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Research Article

Evaluation of Different Culture Media, Fungicides and Bio Control Agents on the Growth of *Phytopthora Capsici* Leonian. Causing Foot Rot of Black Pepper *in Vitro*

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Abstract

Black pepper (*Piper nigrum* L., Piperaceae) is a perennial vine grown for its berries extensively used as spice and in medicine. The drastic drop in the black pepper production in India has been attributed mainly for pronounced mortality of vines by the dreaded disease foot rot caused by *Phytophthora capsici.* An attempt was made to study the effect of different culture media, fungicides and bio control agents on the growth of *Phytophthora capsici.* Out of the eight different culture media used, Oat meal agar, V8 Juice agar, Potato dextrose agar and Rye agar A supported maximum colony diameter (90 mm). Among the fungicides tested, the systemic fungicide Azoxystrobin at 250 ppm, 500 ppm and 1000 ppm concentrations were found highly inhibitory to *Phytophthora capsici*. *In vitro* evaluation of antagonists revealed that *Pseudomonas fluorescens* was most effective in inhibiting the growth of *P. capsici*.

Keywords: Black pepper, *Phytophthora capsici*, oat meal agar, V8 juice agar, potato dextrose agar, rye agar A, Azoxystrobin, *Pseudomonas fluorescens*

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Introduction

Black pepper (*Piper nigrum* L.), the king of spices is one of the most important spice crops cultivated in India. The cultivation and production of black pepper is limited by many diseases of which foot rot caused by *Phytophthora capsici* is the most important and serious disease. All parts of the plant are susceptible and prone to the infection at any stage of the crop creating huge losses of around 25-30%. An attempt was made to study the effect of different culture media, fungicides and bio control agents on the growth of the pathogen *in vitro.*

Material and Methods

A laboratory experiment was conducted at College of Agriculture, UAHS, Shivamogga to know the effect of various culture media on the growth and development of the fungus. Colony diameter and colony characters were taken as the parameters for the study. The media used for the study are given below.

1. V8 Juice Agar
2. Richard's Synthetic Agar
3. Sabouraud Dextrose Agar
4. Oat Meal Agar
5. Corn Meal Agar
6. Carrot Dextrose Agar
7. Potato Dextrose Agar
8. Rye Agar A

All the above media except the Carrot dextrose agar was obtained from HiMedia Laboratories as synthetic media. Carrot dextrose agar was prepared based on the composition obtained from the book 'Diagnostic tools for the identification and detection of *Phytophthora'*-by P. Chowdappa [1].

Twenty ml of each medium was poured into 90 mm diameter Petri dishes and allowed for solidification. After solidification, actively growing hyphae of the fungus were cut into5 mm discs using heat sterilized cork borer and were placed at the centre of each plate. Each experiment was replicated five times and was incubated at 24±1°C for five days. The colony diameter and cultural characters such as colony colour, substrate colour, and colony margin were recorded after fifth day.

Figure 1 Pure culture of *Phytophthora capsici* (Plate.1)

