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EFFICACY OF MIXED FUNGICIDE AGAINST TEN PATHOGENIC FUNGI OF LEAFY VEGETABLES

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Abstract:-Objective: Leafy vegetables account for around 60% of the total vegetable production in the country and prone to several fungal diseases most commonly causing leaf spot and wilting. Therefore wiser use of pesticides will include reduce application rates, identifying new composition and treatment to combat the development of resistance in the pathogens.
Aim: The aim of the present work is to evaluate Captan 70% +Hexaconazole 5% WP a Mixed fungicide against ten pathogenic fungi of leafy vegetables.
Material & methods: The Poisoned food technique was applied for the In-vitro fungicidal assessment and percent inhibition of mycelia growth over the control was calculated. For In-vivo study each leafy vegetable was sown in 12 X 24 M plots in the field. After each ten days of interval, 200 ml of spore suspension of each targeted plant pathogenic fungi was mixed in the soil of respective filed. After 7 days diseases symptoms were developed on the leafy vegetables. Afterwards defined Minimum inhibitory concentration (MIC) in µg/ml of Captan 70% + Hexaconazole 5% WP from tested in-vitro results was sprayed onto the infected leafy vegetables. Finally efficacy of applied mixed fungicide was evaluated by calculating the Percent disease incidence (PDI) and Percent disease reduction (PDR) over control.
Result: In-vitro Captan 70% + Hexaconazole 5% WP revealed 86.36% of inhibition of mycelia growth against all targeted fungal pathogens. MIC of mixed fungicide to all fungal pathogens varied from 1000 µg/ml to 6000 µg/ml. While In-vivo studies revealed Captan 70% + Hexaconazole 5% WP was highly effective in controlling disease incidence as well as 66.68% percent reduction (PDR) against all treated leafy vegetables.
Conclusion: The use of mixed fungicide is very effective against fungal diseases of Leafy vegetables in the field condition as they showed maximum percent diseases reduction (PDR). Therefore such mixed pesticide will include reduce application rates, identifying new composition and treatment to combat the new emerging fungal diseases.

Keywords: Captan 70% + Hexaconazole 5% WP, MIC, leafy vegetables, PDI and PDR.

INTRODUCTION

Out of acknowledge plant flora, near about 30,000 plant diseases are recorded all over the world. Out of which, 5000 diseases are present in India. Therefore on an average each crop plant can be affected by hundred or more than hundred diseases (Agriose, 1997). The current production level is over 90 MT and the total area under vegetable cultivation is around 6.2 million hectares which is about 3% of the total area under cultivation in the country. Leafy vegetables account for around 60% of the total vegetable production in the country and are prone to several fungal diseases most commonly causing leaf spot and wilting. Due to these diseases annually billions of rupees loss occurs throughout the country, though 74% of Indian population is engaged in agriculture.

To control these diseases, the pesticidal compounds being widely used throughout the world which on contrary increasing the agricultural production with increasing pesticide concentration. Older pesticides are eliminated from market due to regulatory changes and new pesticides are becoming expensive, so there is a need to find out more wise way for the safest use of pesticides. The development of new physiological race pathogens to

many of the systemic fungicides is gradually becoming ineffective (Wellman, 1977.) In India, disease control by systemic and non systemic fungicides has become more and more common over past two decades. Presently more than 140 chemical compounds have been registered and sold in the different formulation. Resistance developed by pathogen to fungicides has rendered certain fungicide ineffective (Zhonghau and Michailides, 2005). Thus several broad spectrum mixed fungicides and bacteriocides are recommended for controlling fungal and bacterial diseases respectively. So the use of pesticides has been increasing steadily at an annual rate of about 14 percent since the mid 1950s (Agriose, 1997).

