



In Vitro Inhibitory Effect of Sprint Against Fungal Pathogens.

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ABSTRACT

The in vitro activity of sprint (Carbendazim+Mancozeb) were examined against pathogens viz., *Alternaria solani*, *Colletotrichum lindemuthianum*, *Curvularia lunata*, *Fusarium moniliforme*, *Fusarium oxysporum*, *Fusarium solani*, *Helminthosporium sativum*, *Rhizoctonia solani* causing different vegetable diseases. The MIC values of Carbendazim+Mancozeb fungicide against eight pathogenic fungi of different vegetables were varied and recorded in the range of 50 µg/ml to 3000 µg/ml. The effect of sprint fungicide on the growth rate of mycelium of eight fungal pathogens of different vegetables was most significant ranging from 49.06 to 73.92%. The in-vitro results clearly indicate that, Carbendazim+Mancozeb (Mix fungicide) was most effective as it completely inhibited the radial growth averagely 71.59%.

I. INTRODUCTION

Many fungi have been identified by various workers as causal organism of fungal rot diseases in all parts of the world. Phytopathogenic fungi are living organisms responsible for nearly half of known diseases in crop plants. *Fusarium* spp., are among the most important plant pathogens in the world and are highly variable because of their genetic makeup and changes in environment in which they grow causing morphological changes [10]. Chemical control measures have been tested and found effective in the control of diseases [13,15]. Resistance to systematic fungicides in certain fungi had also been recorded by many workers [6]. Use of modern fungicides greatly contributed to reducing damage caused by a variety of diseases and to increasing not only yields but also quality of crops. The effectiveness of fungicides depends on many factors, e.g. the climatic conditions, type of the product and its active substance and time and method of application [16,8].

Understanding the biology of fungicide resistance, how it develops, and how it can be managed is crucial for ensuring sustainable disease control with fungicides. The benzimidazole fungicide carbendazim was used for the control of many diseases caused by Deuteromycetous

pathogens [4], owing to its systemic properties and its great efficacy in controlling plant diseases. Use of modern fungicides greatly contributed to reducing damage caused by a variety of diseases and to increasing not only yields but also quality of crops. The aim of this work was to examine the effect of fungicide sprint (Carbendazim 25%+Mancozeb 50%) on the growth of phytopathogens.

II. MATERIAL METHODS

Preparation of test fungicide:

One fungicide namely sprint was selected to evaluate its effect on different fungi. The required dilutions of that fungicide were prepared by taking the active ingredient. The poison food technique [9] was adopted in the experiment. The principle involved in this technique is to supplement the nutrient medium with a toxic chemical and then allowing a test fungus to grow on the medium and evaluate the effect of such chemical by measuring the growth of the fungus.

Fungicidal assay:

Prepare 1 lit PDA by using 1000ml water, 200gms potatoes, 20gms Dextrose and 2gms Agar-Agar. 200gm of peeled were cut into 2-3 cm cubes and then were

thoroughly washed with tap water twice to remove the dirt. Then equal quantity of water was added and kept on gas burner to extract the starch, after the extract of the starch it was filtered through muslin cloth and was made upto 1 litres by adding distilled water, then 20gm of dextrose and 20gm of Agar-Agar was added until a homogenous solution was formed. After autoclaving the media, this media was used for further studies, using desired concentration of the fungicides in vitro studies (10ml of the sterile PDA+ 2ml of different concentration of fungicide). Control treatment was maintained without adding any fungicide to the medium. For fungal inoculation agar plugs having fungal isolates with 6 mm diameter taken from 7days old cultures were placed in the centre of each petriplate. Three replications were maintained for each concentration. These plates were incubated at 25±1°C. After incubation for nine days at room temperature, radial growth was measured when fungus attained maximum growth in control plates. The efficacies of the fungicides were expressed as percent inhibition of mycelial growth over control, which was calculated by using the formula [18].

$$I = \frac{C - T}{C} \times 100$$

Where, I = per cent inhibition

C = Colony diameter in control

T = Colony diameter in treatment.

III. RESULTS AND DISCUSSION

The MIC values of Carbendazim+Mancozeb fungicide against eight pathogenic fungi of different vegetables were varied and recorded in the range of 50 µg/ml to 3000 µg/ml (Table3). The pathogen, *F. moniliforme*, *F. oxysporum*, *F. solani*, *R. solani* were found to be most susceptible and revealed MIC values at 50 µg/ml and 60 µg/ml (Table 1) respectively. Whereas *C. lindemuthianum* was found to be most resistant and showed MIC value at 3000 µg/ml (Table 2). While *C. lunata*, *A. solani*, *H. sativum* were inhibited significantly at MIC values- 600 µg/ml, 1500 µg/ml, 2000 µg/ml (Table 2).

