

PERPETUATION, TRANSMISSION AND MANAGEMENT OF CURVULARIA LEAF SPOT OF GLADIOLUS

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ABSTRACT

Systemic and non-systemic fungicides evaluated in pots as well as in field. In field, the minimum disease severity was recorded in Antracol, Score and Tilt (1.33), followed by Dithane M-45, Kavach and Topas (1.67). Moderate disease severity found in Cuman-L, Blitox and Bavistin sprayed plant (2.33). Yield data showed that the highest number of corms/plot, highest weight of the corms/plot and cormels/plot recorded in Score (58.00) (1.40 kg) (577.67) respectively. In the present studies in both the field and pot experiments Score found to be the most promising fungicide against *Curvularia trifolii* f. sp. *gladioli*. The other promising fungicides were Tilt, Kavach, Antracol and Dithane M-45. Bavistin was the least effective fungicide. The fungus *C. trifolii* f. sp. *gladioli* did not survive in pots containing sterilized soil during the off-season. Colonies of fungus *C. trifolii* f. sp. *gladioli* could not be observed from the artificially inoculated soil by dilution plate method.

INTRODUCTION

Gladiolus (*Gladiolus grandiflorus* L.) called the "Queen of bulbous flower crops" is grown in many parts of the world (Kaikal and Nauriyal, 1964). The word gladiolus originally coined by Pliny the Elder (AD 23-79) from the Latin word 'Gladius' meaning 'Sword'. So, it is also known as 'sword lily' on account of the shape of its leaves (Randhawa and Mukhopadhyay, 1986). In Europe, it is commonly called as 'corn flag' (Bose and Yadav, 1989). *Gladiolus* spp. were recognized over 2000 years ago, growing in the fields of Asia minor and were called as 'corn lilies' (Wilfret, 1980). Gladiolus introduced into cultivation at the end of sixteenth century, while it is of comparatively a recent introduction in India. Gladiolus is indeed a boon to a florist. The early-sown gladioli sell at a premium price in the market but have been found to suffer from very high incidence of Curvularia leaf spot in the North Indian plains due to high temperature and humidity prevailing at that time. The disease recorded in India in 1967 from the National Botanical Garden, Lucknow, Uttar Pradesh (Singh, 1968). It causes severe spotting of the stems, leaves, spikes, flowers and even the corms. The characteristic spots are circular and elongated on the leaves, irregular on the spikes. The colour of the spot is tan, surrounded by a darker reddish brown ring and an outer yellow halo. Black spore masses often seen on the central area of the spot. The brown or dead area is sunken. Koche et al. (2009) found that seed treatment with thiram+carbendazim (1:1) at 3 g/kg was superior in controlling the seed-borne mycoflora of soybean seed, including *Curvularia* spp. Rukhsana et al. (2010) evaluated the efficacy

of two systemic fungicides viz., Topsin and Bayleton against seed-borne fungi of sunflower and found these to be significantly effective in the elimination of these fungi. Lakpale (2011) tested efficacy of systemic fungicides Propineb (500 ppm), Hexaconazole (500 ppm) and Epoxiconazole (500 ppm) against spore germination of *C. andropogonis* and found these to be completely inhibitory to spore germination. Dandge (2012) found that Bavistin was the most effective fungicide for *C. lunata*.

Mendiola-Ela (1953) also reported that diseased plant parts left in the field return the fungus to the soil where it becomes the source of the infection. Mandokhot and Chaudhary (1980) observed that *C. clavata*, incitant of leaf spot of maize, produced microsclerotia on soil surface after about 2 months and recorded its survival up to 10 months in soil. Strider (1985) mentioned that Curvularia survives at least a year in soil. Horst (2008) reported that fungus survives in corms.

The disease reduces corm and flower production in gladiolus, it is, therefore, necessary to manage the disease in the field. Control of the disease mainly done with chemicals. Chemical control is one of the most reliable methods to control various plant diseases in an emergency. So, systemic and non-systemic fungicides evaluated in the field to find the most effective fungicide under pot and field conditions. Survival of pathogen *Curvularia trifolii* f. sp. *gladioli* not known under Indian conditions. Moisture level of the soil is likely to have influence on survival of the fungus hence studies on effect of watering frequency on perpetuation of the fungus and transmission of the disease were carried out.