Material & Methods :

I) In-vitro:

For the assessment of in-vitro fungicidal assay, Poisoned food technique (Nene and Thapliyal, 1982) was used. The required concentration of Captan 70% + Hexaconazole 5% WP (mixed fungicide) were prepared as parts per million (ppm) in µg/ml ratio with sterilized double distilled water. Out of this standard concentration, 5 ml of mixed fungicide concentration was taken and added to 45 ml sterilized PDA medium and mixed well. Afterwards PDA medium with fungicide concentration was transferred equally into two sterilized Petri plates and media was allowed to solidify. After complete solidification of the medium, 4 mm diameter disc of 5-7 days old culture of targeted fungi was taken and inoculated into the center of Petri plates in complete aseptic condition. The PDA medium containing Petri plate without fungicide concentration was served as a control. Then all the Petri plates were incubated at 28 ± 2 °C for incubation period and radial growth of colony was measured after 3rd day upto 7th day constantly. The results of mycelial growth were expressed as mean of triplicate. The concentration of Captan 70% + Hexaconazole 5% WP at which the pathogen showed complete inhibition of its mycelia growth was considered as minimum inhibitory concentration (MIC) of fungicide to respective pathogen and percent inhibition of mycelia growth over control was calculated by the formula given by Vincent (1947).

$$I = \frac{100\ (C-T)}{C}$$

Where I = Inhibition of mycelial growth.
C = Mycelial growth in control
T = Mycelial growth in treated.

ii) In vivo:

For the assessment of fungicidal assay at field condition, sowing of each leafy vegetable was carried out in 12×24 m plot in the field. After 10 days of interval, 200 ml spore suspension of each targeted plant pathogenic fungi was mixed in the soil of the field respectively. After 7 days of duration, the diseases symptoms were developed on the leaves of leafy vegetables. Afterwards required minimum inhibitory concentration (MIC) in µg/ml of Captan 70% + Hexaconazole 5% WP from in vitro results were selected for in-vivo study. The define concentration of mixed fungicide was sprayed directly onto the infected leafy vegetables. The fungicide treatment was applied twice at an interval of 10 days for all leafy vegetables. In all cases, leafy vegetable without fungicide treatment served as control and tagged. Simultaneously all treated leafy vegetables were also tagged with respect to tested concentrations. After 10 days of treatment, among each treated leafy vegetable plants, the total number of leaves on each plant and total number of infected leaves on each plant were counted and average in triplicate was recorded. The effectiveness of each fungicide was evaluated by calculating the Percent Diseases Incidence (PDI) and Percent Diseases Reduction (PDR) over control by using following formula,

$$PDI = \frac{\text{Number of diseased leaves on each plant}}{\text{Total number of leaves on each plant}} \times 100$$

And

$$PDR = \frac{\text{PDI in control} - \text{PDI in treatment}}{\text{PDI in control}} \times 100$$

Result:

1) In-vitro Mancozeb assay:

Captan 70% + Hexaconazole 5% WP a Mixed fungicide was tested for the assessment of fungicidal efficacy

and determination of their Minimum Inhibitory Concentration (MIC) against ten different pathogenic fungi of leafy vegetables such as Alternaria brassicae, Alternaria carthami, Alternaria humicola, Collectotrichum lindemuthianum, Fusarium moniliforme, Fusarium oxysporum, Fusarium roseum, Helminthosporium sativum, Pullularia pullulans and Stemphylium verruculosum. The radial growth of the fungal pathogen was recorded as a mean of three replicates at each tested concentration and percent inhibition of mycelial growth over control was tabulated (Table 2). The lowest concentration which showed complete inhibition of mycelial growth was considered as Minimum Inhibitory Concentration (MIC) of fungicide to particular pathogen (Table 1).

The MIC values of Mixed fungicide against ten pathogenic fungi of leafy vegetables were varied from 1000 µg/ml to 6000 µg/ml (Table 1). The pathogen, S. verruculosum, P. pullulans and A. carthami were found to be most susceptible and revealed MIC values at 1000 µg/ml, 2000 µg/ml and 2000 µg/ml respectively. Where as H. sativum and A. brassicae were found to be most resistant and showed MIC values both at 6000 µg/ml. While F. roseum, F. moniliforme, A. humicola, F. oxysporum and C. lindemuthianum were inhibited significantly at MIC values – 2500 µg/ml, 2500 µg/ml, 3500 µg/ml, 3500 µg/ml and 5500 µg/ml (Table 1).

