Table 1; Fig 1 and Fig 2).

The carbendazim at all the tested concentrations was found significantly effective in inhibiting mycelial growth of the *B. cinerea*. The efficacy of captan increased in mycelial inhibition of *B. cinerea* as the concentration increased from 100 ppm to 500 ppm and significantly checked the colony growth at 500 ppm, which was less effective than carbendazim against *B. cinerea*. The carbendazim at all the concentrations (100, 200, and 500 ppm) completely inhibited the growth of *B. cinerea* (Table 2; Fig 1 and Fig 2).

A broad-spectrum non-systemic fungicide, chlorothalonil was also found to be the least effective as reported by Inam *et al.* (1995). While copper oxychloride was ineffective against AR and BC fungi at all the tested concentrations. Fungicides are toxic to fungi in several ways like ceasing mycelial growth and change in metabolic processes, spores may be killed or fail to germinate (Neely 1969). The chlorothalonil does not inhibit the mycelial growth of the pathogen *in vitro* condition, nor does it prevent the seed-to-seedling transmission of the infection. But, chlorothalonil reliably reduces the severity of the ascochyta blight, when used as foliar application during the early + mid flowering stage because inoculum pressure is high in these stages if environmental conditions are favorable for disease development (Kimber and Ramsey, 2001; MacLeod *et al.*, 2002; Chongo *et al*., 2003).

Madhu *et al.* (1986) and Agarwal and Tripathi (1999) described that *B. cinerea* growth was completely inhibited by carbendazim at 10 µg/ml.

Table 1: *Iv vitro* evaluation of different fungicides against *Ascochyta rabiei*

Fungicide	Chemical composition	Conc. Used	Mycelial growth				
			2DAI (mm)	4DAI (mm)	6DAI (mm)	8DAI (mm)	10DAI (mm)
Baycor	Bitertenol WP (25% w/w)	100ppm	0.00	0.00	0.00	0.22	0.72
		200ppm	0.00	0.00	0.00	0.30	0.33
		500ppm	0.00	0.00	0.00	0.05	0.17
Bavistin	Carbendazim 50 WP	100ppm	0.00	0.00	0.00	0.00	0.00
		200ppm	0.00	0.00	0.00	0.00	0.00
		500ppm	0.00	0.00	0.00	0.00	0.00
Captan	Captan 50 WP	100ppm	0.67	0.77	2.17	3.63	6.50
		200ppm	0.00	0.36	1.23	1.77	5.67
		500ppm	0.00	1.54	1.58	1.85	3.00
Dhanucop	Copper oxy chloride 50% WP	100ppm	1.17	4.74	6.50	7.53	13.87
		200ppm	1.00	4.96	7.75	9.22	13.83
		500ppm	1.50	2.54	5.47	8.44	12.00
Kavach	Chlorothalonil 75 WP	100ppm	0.00	1.63	4.34	6.02	10.00
		200ppm	1.00	1.77	4.23	6.52	9.17
		500ppm	0.50	1.61	4.05	6.64	8.67
Control	Control	Control	1.50	4.72	8.03	10.89	15.00
		C.D.	0.40	0.51	0.87	1.29	1.34
		SE(m)	0.14	0.18	0.30	0.45	0.46

Table 2: *Iv vitro* evaluation of different fungicides against *Botrytis cinerea*

Fungicide	Chemical composition	Concn.	Mycelial growth				
			2DAI (cm)	4DAI (cm)	6DAI (cm)	8DAI (cm)	10DAI (cm)
Baycor	Bitertenol WP (25% w/w)	100ppm	0.93	2.80	3.63	4.00	4.10
		200ppm	0.83	2.93	3.73	3.90	4.06
		500ppm	0.93	2.96	3.67	3.93	4.16
Bavistin	Carbendazim 50 WP	100ppm	0.00	0.00	0.00	0.00	0.00
		200ppm	0.00	0.00	0.00	0.00	0.00
		500ppm	0.00	0.00	0.00	0.00	0.00
Captan	Captan 50 WP	100ppm	0.56	2.40	3.37	3.63	4.03
		200ppm	0.36	2.03	3.20	3.93	4.06
		500ppm	0.40	2.06	2.70	3.50	3.83
Dhanucop	Copper oxy chloride 50% WP	100ppm	2.10	4.06	4.16	4.33	4.40
		200ppm	2.16	4.4	3.83	4.00	4.16
		500ppm	1.90	3.73	3.76	4.03	4.03
Kavach	Chlorothalonil 75 WP	100ppm	1.70	2.76	3.30	3.96	4.23
		200ppm	1.06	3.06	3.66	3.96	4.13
		500ppm	0.73	2.76	3.66	4.06	4.20
Control	Control	Control	2.13	4.40	4.40	4.40	4.46
		C.D.	0.28	0.24	0.29	0.27	0.19
		SE(m)	0.097	0.08	0.10	0.09	0.06

