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FUNGICIDAL EFFICACY ON MYCOSIS CONTROL OF FIVE LEAFY VEGETABLE DISEASES

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ABSTRACT

In the present study, *in vitro* potential of three different fungicides like Carbendazim (Systemic fungicide), Mancozeb (Non systemic fungicide) and Captan+Hexaconazole (Mix fungicide) was evaluated against five fungal pathogens of leafy vegetables such as *Alternaria carthami, Alternaria humicola, Fusarium oxysporum, Fusarium roseum, Pullularia pullulans* by Poisoned food technique. Simultaneously the Minimum inhibitory concentration (MIC) of respective fungicides to all five targeted fungal pathogens was also calculated. Out of tested fungicides, Captan+Hexaconazole (Mix fungicide) had marked significant inhibitory effect as it completely inhibited the radial growth averagely 84.38% of all five targeted fungal pathogens in relation to their controls. Whereas remaining, Mancozeb and Carbendazim both were also effective and inhibited the radial growth of all targeted fungal pathogens averagely as 57.01% and 47.59%.

KEY WORDS: Mycosis control, Captan+hexaconazole, Mancozeb, MIC, *Pullularia pullulans*

India has achieved adequate source and good degree of stability of vegetable crop production. Among them leafy vegetables are most essential component of our diet which nourishes with nutrients, minerals and vitamins. For healthy diet, daily minimum consumption should be about 180 g per head, whereas the present consumption of leafy vegetables is less than 20 g per head. Therefore there is an urgent need to explore and cultivate leafy vegetables in India; even though India stand second largest producer followed by China. 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. In India, out of total production, leafy vegetables are prone to several fungal diseases most commonly causing leaf spot and wilting. Annually billions of rupees loss occurs throughout the country due to these diseases, though 74% of Indian population is engaged in agriculture. So plant diseases control or elimination is paramountly important to all concerns. To control these diseases, the pesticidal compounds being widely used throughout the world which on contrary increasing the agricultural production with increasing pesticide concentration. But the future role of pesticide use in agriculture is increasing threatened by several factors like development of resistance in pathogens, accumulation of toxic compounds and loss in food safety. As 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. Thus several broad spectrum 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). Wiser use of pesticides will include reduce application rates, identifying new composition and treatment to combat the development of resistance in the pathogens. In view of this, an *in vitro* study was carried out to find out more effective fungicides for controlling mycosis in leafy vegetables.

MATERIALS AND METHODS

Pathogens

Five plant pathogenic fungi were isolated from five leafy vegetables namely *Brassica oleracea* L., *Carthamus tinctorius* L., *Colocasia esculanta* L., *Rumex vesicarius* L., *Trigonella foenum-graecum* L. The infected leafy vegetables plant parts were separately collected in sterilized polythene bags and were brought in the laboratory. Afterwards, these infected plant part from the advancing margin of lesions were cut into small pieces (2-5mm) and kept in sterile Petri plates separately. The pieces were dipped into 0.1% Mercuric Chloride ($HgCl_2$) solution

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for about one minute. These pieces were transferred to Petri plates containing sterile double distilled water to free them from the chemical trace and saprophytic microorganisms if any. After washing, 2-4 pieces were placed at equal distance on a fresh solidified Potato Dextrose Agar (PDA) medium plates in aseptic condition with the help of sterile forceps and the plates were incubated at 25 $\pm$ 3 °C for 3-7 days. After the incubation period, the fungal cultures were examined under microscope and correctly identified by using standard literature.

Among the different isolated plant pathogenic fungi, only five fungi i.e *Alternaria carthami, Alternaria humicola, Fusarium oxysporum, Fusarium roseum, Pullularia pullulans* were selected as target pathogens. All these five selected pathogens were purified by subculturing them in fresh PDA plates in triplicates for further study.

Fungicides

Three Different types of fungicides were purchased from the Paras Agency, Mondha Market, Aurangabad. Out of these, Carbendazim (Systemic fungicide), Mancozeb (Non systemic fungicide) and Captan+Hexaconazole (Mix fungicide) were tested for their efficacy to control the different leafy vegetable plant pathogens.