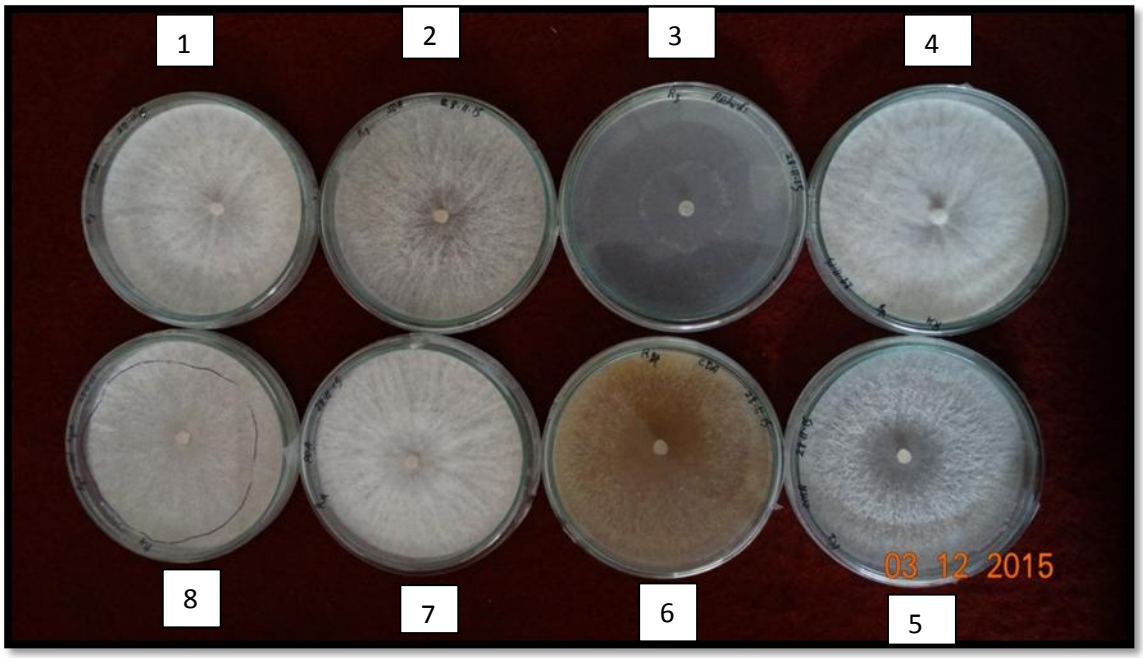

Figure 2 Effect of different culture media on the mycelial growth of *Phytophthora capsici* (Plate. 2)
(1) Oat Meal Agar, (2) Sabouraud Dextrose Agar, (3) Richard's Agar, (4) V8 Juice Agar, (5) Corn Meal Agar, (6) Carrot Dextrose Agar, (7) Potato Dextrose Agar, (8) Rye Meal Agar A

In vitro evaluation of antagonists against Phytophthora capsici

For the evaluation of fungal biocontrol agent, mycelial disc of test fungus, *i.e*., *Phytophthora capsici*, was inoculated at one end of Petri plate and antagonistic fungus opposite to it on the other end by leaving 4 cm gap between them. The biocontrol agent, *Trichoderma harzianum* culture obtained from Agricultural Microbiology lab, College of Agriculture, Shivamogga was used.

Evaluation of bacterial antagonists was taken up by streaking the bacterial antagonists at the centre of the plate and placing the test fungus at both ends of the Petri plate. The culture of bacterial antagonists *viz*, *Pseudomonas fluorescens* and *Bacillus subtilis* used for this study were obtained from Agricultural Microbiology lab, College of Agriculture, Shivamogga. A control was maintained. After a period of incubation, when the growth in control plate

reached maximum (90 mm diameter), the radial growth of the pathogen was measured. Per cent inhibition of growth over control was worked out according to the equation given by Vincent [2].

$$I = \frac{C - T}{C} \times 100$$

Where, I = Percent inhibition, C= Growth in control, T = Growth in treatment.

