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Efficacy of methyl benzimidazole-2-yl carbamate against pathogenic fungi of leafy vegetables

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ABSTRACT

India has achieved self sufficiency and good degree of stability of vegetable crop production. Leafy vegetables are most essential component of our diet which nourishes with nutrients, minerals and vitamins. The aim of the present work is to evaluate methyl benzimidazole-2-yl carbamate against 10 pathogenic fungi of leafy vegetables *in-vitro* and *in-vivo*. The Poisoned food technique was applied for *in-vitro* fungicides assessment and percent inhibition of mycelia growth over control was calculated. For in-vivo study each leafy vegetable sown in 12×24 M plots in the field. After 10 day of interval, 200 ml spore suspension of each targeted plant pathogenic fungi was mixed in the soil of the respectively field. After 7 days, the diseases symptoms were developed on the leaves of vegetables. Afterwards Minimum Inhibitory Concentration (MIC) in µg/ml of Carbendazim from *in vitro* results was selected for *in- vivo* study. The defined concentrations of Methyl benzimidazole-2-yl carbamate was sprayed directly onto the infected leafy vegetables. Efficacy of each fungicide was evaluated by calculating the Percent Diseases Incidence (PDI) and Percent Diseases Reduction (PDR) over control. *In-vitro* Carbendazim revealed 50.99% inhibition of mycelia growth against all of targeted fungal pathogens. MIC of Carbendazim to all fungal pathogens varied from 40-12000 µg/ml. *In vivo* studies, Carbendazim was highly effective in controlling disease incidence. The percent disease reduction (PDR) with Carbendazim treatment was maximum 84.64% in all treated leafy vegetables. The use of Carbendazim is very effective against pathogenic fungi of *C. esculanta, S. oleracia* and *T. foenumgraecum* in the field condition as they showed complete percent disease reduction (PDR).

INTRODUCTION

India has achieved self sufficiency and good degree of stability of vegetable crop production. 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. Vegetables being more succulent and rich in nutrients are prone to variety of diseases right from the sowing to marketing, thereby increasing yield losses during pre and post production periods. 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 (Harlapur et al., 2007). Therefore, there is an urgent need to explore and cultivate leafy vegetables in India; even though India stand second largest producer followed by China. To control these diseases, the pesticides 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 potent fungicides for the safe use. The development of new physiological race pathogens to many of the systemic fungicides is gradually becoming ineffective (Wellman, 1977). Thus several broad spectrum fungicides are recommended for controlling fungal diseases. Resulted, the use of fungicides has been increasing steadily at an annual rate of about 14% since the mid 1950s. Therefore, appropriate fungicide should be used to overcome all these major problems. In spite of use of all available means of plant protection, about 1/3rd of yearly harvested leafy vegetable production of the world is destroyed by many diseases and loss due to this is expected to be nearly 600 crore. Different fungicides are tested against various pathogens (Ravishanker and

Mamatha, 2005; Harlapur et al., 2007) throughout the world.

In the present investigation, methyl benzimidazole-2-yl carbamate (carbendazim) which is systemic fungicide was tested for their mycotoxicity *in-vitro* and *in-vivo*. Minimum inhibitory concentration (MIC), percent diseases incidence (PDI) and percent diseases reduction (PDR) against ten pathogenic fungi of leafy vegetables were determined.

MATERIAL AND METHODS

In-vitro fungicidal assay

For the assessment of *in-vitro* fungicidal assay, poisoned food technique was used. The required concentration of fungicide were prepared as parts per million (ppm) in µg/ml with sterilized double distilled water. Out of this standard concentration, 5 ml of methyl benzimidazole-2-yl carbamate 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 disk of 5-7 day old culture of targeted fungi was taken and inoculated into the center of petri plates in 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 ^{o}C and radial growth of colony was measured after 3rd day up to 7th day constantly. The results of mycelia growth were expressed as mean of triplicates. 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 mycelia growth
C=Mycelia growth in control
T=Mycelia growth in treated.

In- vivo fungicidal assay

For the assessment of fungicidal assay at field condition, each leafy vegetable was sown 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 respectively field. After 7 days, the diseases symptoms were developed on the leaves of vegetables. Afterwards minimum inhibitory concentration (MIC) in µg/ml of carbendazim shown *in-vitro* study considered for *in-vivo* study. The defined concentration of methyl benzimidazole-2-yl carbamate was sprayed directly on 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 of triplicate was calculated. 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:

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

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

RESULTS

Efficacy of carbendazim *in-vitro*

Methyl benzimidazole-2-yl carbamate (carbendazim) a systemic fungicide was tested for the assessment of fungicidal efficacy and determination of their 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*. Percent inhibition of mycelia growth over control was tabulated (Table 2). The lowest concentration which showed complete inhibition of mycelia growth was considered as MIC of fungicide to particular pathogen (Table 1).

