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EFFECT OF FUNGICIDES ON MYCELIAL GROWTH, PYCNIDIAL PRODUCTION AND SPORE GERMINATION OF *ASCOCHYTA RABIEI* (PASS.) LAB.

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ABSTRACT

For the preliminary evaluation of fungicides against *Ascochyta rabiei* (Pass.) Lab., the cause of gram blight, the fungicidal effects on fungal growth, pycnidial production and spore germination were studied *in vitro*. Mycelium of *A. rabiei* was the most sensitive to Benlate + Karathane, Benlate + Daconil and Benlate + Dithane M–45 and least sensitive in descending order to Dithane M–45, Morestan, Brassicol and Quinolate, with intermediate sensitivity to Karathane, Benlate + Brassicol, Benlate, Daconil and Captan. The most effective fungicides in reducing the pycnidial production of *A. rabiei* were Benlate + Daconil, Benlate + Karathane, Benlate and Benlate + Dithane M–45. In general, the most effective fungicides in inhibiting conidial germination were Benlate + Daconil, Benlate, Daconil, Captan and Benlate + Karathane.

INTRODUCTION

Gram blight caused by *Ascochyta rabiei* (Pass.) Lab. appears almost regularly in alarming epidemics in both the barani and irrigated areas of the Punjab (Kausar, 1965). In certain years it causes total failure of the gram crop (Nene, 1981). This disastrous situation is on account of the scarcity or complete absence of genetic resistance in our commercial cultivars. The search for the sources of genetic resistance and their exploitation and incorporation into commercial cultivars is a long way to go. However, the use of fungicides is an immediate alternative control measure of the disease. This paper aims at the preliminary *in vitro* evaluation of fungicides for their comparative effectiveness on the inhibition of mycelial growth, pycnidial production and spore germination of *A. rabiei*, so that *in vitro* evaluated fungicides could further be evaluated for the effective control of gram blight in the field.

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MATERIALS AND METHODS

The effect of eight fungicides : Benlate, Daconil, Brassicol, Captan, Dithane M – 45, Quinolate, Karathane and Morestan alone, as well as in combinations of Benlate + Daconil, Benlate + Brassicol, Benlate + Dithane M – 45, and Benlate + Karathane in 1 : 1 ratio, was studied for their influence on mycelial growth, pycnidial production and spore germination.

(a) *In Vitro Sensitivity of A. rabiei Mycelium to Fungicides*

The sensitivity of *A. rabiei* to different concentrations (5, 10, 20 and 50 ug/ml) of each fungicide alone and in combination was studied using modified Borum and Sinclair's technique (1968). Weighed quantity of fungicide was added to the medium after autoclaving gram seed meal agar (GSMA) (gram flour, 20 gm; glucose, 20 gm; agar agar, 20 gm; and water, 1000 ml). GSMA without fungicide served as control. Fifteen ml of the medium were poured in each of the four 90 cm (diameter) petriplates. After solidification, 6 mm agar plugs containing *Ascochyta rabiei* mycelium were cut from 10 days old GSMA culture plates using a sterile cork borer and placed in the centre of each petriplate. The inoculated pertiplates were incubated at 22 °C and the mean mycelial growth was recorded after 15 days of inoculation.

(b) *In Vitro Effect of fungicides on Pycnidial Production*

For the relative estimation of pycnidial production, 6mm diameter plugs of GSMA from 30 days old culture were cut from each replication of each fungicidal concentration (treatment) with the help of a cork borer and placed on the microscope slide. The plugs were observed under the stereoscope and the average number of pycnidia per 6 mm diameter plug was counted.

(c) *In Vitro Effect of Fungicides on Spore Germination*

The relative effectiveness of different fungicides (either alone or in combination) on spore germination was studied by slide germination method (Anonymous, 1943). Concentrations of each fungicide (1, 5, 10 and 20 µg/ml) were made by adding a definate quality of stock solution to distilled sterilized water. Distilled sterilized water without fungicide served as control. Equal amount of inoculum from 16 days old culture of *A. rabiei* was added to each of the fungicide concentration and stirred well to have a good release of spores

from pycnidial into the solution. Oned rop of each fungicide-spore suspention was placed in a cavity slide and incuba-ted at 22 °C for 24 hours. The experiment was run in triplicate and inhibition of spore germination was recorded.

