EFFICACY OF SOME SYSTEMIC AND PROTECTIVE FUNGICIDES AND FUNGICIDE MIXTURES OF FUNGICIDES AGAINST *FUSARIUM SOLANI* (SACC.) MART. EMEND. SYND. & HANS. CAUSING WILT IN JOJOBA PLANT (*SIMMONDSIA CHINENSIS* (LINK) C. K. SCHNEIDER)

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ABSTRACT: A pathogenic fungus, causing vascular wilt, was isolated from Jojoba plant and was identified as *Fusarium solani* (*Nectria haematococca*). Efficacy of the systemic fungicides (benomyl[x], thiophanate methyl[y]), protective fungicides (propineb[z] captan[m], quintozene[n], mancozeb[o], mancozeb-cu[p]) and fungicide mixtures (thiophanate methyl and mancozeb-copper, propineb and thiophanate methyl, thiophanate methyl and mancozeb, propineb and mancozeb-copper, mancozeb-copper and mancozeb) — 0.5 ml of each fungicide, were evaluated against this pathogen *in vitro* and *in vivo*. *In vitro* studies revealed benomyl, propineb, manocozeb-copper, thiophanate methyl and mancozeb-copper fungicides significantly ($p < 0.05$) suppressed the growth of mycelium compared with the control. However there was no significant difference between these fungicides. Induced growth of mycelium on agar was observed in the mixture of mancozeb-copper and mancozeb, which was significantly ($p < 0.05$) higher than the control.

Some fungicides were applied to one year old Jojoba plants in a greenhouse and the percentage that died was calculated for each treatment. Higher protective values [Protective value (%) = (1 — A/B) 100: A represents the percentage of disease on treated plants: B represents that on untreated plants] were observed on the following treatments: benomyl, propineb, captan, manocozeb and propineb + thiophanate methyl. Of the fungicides tested benomyl and propineb showed higher efficacy and protective value in both *in vivo* and *in vitro* studies.

x. Benlate 50% w. p.
(Lankem Ltd., Sri Lanka)

y. Topsin-M-WP
(J. L. Morison & Jones (Cey.) Ltd., Sri Lanka)

z. Antracol w. p.70%
(Haychem Ltd., Sri Lanka)

m. Orthocide 50% w. p.
(A. Baur & Co. Ltd., Sri Lanka)

n. Morut (80%)
(Haychem Ltd., Sri Lanka)

o. Dithane M - 45
(C. I. C. Chemical Industries, Sri Lanka)

p. Trimiltox
(A. Baur & Co Ltd., Sri Lanka)

Introduction

Oil extract of Jojoba has distinct properties, similar to oil extracted from sperm whale. Female Jojoba plant is commercially important since the oil is extracted from its seed. Seed contains 45 to 55 percent oil which contains no glycerides or glycerol. Due to these properties Jojoba seed totally differs from other conventional oil seed crops viz. soybean, corn, olive and peanut. Jojoba oil has excellent physical and chemical properties and it is used as lubricant, factices, adhesives, cosmetics, medicines, food etc. (Anonymous 1985). Feasibility studies of growing this plant are being done on the east coast of Sri Lanka.

Waterborne fungal pathogens in Jojoba caused by *Fusarium*, *Verticillium*, *Pythium* and *Phytopthora* are recorded in poorly drained and heavy soils (Anonymous 1985). *Fusarium* species are often associated with wounds or with localized lesions caused by *Pythium*, *Phytophthora*, *Botrysphaeria*, *Macrophomina*, *Rhizoctonia* species or by other species of *Fusarium*. There is adequate evidence of root rot caused by *Fusarium solani* in broad bean *(Vicia)* and French bean *(Phaseolus)*. Some Solanaceous crops and cucurbits are also attacked by this pathogen. Death of mature plants by *Fusarium* species was observed in these studies but it has not been reported in Jojoba plant. The pathogen was isolated and identified as *Fusarium solani*. It enters through the roots and affects the vascular system; as a result of this, sudden wilting, defoliation and ultimately death of plant occurs.

Treating individual Jojoba plants is essential since their sex can only be detected when they start flowering at the age of three years or more. This study attempts to find the effects of protective and systemic fungicides or combination of these fungicides against *F. solani in vivo* and also *in vitro*. These studies were done during the period of 1989 — 1990. The same concentration of fungicides were used in both studies.