MATERIALS AND METHODS

Studies on perpetuation, transmission and management of curvularia leaf spot of gladiolus caused by *Curvularia trifolii* f. sp. *gladioli* Parmelee and Luttrell carried out in the Department of Floriculture and Landscaping, Punjab Agricultural University, Ludhiana. The fungus *C. trifolii* f. sp. *gladioli* was isolated from infected gladiolus leaves. Culture of the fungus raised on potato sucrose agar medium (peeled potatoes: 250g, sucrose: 20g, agar-agar: 20g, water 1 litre). The pathogen was recovered from artificially inoculated plants and its identity re-confirmed by comparing with the original culture, which proved the pathogenic nature of the fungus. After confirmation of Koch's postulates, the fungus maintained by sub-culturing on potato sucrose agar slants. The culture was stored in incubator at $25 \pm 1^\circ\text{C}$, *Curvularia lunata* was able to grow wide temperature range $15-37^\circ\text{C}$ (Lal *et al.*, 2014) and renewed every month. The symptoms of the disease on leaves observed after 8 days of inoculation.

The cultivars categorized into resistance classes based upon severity of infection on the leaves as reported earlier (Singh and Singh 2013, Singh *et al.*, 2006): Resistant (0.00-1.00), moderately resistant ($> 1.00-2.00$), moderately susceptible ($> 2.00-3.00$), Susceptible ($> 3.00-4.00$).

Pot and field evaluation of fungicides against leaf spot caused by *Curvularia trifolii* f. sp. *gladioli*

The fungicides, which were effective in checking the growth of *C. trifolii* in vitro in earlier study (Singh *et al.*, 2006), were used in the pot and field trial to evaluate their performance under field conditions. The fungicides tested were as follows:

Sr. No	Common Name	Chemical Name	Concentration(%)
1	Cuman-L	Ziram	0.25
2	Antracol	Propineb	0.20
3	Dithane M-45	Mancozeb	0.20
4	Blitox	Copper oxychloride	0.30
5	Kavach	Chlorothalonil	0.20
6	Score	Difenoconazole	0.10
7	Tilt	Propiconazole	0.10
8	Topas	Penconazole	0.10
9	Bavistin	Carbendazim	0.10
10	Control	-	-

Pot experiment

Three corms of gladiolus variety Sancerre planted in 8-inch diameter pots in October 2012 with three replications of three pots each in a completely randomized design. Pre-inoculation sprays of the above fungicides given when the plants were 70-day-old. The plants artificially inoculated on the subsequent day using spore suspension of the fungus at 4×10^4 conidia/ml of water. High humidity in the field created for 92 h by covering the plants with a clear polythene sheet and by repeated spraying of water. One additional spray of the fungicides given at a fortnightly interval. Control plants sprayed with water. The data on severity of the disease recorded after 4 weeks of inoculation, based on a 0-4 scale, (Singh and Singh, 2013, Singh *et al.*, 2006). Yield data, in terms of corm and cormel yield, recorded at harvest of the crop.

Field experiment

Corms of gladiolus cultivar Sancerre planted in the field at

20630 cm spacing in the month of October 2012 in a randomized block design, with three replications in each of the treatments. There were 42 plants in each of the plots (1.80x1.50 sq. m.). Sprays of the above fungicides given when the plants were 70-day-old. The plants artificially inoculated on the subsequent day using spore suspension of the fungus at 4×10^4 conidia/mL of water. High humidity in the field created for 92 h by covering the plants with a clear polythene sheet and by repeated spraying of water. Two additional sprays of the fungicides given at fortnightly intervals after the onset of the disease. Control plants sprayed with water. The data on severity of the disease, based on a 0-4 scale, recorded after 6 weeks of inoculation. Yield data, in terms of corm and cormels yield, recorded at maturity of the crop.

Effect of irrigation frequency on survival of the fungus *Curvularia trifolii* f. sp. *gladioli* and disease transmission

The soil sterilized by using Formalin (2%) in the month of April 2012. The pots covered with polythene sheet to confine the fumes for effective fumigation. One-month-old culture of the fungus *C. trifolii* f. sp. *gladioli* raised on potato sucrose agar (PSA) mixed in sterilized soil in the month of May 2012 in 8-inch diameter earthen pots. Culture of the fungus collected from four test tubes (approx. 30g) added to each pot. The pots were watered at different time intervals, i.e. on 1, 2, 3, 4, 5 and 6-week schedules. A suitable control was maintained, in which pots were not be watered, except for the natural rainfall. There were four replications with four pots in each of the replicates. The number of viable spores (i.e., conidia) of the fungus in the soil were determined in the month of Oct. 2012 in each of the treatments, using dilution plate method (Johnson and Curl, 1972). Thereafter, healthy corms of gladiolus cultivar Sancerre planted in the pots and data on per cent transmission of the disease recorded.