RESULTS AND DISCUSSION

The effectiveness of fungicides tested in reducing mycelial growth, pycnidial production and spore germination varied and depended upon the fungicide used and its concentration. There was a significant decrease in vegetative growth (Table 1), pycnidial production (Table 2) and spore germination (Table 3) with an increase in the concentration of each of the fungicides. The

Table 1. *In vitro sensitivity of Ascochyta rabiei mycelium to various fungicide concentrations in GSMA after 15 days of growth at 22 °C*

Treatments	Average colony diameter* (mm) at different concentrations (ug/ml)					Mean
	0	5	10	20	50	
Benlate	61.3 a	42.7 f**	36.3 e	24.0 f	0.0 a	32.9 e
Daconil	61.3 a	48.0 h	40.7 g	14.0 c	8.5 b	34.2 f
Brassicol	61.3 a	42.0 f	42.0 h	38.3 j	20.3 g	40.8 i
Captan	61.3 a	38.0 e	36 0 e	28.0 h	16.0 e	36.0 g
Dithane M – 45	61.3 a	55.0 j	39.7 f	27.0 g	13.3 c	38.7 h
Quinolate	61.3 a	50.0 i	46.7 i	38.7 k	30.7 h	45.5 j
Karathane	61.3 a	37.7 e	29.0 d	20.7 d	9.7 b	31 7 d
Morestan	61.3 a	47.0 g	42.3 h	32.7 i	13.0 d	3.93 h
Benlate + Daconil	61.3 a	32.3 c	17.7 b	0.0 a	0.0 a	22.3 b
Benlate + Brassicol	61.3 a	34.7 d	27.0 c	22.3 e	17.0 f	32.5 de
Benlate + Dithane M – 45	61.3 a	21.7 b	18.0 b	12.7 b	10.3 d	24.8 c
Benlate + Karathane	61.3 a	20.3 a	12.3 a	0.0 a	0.0 a	18.8 a
Mean	61.3 a	39.11 b	32.3 c	21.5 d	11.3 e	

* = Average of 4 replications.

** = Any two means in the same column not sharing the same letter differ significantly at 5% level of significance.

Table 2. *In vitro effect of different concentrations of fungicides on pycnidial production of Ascochyta rabiei in GSMA*

Treatment	Average number of pycnidia*/6 mm diameter mycelial disc at different fungicidal concentrations (ug/ml)					Mean
	0	5	10	20	50	
Benlate	367 a	222 b**	171 c	127 b	00 a	177.4 b
Daconil	367 a	282 e	271 i	240 h	141 e	260.2 h
Brassicol	367 a	319 h	306 j	237 j	206 i	297.0 j
Captan	367 a	305 f	256 h	222 f	122 c	254.0 g
Dithane M–45	367 a	230 e	222 f	206 e	130 d	238.0 d
Quinolate	367 a	310 g	306 j	227 g	886 g	279 0 i
Karathane	367 a	256 d	260 g	170 e	160 f	240.6 e
Morestan	367 a	322 b	306 j	270 i	220 j	297.0 j
Benlate + Daconil	367 a	215 a	155 a	00 a	00 a	147.4 a
Benlate + Brassicol	367 a	265 e	213 e	205 e	195 h	249.0 f
Benlate+Dithane M–45	367 a	215 a	200 d	180 d	105 b	213.4 c
Benlate + Karathane	367 a	226 bc	161 b	00 a	00 a	150.8 a
Mean	367 a	263.9 b	234.7 c	177.8 d	121.9 e	

* = Average of 4 replications.

