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In-vitro evaluation of fungicides against *Colletotrichum capsici* causal organism of anthracnose disease of chilli

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Abstract
Chilli is found to be comprised of many plant derived chemical compounds that promote health. Anthracnose of chilli (*Capsicum annuum* L.) caused by *Colletotrichum capsici* (Syd.) Butler and Bisby, is one of the major and devastating diseases of chilli causes severe losses (10-60%) both in yield and quality of the chilli depending upon the varieties. Management strategies for this disease include use of disease free seed, resistant/tolerant cultivars and fungicidal sprays. Chemicals are the most common and practical method to control anthracnose disease. However, fungicide tolerance often arises quickly, if a single compound is relied upon too heavily (Staub, 1991). Fungicides are gaining importance in crop protection in view of their selective properties, low cost and safety to ecosystem. Many fungicides as a chemical have been identified to be effective in the control of plant diseases. Keeping in mind that the anthracnose of chilli, is one of the major and devastating diseases of chilli. Present investigation was aimed to identify the most suitable fungicide to control the incidence of *Colletotrichum capsici.* The present investigation was carried out in the Department of Plant Pathology, college of Agriculture, RVSKVV Gwalior (M.P.) using Completely Randomised Block Design (CRD) with five replication during Kharif 2015-16. Six chemicals (Carbendazim 50WP, Metalaxyl+Mancozeb (Redomil Gold), Mancozeb 75WP, Thiophanate methyl, Copper oxychloride, Tricyclazole 75WP,) will be evaluated against *C. capsici* under *in vitro* condition by adoption of Poisoned Food Technique with different concentration viz. 20, 50, 100, 250, 500 and 1000 µg/ml, respectively and untreated was considered as a control in all the replication with different concentration. Findings concluded that the minimum mycelial growth was recorded in 1000 µg/ml compared to rest of concentrations. Thiophanate methyl and Copper oxychloride were the best toxic fungicides inhibiting the mycelial growth *in vitro* at all the six concentrations. In fungicides, Thiophanate methyl inhibited highest mycelial growth, which was at par with Copper oxychloride.

Keywords: *Colletotrichum capsici*, poisoned food, fungicides, mycelial growth

Introduction
Chilli is found to be comprised of many plant derived chemical compounds that promote health. In India, the major chillies growing states are Andhra Pradesh, Karnataka, Maharashtra, Orissa, Tamil Nadu, Madhya Pradesh and Rajasthan. India accounts for 1.2 million tonnes of production annually, and is the largest producer in terms of international trade, exporting 25 per cent of its total production (Anon. 2010) [1]. In India it is grown in 775 thousand ha area with production of 1.4 metric MT and productivity of 1.9 ton per ha. In Madhya Pradesh chilli is grown under an area of 54.41thousand ha with production of 93.57 thousand MT and productivity 0.98 ton per ha (Anon-2013-14). Anthracnose of chilli (*Capsicum annuum* L.) caused by *Colletotrichum capsici* (Syd.) Butler and Bisby, is one of the major and devastating diseases of chilli causes severe losses (10-60%) both in yield and quality of the chilli depending upon the varieties (Bansal and Grover, 1969) [3]. On the leaves, small, circular spots appear. Severely infected leaves fall off leading to defoliation. The infection of growing tips lead necrosis of branches which progresses backward on the diseased branches (Dieback Stage).Black dots (Acervuli) are formed all over the necrotic surface of the affected twigs, later on. On fruits, dark brown to black sunken spots, circular or angular shape with concentric rings of acervuli that are often wet and produce pink to orange conidial (Spores) masses are evident. Management strategies for this disease include use of disease free seed, resistant/tolerant cultivars and fungicidal sprays.

Chemicals are the most common and practical practical method to control anthracnose disease. However, fungicide tolerance often arises quickly, if a single compound is relied upon too heavily (Staub, 1991) [9]. Fungicides are gaining importance in crop protection in view of their selective properties, low cost and safety to ecosystem. Many fungicides as a chemical have been identified to be effective in the control of plant diseases. Keeping in mind that the anthracnose of chilli, is one of the major and devastating diseases of chilli. Present investigation was aimed to identify the most suitable fungicide to control the incidence of *Colletotrichum capsici.*

Material and Methods

The present investigation was carried out in the Department of Plant Pathology, college of Agriculture, RVSKVV Gwalior (M.P.) using Completely Randomised Block Design (CRD) with five replication during Kharif 2015-16.Following six chemicals (Carbendazim 50WP, Metalaxyl+Mancozeb (Redomil Gold), Mancozeb 75WP, Thiophanate methyl, Copper oxychloride, Tricyclazole 75WP,) will be evaluated against *C. capsici* under *in vitro* condition by adoption of Poisoned Food Technique with different concentration viz. 20, 50, 100, 250, 500 and 1000 µg/ml, respectively and untreated was considered as a control in all the replication with different concentration.