The effect of Mixed fungicide on the growth rate of mycelium of ten fungal pathogens of leafy vegetables was most significant (Table 2). The percent inhibition of mycelial growth of H. sativum, F. moniliforme and P. pullulans were found to be maximum, 92.92%, 89.54% and 88.09% respectively, among all tested concentrations. While the percent inhibition of mycelial growth of A. carthami, F. roseum, F. oxysporum and A. humicola were to be significant as 85.44%, 85.23%, 81.96%, and 81.18% respectively. On contrary, S. verruculosum, A. brassicae and C. lindemuthianum revealed least percent inhibition of mycelial growth as 80.14%, 80.03% and 79.02% (Table .2).

Table : 1. MIC of Captan 70% + Hexaconazole 5% WP against plant pathogenic fungi in µg/ml.

Pathogen	Mancozeb
Alternaria brassicae	6000*
Alternaria carthami	2000
Alternaria humicola	3500
Collectotrichum lindemuthianum	5500
Fusarium moniliforme	2500
Fusarium oxysporum	3500
Fusarium roseum	2500
Helminthosprium sativum	6000
Pullularia pullulans	2000
Stemphylium verruculosum	1000

* All values expressed in mean of three replicates.

Table : 7. Inhibitory effect of Captan+ Hexaconazole on the mycelial growth of targeted fungi.

Pathogen	Control	Growth rate (mm) and percent inhibition of mycelail growth at various concentration in µg/ml												Mean of % inhibition
		500	1000	1500	2000	2500	3000	3500	4000	4500	5000	5500	6000	
A.b.	81	47 (41.47)	28 (65.43)	24 (70.37)	18 (77.77)	16 (80.24)	14 (82.71)	12 (85.18)	11 (86.41)	10 (87.65)	08 (90.12)	06 (92.59)	-	80.03±0.79
A.c.	71	24 (66.19)	07 (90.14)	- (100)										85.44±0.85
A.h.	82	29 (64.63)	24 (70.73)	20 (75.60)	16 (80.42)	11 (86.58)	08 (90.24)	- (100)						
C.l.	88	51 (42.04)	29 (67.04)	25 (71.59)	23 (73.86)	19 (78.40)	17 (80.68)	12 (86.36)	10 (88.63)	09 (89.77)	08 (90.90)	-		81.18±1.24
F.m	88	18 (79.54)	12 (86.36)	09 (89.77)	07 (92.04)	- (100)						(100)		79.02±1.41
F.o.	84	32 (61.90)	22 (73.80)	18 (78.57)	15 (82.14)	11 (86.90)	08 (90.47)	- (100)						89.54±0.94
F.r.	84	22 (73.80)	19 (77.38)	12 (85.71)	09 (89.28)	- (100)								81.96±0.73
H.s.	90	34 (62.22)	22 (75.55)	17 (81.11)	15 (83.33)	13 (85.55)	12 (86.66)	11 (87.77)	10 (88.88)	09 (90)	09 (90)			85.23±0.71
P.p.	84	22 (73.80)	11 (86.90)	07 (91.66)	- (100)							08 (91.11)		92.92±1.05
S.v.	68	27 (60.29)	- (100)										- (100)	88.09±0.95
														80.14±0.76

* Mean diameter of mycelial growth in mm at varied concentration (µg/ml) and figure in parenthesis represents percent inhibition of mycelial growth at varied concentration. Where A.b. = A. Brassicae, A.c.= A. carthami, A.h.= A. humicola, C.l.= C. lindemuthianum, F.m.= F. moniliforme, F.o.= F. oxysporum, F.r. = F. roseum, H.s.= H.sativum, P.p.= P. pulluans, S.v.= S. verruculosum.

B) In vivo Captan + Hexaconazole assay:

For the assessment of fungicidal efficacy in vivo, MIC values in µg/ml of Captan 70% + Hexaconazole 5% WP from the in-vitro test were used. These concentrations were directly sprayed onto the leafy vegetable plants twice after 10 days of interval and after 30 days of treatment the effectiveness of fungicide were recorded as percent disease incidence (PDI) and percent disease reduction (PDR) (Table 3).