** = Any two means in the same column not sharing the same letter differ significantly at 5% level of significance.

most effective fungicides (or their combinations) in reducing all the three parameters, i. e. growth, pycnidial production and spore germination were Benlate + Daconil and Benlate + Karathane, whereas Benlate + Dithane M–45 was the most effective in reducing mycelial growth and pycnidial production but intermediate in effectiveness as regards the inhibition of spore germination. Benlate exhibited intermediate effectiveness in the inhibition of mycelial growth but was the most effective in reducing pycnidial production and spore germination. Both Karathane and Benlate + Brassicol were intermediate in their effectiveness in reducing the mycelial growth, pycnidial pro-

duction and spore germination, whereas Dithane M – 45 was intermediate in its effectiveness on pycnidial production, but least effective on mycelial growth and spore germination. Similarly, Morestan was intermediate in its effectiveness for spore germination but least effective regarding mycelial growth and pycnidial production. Quinolate and Brassicol were the least effective fungicides in the inhibition of mycelial growth, pycnidial production and spore germination.

Table 3. *In vitro effect of different concentrations of fungicides on conidial germination of Ascochyta rabizi*

Treatments	Per cent spore germination* at different concentrations (ug/ml)					Mean
	0	1	5	10	20	
Benlate	85.3* a	32.3 bc**	10.3 a	0.0 a	0.0 a	25.6 ab
Daconil	85.3 a	34.6 cd	12.3 b	0.0 a	0.0 a	26.6 d
Brassicol	85.3 a	70.0 i	42.0 i	25.0 e	15.0 c	47.5 e
Captan	85.3 a	35.0 de	15.0 c	0.0 a	0.0 a	27.1 b
Dithane M – 45	85.3 a	45.0 h	30.0 h	20.0 d	8.0 b	37.9 d
Quinolate	85.3 a	78.0 j	65.0 j	52.0 j	40.0 d	64.1 f
Karathane	85.3 a	43.0 g	28.6 g	10.3 b	0.0 a	35.5 e
Morestan	85.3 a	37.0 ef	25.6 f	12.0 b	0.0 a	32.0 c
Benlate + Daconil	85.3 a	26.0 a	8.0 a	0.0 a	0.0 a	23.9 a
Benlate + Brassicol	85.3 a	38.0 f	23.0 e	16.0 c	0.0 a	32.5 c
Benlate + Dithane M – 45	85.3 a	30.0 b	28.0 fg	15.0 c	0.0 a	31.7 c
Benlate + Karathane	85.3 a	32.0 b	20.0 d	0.0 a	0.0 a	27.5 b
Mean	85.3 a	41.8 b	25.6 b	12.5 c	5.3 d	

* = Average of 3 replications.

** = Any two means in the same column not sharing the same letter differ significantly at 5% level of significance.

Some of the fungicides tested showed a different pattern of fungitoxicity on mycelial growth, pycnidial production and spore germination. For example, Daconil and Captan were the most effective in the inhibition of spore germina-

tion (Table3) but comparatively less effective to check mycelial growth (Table 1) and pycnidial production (Table 2). Similarly, Benlate was more effective in reducing pycnidial production (Table 2) and spore germination (Table 3) but less effective in the inhibition of mycelial growth (Table 1). Slower uptake, detoxification or metabolic activities of the fungus may explain the differences in fungicide tolerance among morphologically different structures such as mycelium, pycnidia and spores. Sensitivity of certain organs to fungicides might be attributed to an alteration in the fungitoxicant in their certain physiological or metabolic processes, particularly those involved in nucleic acid synthesis and respiration. Physical or metabolic transformation of a fungicide to a more or less toxic product may add or reduce the effects of the fungitoxicant on certain organs.

The comparative effectiveness of Dithane M--45, Captan, Benlate, Brassicol and Morestan (alone or in combination) in reducing mycelial growth and pycnidial production of *A. rabiei* has also been reported by Jamil (1981) and is an confirmity with the present results. The comparative effectiveness of Captan, Dithane M–45 and Brassicol in reducing spore germination has been reported by Vir and Grewal (1974). Similarly, effective spore inhibition of *A. rabiei* by Benlate, Dithane M–45 and Daconil has been reported (Anonymous, 1980).

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