Methodology

In the present study above fungicides were evaluated against *Colletotrichum capsici* by adopting poisoned food technique.

Poisoned Food Technique

The potato dextrose agar medium was prepared and sterilized, than under aseptic condition the required quantity of each fungicides or chemicals were incorporated into 80 ml sterilized PDA filled in flask of 250 ml capacity from each flask 4 sterilized petriplates of 90mm diameter were poured. This poured petriplates were inoculated at the center with a 7 mm fungal disc from seven days old culture of the test organism. Control (without chemicals) was maintained for comparison. The petriplates were incubated at 25+2℃. The radial growth of the fungus was measured at 3, 5, 7 days after inoculation (Nene and Thapliyal 1993) [8].

Preparation of fungicides solution with different concentration

The chemicals or fungicides were used in powder form for their evaluation against *C. capsici* under different concentration 20, 50, 100, 250, 500, 1000 µg/ml. by poison food technique. For making 20 µg/ml. solution of the respective chemicals powder was incorporated into 80 ml. of PDA media in the flask of 250 ml. which was already autoclaved and pinch of streptomycin sulphate was mixed just before the pouring and remaining concentration of solution was prepared with mixed the same quantity of sterilized PDA to the respective chemicals with different concentration (50, 100, 250, 500, 1000 µg/ml.) respectively.

Result and Discussion

The basic approach before recommending chemical control against a particular disease is to evaluate the fungicides against pathogen under laboratory conditions at different concentrations. Increase in concentrations of fungicides caused a decrease in mycelial growth of the fungus thereby resulting in increased inhibition. *In vitro* toxicity test revealed that at seven days after of inoculation fungicide Thiophante Methyl was highly toxic with minimum mycelial growth of *C. capsici*, which was at par with copper oxychloride. Among the concentrations, minimum mycelial growth was recorded in 1000 µg/ml, which was at par with 500µg/ml compared to rest of concentrations. Thiophanate Methyl and copper oxychloride showed minimum mycelial growth. Results revealed that Thiophanate Methyl and copper oxychloride were the best toxic fungicides to inhibt the mycelial growth *in vitro* at all the six concentrations. All the fungicides were significantly inhibited the mycelia growth of *C. capsici* at all concentrations after seven days of inoculation. Haq and co-workers (2013) [5] were evaluatedthe efficacy of different fungicides (Dithane M-45, Alliet, Carbendazim, Acrobit and Antracol) against *C. capsici* at different concentrations after three time intervals under *In vitro* studies. All fungicides exhibited differential effects in inhibiting the mycelial growth of C. *capsici.* Among these fungicides, Carbendazim was the most effective in inhibiting the mycelial growth after 4, 8 and 12 days followed by Acrobit, Dithan M-45 and Antracol. Mesta (1996) [6] reported that among the non-systemic fungicides Mancozeb, Captan and Chlorothalonil were found to be highly effective in inhibiting the growth of *C. capsici* at 3000 µg/ml concentration and among the systemic fungicides Carbendazim, Bitertinol and Tridemefon were found effective at 1000 µg/ml concentration. Gopinath and Co-workers (2006) [4] tested the efficacy of 3 Triazole fungicide viz. Hexaconazole (0.1%), Propiconazole (0.1%) and Triadimefon (0.1%) against *C. capsici* by poison food technique. Similar result were obtained by Mali and Joi (1985) [6] who reported Difolatan (Captafol), Thiram and Vitavax (Carboxin) as most effective against colony growth and sporulation of *C. capsici.*

Table 1: Inhibition per cent of *Colletotrichum capsici* by fungicide amended medium at three days after inoculation

