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DEGRADATION OF MANCOZEB IN SOIL AND ITS EFFECT ON THE GROWTH OF HORSE GRAM PLANTS

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ABSTRACT

Manganese and zinc form a coordination complex to give Mancozeb that is slightly soluble in water and not corrosive in a dry state. Since the rate of decomposition is slow it is of concern as it is used as a foliar spray, in seed treatment and as soil drench often as a protective fungicide, the concern arises over the occurrence of such substances as residue could pose a problem following inhalation of the dust or even dermal contact. The present study emphasizes on the prevalence of native fungi in soils treated with Mancozeb and the capacity of Aspergillus niger to withstand concentrations of the chemical when used in field soils.

KEYWORDS: Fungicides, Macozeb, Aspergillus niger, Dolichos biflorus

INTRODUCTION

A fungicide is a specific type of pesticides that controls fungal disease by specifically inhibiting or killing the fungus causing the disease. Fungicides are extensively used in industry, agriculture, home and garden for a number of purposes including protection of seed grain during storage, shipment and germination, protection of mature crops, berries, seedlings, flowers and grasses in the field, in storage and during shipment, suppression of mildews that attack painted surfaces, control of slime in paper pulps, and protection of carpet and fabrics in the home. Even though fungicides have many uses in different fields they do have certain harmful effects. Fungicide resistance is a stable and heritable trait that results in a reduction in sensitivity to a fungicide by an individual fungus. Apart from fungicide resistance exhibited by the fungi, fungicides have a problem of being persistent in the environment for a long time bringing about bio-accumulation and bio-magnification. The problems associated with the use of these chemicals can be minimized by a process known as detoxification of pesticides which involves abiotic and biotic transformation of pesticides into relatively harmless substances. The fungicide used in the present study is mancozeb. It belongs to ethylene bis dithiocarbamates. The IUPAC name is manganese ethylenebis (dithiocarbamate) (polymeric) complex with zinc salt [$(C_4H_6MnN_2S_4)$*$(C_4H_6N_2S_4Zn)$]. It is also called as Dithane M-45, Fore, Mancofol, Manzate,200, Manzeb.

As it has been found that fungi are capable of degrading fungicides, the most common fungi is *Aspergillus* species which has many features with respect to degradation. *Aspergillus* is a very widely distributed saprophytic fungus occuring in almost all dead and decaying matter like rotten vegetables, butter, bread, rice, syrups, damp fruits, jams, jellies, leather goods, rotten wood etc. The present study exploits this inherent ability of *Aspergillus niger* to degrade the fungicide mancozeb and to determine the tolerance level of the fungicide by *Aspergillus niger.* Further the study also involves the effect of mancozeb and *Aspergillus niger* on the growth of horse gram (*Dolichos biflorus*).

MATERIALS AND METHOD

The microorganism used in the study was *Aspergillus niger*. The fungi was isolated from the soil samples collected from fields of Hoskote, Bangalore, India. The organism was maintained as pure culture on MRBA for subsequent use in the various experiments. The fungicide considered in the present study includes Mancozeb. It was purchased from Hebbal, Bangalore, India. The media used for testing the tolerance of Mancozeb by the fungus was MRBA. The media was further modified for the study- MRBA-1: media without a nitrogen source and carbon source i.e peptone and dextrose and MRB Broth: Broth was prepared using original media ingredients without ag

Soil sample treated with fungicide was collected and soil microorganisms were isolated and untreated soil taken from fields served as control.1 gram of soil sample was weighed. Soil sample was serially diluted. After serial dilution, 1 ml suspension of dilutions 10^{-2}, 10^{-3} and 10^{-4} were added to plates in duplicates. 20 ml of molten, cooled MRBA medium was added to the plates and swirled gently to obtain uniform distribution of cells or spores. The medium was allowed to solidify. The plates were incubated at room temperature (25^{0} c) for 5-7 days. Fungi were stained using lactophenol cotton blue and preliminary identification was done.

Solid and liquid media was used to check for fungicide tolerance in duplicates. In each state, two sets of media were prepared. First set where Mancozeb was added to already sterilized media and second set where Mancozeb was added to the media and then sterilized. For solid media MRBA was prepared and for liquid media MRB broth was used. Different concentrations (0.25%, 0.50%, 0.75% and 1%) of the Mancozeb was weighed and added to both solid and liquid media. For the first set in solid media, respective concentrations of Mancozeb was added to a test tube, then 20 ml of sterilized media was poured into it. It was then shaken thoroughly in order to mix the fungicide and the media. This was poured into respective labeled petriplates and allowed to solidify. Similarly for the second set in solid media, respective concentration of Mancozeb was added to a test tube, MRBA was added and then sterilized for 15 mins at 15 lbs pressure. After autoclaving, required amount of streptomycin was added to cooled (45^{o}c) media and poured into petriplates. Likewise, for the first set of liquid media 100 ml of the broth was taken in a conical flask, sterilized and then required concentration of Mancozeb was added. For the second set, respective concentration of Mancozeb was added to 100 ml of broth and then sterilized. Inoculation with *Aspergillus niger* was done by point inoculation on solid media having different concentration of Mancozeb and for liquid media, a loopful of the organism was taken. The plates and conical flasks were incubated at room temperature (25^{o}c) for 10 days. On the 10^{th} day, increase in growth in terms of diameter for solid media and mycelial weight in case of broth culture was recorded.