Materials and Methods

Isolation of pathogen

Segments of root (2 cm) with suspected *Fusarium* lesions were surface sterilized by immersion in 1.8% sodium hypochloride for two minutes. The segments were then blotted dry on sterile filter paper and were cut into tiny pieces and placed into dichloramphenicol (200 ppm) peptone agar (DCPA). This medium has recently been described as a selective medium for the isolation of *Fusarium* spp (Andrews and Pitt 1986). Inoculated plates were incubated at 25°C near uv light.

Preparation of inoculum for pathogenicity test

Rice chaff (50 g) and maize meal (10 g) was ground and 100 ml of distilled water was added and autoclaved for 30 min, at 121 °C, 14 psi (Burgess and Liddel 1982). Isolates were inoculated on to this medium and incubated for four weeks, then dried and crumbled into fine particles.

Koch's postulates

Prepared inoculum was added to the soil of year old healthy plants in pots (9.5 cm diameter, 23.5 cm height) growing in the greenhouse. Discoloration and wilting began to appear four weeks after inoculation. Affected plants were compared with healthy plants. To establish Koch's postulates, the fungus was reisolated and compared with an original isolate.

In vitro study (Supplement with fungicides)

Two systemic fungicides, benomyl (472 ppm) and thiophanate methyl (1000 ppm), five protective fungicides, mancozeb (2780 ppm), propineb (2000 ppm), quintozene (7000 ppm), mancozeb-cu (6000 ppm) and captan (2520 ppm) and selected mixtures thiophanate methyl + mancozeb, thiophanate methyl + mancozeb-cu, propineb + thiophanate methyl, mancozeb-cu + mancozeb, propineb + mancozeb and propineb + mancozeb-cu were prepared from commercially available recommended products. All the above fungicides were dissolved or suspended in sterilized water. One ml of fungicide was added to 99 ml of DCPA (50°C), mixed and poured into 10 cm sterilized petri dishes. In mixtures, 0.5 ml of each fungicide was added.

Each treatment was replicated six times. Twenty four hours after pouring, a mycelial disc (1 cm diameter) was cut with cork borer from the edge of an actively growing colony of *F. solani*, and placed on the medium in the centre of each fungicide amended plate. All the cultures were incubated in 25°C for five days. Radial mycelial growth (average of two diameters at right angles) was recorded for five consecutive days. Growth patterns of the mycelium on amended fungicide plates were compared with unamended plates.

Mean mycelial growth (diameter) on the fifth day was used for the comparison between different treatments, as Lynne *et al.* (1986) reported that 5-7 days is the most appropriate time for differentiation between treatments.

In vivo study

Year old plants grown in polythene bags (9.5 cm diameter, 23.5 cm height) were used in the study. Prepared colonized chaff inoculum was added to each bag. Ten millilitres of fungicide solution per plant was applied two days before and one day after inoculation respectively. Each treatment was applied to four healthy plants. The treated plants were grown in a greenhouse and disease severity was assessed one month after inoculation. Number of affected plants in each treatment was counted and its protective value was evaluated by using the following formula:

Protective value (%) = { 1 - A/B} 100

A represents % of disease on treated plants.

B represents % of disease on untreated plants (Yamada *et al.* 1986)

Results and Discussion

Individual effects of fungicides

In vitro studies revealed that one systemic (benomyl) and two protective (propineb, mancozeb-cu) fungicides significantly ($p<5.05$) suppressed the growth of mycelium compared to the controls (Table 1). However, there were no significant differences between these three fungicides. Mycelial growth suppression on benomyl, mancozeb-cu and propineb was 57.5% 46.6% and 52.6% respectively.

From *in vivo* studies, mancozeb, propineb, captan and benomyl showed the highest protective values (75–100) (Table 2). Fungicides which showed significantly better performance *in vitro* did not act in the same manner when they were applied in soil. There are other records of the lack of correlation between the fungitoxicity of compounds *in vitro* and in vivo (Yamada *et al.* 1986). Thiophanate methyl treated plots showed very low protective value (0%) similar to the untreated plots. Although the performance of captan and mancozeb were not significant in reducing the growth of mycelium *in vitro*, a protective value of 75% was recorded in the field. The protective value of mancozeb-cu treated plots was 50%.