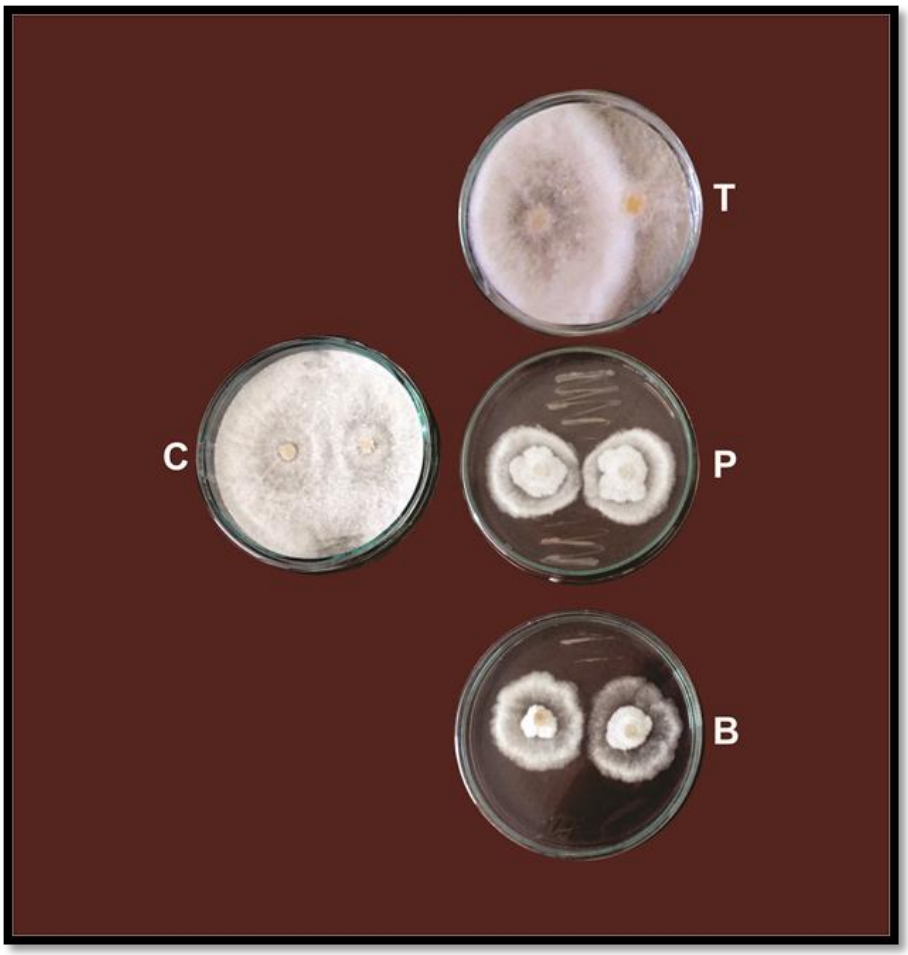

Figure 3 Effect of antagonists against *Phytophthora capsici* (Plate. 3) T- *Trichoderma harzianum* P- Pseudomonas fluorescens, B- Bacillus subtilis, C- Control

In vitro evaluation of fungicides against Phytophthora capsici

The efficacy of different non-systemic and systemic fungicides in inhibiting the mycelial growth of *Phytophthora capsici*was assayed *in vitro* by following poisoned food technique [3]. Different concentrations *viz.*, 250, 500 and 1000 ppm for systemic fungicide and 1000, 2000 and 3000 ppm for non-systemic fungicides were used.

Systemic Fungicides		
Sl. No.	**Trade Name/ Common name**	**Chemical name**
1.	Acrobat 50% WP	Dimethomorph (50% WP)
2.	Aliette 80 WP	Fosetyl-Al 80% WP
3.	Synergy/ Akomin	Potassium phosphonate
4.	Amistar	Azoxystrobin 23% SC

Non-systemic fungicides/ Combi products		
Sl. No.	**Trade Name/ Common name**	**Chemical name**
1.	Equation Pro	Famoxadone (16.6% w/w) + Cymoxanil (22.1% SC)
2.	JU- Redomil	Metalaxyl 8% + Mancozeb 64% WP
3.	Sectin 60 WG	Fenamidone (10%) + Mancozeb 50% WG
4.	Curzate M8	Cymoxanil (8%) + Mancozeb (64%)
5.	Kocide™ 2000	Copper hydroxide (53.8% w/w)
6.	Bordeaux mixture (1%)	Copper sulphate + hydrated lime

Required quantity of individual fungicides were prepared and added separately into molten and cooled potato dextrose agar medium so as to get the desired concentrations of the fungicides. Later, 20 ml of the poisoned medium was poured into sterilized Petri plates. Mycelial disc of five mm size was cut from five days old actively growing cultures of *Phytophthora capsici* using a sterilized cork borer and one such disc was placed at the center of each agar plate. Control was maintained without adding any fungicide to the medium. Three replications were maintained for each concentration. After incubation for five days at room temperature, radial growth was measured when fungus attained maximum growth in control plates. The efficacies of the fungicides were expressed as percent inhibition of mycelial growth over control, which was calculated by using the formula of Vincent [2],