In order to check the effect of the Mancozeb on the growth of *Aspergillus niger* with nutrient stress MRBA and MRB broth was prepared without dextrose and peptone along with the respective concentration of Mancozeb (0.25%, 0.50%, 0.75%, 1%) which were considered tolerated by the microorganism, MRBA-1 and the normal media having all the nutrients along with the tolerance concentration of Mancozeb was MRBA. Both solid as well as broth was prepared in duplicates. In the first set, MRBA-1, 1% of mancozeb was added to 20 ml of sterilized MRBA-1 media and poured into petriplates. Similarly, 1% of mancozeb was added to 20 ml of sterilized MRBA media and poured into petriplates. For the second set in solid media, 0.75% of mancozeb was added to a test tube. Then 20 ml of sterilized MRBA-1 media was added to it, mixed well and poured into petriplates. 0.75% of mancozeb was added to a test tube and 20 ml of sterilized MRBA media was added, mixed well and poured into labelled petriplates. To the first set of liquid media 1% mancozeb was added to 100 ml of sterilized MRB broth-1 media and for control liquid media, 1% mancozeb was added to 100 ml of

sterilized MRB broth. 0.25% of mancozeb was added to 100 ml of MRB broth-1 and sterilized. For control liquid media, 0.25% of mancozeb was added to 100 ml of MRB broth and sterilized. Inoculation with *Aspergillus niger* was done by point inoculation on solid media and a loopful of organism was added into liquid media. Plates/conical flasks were incubated at room temperature (25^{o}c) for 10 days. On the 10^{th} day, increase in growth in terms of diameter for solid media and the mycelial mat weight for liquid media was recorded and tabulated.

The study extended to find out the effect of Mancozeb and *Aspergillus niger* on the growth of horse gram plants. Soil collected was sterilized in an autoclave at 15 lbs, 121^{o}c for 1-2 hrs. The disposable cups were filled with the sterilized soils. The test was done in triplicates. Horse gram seeds about 10 seeds/cup were sown in the cups. Regular watering and a natural environment were maintained. The 4 different sets maintained were: T1- Control, T2- *A. niger*, T3- Mancozeb, T4- Mancozeb+*A. niger* After 2 days of sowing seeds, *Aspergillus niger* was inoculated to T1 and T4 plants on the third day. 5 ml of the broth containing *Aspergillus* was inoculated in to the soil. The fungus was then allowed to colonize and grow in soil. On the 5^{th} day, fungicide at 1% concentration was prepared and sprayed on T3 and T4 plants. Control plants were untreated. Growth parameters were recorded at regular intervals of 10 days. On the 10^{th} day, plants were harvested and growth parameters in terms of shoot length (cm), root length (cm), number of leaves, disease incidence and death were recorded and tabulated. Similarly, harvesting of plants was done on the 20^{th} and 30^{th} day to record the effects of fungicides and *Aspergillus* on the growth of the plants.

RESULTS AND DISCUSSION

Isolation of Soil Microflora

The fungi isolated on MRBA from the control soil sample and soil treated with Mancozeb at different dilutions (10^{-2},10^{-3}, 10^{-4}) included *A. niger, A. flavus, Fusarium, Cocoidiodes, Cladosporium, Alternaria, and Penicillium.* (Table-1).

Test for tolerance of Mancozeb in MRBA by Aspergillus niger. In the first set of experiments, Mancozeb at a concentration of 1% showed the growth of *A. niger* followed by 0.25%, 0.50% and 0.75% when compared to the control. (Table-2a). In the second set of experiment, in the 1% concentration of Mancozeb no increase in growth was recorded while 0.25%, 0.50% and 0.75% recorded a growth of 5.6, 1.9 and 2.7 cms respectively. (Table- 2b)

Test for tolerance of Mancozeb in MRB broth by Aspergillus niger. In the first set of experiments sterilized broth with Mancozeb at different concentrations recorded growth in terms of weight of the fungal mat. The results were recorded after 10 days of incubation. Growth of the mycelial mat was observed in all concentrations of Mancozeb with significant results only at 0.25% concentration (Table- 3a).