The effectiveness of different fungicidal treatments on percent disease incidence (PDI) and percent disease reduction (PDR) revealed that mixed fungicide was highly effective in controlling the disease incidence. The percent of disease reduction with Captan 70% + Hexaconazole 5% WP treatment was potent, averagely as a 66.68% (Table 3).

Table : 3. In-vivo effect of Captan + Hexaconazole on leafy vegetable disease reduction.

Leafy vegetables	Control		Treated		PDI in control	PDI in treated	PDR (%
	No. of infected leaf	Total no. of leaf	No. of Infected leaf	Total no. of leaf			
A. gaevolens	8	16	1	16	50	6.25	87.50
B. oleraceae	5	9	2	9	55.55	22.22	60
C. tinctorius	6	15	3	16	40	18.75	53.12
C. esculanta	3	5	1	6	60	16.66	72.23
C. sativum	6	19	3	19	31.57	15.78	50.01
R. vesicarious	9	29	4	28	31.03	14.28	53.98
S. oleracia	5	14	1	12	35.71	8.33	76.67
T. foenum- graecum	5	12	1	12	41.66	8.33	80

All values of mean of triplicate; where PDI = Percent diseases incidence and PDR = Percent diseases reduction.

DISCUSSION:

In preliminary assessment, Captan + Hexaconazole was tested for their fungitoxicity against ten fungal pathogens of leafy vegetables namely, A. brassicae, A. carthami, A. humicola, C. lindemuthianum, F. moniliforme, F. oxysporum, F. roseum, H. sativum, P. pullulans, S. verruculosum. Fungi are regarded as one of the chief causative agents of plant diseases (Cambell et al. 2000). Among the pathogens, S. verruculosum, P. pullulans and A. carthami were found to be most susceptible on contrary, H. sativum and A. brassicae were found to be most resistant. Similar work was previously reported by several workers (Tu and Jarvis, 1979; Ravishanker and Mamatha, 2005; Harlapur et al., 2007). In the present vitro study, Captan+hexaconazole was found to be most effective one as it completely inhibited the radial growth averagely 86.36% of all tested pathogens. Similar finding was recorded by Pandu et al. (1986) and Xiujian et al., (2000) also reported fungicidal efficacy of carbendazim, captan, benomyl, triademefon, propicanzole. The fungicide Mancozeb and Captan being recommended for management of diseases like seedling blight of A. falcataria (Srivastava and Soni, 1993), leaf spot diseases of Populus deltoids caused by Alternaria alternata (Dey and Debata, 2000); leaf spot and blight of Syzygium cumini caused by Cylindrocladium quinqueseptatum (Mehrotra and Mehrotra, 2000) followed by Rodomil and Bayleton were effective against F. solani.

In the present investigation, it was recorded that there were variation in Minimum inhibitory concentration (MIC) of Captan+hexaconazole against ten fungal pathogens of leafy vegetables. The MIC values of Mixed fungicide fungicide against ten pathogenic fungi of leafy vegetables were varied from 1000 µg/ml to 6000 µg/ml (Table 1). The pathogen, S. verruculosum, P. pullulans and A. carthami were found to be most sensitive and revealed MIC values at 1000 µg/ml and 2000 µg/ml except H. sativum and A. brassicae which showed high resistant at 6000 µg/ml. Bains and Mohan (1982) reported that heterogeneous population of resistant and sensitive nuclei in the isolate might be responsible for variation in the MIC of fungicides. Similarly variation in sensitivity and resistant of different fungal pathogens to fungicides was reported by several workers (Dekker and Gielink, 1979; Jones and Ehret, 1981; Gangawane and Saler, 1981).

Captan+hexaconazole in the field condition for reducing the diseases incidence on leafy vegetables, and showed average disease reduction i.e 66.68% at field condition. Captan+hexaconazole were efficient at the field condition to control leaf spot and blight of Michelia champaca caused by Rhizoctonia spp. (Mehrotra, 1992) and post emerging damping off Eucalyptus hybrid by Verticillium sp.(Harsh et al. 1992). Siddaramaiah et al. (1980 b) have

recommended Bavistin and Banlate for managing Cercospora leaf spot of Carthamus tinctorius which agree with our results.

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