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Evaluation of fungicides against phyllosphere mycoflora of foliage plants

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ABSTRACT : Five fungicides representative of sulphur group were selected for evaluation of controlling mycelial growth against the fungal forms present on the phyllosphere. Firstly the study was made to screen a range of fungicides for their ability to inhibit mycelial growth of pathogens in vitro, secondly the aim was to determine the efficacy of some of these fungicides against four *Fusarium sps.* found in the phyllosphere of foliage plants. All the fungicides were tested for efficacy against the isolates cultured on the solid PDA's medium and Richard's liquid medium. Study also focused on the effect of experimental fugicides carbendazim and selected anlongs against specific phyllosphere fungi. Physical parameters are used for suppression of fungal spores germination and effect of mycelial growth of *F. pallidospermum* in order to develop standarised conditions that were required in invitro fungicides evolutions. These fungicides had not been for activity against *Fusarium sps*.

Keywords : Fungicides, Mycelial growth, phyllosphere, *Fasarium sps*

INTRODUCTION

Fungicides have become more popular these days. Everyday some new fungicides are being introduced and evaluated in the various plant pathological laboratories. Their application in the practical fields can only be suggested against the virulent pathogens after a successful laboratory evaluation. It therefore, needs a constant watch and effort to evolve new fungicides along with some important non-chemical methods of controlling the diseases. A primary study with different fungicides was made to evaluate them in laboratory against all the fungal forms. The most effective fungicides were also considered to be used as pre-inoculation and post-incoluation dip treatment against the fungi. Basically fungicides are chemical compounds or biological organism use to kill or inhibit mycelial growth or fungal spore. The most common active ingredient is sulphur, present at 0.08% in weaker concentration and as high as 0.5 to 1.5% for more potent fungicides. A fungicide must be co-exist with the cells of the living tissue and must exhibit a selectivity which discriminates between the living tissue of the host and of the pathogen (Crowdy, 1970). The selectively is measured inturnof a therapeutic index. Therapeutic index is calculated by the dividing the minimum curative dose by the maximum tolereated (by host) dose. However, a single therapeutic index is assigned to each therapeutant due to different susceptibilities of the different plant parts to the toxic damage (Diamond, 1962).

The fungicides are also apply to the aerial parts of the plant to control an air borne epidemic which usually develop rapidly. The applications are made according to the pre determine schedule so as to cover all potentially infective parts of the plant. Systemic fungicide by virtue of penetration and improved distribution suffer less surface weathering and consequently so better disease control (Evans, 1971).

A fungicide frequently is given different names by different commercial firms. It becomes, therefore, very difficult for a person working with a fungicides to remember these names and also to try to find out the active inredients present in commercial formulations. The carbamet (organic sulpher) fungicides form a very important group among fungicides. Most of these are foliage fungicides, while some are used for soil and seed treatment, Tisdale, and Flenner, (1942) first demonstrated the fungicidal possibilities of the carbonates in 1931 in the laboratories of E.I. Du Pont company, the U.S.A. but the commercial production started about a decade later.

MATERIALS AND METHODS

The vital role of fungicides have been enriching the various disciplines of plant pathology. Its role in modernizing the agriculture in India has been with in great detail by Mehta (1971). For the last six decades, production of new fungicides and their application has gained considerable momentum. The more virulent fungal pathogen are posing a challenge and therefore, there is a constant need to evolve new and more efficient fungicides. According to the latest information available, about 200 fungicides are in the world market and many more are under test and trial.

Due to the higher cost of chemicals, the cost of production of various fungicides is also increasing. It is therefore, essential that wastage is avoided and the performance is evaluated in the laboratory before being advised for field trial. The precision of the more highly standardized laboratory test is greater between the laboratory tests and the field tests. So, it is essential to screen out the efficiency of the fungicides in the laboratory.

A standardized method for evaluating protectant chemicals was suggested in 1943 by a committee on standardization of the fungicidal test of the American

phytopathological society (committee on standardization of fungicides test 1943). The method involves application of fungicides to chemically clean glass slide by means of a precision technique such as settling tower or horizontal sprayer. For this purpose we used glasswares were chemically cleaned and stored in dustproof condition. They were also cleaned with potassium dichromate sulphuric acid cleaning solution followed by washing in clean distilled water. This is then suitably sterilized and stored for later use. The glass slides used are the standared (x1") size.