The effect of Carbendazim+Mancozeb fungicide on the growth rate of mycelium of eight fungal pathogens of different vegetables was most significant ranging from 49.06 to 73.92% (Table 1 and Table 2). The percent inhibition of mycelial growth of *H. sativum*, *A. solani* and *C. lunata* were found to be maximum 73.92%, 68.58% and 67.16% respectively, among all tested concentrations. While the percent inhibition of mycelial growth of *F. moniliforme*, *F. oxysporum* and *R. solani* were found to be significant

Table 1. Inhibitory effect of Sprint on the mycelial growth of targeted fungi

Pathogen	Control	Growth rate in (mm) and percent inhibition of mycelial growth at various concentration in (µg/ml)						Mean of % inhibition
		I						
		10	20	30	40	50	60	
F. m	89.6	90 (0.4)	46.6 (47.9)	24.6 (72.5)	10 (88.8)	06 (93.3)		66.03±0.79
F. o	89.6	90 (0.4)	49.3 (44.9)	39.3 (56.1)	14.3 (84)	11 (87.7)	06 (93.3)	60.68±0.34
F. s	89.6	81.3 (0.9)	43.3 (51.6)	15.6 (82.5)	11.3 (87.3)	10.6 (88.1)	08 (91)	51.73±0.14
R. s	89.6	88.6 (0.1)	56.6 (36.8)	43.3 (51.6)	21.3 (76.2)	08 (91)	06 (93.3)	58.16±0.67

Mean diameter of mycelial growth in mm at varied concentration (µg/ml) and figure in parenthesis represents percent inhibition of mycelia growth at varied concentration Where F. m= *Fusarium moniliforme*, F. o= *Fusarium oxysporum*, F. s= *Fusarium solani*, R. s= *Rhizoctonia solani*.

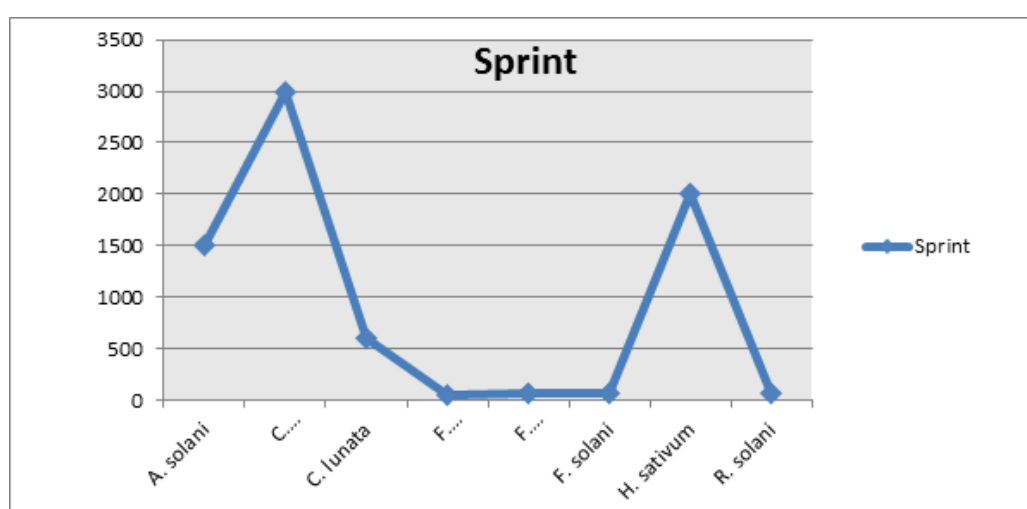
Table 2. Inhibitory effect of Carbendazim+Mancozeb on the mycelial growth of targeted fungi.

Pathogen	Contrl	Growth rate in (mm) and percent inhibition of mycelial growth at various concentration in (µg/ml)															Mean of % inhibition
		II															
		100	200	300	400	500	600	1000	1100	1200	1300	1400	1500	2000	2500	3000	
A. s	89.6								38 (57.5)	34.6 (61.3)	34.6 (61.3)	27.3 (69.5)	06 (93.3)				68.58±66
Col. l								76 (15.1)						50 (44.1)	43.3 (51.6)	06 (93.3)	1.21
Cur. l	89.6	70 (21.8)	30.3 (66.1)	26.6 (70.3)	23.3 (73.9)	20 (77.6)	06 (93.3)						52.6 (41.2)				49.06±
H. s	89.6					41.6 (53.5)		30.6 (65.8)									1.93
	89.6												14 (84.3)	07 (92.1)			67.16±
																	0.74
																	73.92±
																	0.98

Mean diameter of mycelial growth in mm at varied concentration (µg/ml) and figure in parenthesis represents percent inhibition of mycelia growth at varied concentration. Where A. s= *Alternaria solani*, Col. l= *Colletotrichum lindemuthianum*, Cur. l= *Curvularia lunata*, H. s= *Helminthosporium sativum*,

Table 3. MIC of fungicide sprint against plant pathogenic fungi in µg/ml.