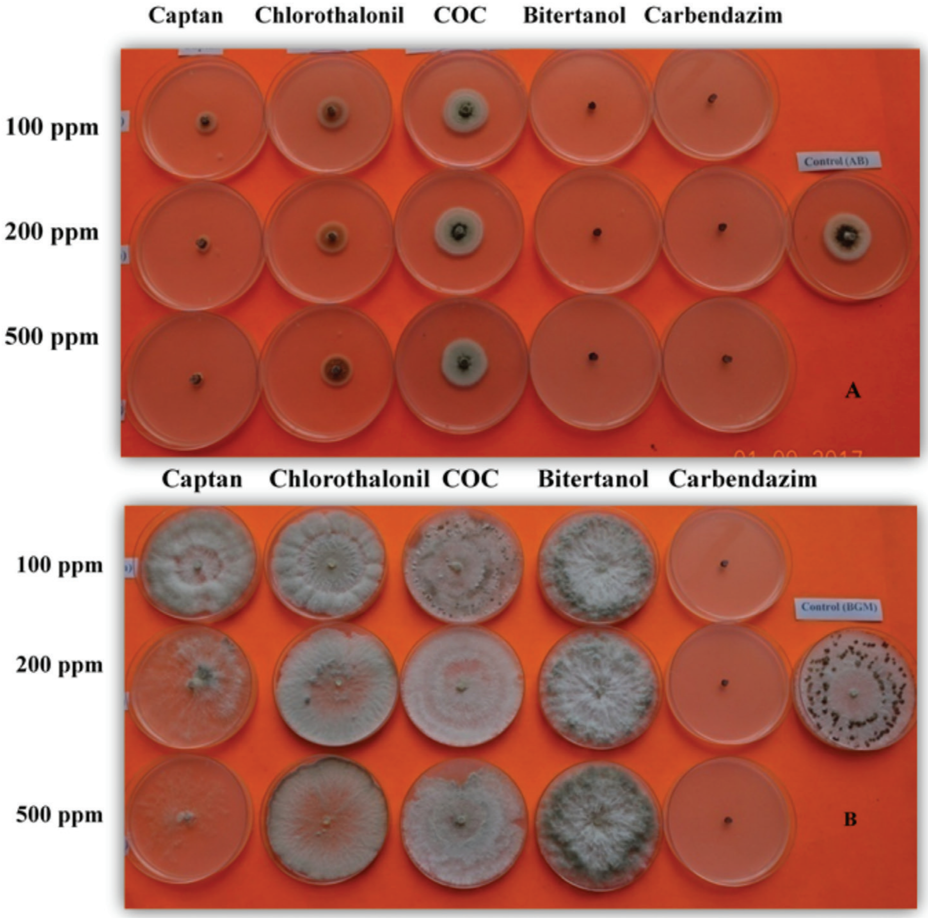

Fig. 1. Fungistatic effects on the mycelial growth of *A. rabiei* (A) and *B. cinerea* (B) in the presence of fungicide as compared with control