Concentrations Fungicides	Inhibition percentage of *Colletotrichum capsici* *						
	20 (µg/ml)	50 (µg/ml)	100 (µg/ml)	250 (µg/ml)	500 (µg/ml)	1000 (µg/ml)	MEAN A
Carbendazim 50WP	78.66(64.78)	81.33(64.76)	84.25(66.69)	88.66(70.49)	87.75(69.69)	90.37(72.17)	85.17(68.10)
Metalaxyl+Mencozeb 75WP	68.78(56.11)	69.67(56.73)	70.90(57.39)	72.04(58.10)	74.85(60.04)	86.88(68.82)	73.85(59.53)
Mancozeb 75WP	59.24(50.33)	64.77(53.64)	68.91(56.16)	69.88(56.75)	76.60(61.17)	85.76(67.89)	70.86(57.66)
Thiophanate methyl	91.67 (73.42)	91.95(73.76)	92.82(74.62)	96.32(81.40)	98.00(86.31)	98.67(87.01)	94.91(79.42)
Copper oxychloride	85.71(78.46)	91.33(75.01)	91.58(73.23)	93.63 75.38)	96.17(81.21)	98.82(87.19)	92.87(78.41)
Tricyclazole 75WP	83.25 (66.32)	84.09(66.58)	88.15(69.88)	88.37(70.34)	90.00(73.72)	90.63(72.41)	87.42(69.88)
Control	0.00 (0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
MEAN B	66.76(55.63)	69.02(55.78)	70.94(56.85)	72.70(58.92)	74.77(61.73)	78.73(65.07)	
Factors	SE(m)			C.D.			
Factor(A)	1.18			3.29			
Factor(B)	1.09			3.04			
Factor(A X B)	1.00			2.78			

*Mean of five replications, () Data in parentheses are arcsine transformed values

Table 2: Inhibition per cent of *Colletotrichum capsici* by fungicide amended medium at five days after inoculation

Concentrations Fungicides	Inhibition percentage of *Colletotrichum capsici* *						
	20 (µg/ml)	50 (µg/ml)	100 (µg/ml)	250 (µg/ml)	500 (µg/ml)	1000 (µg/ml)	MEAN A
Carbendazim 50WP	83.11(65.87)	87.28(69.19)	88.11(70.10)	90.83(72.65)	91.27(73.00)	91.73(73.43)	88.72(70.70)
Metalaxyl+Mencozeb 75WP	66.28(54.51)	71.06(57.51)	73.93(59.34)	77.43(61.67)	80.48(63.90)	85.71(67.88)	75.82(60.80)
Mancozeb 75WP	58.68(50.01)	62.58(52.34)	69.57(56.57)	72.27(58.29)	83.56(66.15)	92.48(74.31)	73.19(59.61)
Thiophanate methyl	87.88(79.77)	88.11(70.01)	91.14(72.83)	98.67(87.01)	98.67(87.01)	100.00(90.00)	94.08(81.10)
Copper oxychloride	84.39(66.76)	87.28(69.19)	98.57(86.90)	98.67(87.01)	98.82(87.19)	98.86(87.24)	94.43(80.71)
Tricyclazole 75WP	76.90(61.31)	82.55(65.35)	84.07(66.52)	91.07(72.77)	91.90(73.67)	94.90(78.32)	86.90(69.66)
Control	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
MEAN B	65.32(54.03)	68.41(54.80)	72.20(58.89)	75.56(62.77)	77.81(64.42)	80.53(67.31)	
Factors	SE(m)			C.D.			
Factor(A)	0.90			2.51			
Factor(B)	0.83			2.32			
Factor(A X B)	0.76			2.12			

*Mean of five replications**, ()** Data in parentheses are arcsine transformed values

Table 3: Inhibition per cent of *Colletotrichum capsici* by fungicide amended medium at seven days after inoculation

