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Mycosis Control of Leafy Vegetables by Chemotherapy

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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 brassicae, Collectotrichum lindemuthianum, Fusarium moniliforme, Helminthosporium sativum, Stemphylium verruculosum* 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 84.33% of all five targeted fungal pathogens in relation to their controls. Whereas remaining, Mancozeb and Carbendazim both were significantly effective and inhibited the radial growth of all targeted fungal pathogens averagely as 60.52% and 54.4%.

Key Words: Mycosis control, Chemotherapy, Captan+Hexaconazole (Mixed fungicide), MIC

Introduction

India has achieved self sufficiency 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; where as 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. 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. 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. So the use of pesticides has been increasing steadily at an annual rate of about 14 percent since the mid 1950s [1]. 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 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 fresh solidified Potato Dextrose Agar (PDA) medium plates in aseptic condition with the help of sterile foreceps. Then the plates were incubated at 25 $\pm$ 3 ^{o}C for 3-7 days and examined daily for the growth occurrence of the fungus. 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 such as *Alternaria brassicae, Collectotrichum lindemuthianum, Fusarium moniliforme, Helminthosporium sativum* and *Stemphylium verruculosum* 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 [10] 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 petri plate containing PDA medium without fungicide concentration was served as control. Then all the Petri plates were incubated at 28 ± 2 ^{o}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 [17].

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

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

Results

The MIC values of carbendazim fungicide against five pathogenic fungi of leafy vegetables were varied from 40 µg/ml to 12,000 µg/ml in which, *C. lindemuthianum* was found to be most susceptible and revealed MIC values at 40 µg/ml with maximum 80.07% inhibition of its mycelial growth. On contrary, *H. sativum* was found to be most resistant and showed MIC values at 12,000 µg/ml with 38.24% inhibition of myceliel growth (Table. 1 and 2).

The MIC values of mancozeb fungicide against five pathogenic fungi of leafy vegetables were in the range of 1000 µg/ml to 6500 µg/ml in which, *H. sativum* was found to be most sensitive to mancozeb and revealed MIC values at 1000 µg/ml with 81.39% maximum percent inhibition of its mycelial growth. Whereas, *S. verruculosum* was found to be most resistant and showed MIC values at 6500 µg/ml with 59.70% inhibition of mycelia 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 1000 µg/ml to 6000 µg/ml. Among targeted pathogens, *S. verruculosum* was found to be most sensitive and revealed MIC values at 1000 µg/ml with 80.14% maximum percent inhibition its mycelial growth. Whereas, *H. sativum* was found to be most resistant and showed MIC values at 6000 µg/ml with 92.92% inhibition of mycelia growth (Table. 1 and4).

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

Pathogen	Carbendazim	Mancozeb	Captan+Hexaconazole
Alternaria brassicae	*1000	5000	6000
Collectotrichum lindemuthianum	40	6000	5500
Fusarium moniliforme	80	4500	2500
Helminthosprium sativum	12000	1000	6000
Stemphylium verruculosum	300	6500	1000

* All values expressed in mean of three replicates

Table : 2. Inhibitory effect of carbendazim 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						II									III	
		20	40	60	80	90	100	200	300	400	500	600	700	800	900	1000	12000	
A.b.	84	*80 (4.76)	75 (10.71)	72 (17.85)	64 (23.80)	63 (25)	62 (26.10)	54 (37.71)	48 (42.85)	42 (50)	33 (60.71)	24 (71.42)	13 (84.52)	10 (88.09)	07 (91.66)	- (100)		40.52±0.74
C.l.	64	24 (62.50)	19 (70.31)	08 (87.50)	- (100)													80.07±0.55
F.m	75	59 (21.33)	42 (44)	21 (72)	- (100)													52.99±0.94
H.s.	84										80 (4.76)					78 (7.14)	- (100)	38.24±0.75
S.v.	72						46 (36.11)	29 (73.61)	- (100)									60.18±0.87

* 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, C.l.= C. lindemuthianum, F.m.= F. moniliforme, H.s.= H.sativum, S.v.= S. verruculosum.*