Fungicidal Assay

For the assessment of *in-vitro* fungicidal assay, Poisoned food technique (Nene and Thapliyal, 1982) was used. The required concentration of 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 each 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 in the center of Petri plates in complete aseptic condition. The PDA medium containing Petri plate without fungicide concentration was served as 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 fungicide 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.

RERULTS

The MIC values of Carbendazim fungicide against five pathogenic fungi of leafy vegetables were varied from 90 µg/ml to 700 µg/ml in which, *P. pullulans* and *F. roseum* were found to be most susceptible and revealed MIC values at 90 µg/ml with maximum 50.89% and 58.82% inhibition of its mycelial growth. On contrary, *A. humicola* was found to be most resistant and showed MIC values at 700 µg/ml with 36.41% inhibition of its mycelial growth (Table 1 and 2).

The MIC values of Mancozeb fungicide against five pathogenic fungi of leafy vegetables were in the range of 3000 µg/ml to 8500 µg/ml in which, *A. carthami* was found to be most sensitive to Mancozeb and revealed MIC values at 3000 µg/ml with 48.18% maximum percent inhibition of its mycelial growth. Whereas, *F. roseum* was found to be most resistant and showed MIC values at 8500 µg/ml with 59.65% inhibition of its mycelial growth (Table 1 and 3).

The MIC values of Captan+Hexaconazole mixed fungicide against five pathogenic fungi of leafy vegetables were also varied in the range of 2000 µg/ml to 3500 µg/ml. Among targeted pathogens, *P. pullulans* and *A. cathami* were found to be most sensitive and revealed MIC values at 2000 µg/ml with 88.09% and 85.44% maximum percent inhibition its mycelial growth. Whereas, *F. oxysporum* was found to be most resistant and showed MIC values at 3500 µg/ml with 81.96% inhibition of its mycelial growth (Table 1 and 4).

These *in vitro* results clearly indicates that, Captan+Hexaconazole which was mixed fungicide was more effective as it completely inhibited the radial growth averagely 84.38% of all five targeted fungal pathogens than Mancozeb and Carbendazim which both were significantly effective & inhibited the radial growth of all targeted fungal pathogens averagely as 57.01% and 47.59%.

Table 1: MIC of various fungicides against plant pathogenic fungi in µg/ml

Pathogen	Carbendazim	Mancozeb	Captan+Hexaconazole
Alternaria carthami	500	3000	2000
Alternaria humicola	700	4500	3500
Fusarium oxysporum	100	3500	3500
Fusarium roseum	90	8500	2500
Pullularia pullulans	90	6000	2000

* All values expressed in mean of three replicates.

DISCUSSION

In the present study, three different fungicides were tested for their fungitoxicity against five fungal pathogens of leafy vegetables namely, *Alternaria carthami, Alternaria humicola, Fusarium oxysporum, Fusarium roseum, Pullularia pullulans*. Fungi are regarded as one of the chief causative agents of plant diseases (Cambell et al., 2000). Among pathogens, *P. pullulans* revealed more susceptibility while *F. roseum* showed most resistance against all three tested fungicides. Similar susceptibility and resistance in the pathogens was previously reported by several workers (Ravishanker and Mamatha, 2005; Harlapur et al., 2007). In the present *in vitro* study, Captan+hexaconazole was found to be most effective one as it completely inhibited the radial growth averagely 84.38% of all five targeted pathogens. While Mancozeb and Carbendazim was significantly effective and revealed averagely 57.01% & 47.59% inhibition of all five targeted fungal pathogens. Similar finding was recorded by Tiwari et al. (1988) and reported that significant efficacy of Mancozeb in reducing the growth of *C. paradoxa*. Pandu et al. (1986) and Xiujian et al., (2000) also reported fungicidal efficacy of carbendazim, captan, benomyl, triademefon, propicanzole and suggested that systemic fungicide were more effective than non systemic fungicide against *C. fimbriata* Eillis and Halsted. Vijaya et al. (2007) also reported that carbendazim, propiconazole (systemic fungicides) and captan, mancozeb (non systemic fungicides) were more effective at 0.05% and 0.1% concentration against *Ceratocystis paradoxa.* 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 study, it was recorded that there were variation in Minimum inhibitory concentration (MIC) of fungicides against five fungal pathogens of leafy vegetables. The MIC of all tested fungicides, ranges from 90-8500 µg/ml. Separately carbendazim revealed MIC in the range of 90-700 µg/ml against all targeted fungal pathogens. 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. Therefore Mancozeb revealed MIC ranging from 3000-8500 µg/ml against five tested pathogens of leafy vegetables in which *A. carthami* was most susceptible pathogens. It indicates that Mancozeb was significantly