$$I = \frac{C - T}{C} \times 100$$

Where, I = Percent inhibition, C= Growth in control, T = Growth in treatment

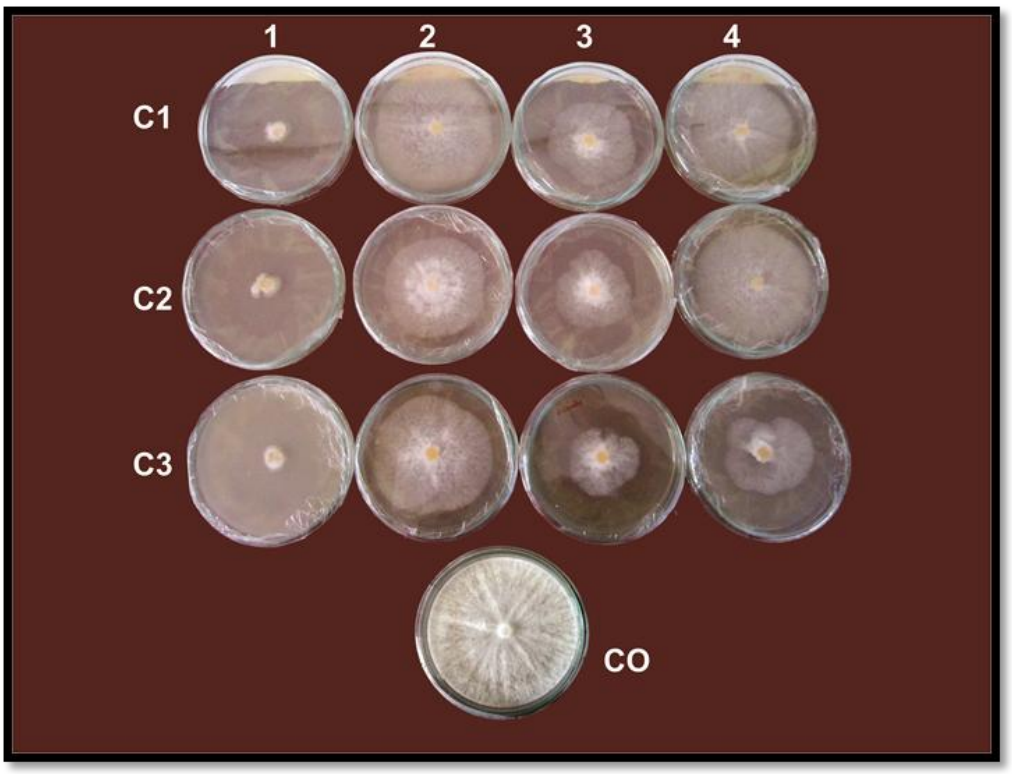

Figure 3 Effect of different concentrations of systemic fungicides on *Phytophthora capsici* (Plate. 4)
(1) Azoxystrobin 23% SC, (2) Dimethomorph (50% WP), (3) Potassium phosphonate, (4) Fosetyl-Al 80% WP, C1- 250 ppm, C2- 500 ppm, C3- 1000 ppm, CO- Control

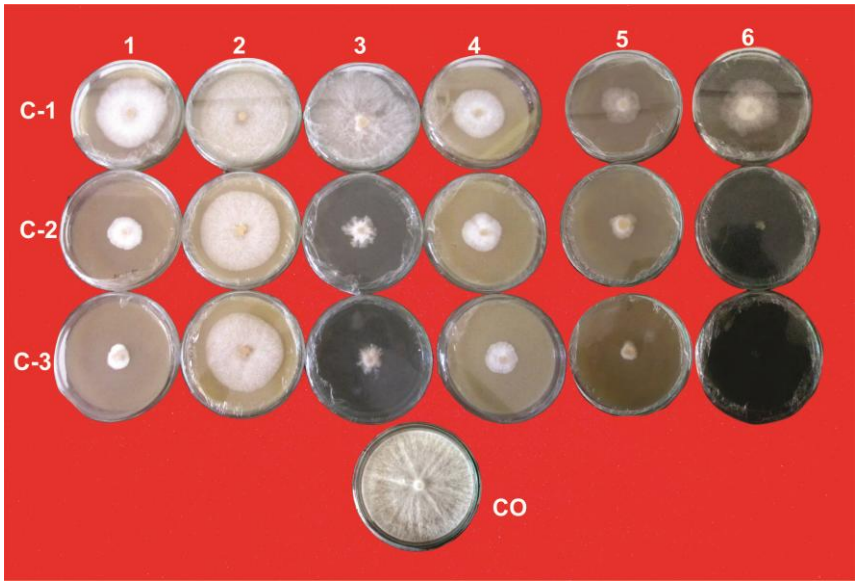

Figure 4 Effect of different concentrations of non-systemic fungicides and combi- products on *Phytophthora capsici* (Plate.5)
(1) Metalaxyl 8% + Mancozeb 64% WP, (2) Cymoxanil (8%) + Mancozeb (64%), (3) Copper hydroxide (53.8% w/w), (4) Fenamidone (10%) + Mancozeb 50% WG, (5) 1% Bordeaux mixture, (6) Famoxadone (16.6% w/w) + Cymoxanil (22.1% SC), C1- 1000 ppm, C2- 2000 ppm, C3- 3000 ppm, CO- Control

