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EFFICACY OF MANGANESE ETHYL BISDITHIO-CARBAMATE AGAINST TEN PATHOGENIC FUNGI OF LEAFY VEGETABLES

Vishal Narayan Shinde
Department of Botany, ADMSP's Late Annasaheb R. D. Deore, Arts & Science College, Mhasdi,

Abstract:

Objective: At present, there are 1.5 millions of plant species exist on the earth playing a role of prime source of food, fibers and drugs. India has achieved self sufficiency and good degree of stability of vegetable crop production out of which leafy vegetables are most essential component of our diet which nourishes with nutrients, minerals and vitamins.

Aim: The aim of the present work is to evaluate Manganese ethyl bisdithio-carbamate (Mancozeb) a Non Systemic fungicide against ten pathogenic fungi of leafy vegetables.

Material & methods: The Poisoned food technique was applied for the *In-vitro* fungicide 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 Mancozeb from tested *in-vitro* results was sprayed onto the infected leafy vegetables. Finally efficacy of Manganese ethyl bisdithio-carbamate was evaluated by calculating the Percent disease incidence (PDI) and Percent disease reduction (PDR) over control.

Result: *In-vitro* Mancozeb testing 58.76% of inhibition of mycelia growth against all tested targeted fungal pathogens. MIC of Mancozeb to all fungal pathogens varied from 1000 µg/ml to 8500 µg/ml. *In-vivo* studies, Mancozeb was highly effective in controlling disease incidence. The percent reduction (PDR) with Mancozeb treatment was maximum i.e. 57.40% in all treated leafy vegetables.

Conclusion: The use of Mancozeb is very effective against fungal diseases of *A. gaevolens, C. esculanta, S. oleracia, T. foenum-graecum* in the field condition as they showed complete percent diseases reduction (PDR). Therefore wiser use of pesticides will include reduce application rates, identifying new composition and treatment to combat the new emerging fungal diseases

Key words: Manganese ethyl bisdithio-carbamate, Fungicidal Assay, MIC, leafy vegetables, Percent Diseases Reduction (PDR).

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INTRODUCTION:

At present, there are 1.5 millions of plant species exist on the earth playing a role of prime source of food, fibers and drugs. India has achieved self sufficiency and good degree of stability of vegetable crop production out of which leafy vegetables are most essential component of our diet which nourishes with nutrients, minerals and vitamins. But these leafy vegetables being more succulent and rich in nutrients are prone to variety of diseases mostly to wilting and leaf spot right from the sowing to till marketing, thereby increasing yield losses during pre and post production periods. Due to these diseases annually billions of rupees loss occurs throughout the country. Therefore there is 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 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). 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). 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 crores.

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 Mancozeb were prepared as parts per million (ppm) in µg/ml ratio with sterilized double distilled water. Out of this standard concentration, 5 ml of Manganese ethyl bisdithio-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 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 ^{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 Mancozeb 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).

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

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 Mancozeb from *in vitro* results were selected for *in vivo* study. The define concentration of Manganese ethyl bisdithio-carbamate 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:

Manganese ethyl bisdithio-carbamate (Mancozeb) a non-systemic 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).

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The MIC values of mancozeb fungicide against ten pathogenic fungi of leafy vegetables were varied from 1000 µg/ml to 8500 µg/ml (Table 1). The pathogen, *H. sativum* and *A. carthami* were found to be most sensitive and revealed MIC values at 1000 µg/ml and 3000 µg/ml respectively. On contrary, *F. roseum* and *S. verruculosum* were found to be most resistant and showed MIC values at 8500 µg/ml and 6500 µg/ml respectively. While *F. oxysporum, F. moniliforme, A. humicola, A. brassicae, C. lindemuthianum* and *P. pullulans* were inhibited significantly at MIC values such as 3500 µg/ml, 4500 µg/ml, 4500 µg/ml, 5000 µg/ml, 6000 µg/ml and 6000 µg/ml respectively (Table 1).