RESULTS AND DISCUSSION

Pot evaluation of fungicides against the pathogen *Curvularia trifolii* f. sp. *gladioli*

Data of the experiment on effectiveness of fungicides in the pots shown in Table 1. All the fungicides, except Bavistin (0.1%) reduced the severity of the disease significantly over the control. The number of corms/plant also not significantly influenced by the fungicidal treatments, though Score proved better than others did. Similarly, the corm weight/plant also not significantly affected by the fungicidal treatments. The propagation of gladiolus normally done through corms and cormels. However, it is always more economical to propagate the material through the cormels. Hence, the higher number of cormels produced in some of the fungicidal treatments will help in faster and cost-effective propagation of gladiolus. The weight of cormels/plant (g) was more in all the treatments than the control, but it was not significantly different at 5 per cent level of significance.

Singh *et al.* (2006) evaluated Dithane M-45 at different concentrations against *C. trifolii* f. sp. *gladioli* and found that Dithane M-45 exhibited promising efficacy, giving complete inhibition of growth at $200 \mu\text{g/mL}$ concentrations. Thenge *et al.* (2008) observed that mancozeb at 0.2% completely inhibited growth of *C. lunata*. Sumangala *et al.* (2008) tested

Table 1: Evaluation of fungicides against the pathogen (*Curvularia trifolii* f. sp. *gladioli*) in pots

S. No.	Fungicide	Chemical Name	Concn.(%)	Infection grade* (0-4 scale)	Corm Number /plant	Corm weight/ plant(g)	Cormel number/ plant	Cormel weight/ plant(g)
1	Cuman –L	Ziram	0.25%	2.33	1.07	21.00	6.78	0.56
2	Antracol	Propineb	0.2%	2.00	1.15	19.00	6.67	0.44
3	DithaneM-45	Mancozeb	0.2%	2.00	1.18	21.11	7.11	0.56
4	Blitox	Copper oxychloride	0.3%	2.67	1.00	19.33	6.44	0.44
5	Kavach	Chlorothalonil	0.2%	2.00	1.18	19.11	7.11	0.44
6	Score	Difenoconazole	0.1%	1.67	1.26	24.45	8.00	0.45
7	Tilt	Propiconazole	0.1%	2.00	1.11	20.33	6.89	0.56
8	Topas	Penconazole	0.1%	2.00	1.07	19.44	6.67	0.44
9	Bavistin	Carbendazim	0.1%	3.00	1.04	19.89	6.56	0.44
10	Control	-	-	3.67	1.07	18.89	5.11	0.22
	C.D. 5 %	-	-	0.82	NS	NS	1.17	NS
	Correlation coeff (r)	-	-	-	-0.66	-0.48	-0.88	-0.70

Table 2: Field evaluation of fungicides against the pathogen (*Curvularia trifolii* f. sp. *gladioli*)

S.No.	Fungicide	Chemical Name	Conc.(%)	Infection grade*(0-4 scale)	Corm Number /plot	Corm weight/ plot(kg)	Cormel number /plot	Cormel weight/ plot(g)
1	Cuman –L	Ziram	0.25	2.33	54.00	1.25	471.00	49.00
2	Antracol	Propineb	0.2	1.33	55.33	1.28	489.33	50.00
3	DithaneM-45	Mancozeb	0.2	1.67	54.33	1.22	342.00	46.00
4	Blitox	Copper oxychloride	0.3	2.33	50.00	1.13	401.00	41.30
5	Kavach	Chlorothalonil	0.2	1.67	56.33	1.25	460.00	46.00
6	Score	Difenoconazole	0.1	1.33	58.00	1.40	577.67	61.67
7	Tilt	Propiconazole	0.1	1.33	57.67	1.32	512.67	53.67
8	Topas	Penconazole	0.1	1.67	52.67	1.19	301.67	37.00
9	Bavistin	Carbendazim	0.1	2.33	53.00	1.24	302.67	39.00
10	Control	-	-	3.00	47.33	0.94	217.00	22.00
	C.D. 5 %	-	-	0.99	NS	0.13	188.37	18.54
	Correlation coeff (r)	-	-	-	-0.88	-0.83	-0.70	-0.80