A standared solid Potato Dextrose Medium and liquid Richard's medium are used. Potato Dextrose Agar Medium was prepared in flask and sterilized. To this medium was added the requisite quantity of fungicide (Mancozeb). So as to get certain final concentration. A series of concentration was prepared. The fungicide was thoroughly mixed by stirring. The medium was then poured into petriplates and stored in the refrigerator till required. A culture of the test fungus was grown on PDA for a certain period (generally 7 days) at the optimum temperature for growth. Small disc (0.7 cm) of the centre was out with a sterilized cork borer and transferred asoptically in the centre of a petridish contraining the medium with a certain amount of fungicides. Suitable checks kept where the culture disc are grown under the same condition on PDA without fungicides. The fungus colony diameter measured every 24 hours. The colony diameter, compared with check, has taken as a measured of fungi toxicity. Same process was repeated for other fungicides *i.e.*, Carbendazim, Ziram, Thiram, Maneb, for observing different fungus growth.

The data obtained on the effect of different concentration of the test chemical on the germination or on growth were plotted on a graph-paper to obtain a dosage response curve (DR curves). The dosage response curve graph papers either on ordinary graph paper, semi-log paper on log problem graph papers. Usually for DR cruves ordinary graph papers are used and hence not suitable for statistical analysis.

Frequently however relative fungi toxicity was measured as the minimum concentration at which no growth or germination was obtained. This is called minimum inhibitory concentration (m.i.c.).

Liquid medium such as Richard's medium was also used for observing effecting of fungicides in vitro. Isolates from any fungus which grow well on a liquid can be used as the test fungus were kept at suitable temperature for incubation of the rating has done on 1-10 scale (Gottlieto *et. al.*, 1950) or the actual dry weight of the fungus has taken after filtering the liquid out. The data which were observed in the present study are based on different concentration of fungicides and five different incubation period. After incubation at 25 ± 1°C for 4 days in an alternate light and dark regime of 12 hrs. growth inhibition of the pathogens is calculated.

RESULT

In the present course of investigation fungal forms isolated fromthe phyllosphere of (05) foliage plants were as follows : 10 on Agaloenema pictum, 11 on Diffenbechia picta, 11 on Dracaena marignata, 12 on Maranta cherrymeri and 11 on Sensieviera trifasicata which were evaluated by five different types of fungicides *viz.*, Carbendazim, Mancozeb, Maneb, Thiram and Ziram at three different concentration in vitro. The efficacy of fungicides was tested by challenging selected fungal form (*Fusarium sps.*) of phyllosphere fungi in vitro using conc. of 0.5%, 1.0% and 1.5% of fungicides.

From the comparative study of mycelial growth in the two medium (*i.e.*, liquid and solid medium) in table iit is clear that the rate of growth was higher in 10 days for all the four sps. *Fusarium* (*viz.*, *F. equset, F. moniliformae, F. pallidospermum and F. solan*). In 5 days the rate of mycelial growth was low in comparison to 10 days on both the medium. The minimum growth was observed in 15 ddays for all 4 sps. of *Fusarium.*

Thus from the observation given in the Table 1 it is quite clear that the dry weight of mycelium were maximum in 10 days. On the contrary the dry weight of mycelium were minimum in 15 days and in 5 days the weight of mycelium were in between 10 and 15 days.

Table 1 : Showing growth rate of four *Fusarium sps.* on two medium *i.e.*, Richard's medium and PDA medium.

Richard's medium					PDA medium			
Organisms	Mycelial dry weight in mg			Presence in days in the medium	Mycelial dry weight in mg			Presence in days in the medium
	5 days	10 days	15 days		5 days	10 days	15 days	
F. equseti	52.50	95.30	32.50	1-9	42.30	78.35	28.50	1-9
F. moniliformae	45.30	88.55	26.95	1-8	41.30	65.55	33.60	1-8
F. Pallidospermum	51.50	94.30	27.00	1-7	45.65	68.50	22.30	1-7
F. Solani	40.30	71.55	11.15	1-7	38.40	72.35	27.35	1-7

Thus from the above observation it becomes clear that the most effective fungicides were Thiram and Ziram Macozeb was the second best. The efficacy of these three fungicides were also examined under laboratory conditions. Observations were taken at a regular interval of time and the results obtained have been presented by Histogram. Carbendazim showed a broad spectrum of fungitoxic activity being effective againt four sps. of *Furasium*.

The minimum effective concentration of the most effective fungicides as recorded during the present course of investigation was also determined in the same was as employed above. The result obtained with the give fungicides *viz.*, Carbendazim, Maneb, Macozeb, Thiram and Ziram have been presented in the Table 2 and 3.

Five fungicides *viz*., Carbendazim, Mancozeb, Maneb, Thiram and Ziram at concentration of 25, 50, 100, 150, 250, 500 and 750 µg/ml each were evaluated to study their effect on spore germination of *Fusrium pallidospermum*. This result are presented in Table 2 and 3.

