Pak. J. Agri. Sci., Vol. 21 (1-2), 1984

EVALUATION OF SYSTEMIC FUNGICIDES FOR THE CONTROL OF GRAM BLIGHT

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ABSTRACT

During *in vitro* evaluation of nine systemic fungicides *Ascochyta rabiei* mycelium was the most sensitive to Tilt, TBZ, Thiophanate methyl and least sensitive, in decending order to Sicarol, Bayleton, Saprol and Afugan with an intermediate sensitivity to Benomyl and Carboxin. Tilt and TBZ completely inhibited the mycelial growth even at 5 ug/ml, whereas with Thiophanate methyl and Benomyl, complete inhibition was obtained only at 50 ug/ml dosage rate.

The most effective spray fungicides in reducing disease rating of Ascochyta blight were Tilt and TBZ, whereas the most effective fungicides in reducing per cent pod infection and per cent seed with symptom lesions of *A. rabiei* were Tilt, TBZ and Benomyl. Gram plots sprayed with TBZ, Thiophanate methyl, Benomyl, Tilt, Carboxin and Sicarol gave, respectively, 201, 196, 177, 144, 119 and 110 per cent higher grain yield over the non-sprayed control. Tilt phytotoxicity to gram flowers resulted in lower yield than that of TBZ.

INTRODUCTION

Gram blight caused by *Ascochyta rabiei* (Pass.) Lab. is typically an epidemic disease which appears almost regularly in alarming epidemics in both the barani and irrigated areas of the Punjab. The fungus infects all above ground parts of the gram plant (*Cicer arietinum* L.) i. e. foliage, stem and pods and the disease is perpetuated from season to season either through infected gram seeds or through infected plant debris lying on the surface of the fields. In some years with favourable season it causes total failure of the gram crop. This disastrous situation derives from lack of suitable resistant commercial varieties and calls for extensive search for resistant germplasm to breed stable resistant varieties for general cultivation.

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However, use of chemicals, though expensive than genetic resistance, may also be resorted to as appropriate alternative control measure for this disease, until resistant varieties become available. This paper reports on (i) the *in vitro* evaluation of systemic fungicides against the colony growth of *A. rabiei* and (ii) *in vivo* control of gram blight with systemic fungicides.

MATERIALS AND METHODS

I) *In-vitro sensitivity of A. rabiei mycelium to systemic fungicides*

The sensitivity of *A rabiei* mycelium to 5, 10, 20 and 50 ug/ml of each of the nine test fungicides was studied using a modification of Borum and Sinclair,s technique (1968). The *in-vitro* fungicidal concentrations were obtained by adding the appropriate amounts of fungicide stock solutions to autoclaved(15 p. s. i./ 15 min.) chickpea agar (Chickpea meal 20 gm, glucose 20 gm and agar agar 20 gm per 1000 ml water) cooled to about 45 C. Chickpea agar without fungicides served as control. Fifteen ml of non-amended (control) or amended chickpea agar for each treatment were poured into four, 9 cm culture plates. After the agar had solidified, 6 mm agar plugs containing *A. rabiei* mycelium were cut from 15 day-old chickpea agar culture plates using a sterile cork borer and placed in the centre of each test plate. The mean diameter of mycelial growth was recorded after 23 days. There were two runs of each experiment.

II) *Field evaluation of fungicides for the control of gram blight*

A blight susceptible local chickpea cultivar V-1108 was planted in four rows subplots (12′ x 4′ treatments) with 30 cm row to row and 15 cm plant to plant distance. There were six fungicide treatments and a non-sprayed control arranged in a four replicated randomized complete block design with 60 cm distance between treatments and 90 cm between replications. The plants were sprayed with each of the six test fungicides (Table 2) at the rate of 450 gm (a. i.)/acre, per 100 gallons of water with a hand sprayer at 15 days interval beginning at 140 days after planting (mid February). A spore suspension of *A. rabiei* (approx. 2×10^4 spores/ml) in tap water was also sprayed 24 hours after each fungicidal spray. A disease severity rating was made 20 days after the last fungicidal spray, when the plants were near physiological maturity, using

0-5 grade scale of Morral and McKenzie (1974). At maturity the pods of chickpea from individual treatments were hand picked and kept separated. A 500 pod sample from each replication of each treatment was evaluated for per cent pod infection. All the pods from each replication of each treatment were hand threshed, weighed and compared with other treatments. A sample of 500 seeds from a seed lot of each treatment was drawn and was visually evaluated for per cent seeds with symptom lesions of *A. rabiei* and compared with those from other treatments.