In the second set of experiments with sterilized concentrations of Mancozeb in MRB broth, growth of organism at a concentration of 0.25% of mancozeb showed very little growth. (Table-3b).

Test for tolerance of A. niger with nutrient stress in MRBA and MRBA-1

The results recorded in the first set with sterilized MRBA and 1% concentration of Mancozeb showed a growth of 2.7cms. The experiments involving media without peptone and dextrose did not show any increase in growth. (Table-4a)

In the second set of experiments involving sterilized media with concentration of fungicide, Mancozeb did not show any increase in growth. The media devoid of peptone and dextrose also did not record any increase in growth. (Table-4b) (Plate-I)

Test for tolerance of A. niger with nutrient stress in MRB broth. In the MRB broth, Mancozeb recorded a mat weight of 4.3g at 1% concentration. MRB broth without peptone and dextrose did not record any increase in growth.(Table- 5a)

In the second set of experiment, significant mat weight was recorded at 0.25% of Mancozeb with 3.42g. The media without peptone and dextrose also did not show any increase in growth.(Table-5b) (Plate-2)

Effect of fungicides and Aspergillus niger on the growth of horse gram (Dolichos biflorus) plants.

The results recorded at the 10 day duration of growth in the different treatments did not show any disease incidence nor there was death of plants. The results on an average of five plants in triplicates were considered. The plants treated with Mancozeb showed greater root length. The number of leaves recorded in all treatments also remained the same.(Table-6).

Similarly, results were recorded at the 20 day duration of growth in the different treatments. T4 (Mancozeb+*A.niger*) showed greater root length. The number of leaves was found to have reduced in T2 (*A. niger)* when compared to the control.(Table-7).

Results of the 30 day old horse gram plants recorded showed significant shoot length, root length and number of leaves were noticed in T1(Control).(Table-8).

In an attempt to compare the above results, broth with unsterilized mancozeb showed an increased mat weight of *A. niger* at 0.25% and at the same concentration Mancozeb showed very insignificant growth in sterilized form. These findings help us identify Manganese and Zinc important supplements in media for growth of *A. niger.*

TABLE 1

ISOLATION OF MICROFLORA FROM FUNGICIDE TREATED SOILS

FUNGICIDE	NO OF COLONIES	COLOUR	ORGANISM
Mancozeb	52	tan	*Cocoidiodes*
		greenish yellow	*A.flavus*
		Black	*A.niger*
		Green powdery	*Cladosporium*
		Pinkish white	*Fusarium*
		Greyish green	*Alternaria*
	2		
	13	Pinkish white	*Fusarium*
	3	Green powdery	*Cladosporium*
	1	Dark blackish green	*Cladosporium*
	1	light green	*Penicillium*

TABLE 2

TEST FOR TOLERANCE OF FUNGICIDE USING A.niger ON MRBA

2a: Fungicide at different concentrations + sterilized media

Fungicide	/concentration	0.25%	0.50%	0.75%	1%
		Growth in cms			
Mancozeb		2.4	1.8	0.6	0.9

2b: Sterilized media with different concentrations of fungicide

Fungicide	/concentration	0.25%	0.50%	0.75%	1%
		Growth in cms			
Mancozeb		5.6	1.9	2.7	No increase
					in growth

TABLE 3

TEST FOR TOLERANCE OF FUNGICIDE USING A.niger ON MRB Broth

3a: Fungicide at different concentrations + sterilized media

					Weight in grams
Fungicide		Concentration	Fresh weight	Dry weight	Total biomass
Mancozeb		0.25%	4.04	0.97	3.07
		0.50%	3.75	1.21	2.54
		0.75%	0.23	0.05	0.18
		1%	0.69	0.11	0.58

3b: Sterilized media with different concentrations of fungicide

					Weight in grams
Fungicide		Concentration	Fresh weight	Dry weight	Total biomass
Mancozeb		0.25%	1.6	0.37	1.23
		0.50%	-	-	-
		0.75%	-	-	-
		1%	-	-	-