Concentrations Fungicides	Inhibition percentage of *Colletotrichum capsici* *						
	20 (µg/ml)	50 (µg/ml)	100 (µg/ml)	250 (µg/ml)	500 (µg/ml)	1000 (µg/ml)	MEAN A
Carbendazim 50WP	77.84(61.95)	81.18(64.34)	81.79(64.95)	82.46(65.38)	83.83(66.53)	88.80(70.50)	82.65(65.61)
Metalaxyl+Mencozeb 75WP	60.46(51.05)	70.97(57.49)	73.43(59.02)	73.86(59.27)	78.50(62.43)	84.09(66.52)	73.55(59.30)
Mancozeb 75WP	59.78(50.69)	66.32(54.54)	70.92(57.37)	74.41(59.64)	78.02(62.06)	84.96(67.33)	72.40(58.61)
Thiophanate methyl	83.32(65.95)	89.07(70.76)	90.86(72.46)	91.81(73.55)	96.60(81.74)	97.64(84.38)	91.55(74.81)
Copper oxychloride	85.86(67.96)	87.85(69.64)	87.06(77.18)	89.01(71.23)	95.91(80.94)	98.18(85.07)	90.65(75.34)
Tricyclazole 75WP	79.56(63.14)	79.65(63.27)	79.69(63.28)	80.33(63.73)	81.28(64.55)	87.35(69.51)	81.31(64.58)
Control	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
MEAN B	63.83(51.53)	67.86(47.15)	69.11(56.32)	70.27(56.11)	73.45(59.75)	77.29(63.33)	
Factors	SE(m)			C.D.			
Factor(A)	0.86			2.41			
Factor(B)	0.80			2.23			
Factor(A X B)	0.73			2.03			

*Mean of five replications, () Data in parentheses are arcsine transformed values

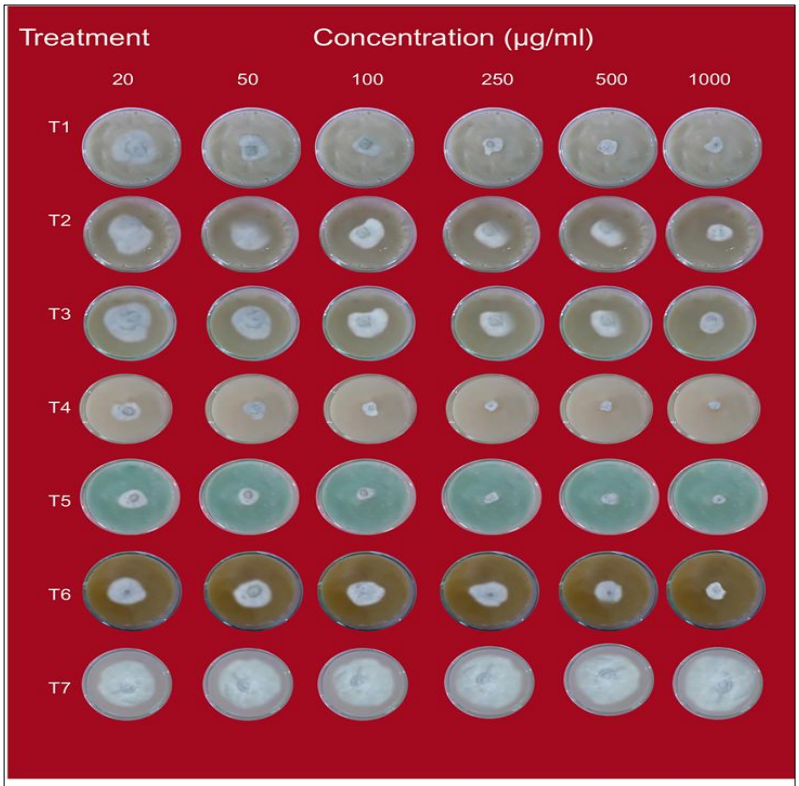

Plate:10 In-vitro evaluation of fungicide against *C.capsici* after seven days of inoculation