Table 2 : Showing effect of different fungicides on spore germination of *Fusarium pallidospermum.*

SI.No.	Fungicides	Germination percentage in concentration (µg/ml)							
		25	50	100	150	200	250	500	750
1.	Carbendazim (Bavistin 50 wp)	28	23	9	0.0	0.0			
2.	Mancozeb (Indofil M-45)	62	53	42	35	28	22	0.0	0.0
3.	Maneb	76	67	60	52	45	28	12	0.0
4.	Thiram	84	75	68	56	48	30	15	6
5.	Ziram	86	79	71	59	51	35	19	10
6.	Control	90							

Table 3 : Showing effect of different fungicides on mycelial growth of *Fusarium pallidospermum*.

SI.No.	Fungicides	Percent of inhibition concentration ppm								
		200	250	300	500	700	1000	1250	1500	Mean
1.	Carbendazim	90.6 (72.2)**	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	98.8 (87.7)
2.	Mancozeb	60.2 (50.6)	64.1 (53.2)	66.8 (54.8)	79.8 (54.6)	90.7 (72.3)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	82.7 (69.5)
3.	Maneb	44.4 (41.7)	46.0 (42.7)	46.3 42.9)	66.0 54.3)	77.6 (61.7)	88.8 (70.5)	100.0 (90.0)	100.0 (90.0)	71.1 (67.7)
4.	Thiram	32.5 (34.7)	43.2 (41.12)	51.3 (45.7)	71.0 (57.4)	75.6 (60.4)	86.1 (68.1)	92.7 (72.3)	100.0 (90.0)	69.0 (59.0)
5.	Ziram	25.2 (30.1)	34.8 (36.1)	41.4 (40.0)	62.2 (52.0)	69.1 (56.2)	77.3 (61.5)	83.7 (66.2)	87.3 (69.1)	60.1 (51.4)
	Mean	**50.6 (45.9)**	**57.6 (52.6)**	**61.2 (54.7)**	**75.8 (61.7)**	**82.6 (68.1)**	**90.4 (76.0)**	**95.3 (82.1)**	**97.4 (85.8)**	

	S.Em ±	CD at 5%
Treatment (Fungicides) (A)	0.1	0.5
Concentration (B)	0.2	0.6
Treatment (A) × (B)	0.5	1.5

*Each value is an average of three replication. **Value given in parenthesis are after angular transformation control uas 90.68 m.m.

It is evident from data in tables that all the treatments were found effective in suppression of germination of *Fusarium pallidospermum* at variable concentration. Among the fungicides carbendazim was found most effective and 100 percent inhibition was obtained at 15 ppm whereas Mancozeb gave the similar results at 500 ppm. Maneb and Thiram showed some what similar magnitude of inhibition. Ziram was found least effective in suppression of spore germination.

DISCUSSION

Phyllosphere mycoflora forecaste the possible incidence of diseases to the plants and hence considered the importance of these aspects diseases of foliage plants in nursery and gardens have been recorded. The phyllosphere of a plant does not remain a constant figure throughout the year which is quite evident from month wise study. From such a study the impact of changing season and meteorological conditions on the phyllosphere complex becomes more and more clear. Preece and Dickison (1971) have summarized the work concerning phyllosphere (phylloplane) fungi and have laid emphasis over spore trapping mechanism by leaves as well as the landing of spores on the leaf surfaces.

From the persual of the present investigation it has become evident that the phyllosphere mycoflora of foliage plants (*i.e.*, Agaloenema, Diffenbechia, Dracaena, Maranta, Philodendron, and Sensieviera) consists of a large variety

of fungal forms, of which some are pathogenic causing diseases. Though the pathogenic forms remain over the leaf surfaces they fail to cause any damage till the leaves reaches to the scenescene stage. Saprophytic activities of micro-organisms on leaf surfaces have been described by last (1955). Kerling (1958, 64), Lehman (1950), Cross (1963), Last and Deighton (1965). Sinha (1964), Leben and Daft (1967), Dickinson (1967), Sharma and Sinha (1968), Sinha (1971), Prasad (1976), Jha (1977) and Jamil (1998).

The aim of this study was, firstly to screen a range of fungicides including new chemical formulations for their ability to inhibit mycelial growth in vitro : secondly, the aim was determine the efficacy of some of these fungicides againstthe present isolates, live fungicides (*i.e.*, Carbendazim, Thiram, Mancozeb, Maneb, Ziram) representative of sulpher groups were selected for in-vitro evaluation against the responsible pathogens. These fungicides were significantly inhibited mycelial growth at different concentration and incubation period respectively.

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