Table 1. Mean difference of mycelial growth (cm) of *Fusarium solani* in different combinations of treatments.

	control	mancozeb	quintozene	mancozeb-cu	propineb	thiophanate methyl	captan	benomyl	mancozeb + thiophanate methyl	mancozeb-cu + thiophanate methyl	propineb + thiophanate methyl	mancozeb + mancozeb-cu	mancozeb + propineb	maneozeb - cu propineb
control	0.000													
mancozeb	0.575													
quintozene	0.592	0.017												
mancozeb-cu	* - 2.02	* - 1.45	* - 1.43											
propineb	* - 2.28	* - 1.71	* - 1.69	- 0.26										
thiophanate methyl	0.57	0.00	- 0.02	* 1.45	* 1.71									
captan	0.28	- 0.29	- 0.31	* 1.75	* 2.0	0.29								
benomyl	* - 3.48	* - 1.92	* - 1.9	- 0.46	* - 0.26	* - 1.92	* - 2.2							
mancozeb + thiophanate methyl	0.57	0.00	- 0.02	* 1.45	* 1.71	0 00	- 0.29	* 1.92						
mancozeb-cu + thiophanate methyl	* - 1.9	* 1.35	* - 1.33	- 0.1	0.35	* - 1.35	* - 1 65	0.56	* - 1.35					
propineb + thiophanate methyl	0.81	0.23	0.22	1.22	* 1.47	- 0.23	0.53	* 1.68	- 0.23	1.12				
mancozeb + mancozeb-cu	* 1.22	* 1,79	* 1.81	* 3.2	* 3.50	* 1.79	* 1.49	* 3.71	* 1.79	* 3.1	2.02			
mancozeb + propineb	- 0.37	- 0.95	- 0.97	2.40	* 2.66	0.95	0.65	* 2.87	0 95	* 2.30	* 1.18	- 0 84		
maneozeb - cu propineb	0 44	- 0 13	- 0.15	* 1.58	* 1.84	0.13	- 0.16	* 2.05	0.13	* 1 48	0.367	- 1.68	- 0.82	-
	control	mancozeb	quintozene	mancozeb-cu	propineb	thiophanate methyl	captan	benomyl	mancozeb + thiophanate methyl	mancozeb + thiophanate methyl	propineb + thiophanate methyl	mancozeb + mancozeb-cu	mancozeb + propineb	mancozeb - cu propineb

Comparison (Analysis of variance) significant at 0.05% level are indicated by *, alpha=0.05, confidence=0.95, MSE=0.243365, critical value of studentized range=4.913, minimum significant difference=0.9896.

Table 2. Protective effects of some selected, systemic and mixtures of fungicides against *Fusarium* wilt in Jojoba plants

Fungicide	Protective value (%)	
	1 month after inoculation	2 months after inoculation
control	75	00
mancozeb	100	5
quintozene	66.6	50
mancozeb-cu	100	50
propineb	100	75
thiophanate methyl	33.3	00
captan	100	75
benomyl	100	100
mancozeb+ thiophanate methyl	100	25
mancozeb-cu+ thiophanate methyl	100	50
propineb+ thiophanate methyl	66.6	75
mancozeb+ mancozeb-cu	100	25
mancozeb+ propineb	100	50
mancozeb-cu+ propineb	100	00

Protective value (%) = [1 – (A/B) 100]

A = represents the percentage of disease on treated plants.

B = represents that on untreated plants.

Lower protective values *in vitro* studies may be either due to the degradation of fungicides when applied to the soil or the fungicide may be nullified by root exudates of host plant. Higher protective values *in vitro* studies may result from a decrease in virulence of the fungus in the soil.

Mixture of fungicides

The mixture of thiophanate methyl and mancozeb-cu mixture has significantly ($p<0.05$) suppressed the growth of mycelium when compared to the control, whereas mancozeb-cu+mancozeb mixture has significantly ($p<0.05$) promoted the growth of mycelium compared to the control *in vitro* studies (Table 1). This study shows that mixtures of fungicide can increase the severity of disease. Using fungicide mixture may therefore be deleterious. *In vivo* studies revealed that mixtures of mancozeb-cu+mancozeb showed lower protective value (25%) (Table 2). However, this mixture significantly stimulated growth *in vitro*.

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