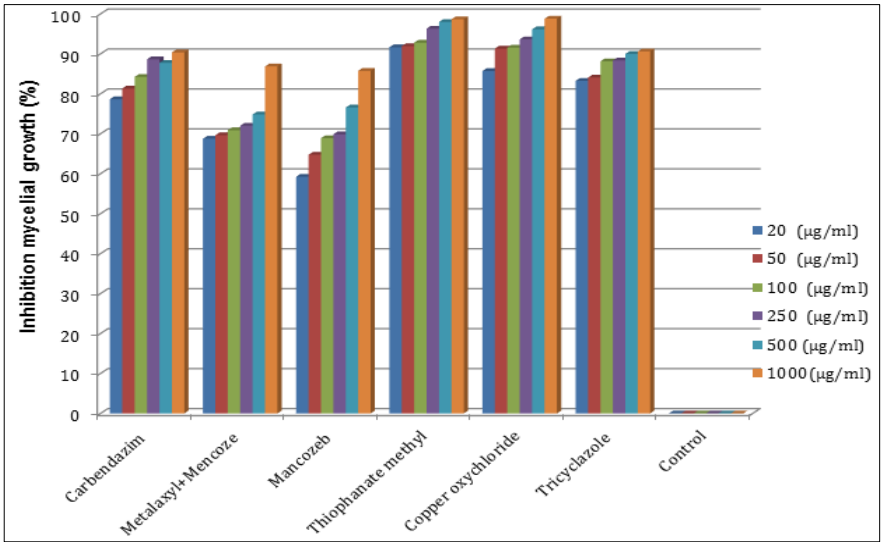

Fig 1: Inhibition per cent of *Colletotrichum capsici* by fungicide amended medium at three days after inoculation

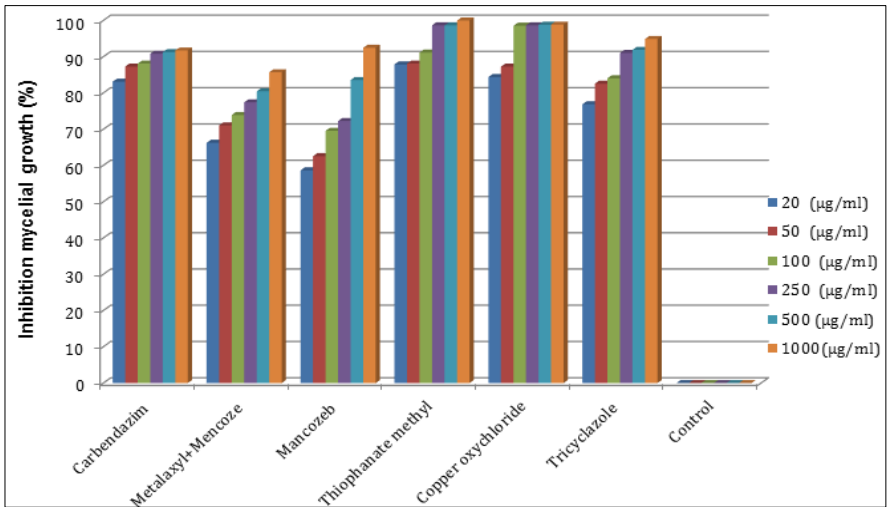

Fig 2: Inhibition per cent of *Colletotrichum capsici* by fungicide amended medium at five days after inoculation

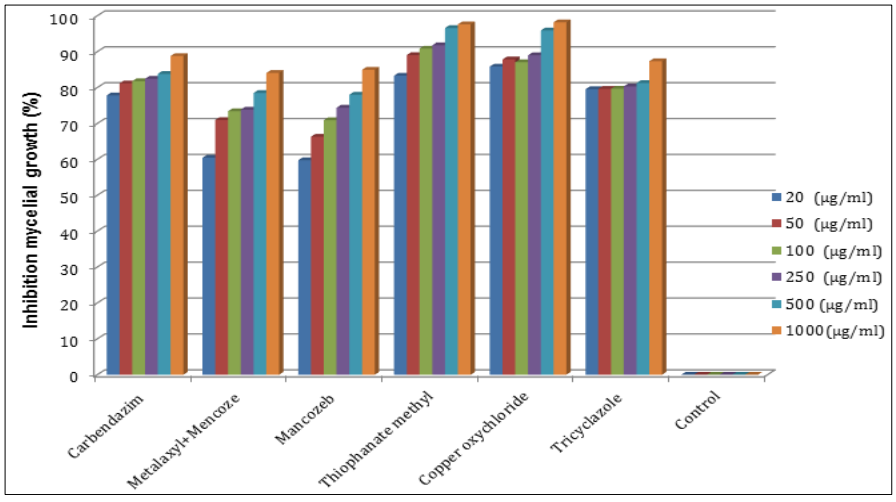

Fig 3: Inhibition per cent of *Colletotrichum capsici* by fungicide amended medium at seven days after inoculation

Conclusion

Results concluded that the minimum mycelial growth was recorded in 1000 µg/ml compared to rest of concentrations. Thiophanate methyl and Copper oxychloride were the best toxic fungicides inhibiting the mycelial growth *in vitro* at all the six concentrations. In fungicides, Thiophanate methyl inhibited highest mycelial growth, which was at par with Copper oxychloride.

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