TABLE 4

TEST FOR TOLERANCE WITH NUTRIENT STRESS IN SOLID MEDIA

4a

1 MRBA

Fungicide		Concentration	Growth in cms
Mancozeb		1%	2.7

2 MRBA-1

Fungicide		Concentration	Growth in cms
Mancozeb		1%	No increase in growth

4b

1 MRBA

Fungicide		Concentration	Growth in cms
Mancozeb		0.75%	No increase in growth

2 MRBA-1

Fungicide		Concentration	Growth in cms
Mancozeb		0.75%	No increase in growth

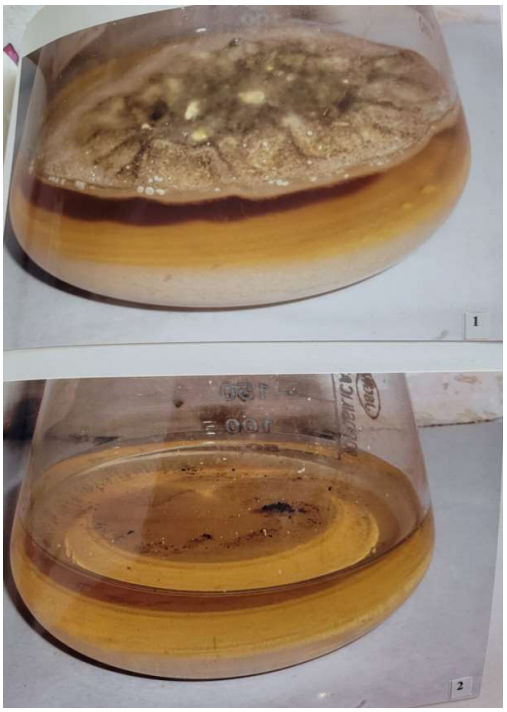

PLATE- 1-Tolerance of *A. Niger* with nutrient stress in 1% (1-Mancozeb in MRB Broth, 2-MRB Broth-1)

TABLE 5

TEST FOR TOLERANCE WITH NUTRIENT STRESS IN MRB BROTH

5a

1 MRB Broth

Fungicide		Concentration	Fresh weight	Dry weight	Total biomass	
Mancozeb		1%	6.17	1.87	4.3	

2 MRB Broth-1

Fungicide		Concentration	Fresh weight	Dry weight	Total biomass	
Mancozeb		1%	-	-	-	-

5b

1 MRB Broth

Fungicide		Concentration	Fresh weight	Dry weight	Total biomass	
Mancozeb		0.25%	4.58	1.16	3.42	

2 MRB Broth-1

Fungicide		Concentration	Fresh weight	Dry weight	Total biomass	
Mancozeb		0.25%	-	-	-	

Plate-2: Tolerance of *A. niger* with nutrient stress in 0.25% concentration of sterilized fungicide (1. Mancozeb in MRB Broth, 2. Mancozeb in MRB Broth-1)

TABLE 6

EFFECT OF FUNGICIDE A AND A.niger ON THE GROWTH OF HORSE GRAM PLANTS 10 DAY OLD TREATED PLANTS

Treatments/ parameters	Shoot length(cms)	Root length(cms)	No of leaves	Disease symptoms
T1	7.52	3.18	2	-
	7.34	2.5	2	-
	8.36	7.66	2	-
T2	7.98	4	2	-
	8.24	6.22	2	-
	7.44	3.64	2	-
T3	6.6	1.8	2	-
	8.2	3.48	2	-
	7.72	9.04	2	-
T4	7.78	3.28	2	-
	7.58	2.98	2	-
	7.04	1.96	2	-

TABLE 7

20 DAY OLD TREATED PLANTS

Treatments/ parameters	Shoot length(cms)	Root length(cms)	No of leaves	Disease symptoms
T1	9.02	5.16	5	-
	8.8	1.87	5	transparent lines on one leaf
	9.48	3.32	5	-
T2	8.5	4.06	5	-
	9.18	2.5	5	-
	8.94	3.06	4.8	1 leaf wrinkled on 1 side
T3	9.1	4.2	5	-
	9	2.2	5	1 small discolored wrinkled leaf
	8.78	3.34	5	-
T4	9.9	2.66	5	-
	9.2	5.08	5	-
	7.26	2.73	4	-

TABLE 8

30 DAY OLD TREATED PLANTS

Treatments/ parameters	Shoot length(cms)	Root length(cms)	No of leaves	Disease symptoms
T1	10.4	3.5	6.2	-
	11.5	6.1	7.66	-
	10.1	3.76	6.66	-
T2	10	2.5	5.33	-
	9.37	2.22	6	-
	11.07	3.15	5.75	-
T3	9.86	1.84	5.2	-
	6.94	2	4	-
	10.26	4	4.4	-
T4	8.62	3.2	6.6	-
	10.28	2.02	5.8	-
	10.1	4.5	6.66	-

(AVERAGE OF 5 PLANTS IN 3 REPLICATES)

CONCLUSION

In an attempt to check the effect of a fungicide like Mancozeb that is used commonly, the growth of a native flora, isolated from treated soils proves to be a promising fungi as it able to utilize the components in the fungicide thus exhibiting that nutrient stress and tolerance and its ability to grow in fungicide treated soils and further the healthy growth of horse gram in combination of the fungicide and fungus proves that this study relates to break down of the fungicide to reduce toxicity to the plant as well as to the soil by remaining as a residue.

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