three non-systemic fungicides and found that mancozeb was the most effective fungicide followed by chlorothalonil at 0.2 % concentration. Falloon, 1976 found that mancozeb and thiram were inhibitory to *C. trifolii* but benomyl, chlorothalonil and carboxin gave no zone of inhibition on growth of *C. trifolii*. Gupta *et al.* (2001) evaluated fungicides against leaf spot pathogen *C. lunata* (*Cochliobolus lunatus*) in the laboratory. The fungicides tested were Dithane M-45 (mancozeb), Kavach (chlorothalonil), Blitox-50 (copper oxychloride), Bayleton (triadimefon) and Bavistin (carbendazim). The fungicide Dithane M-45 was the most effective against the fungus followed by Kavach. Singh *et al.* (2006) reported Blitox and Cuman-L to be moderately effective against *C. trifolii*. Zenghai *et al.* (2002) found that the most toxic fungicide against *C. lunata* was thiram, followed by chlorothalonil, zineb and mancozeb. Thenge *et al.* (2008) observed that Chlorothalonil (0.2%) was less effective as compared to the other fungicides against the disease. Kumar (1998) tested Dithane M-45, Kavach, Blitox, and Bavistin against foliar pathogens *in vitro*. Among all the fungicides tested Dithane M-45 proved to be the most effective and at 200 µg/mL it inhibited 84.4 per cent colony growth of *C. lunata*. The next best fungicide was Kavach, whereas Bavistin was least effective for the fungus. Grewal and Payak, 1976 evaluated *in vitro* efficacy of fungicides against *Curvularia pallescens* and found that Dithane M-45, Difolatan and

Dithane Z-78 were effective against the pathogen. Arun and Tomar, (2005) evaluated the efficacy of several fungicides at a 2% concentration, against leaf spot disease of mung bean caused by *Curvularia lunata* (*Cochliobolus lunatus*). They found that Cuman was the most effective treatments in pot conditions. Saikia (1982) reported that Cuman completely checked growth of *C. eragrostidis*.

Jiang *et al.* (2004) reported that Score was highly inhibitory to *C. lunata*, whereas Tilt had less effect than the former fungicide. Sumangala *et al.* (2008) evaluated systemic fungicides and reported that maximum inhibition of mycelial growth obtained from difenoconazole and propiconazole at 0.1 % concentration. Olufolaji, (1996) reported efficacy of the mancozeb (Dithane M-45) and propiconazole (Tilt) against *C. cymbopogonis*, causal agent of leaf spot of sugarcane. *In vitro* tests showed that the fungicides were toxic to the pathogen at various concentrations and at different developmental stages. Propiconazole prevented germination up to 36 h after inoculation at 0.5% (5g/litre). Propiconazole inhibited mycelial growth and sporulation at up to 3-5 g/litre. Mancozeb did not give disease control *in vitro*. At 5 g/litre, propiconazole totally inhibited mycelial growth and spore formation. Singh *et al.*, (2006) reported that Score, Topas, and Tilt exhibited promising efficacy, giving complete inhibition of growth at 10, 10, and 10 µg/mL concentrations, respectively against *C. trifolii*. Rajak and Pandey (1987) reported that carbendazim provided best

inhibition of *C. lunata* causing soft rot of snake gourd but Subrahmanya *et al.* (1988) reported Bavistin (carbendazim) was less effective against *C. lunata* on sorghum seed stored under different conditions. Similarly, Singh *et al.* (1997) also found that carbendazim was least effective for *Curvularia* leaf spot of brinjal. Kamal *et al.* (2001) reported that Bavistin was ineffective against *C. lunata*. Singh *et al.* (2006) also reported in their study that Bavistin was the least effective fungicide against *C. trifolii*. Deshpande (1993) reported that Carbendazim at 200 ppm was effective against *C. lunata*. Joshi and Hegde, (1977) observed synergistic effect of fungicides against *C. lunata*. Maximum synergism was apparent between Bavistin at 20 ppm, followed by the same fungicides at 10 ppm. Singh *et al.* (2006) reported that Antracol exhibited promising efficacy at different concentrations against *C. trifolii*, giving complete inhibition of growth at 200, 200, 10, 10 and 10 µg/mL concentrations.