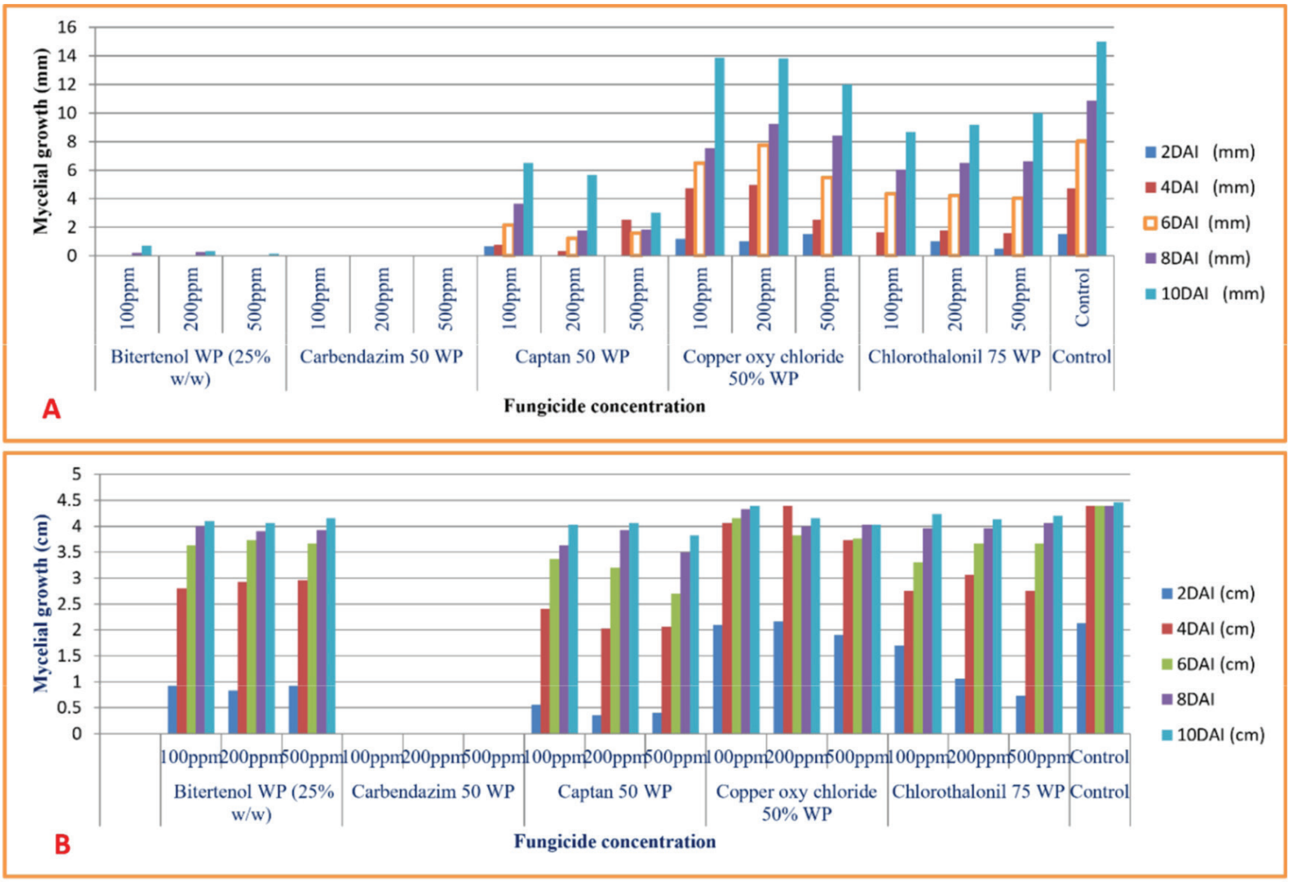

Fig. 2. In-vitro evaluation of the efficacy of fungicides at different concentrations agains (A) *Ascochyta rabiei* and (B) *Botrytis cinerea*

It could be possible to reduce the infection risk of both the fungus by using carbendazim spraying of chickpea crops at cloudy and high humidity conditions that favour disease occurrence in temperate climates. Foliar, seed, or soil treatment with carbendazim *needs to be* checked against ascochyta blight and Botrytis gray mold diseases. Carbendazim belongs to the benzimidazole group of fungicides, which interferes with energy production and cell wall synthesis in fungi (Nene *et al.,* 2012). The fungal inhibition of carbendazim is due to the induction of nuclear instability by disturbing mitosis and meiosis (Konde *et al.,* 2008).

CONCLUSION

In the present study, laboratory testing of five fungicides at three different concentrations (100 ppm, 200 ppm, and 500 ppm) through poisoned food technique revealed that carbendazim at all the concentrations, captan @ 500 ppm showed effectiveness in inhibiting the fungal growth of *A. rabiei* and *B. cinerea*. The systemic fungicide, carbendazim was proved to be the best among the tested fungicides which completely inhibited the fungal growth in all concentrations against both the fungus. This study can help to simplify the concept of chemicals against the Ascochyta blight and Botrytis gray mold disease-causing pathogens. Screening/(Evaluation) of these chemicals is needed for additional confirmation under greenhouse and field trials.

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