RESULTS AND DISCUSSION

A. rabiei varied in its *in-vitro* sensitivity to the nine fungicides tested (Table 1). There was a significant decrease in vegetative growth with increase of fungicide concentration for each fungicide. *A. rabiei* was the most sensitive to Tilt, TBZ, and Thiophanate methyl and least sensitive, in descending order, to Sicarol, Bayleton, Saprol and Afugan, with intermediate sensitivity to Benlate and Vitavax. Tilt and Thiabendazole completely inhibited the mycelial growth at 5 ug/ml, whereas with Thiophanate methyl and Benlate complete inhibition was obtained at 50 ug/ml dosage rate. The mean mycelial growth on plates containing Thiophanate methyl was significant (1% level) below that of Benomyl plates at all rates except at 50 ug/ml. There were no significant differences in mean mycelial growth between culture plates containing Bayleton, Saprol, Afugan and non-amended control except at 20 and 50 ug/ml.

Thiabendazole, Thiophanate methyl and Benomyl are chemically related (Benzimidazole) compounds which show systemic fungitoxic activity in many crop plants (Ervin, 1969; Erwin *et al.*, 1969 and Fuchs *et al.*, 1970). The significant difference between *in-vitro* growth of *A. rabiei* mycelium on agar containing Thiabendazole, Thiophanate methyl and Benomyl might be due to their differential conversion rate to MBC and its uptake by the fungus(Fuchs *et al.*, 1972). The fungitoxic conversion product of Thiophanate methyl into MBC has been reported to be affected by both fungus metabolic (Vonk aud Sijpesteijn, 1971) and plant metabolic activity (Erwin, 1973). The similar fungitoxic spectrum of three Benzimidazole fungicides *in vitro* has also been reported (Bartels-Schooley and MacNeill, 1971; Bollen, 1972). This fact, plus the fact that resistant mutants to one of the three fungicides are usually resistant to others (Bartels-Schooley and MacNeill, 1971; Hastie and Georgopoulos, 1971) suggest that they share a common mode of action, although there may be secondary sites of action (Bartels-Schooley and MacNeill,1971), that condition quantitative difference in their activity. The greater effectiveness of Thiabendazole in inhibiting mycelial growth of *A. rabiei* as compared to Benomyl and Thiophanate methyl may be attributed to such secondary sites of action. Besides, interfering with DNA synthesis or cell divisions like other Benzimidazoles, TBZ has been reported to be interfering with such secondary sites involving vitamin B-12 synthesis in which TBZ act as an antimetabolite for the precursor (Stuzenberges and Parle, 1973).

Table 1. *In vitro sensitivity of Ascochyta rabiei mycelium to various systemic fungicide concentrations in GSMA after 28 days of growth at 22 °C*

Treatments	Average colony diameter (mm) at different concentrations (ug/ml)			
	5	10	20	50
Tilt	0.0	0.0	0.0	0.0
TBZ (Thiabendazole)	0.0	0.0	0.0	0.0
Thiophanate methyl (Topsins M.)	66.0	42.0	10.0	0.0
Benlate (Benomyl)	86.0	80.0	36.0	0.0
Vitavax (Carboxin)	80 0	75.0	58.0	40.0
Sicarol (Pyracarbolid)	83.0	70.0	66.0	64.0
Bayleton (Triadimefon)	88.0	88.0	77.0	70.0
Saprol (Triforine, Cela W 524)	88.0	86.0	82.0	74.0
Afugan (Pyrazophos)	89.0	87.0	87.0	77.0
Control	90.0	90.0	90.0	90.0

LSD (0.01) between fungicides = 2.30.

LSD (0.01) between doses = 1.80.

Tilt is a new systemic fungicide with a broad spectrum of effectiveness against fungi belonging to class Ascomycetes, Basidiomycetes and Fungi Imperfecti (unpublished data). We report Tilt to be as toxic as Thiabendazole in the inhibition of mycelial growth of *A. rabiei*. Carboxin, Sicarol Bayleton, Saprol and Afugan exhibited decreasing effectiveness in the inhibition of *A. rabiei* mycelium probably because these fungicides are either specific for the control of powdery mildew fungi or rust and smut fungi or both (Nene and Thapliyal, 1979).