Table : 3 Inhibitory effect of mancozeb on the mycelial growth of targeted fungi

Pathogen	Cont	Growth rate (mm) and percent inhibition of mycelial growth at various concentration in µg/ml													Mean of % Inhibition
		500	1000	1500	2000	2500	3000	3500	4000	4500	5000	5500	6000	8500	
A.b.	67	34 (49.25)	30 (55.22)	26 (61.19)	22 (67.16)	18 (71.64)	14 (85.07)	08 (88.05)	06 (91.04)	- (100)					74.77 ±0.73
C.l.	72	57 (20.83)	54 (25)	52 (27.77)	39 (45.83)	37 (48.16)	35 (51.38)	33 (54.16)	32 (55.55)	29 (59.72)	26 (63.88)	16 (77.77)	- (100)		52.54±1.11
F.m	89	81 (8.98)	78 (12.35)	74 (16.85)	69 (22.47)	66 (25.84)	60 (32.58)	52 (41.57)	47 (47.19)	- (100)					
H.s.	86	32 (62.79)	- (100)												34.20 ±0.98
S.v.	59	38 (35.59)	35 (40.37)	33 (44.06)	29 (50.84)	27 (54.33)	26 (55.93)	25 (57.62)	23 (61.01)	22 (62.71)	20 (66.10)	18 (69.49)	13 (77.96)	- (100)	81.39±0.40
															59.70±1.37

* 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, C.l.= C. lindemuthianum, F.m.= F. moniliforme, H.s.= H.sativum, S.v.= S. verruculosum.*

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	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)	- (100)	80.03±0.79
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)	- (100)		79.02±1.41
F.m	88	18 (79.54)	12 (86.36)	09 (89.77)	07 (92.04)	- (100)							- (100)	89.54±0.94
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)	08 (91.11)		92.92±1.05
S.v.	68	27 (60.29)	- (100)											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, C.l.= C. lindemuthianum, F.m.= F. moniliforme, H.s.= H.sativum, S.v.= S. verruculosum.*

These *in vitro* results clearly indicates that, captan+hexaconazole which was mixed fungicide was more effective as it completely inhibited the radial growth averagely 84.33% of all five targeted fungal pathogens than mancozeb and carbendazim which both were significantly effective and inhibited the radial growth of all targeted fungal pathogens averagely as 60.52% and 54.4%.

Discussion

In the present study, three different fungicide were tested for their fungitoxicity against five fungal pathogens of leafy vegetables namely, *A. brassicae, C. lindemuthianum, F. moniliforme, H. sativum, S. verruculosum*. Fungi are regarded as one of the chief causative agents of plant diseases [3]. Among all tested fungicides the pathogen *F. moniliforme* was found to be most susceptible against tested fungicides. Whereas *H. sativum* was most resistant against tested fungicides. Similar work was previously reported by several workers [12 and 7]. In the present *vitro* study, captan+hexaconazole was found to be most effective one as it completely inhibited the radial growth averagely 84.33% of tested pathogens. While mancozeb and carbendazim was significantly effective and revealed averagely 60.52% and 54.20% inhibition of all five tested fungal pathogens. Significant efficacy of Mancozeb in reducing the growth of *C. paradoxa* [15]. 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 [11and18]. Carbendazim, propiconazole from systemic fungicide and captan, mancozeb from non systemic fungicide were more effective at 0.05% and 0.1% concentration against *Ceratocystis paradoxa* [16]. The fungicide mancozeb and captan being recommended for management of diseases like seedling blight of *A. falcataria* [14], leaf spot diseases of *Populus deltoids* caused by *Alternaria alternata* [5]; leaf spot

and blight of *Syzygium cumini* caused by *Cylindrocladium quinqueseptatum* [9] 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 fungicide against five fungal pathogens of leafy vegetables. The MIC of all tested fungicides, ranges from 40-12000 µg/ml. Separately carbendazim revealed MIC in the range of 40-1000 µg/ml against all targeted fungal pathogens except *H. satvium* which showed high resistant at 12000 µg/ml. Heterogeneous population of resistant and sensitive nuclei in the isolate might be responsible for variation in the MIC of fungicides [2]. Therefore, mancozeb revealed MIC ranging from 1000-6500 µg/ml against five tested pathogens of leafy vegetables in which *H. satvium* was most susceptible pathogens. It indicates that mancozeb was significantly effective against that fungal pathogen which showed resistant against carbendazim. Similarly captan+hexaconazole fungicide revealed MIC in the range of 1000-6000 µg/ml in which *S. verruculosum* was most sensitive and *H. sativum* and *A. brassicae* were most resistant pathogens. Similarly variation in sensitivity and resistant of different fungal pathogens to fungicides was reported by several workers [4,8,6 and13].

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