Field evaluation of fungicides against the pathogen (*Curvularia trifolii* f. sp. *gladioli*)

Data of the experiment on field efficacy of fungicides shown in Table 2. The minimum disease severity was recorded in Antracol, Score and Tilt (1.33), followed by Dithane M-45, Kavach and Topas (1.67). Moderate disease severity found in Cuman-L, Blitox and Bavistin sprayed plant (2.33). The maximum disease severity observed in the control (3.00).

It is evident from yield parameter, viz. number of corms, weight of corms, number of cormels and weight of cormels shows that higher disease severity resulted in lesser yield of corms and cormels (both number and weight). The correlation was high (ranging from -0.70 to -0.88) for the yield parameter. Magie, (1948, 1949 and 1963) reported that Dithane was effective in preventing *Curvularia* infection. Magie and Price, (1975) could control *Curvularia* leaf spot of gladiolus through weekly sprayings of mancozeb. Misra (1978) reported best control of *C. trifolii*, on gladioli cv. Glory, with fortnightly sprays of Dithane M-45 (mancozeb) at 0.2% concentration. Gadage and Patil, (1977) tested fungicides against *Curvularia* leaf spot of cotton and found that Dithane M-45 was effective when used as a curative fungicide. Arshad *et al.* (2009) observed that Dithane M-45 was effective against grain discoloration disease of rice. Gadage and Patil, (1977) tested fungicides against *Curvularia* leaf spot of cotton. In the prophylactic spray, the fungicides effective in order of their merit were Blitox and Ziram. In the curative spray, the fungicides found to be effective were Blitox and Ziram. Singh *et al.* (1997) found that *Curvularia* leaf spot of brinjal was significantly controlled with mancozeb + thiophanate-methyl; followed by chlorothalonil (Kavach) and thiophanate-methyl (Topsin-M). Moore, (1979) mentioned that foliar applications of fungicides chlorothalonil (daconil) and benzimidazole were effective against *C. trifolii*. Kumar, (1998) tested Dithane M-45, Kavach, Blitox, and Bavistin against foliar pathogens. In the testing under field conditions (*in vivo*), Dithane M-45 at 0.20 per cent concentration proved to be the best against *C. lunata* and it totally inhibited the disease in the field. The other effective fungicides were Kavach and Blitox-50. However, Bavistin was the least effective against these leaf spot fungi but Arshad *et al.* (2009) observed that carbendazim found to be the best against grain discoloration disease of rice. Arun and Tomar (2005) evaluated the efficacy

of several fungicides at a 2% concentration, against leaf spot disease of mung bean caused by *Curvularia lunata* (*Cochliobolus lunatus*). They found that Cuman was the most effective treatments in field conditions. Shakir *et al.* (1998) also reported that Topsin-M was effective against *C. lunata*.

In the present studies in both the field and pot experiments Score found to be the most promising fungicide against *Curvularia trifolii* f. sp. *gladioli*. The other promising fungicides were Tilt, Kavach, Antracol and Dithane M-45. Bavistin was the least effective fungicide.

Effect of irrigation frequency on survival of the fungus *Curvularia trifolii* f. sp. *gladioli* and disease transmission

The fungus *C. trifolii* f. sp. *gladioli* did not survive in pots containing sterilized soil during the off-season. Colonies of fungus *C. trifolii* f. sp. *gladioli* could not be observed from the artificially inoculated soil by dilution plate method. Loss of spores of the test fungus from soil may be due to their disintegration. It shows that the fungus does not survive in soil under natural rainfall conditions and at different irrigation frequency levels. In the present study, the experiment conducted in pots. Since the temperature in the soil in the pots becomes high during the hot summers, it may be affecting their viability. Disease transmission also not observed during the crop season as none of the plants infected with *C. trifolii* f. sp. *gladioli*. Amin and Abudalla (1980) also reported similar findings that the fungus *C. lunata* var. *aeria* disappeared from the soil samples after six weeks of inoculation, irrespective of soil amendments. Kilpatrick, (1958) isolated *C. trifolii* from over-wintered clover leaves and observed that in nature the fungus apparently survives until leaves decompose. Mendiola, (1953) reported that diseased plant parts left in the field return the fungus to the soil where it becomes the source of the contamination.

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