Pathogens	Sprint
<i>Alternaria solani</i>	1500
<i>Colletotrichum lindemuthianum</i>	3000
<i>Curvularia lunata</i>	600
<i>Fusarium moniliforme</i>	
<i>Fusarium oxysporum</i>	50
<i>Fusarium solani</i>	60
<i>Helminthosporium sativum</i>	60
<i>Rhizoctonia solani</i>	2000
	60

**Figure 1.** MIC of fungicide sprint against pathogens of vegetables.

Fungicides are important tools for managing diseases in many crops. Unlike insecticides and some herbicides which kill established insects or weeds, fungicides are

most commonly applied to protect healthy plants from infection by fungal plant pathogens. Chemical control measures have been tested and found effective in the

control of diseases [13,15]. Certain protective fungicides although hazardous to environment are still used for the control of fungal diseases [11,17,18]. Fungicides may act on or interrupt the metabolic system of the pathogen [1].

The effectiveness of a fungicide depends on its innate toxicity, permeation, and other factors like climate conditions, type of the product and its active substances and time and method of applications. To be effective, fungicides must be applied before infection become established and in a sufficient spray volume to achieve thorough coverage to the plant or treated area. The protection of fungicide is temporary, because they are subjected to weathering and breakdown over time. They also must be reapplied to protect new growth when disease threatens. Poor disease control with fungicides can be used from several causes including insufficient application rate inherently low effectiveness application method and excessive rainfall.

One of the common problems in the chemical control of plant pathogens is the ability of these pathogens to develop resistance [3,5,12]. Fungicide resistance will develop faster if the initial frequency of resistant genes in a pathogen population is greater, if selection for those genes in the presence of fungicide is stronger, or if selection against resistance genes in the absence of fungicide is weaker. Resistance management programs rely on reducing selection pressure by keeping disease pressure low, applying fungicides in mixtures or alteration with fungicide from a different mode of action group, and limiting the number of application per crop season. Fungicides are often combined in mixture for three main reasons: i) to widen the spectrum of antifungal activity to control several diseases occur simultaneously in a crop; ii) to exploit additive and synergistic interactions between fungicides, by which the overall activity is increased and the concentrations of the compounds can be reduced without loss of activity and, iii) to delay the selection process of resistant individuals in a pathogen population to one component of the mixture. In the present study, fungicides used in mixture are Carbendazim+Mancozeb (Table 2). Similar findings were recorded by Harlapur [7] revealed that mancozeb @ 0.25 per cent found most effective in inhibiting the growth of *E. turcicum*. Propiconazole @ 0.1 per cent, carboxin power @ 0.1 per cent and zineb @ 0.25 per cent were found equally effective which can

be used as an alternative to mancozeb. The use of Benomyl, Carbendazim and Mancozeb significantly inhibited *Physoderma maydis* on maize (Brown spot) reported by Osunlaja[14] and there was complete inhibition of sporangia germination at 10,000 ppm i.e. of the fungicides. Carbendazim and Carbendazim + Mancozeb gave 100 % inhibition of mycelial growth of *F. solani* at 0.2 and 0.3% concentrations [2]. The present work revealed that the selected fungicides were potent inhibitors with the minimum inhibitory concentration (MIC) of these fungicides against eight fungal pathogens which were isolated from different vegetables.

IV. CONCLUSION

Carbendazim+Mancozeb revealed average efficacy of 71.59% against all targeted fungi. The minimum inhibitory concentration (MIC) of fungicide against all fungal pathogens was found to be variable. In Carbendazim+Mancozeb assay, MIC value ranges from 50 µg/ml to 3000 µg/ml and among the targeted pathogens, *H. sativum* was highly susceptible and revealed MIC at 2000 µg/ml, while *C. lindemuthianum* was highly resistant and inhibited at 3000 µg/ml. From the present work it is concluded that fungicide spray inhibited the fungal growth invitro and in the further invivo studies it will be used.

V. REFERENCES

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