Although all plots showed variably blight symptoms, the plots sprayed with the test fungicides had significantly lower disease rating and lower per cent pod infections than the non-sprayed control (Table 2). The most effective fungicides in reducing disease rating were Tilt and Thiabendazole, although the effectiveness of the two was not significantly different from each other. Thiophanate methyl, Benomyl, Carboxin and Sicarol exhibited the same effectiveness in reducing the disease rating of gram blight. The most effective fungicides in reducing per cent pod infection, in desending order, were Tilt, Thiabendazole and Benomyl. There was no significant difference in per cent pod infection of plots sprayed with Benomyl and Carboxin and plots sprayed with Thiophanate methyl, Carboxin and Sicarol. Plants sprayed with Tilt were comparatively rather lush green as if nitrogen fertilizer was applied to them. However, they

Table 2. *Effect of spray of systemic fungicides on disease severity, per cent pod infection, seeds with symptom lesions of Ascochyta rabiei and grain yield*

Treatments	Disease rating	Per cent pod infection	Per cent seed infection	Average yield/ plot (12'x4) gm	Per cent yield increase over control
Tilt	1.0a*	5.5a	12.5a	917.0c	144.0
TBZ (Thiabendazole)	1.3a	10.0b	15.5a	1130.0e	201.0
Thiophanate methyl (Tospin M.)	3.0b	26.8d	24.0c	1113.0e	196.0
Benlate (Benomyl)	2.3b	21.5c	16.6ab	1039.0d	177.0
Vitavax (Carboxin)	2.8b	24.0cd	17.8b	824.0b	119.0
Sicarol (Pyracarbolid)	2.8b	25.8d	20.5bc	790.0b	110.0
Control	4.0c	73.8e	60.0d	370.0a	–

*Figures with the same letters do not differ significantly at 5% level of significance.

were slightly stunted and showed some injury to flowers which did not set seeds. Tilt induced stunting have also been found in sunflowers and broad-bean plants drenched with aqueous solution of the fungicide (Ahmad, 1983; Majid and Ilyas, 1983). Though all the plots sprayed with test fungicides remained green much longer than non-sprayed plots which died and defoliated much earlier; maturity of the plots sprayed with Tilt was delayed by 7-10 days.

Although the seeds from all fungicide treated plots had significantly lower percentage of seeds with symptom lesions of *A. rabiei.*, the most effective treatments were Tilt, Thiabendazole and Benomyl, which statistically exhibited the same effectiveness (Table 2). There was no significant difference (1% level) between Carboxin and Sicarol and between Thiophanatemethyl and Sicarol in reducing per cent seed with lesions of *A. rabiei.* The difference in the effectiveness of three benzimidazoles in reducing seed with symptom lesions may be due to the differences in the uptake of the three fungicides or their fungitoxic conversion product (MBC) by the pods of gram plants. Gram plants sprayed with Thiabendazole and Thiophanatemethyl gave significantly higher yield (201 and 196 per cent, respectively), followed by those sprayed with Benomyl, Carboxin and Sicarol which significantly gave 177, 144, 119, and 110 per cent increase over the non-sprayed control. There was no significant difference between the yield of plots sprayed with Thiabendazole and Thiophanatemethyl and between plots sprayed with Carboxin and Sicarol. Although Tilt exhibited the same effectiveness in inhibiting *A. rabiei* mycelium and decreased disease rating as Thiabendazole, its spray resulted in significantly lower yield than the plots sprayed with Thiabendazole. The reduction in the yield of Tilt sprayed plots was on account of its toxic effect on some of the flowers, which did not set pods and seeds. The toxic effect of Tilt can be avoided by reducing the dosage rate of fugicide. Although the eradication of seed-borne *A. rabiei* with Benomyl, Thiabendazole, Thiophanatemethyl and Bayleton (Triadimefon) seed treatments have been reported (Kniser *et al.*, 1973; Malik *et al.*, 1983). The effect of such treatment on increased seedling emergence and decreased blight incidence have also been reported (Kaiser *et al.*, 1973), yet there is no information available on the effect of foliar spray of these fungicides for the control of gram blight. This paper reports on the effect of TBZ, Thiabendazole, Benomyl, Carboxin, and Sicarol spray which significantly controlled the blight disease and increased yield over the non-sprayed control.

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