The effect of Mancozeb 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* and *A. brassicae* were found to be maximum, 81.39% and 74.77% respectively, among all tested concentrations. While the percent inhibition of mycelial growth of *A. humicola, S. verruculosum, F. roseum, P. pullulans, F. oxysporum* and *C. lindemuthianum* were found to be significant as 66.95%, 59.70%, 59.65%, 56.15%, 54.14% and 52.54% respectively. On contrary, *A. carthami* and *F. moniliforme* revealed minimum MIC of mycelial growth as 48.18% and 34.20% (Table .2)

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

Pathogen	Mancozeb
Alternaria brassicae	5000*
Alternaria carthami	3000
Alternaria humicola	4500
Collectotrichum lindemuthianum	6000
Fusarium moniliforme	4500
Fusarium oxysporum	3500
Fusarium roseum	8500
Helminthosprium sativum	1000
Pullularia pullulans	6000
Stemphylium verruculosum	6500

* All values expressed in mean of three replicates

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Table : 2. 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.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
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
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)					34.20 ±0.98
F.o.	89	83 (6.74)	73 (17.97)	60 (32.58)	34 (61.79)	23 (74.57)	13 (85.39)	- (100)	38 (56.81)	35 (60.22)	32 (63.63)	29 (67.04)	27 (69.31)	- (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)	34 (61.36)	30 (65.90)	22 (75)	17 (80.68)	- (100)	- (100)	59.65 ±1.41
H.s.	86	32 (62.79)	- (100)	56 (36.36)	49 (44.31)	44 (50)	40 (54.54)	37 (57.95)	23 (61.01)	22 (62.71)	20 (66.10)	18 (69.49)	13 (77.96)		81.39 ±0.40
P.p.	88	70 (20.45)	64 (27.27)	36 (44.06)	33 (50.84)	29 (54.33)	27 (55.93)	25 (57.62)							56.15 ±1.24
S.v.	59	38 (35.59)	35 (40.37)												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, 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* Mancozeb assay:

For the assessment of fungicidal efficacy *in vivo,* MIC values in µg/ml of Mancozeb from the *in-vitro* test were used. These concentrations were directly sprayed onto the leafy vegetable

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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 Mancozeb was highly effective in controlling the disease incidence. The percent of disease reduction with Mancozeb treatment was potent, averagely as a 57.40% (Table 3).

Table : 3. In-vivo effect of Mancozeb 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	Mancozeb	8	16	2	17	50	11.76	76.48
B. oleraceae	Mancozeb	5	9	3	10	55.55	30	45.99
C. tinctorius	Mancozeb	6	15	3	15	40	20	50
C. esculanta	Mancozeb	3	5	1	5	60	20	66.66
C. sativum	Mancozeb	6	19	4	16	31.57	25	20.81
R. vesicarious	Mancozeb	9	29	5	29	31.03	17.24	44.44
S. oleracia	Mancozeb	5	14	1	12	35.71	8.33	76.67
T. foenum-graecum	Mancozeb	5	12	1	11	41.66	9.09	78.18

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

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DISCUSSION:

In preliminary assessment, Manzoceb 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 pathogen, *H. sativum* and *A. carthami* were found to be most sensitive on contrary, *F. roseum* and *S. verruculosum* were found to be most resistant against tested Mancozeb. 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, Mancozeb was significantly effective and revealed averagely 58.76% inhibition of all tested pathogens. Similar finding was recorded by Tiwari *et al.* (1988) and reported that significant efficacy of Mancozeb in reducing the growth of *C. 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 investigation, it was recorded that there were variation in Minimum inhibitory concentration (MIC) of Mancozeb against ten fungal pathogens of leafy vegetables. The MIC values of Mancozeb fungicide against ten pathogenic fungi of leafy vegetables were varied from 1000 µg/ml to 8500 µg/ml (Table 1). The pathogen, *H. sativum* and *A. carthami* were found to be most sensitive and revealed MIC values at 1000 µg/ml and 3000 µg/ml except *F. roseum* which showed high resistant at 8500 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).

Mancozeb in the field condition for reducing the diseases incidence on leafy vegetables, and showed average disease reduction i.e 57.40% at field condition. Siddaramaiah *et al.* (1980a) reported similar result as Carbendazim and Mancozeb 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.* Siddaramaiah *et al.* (1980 b) have recommended Bavistin and Banlate for managing *Cercospora* leaf spot of *Carthamus tinctorius* which agree with our results. Carbendazim and 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).