Results and Discussion

Oat meal agar, V8 Juice agar, Potato dextrose agar and Rye agar A supported maximum colony diameter of 90.00 mm. It was followed by Carrot dextrose agar, Sabouraud dextrose agar and Corn meal agar. Although Sabouraud dextrose agar supported more colony diameter (69.5) as compared to corn meal agar (66.9 mm), the growth of mycelia was abundant and thick in CMA than in Sabouraud Dextrose Agar. However, Richard's synthetic agar did not support the growth of *Phytophthora capsici* and performed poorly (Plate.2). Similar reports on cultural studies of *Phytophora capsici* has been reported earlier [4-7].

In vitro evaluation of bio-control agents against Phytophthora capsici

The present investigation assessed the antagonistic effect of different antagonists by dual culture technique. Per cent mycelial inhibition of *P. capsici* over control under *in vitro* condition ranged from 36.56 to 56.39 per cent. Significantly, highest inhibition of radial growth of mycelia of *P. capsici* was recorded in plates treated with *Pseudomonas fluorescens* (56.39) and least inhibition (36.56) was seen when *Trichoderma harzianum* was used (Plate.3). The use of *Bacillus subtilis* also showed considerable (52.09) mycelia inhibition (**Table 1**).

The antagonism of *Trichoderma* spp. against many fungi is mainly due to production of acetaldehyde [8, 9]. This may also be the reason for antagonistic effect of native isolates of *Trichoderma* against *P. capsici*. The antagonistic nature of *Bacillus subtilis* and *Pseudomonassp* has been reported earlier in 1989 [10].

Similar results wherein the efficacy of *Trichoderma* spp. and *Pseudomonas* sp. against the pathogen *P. capsici* was previously reported by many scientists [6, 7, 11-21].

Table 1 *In vitro* evaluation of bioagents against*Phytophthora capsici*

Bioagent	Inhibition of mycelial growth (%)
Pseudomonas fluorescens	56.39 (48.67)*
Bacillus subtilis	52.09 (46.20)
Trichoderma harzianum	36.56 (37.20)
S.Em. ±	0.56
CD @ 1%	1.70

*Figures in parenthesis are Arcsine transformed values

Table 2 *In-vitro* Evaluation of systemic fungicides against *Phytophthora capsici*

Sl. No.	Fungicides		Mean per cent inhibition			Mean
			Concentration (ppm)			
	Common name	**Trade name**	**250**	**500**	**1000**	
1	Azoxystrobin 23% SC	Amistar	81.45(64.49)	81.34 (64.41)	83.78 (66.25)	82.18 (65.04)
2	Dimethomorph (50% WP)	Acrobat 50% WP	17.66 (24.80)*	23.89 (29.25)	18.78 (25.67)	20.11 (26.64)
3	Potassium phosphonate	Synergy/ Akomin	34.56 (35.99)	42.11 (40.40)	48.45 (44.11)	41.70 (40.22)
4	Fosetyl-Al (80% WP)	Aliette 80 WP	18.00 (25.03)	19.56 (26.21)	38.44 (38.30)	25.33 (30.18)
S.Em.±			1.20	1.46	1.24	0.77
CD @ 1%			3.49	4.28	3.61	2.24

*Figures in parenthesis are Arcsine transformed values

Table 3 *In-vitro* evaluation of non systemic fungicides and combi products against *Phytophthora capsici*