Table 2: Inhibitory effect of Carbendazim on the mycelial growth of targeted fungi

Pathogens	Control	Growth rate (mm) and percent inhibition of mycelail growth at various concentration in µg/ml												Mean of % inhibition
		I						II						
		20	40	60	80	90	100	200	300	400	500	600	700	
A.c.	78	74 (5.12)	68 (12.82)	62 (20.21)	55 (34.61)	51 (34.61)	47 (39.74)	33 (57.69)	18 (76.89)	09 (88.46)	- (100)			39.57±0.90
A.h.	81	78 (3.70)	74 (8.06)	68 (16.04)	64 (20.28)	61 (24.69)	59 (27.16)	48 (40.74)	35 (56.79)	27 (66.66)	17 (79.01)	09 (88.88)	- (100)	36.41±0.79
F.o.	75	63 (16)	48 (36)	31 (58.66)	14 (74.66)	10 (86.66)	- (100)							52.26±0.89
F.r.	68	46 (32.35)	34 (50)	22 (67.64)	11 (83.82)	- (100)								58.82±1.05
P.p.	74	60 (18.19)	48 (35.13)	29 (60.81)	12 (83.78)	- (100)								50.89±0.84

* 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.c.= A. carthami, A.h.= A. humicola, F.o.= F. oxysporum, F.r. = F. roseum, P.p.= P. pulluans.*

Table 3 :Inhibitory effect of Mancozeb 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	8500	
A.c.	46	40 (13.04)	35 (23.91)	33 (28.26)	22 (52.17)	13 (71.73)	- (100)								48.18 ±0.84
A.h.	63	37 (41.26)	31 (50.79)	26 (58.73)	24 (61.90)	21 (66.66)	18 (71.42)	16 (74.03)	14 (77.77)	- (100)					66.95±0.87
F.o.	89	83 (6.74)	73 (17.97)	60 (32.58)	34 (61.79)	23 (74.57)	13 (85.39)	- (100)							54.14±0.97
F.r.	88	72 (18.18)	59 (32.95)	54 (38.36)	50 (43.18)	48 (45.45)	46 (47.72)	41 (53.40)	38 (56.81)	35 (60.22)	32 (63.63)	29 (67.04)	27 (69.31)	- (100)	59.65±1.41
P.p.	88	70 (20.45)	64 (27.27)	56 (36.36)	49 (44.31)	44 (50)	40 (54.54)	37 (57.95)	34 (61.36)	30 (65.90)	22 (75)	17 (80.68)	- (100)		56.15±1.24

Table 4: 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	
A.c.	71	24	07	-					85.44±0.85
		(66.19)	(90.14)	(100)					
A.h.	82	29	24	20	16	11	08	-	
		(64.63)	(70.73)	(75.60)	(80.42)	(86.58)	(90.24)	(100)	81.18±1.24
F.o.	84	32	22	18	15	11	08	-	
		(61.90)	(73.80)	(78.57)	(82.14)	(86.90)	(90.47)	(100)	81.96±0.73
F.r.	84	22	19	12	09	-			
		(73.80)	(77.38)	(85.71)	(89.28)	(100)			85.23±0.71
P.p.	84	22	11	07	-				
		(73.80)	(86.90)	(91.66)	(100)				88.09±0.95

Note for Table 3 & 4 :* 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.c. = A. carthami, A.h. = A. humicola, F.o. = F. oxysporum, F.r. = F. roseum, P.p. = P. pulluans.*

effective against those fungal pathogen which showed resistant against carbendazim. Similarly captan+ hexaconazole fungicide revealed MIC in the range of 2000-3500 µg/ml in which *P. pullulans* and *A. carthami* were most sensitive and *F. oxysporum* was most resistant pathogens. 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; Nasreen, 1982).Therefore it was concluded that Captan+Hexaconazole (Mixed fungicide) was more effective against fungal diseases of leafy vegetables than the others.

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