REFERENCES:

1. Agriose, G. N. 1997. Plant Pathology (4th ed.) Academic Press Londan.
2. Bains, S. S. and C. Mohan. 1982. Location and behavior of *Helminthosporium maydis* isolate, sensitive and tolerant to Diathane M-45. Indian Phythopath. **35** : 585-589.

3. Cambell, N. A., L. G. Mitchell, and J. B. Rece. 2000. Biology concepts and connections. 3rd ed. Addison Wesley Longman, Inc. New York. **p**. 672-674.
4. Dekker, J. and A. J. Gielink. 1979. Acquired resistance to pimaricin in *Cladosporim cucumerinum* and *Fusarium oxysporum f. sp. narcicci* associated with decreased virulence. Neth. J. Plant Pathol. **86** : 67-73.
5. Dey, A. and D. K. Debata. 2000. Studies on leaf spot disease of *Popules deltoids* Marsh caused by *Alternaria raphani.* Indian forester. **126** : 1013-1014.
6. Gangawane, L. V. and R. S. Saler. 1981. Resistance to fungicide in *Aspergillus flavus.* Neth. J. Plant Pathol. **87** : 254.
7. Harlapur, S. I., M. S. Kulkarni, M. C. Wali and S. Kulkarni. 2007. Evaluation of plant extracts, Bio-agent and fungicides against *Exserohilum turcicum* (Pass.) Lonard and Suggs. causing Triticum leaf blight of maize. Karnataka J. of Agri. Sci. **20(3)** : 541-544.
8. Harsh, N. S. K., V. S. Dadwal and Jamaluddin. 1992. A new post emerging damping off diseases of Eucalyptus seedling. Indian Forester. **118** : 279-283.
9. Jones, A. L. and G. R. Ehert, 1981. Resistance of *coccnyces liemalis* to benzimidazole fungicide. Plant diseases. **64** : 767-769.
10. Mehrotra, A. and M. D. Mehrotra. 2000. Leaf spotting blight, a new diseases of *Syzygium cumini* by two *cylindrocladium species* from India. Indian J. forestry. **23** : 496-500.
11. Mehrotra, M. D. 1992. Rhizoctonia leaf spotting and blight of *Michelia champaca,* a new nursery disease and its management. Indian Forester. **18** : 227-292.
12. Nene, Y. L. and P. N. Thapliyal. 1982. Fungicides in plant disease control. Oxford and IBH Publishing House, New Delhi. **p**. 163.
13. Ravishanker, R. V. and T. Mamatha. 2005. Seedling disease of some important forest tree species and their management. Working papers of the finnish forest Res. Institute **11**: 51-63.
14. Siddaramaiah, A.I., R. P. Bhat, S. A. Desai and B. K. Hedge. 1980 b. Chemical control of Cercospora leaf spot of safflower. J. Oil Seed. p. 12-13.
15. Siddaramaiah, A.I., S. kulkarni and A.B. Basvarajaiah. 1980 a. Control of leaf spot diseases of *Wrightia tinctoria* Br. in the forest nursery. Indian Forester. **106** : 771-774.
16. Srivastava, K. K. and K. K. Soni. 1993. Seedling blight of *Albizzia falcataria* and its control. Annu. Forestry. **1** : 82-84.
17. Tiwari, D. K., R. C. Srivastava, N. Katiyar and B. Lal. 1988. Chemical rot of Thielaviopsis rot of papaya. Indian Phytopathol. **41** : 491-492.
18. Tu, J. C. and W. R. Jarvis. 1979. Response of *Collectotrichum lindumuthanium* to benomyl. Canidian J. Plant Pathol. **1**: 12-16.
19. Vincent, J. M. 1947. Distortion of fungal hyphae in the presence of certain inhibitors. Nature **150** : 850-853.
20. Wellman, R. H. 1977. Problem in development resistant and use of fungicide. Annua. Rev. Phytopathol. **15** : 155-163.