Sl. No.	Fungicides		Mean per cent inhibition			Mean
			Concentration (ppm)			
	Common name	**Trade name**	**1000**	**2000**	**3000**	
1	Metalaxyl 8% + Mancozeb 64% WP	JU- Redomil	32.78(34.91)	62.22(52.07)	77.41(61.65)	57.47(49.55)
2	Cymoxanil (8%) + Mancozeb (64%)	Curzate M8	15.56(23.21)	26.11(30.72)	31.11(33.90)	24.26(29.29)
3	Copper hydroxide (53.8% w/w)	Kocide™ 2000	6.67(14.85)	70.37(57.03)	73.15(58.80)	50.06(43.60)
4	Fenamidone (10%) + Mancozeb 50% WG	Sectin 60 WG	52.96(46.70)	67.96(55. 53)	73.33(58.91)	64.75(53.71)
5	Famoxadone (16.6% w/w) + Cymoxanil (22.1%SC)	Equation Pro	62.04(51.97)	74.63(59. 76)	86.11(68.30)	74.26(59.95)
6	Copper sulphate + hydrated lime	Bordeaux mixture (1%)	42.96(40.95)	97.77(84.37)	100.0(89.05)	80.24(70.47)
	S.Em.±		1.37	2.80	1.54	13.34
	CD @ 1%		4.19	8.54	4.71	NS

*Figures in parenthesis are Arcsine transformed values

In vitro evaluation of different fungicides against Phytophthora capsici

The data on different fungicides screened *in-vitro* at three different concentrations for systemic and contact fungicides and their per cent mycelial inhibition over control are presented in **Table 2** and **3**. Data from the tables revealed that, the efficacy of different fungicides, concentrations and their interaction on per cent inhibition of mycelial growth of *P. capsici* differed significantly.

The highest per cent mycelial inhibition was recorded with Azoxystrobin fungicide with a mean inhibition of 82.18% and it was superior over all the other systemic fungicides tested (Plate.4). It was followed by 1% Bordeaux mixture and Equation pro with a mean per cent mycelial inhibition of 80.24% and 72.47% respectively. Metalaxyl + Mancozeb combination (Redomil JU) (58.02%), Fenamidone + Mancozeb combination (Sectin) (64.51%), Kocide (49.57%) and even Akomin (Potassium phosphonate)- a plant tonic (41.70%) showed increased per cent mycelial inhibition as the concentration increased (Plate.5).

Bordeaux mixture recorded almost 100% mycelial inhibition at 2000 and 3000ppm concentrations. Minimum mean per cent mycelial inhibition was observed with fungicides Dimethomorph (20.11%) and Cymoxanil + Mancozeb combination (Curzate M8) (24.44%) (Plates 4 and 5). However, all the fungicides screened *in-vitro* against *P. capsici* at three different concentrations were significantly superior to control and significantly differed with each other.

The fungicidal [7] nature of Akomin, a plant tonic generally being recommended for plantation crops was identified earlier. The laboratory evaluation of Ridomil against *Phytophthora parasitica* var. *nicotianae* revealed significant reduction in growth and sporulation of fungus at 0.1, 0.2, 0.3 and 0.4 per cent concentration [22]. The present study is also in agreement with an investigation carried out by Susheela Bhai and Anjali in 2015 [23], using strobilurin fungicides. When the concentration was increased from 500 up to 6000 ppm, 100% inhibition of mycelial growth was obtained at 6000 ppm for a strobilurin fungicide- Ergon 44.3% (w/w) [Kresoxim methyl 500 g L-1 SC].

Conclusions

Out of the eight different culture media tested, Oat meal agar, V8 Juice agar, Potato dextrose agar and Rye agar A supported maximum colony diameter of 90.00 mm. Among the test antagonists, *Pseudomonas fluorescens* was most effective in inhibiting the growth of *P. capsici*. Least inhibition was noticed when *Trichoderma harzianum* was used.

Out of the ten fungicides tested *in vitro*, the systemic fungicide Azoxystrobin at all three concentrations (250 ppm, 500 ppm and 1000 ppm), the combi products Fenamidone + Mancozeb (Sectin) and Famaxodone+ Cymoxanil (Equation pro) at all the three concentrations (1000 ppm, 2000 ppm and 3000 ppm) were found highly inhibitory against *Phytophthora capsici*. Bordeaux mixture (1%) at 2000 ppm and 3000 ppm gave 100% mycelial inhibition of the fungus. Potassium phosphonate at 1000 ppm were also inhibitory to the pathogen. The least effective was dimethomorph followed by Cymoxanil + Mancozeb combination (Curzate M8).

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