The MIC value of carbendazim fungicide against ten pathogenic fungi of leafy vegetables was varied from 40 µg/ml to 12,000 µg/ml (Table 1). The pathogens *C. lindemuthianum* and *F. moniliforme* were found to be most susceptible and revealed MIC values at 40 µg/ml and 80 µg/ml respectively. While *H. sativum* and *A. brassicae* were found to be most resistant as showed highest MIC values (12,000 µg/ml and 1000 µg/ml respectively).

The effect of carbendazim fungicide on the growth rate of mycelium of ten pathogenic fungi of leafy vegetables was most significant (Table 2). The percent inhibition of mycelia growth of *C. lindemuthianum* and *S. verruculosum* were found to be maximum (i.e. 80.07% and 60.18%) respectively to all tested concentrations. The percent inhibition of mycelia growth of *F. roseum, F. moniliforme, F. oxysporum, P. pullulans, A. brassicae* and *A. carthami* was significantly inhibited. Lowest percent of mycelia growth was observed in *H. sativum* and *A. humicola*.

Table 1. MIC of carbendazim against plant pathogenic fungi in µg/ml.

Pathogen	Carbendazim
Alternaria brassicae	*1000
Alternaria carthami	500
Alternaria humicola	700
Collectotrichum lindemuthianum	40
Fusarium moniliforme	80
Fusarium oxysporum	100
Fusarium roseum	90
Helminthosprium sativum	12000
Pullularia pullulans	90
Stemphylium verruculosum	300

*all values expressed in mean of triplicates.

Table 2. Inhibitory effect of carbendazim on the mycelia 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						III									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
A.c.	78	74 (5.12)	68 (12.82)	62 (20.21)	55 (29.48)	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
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
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
H.s.	84										80 (4.76)					78 (7.14)	- (100)	38.24±0.75
P.p.	74	60 (18.19)	48 (35.13)	29 (60.81)	12 (83.78)	- (100)												50.89±0.84
S.v.	72						46 (36.11)	29 (73.61)	- (100)									60.18±0.87

*Mean diameter of mycelia growth in mm at varied concentration (µg/ml) and figure in parenthesis represents percent inhibition of mycelia 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,* and *S.v.*= *S. verruculosum*

***In vivo* fungicidal assay**

or the assessment of fungicidal efficacy *in-vivo,* MIC in µg/ml of carbendazim shown *in-vitro* study considered and applied for *in-vivo* study the effectiveness of fungicide were recorded as percent disease incidence (PDI) and percent disease reduction (PDR).

The effectiveness of different fungicidal treatments on percent disease incidence (PDI) and percent disease reduction (PDR) revealed that carbendazim was highly effective in controlling the disease incidence. The percent of disease reduction exhibited by carbendazim treatment was significant with an average 84.64% (Table 3). The PDR was occurred from 68-100 percent in selected plants. Hundred percent PDR was observed in *C. esculanta, S. oleracia* and *T. foenumgraecum* after spraying of carbendazim.

Table 3. *In-vivo* effect of carbendazim on leafy vegetable disease reduction.

Leafy vegetables	Fungicides	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	Carbendazim	8	16	1	15	50	6.66	86.68
B. oleraceae	Carbendazim	5	9	1	10	55.55	10	81.99
C. tinctorius	Carbendazim	6	15	2	16	40	12.50	68.75
C. esculanta	Carbendazim	3	5	0	5	60	00	100
C. sativum	Carbendazim	6	19	2	17	31.57	11.76	62.74
R. vesicarious	Carbendazim	9	29	2	28	31.03	7.14	76.99
S. oleracia	Carbendazim	5	14	0	13	35.71	00	100
T. foenum-graecum	Carbendazim	5	12	0	9	41.66	00	100

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

DISCUSSION

In preliminary assessment, carbendazim was tested for their fungitoxicity against ten fungal pathogens of leafy vegetables namely (Cambell et al. 2000). Among the pathogen *A. carthami, F. moniliforme* and *S. verruculosum* were found to be most susceptible against tested fungicides whereas *A. brassicae* and *H. sativum* were most resistant to fungicide. Similar finding was reported by several researchers (Tu and Jarvis, 1979; Ravishanker and Mamatha, 2005; Harlapur et al., 2007). 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. The fungicide mancozeb and captan being recommended for management of diseases like seedling blight of *A. falcataria*, 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 MIC of carbendazim against ten fungal pathogens of leafy vegetables. 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).

Carbendazim in the field condition reduced the diseases incidence on leafy vegetables. Siddaramaiah et al. (1980) reported similar result as carbendazim is effective against a wide range of fungal pathogen and has been used to control many disease such as leaf spot of *W. tinctoria* caused by *Cercospora wrightii*.

CONCLUSION

The present study demonstrated that the use of carbendazim is very effective against pathogenic fungi of *C. esculanta, S. oleracia* and *T. foenumgraecum* in the field condition as they showed complete percent disease reduction (PDR).

CONFLICT OF INTEREST

None declared.

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