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IN VITRO EVALUATION OF FUNGICIDES AGAINST RUBBER ISOLATE OF *THANATEPHORUS CUCUMERIS* AND MANAGEMENT OF *THANATEPHORUS* FOOT ROT OF *HEVEA* SEEDLINGS

C K Jayasinghe, E B Fernando and B I Fernando

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ABSTRACT

Thanatephorus cucumeris, the causal agent of target leaf spot disease of *Hevea* also attacks the hypocotyls of germinated seeds and cause damping off and foot rot of seedling in *Hevea* nurseries in Sri Lanka.

Sixteen fungicides were screened for their abilities to reduce the saprophytic growth of *T. cucumeris* on agar and in soil while five fungicides were tested to control foot rot of *Hevea* seedlings in pots. Five fungicides (benomyl, metalaxyl 8% + mancozeb 64%, tebuconazole, thiram and tridemorph) were found to be effective in inhibiting fungal growth 100% (EC 100%) on agar at a very low concentration of 50 μg/ml. Three fungicides, *viz*. captan, mancozeb, oxadixyl 10% + mancozeb 56% achieved EC 100 at 100 μg/ml while five more fungicides (chlorothalonil, oxadixyl 10% + propineb 56%, pencycuron, propineb and triadimenol) showed EC 100 between 200-800 μg/ml. However, only four fungicides (benomyl, pencycuron, propineb and tebuconazole) reached the EC 100 within the tested range when assessed in the presence of soil indicating that these are the potential fungicides in management of rubber isolate of *T. cucumeris*.

In pot culture trials two fungicides, pencycuron and benomyl were found to be effective in controlling the disease. However, the disease was completely checked by traditional burning of soil.

Key words: disease management, fungicide screening, *Hevea brasiliensis*, *Thanatephorus cucumeris*.

INTRODUCTION

During the heavy South West monsoon periods of 1991 and 1992 our attention was drawn to destruction of *Hevea* seedlings in the seed germination beds in several districts in Sri Lanka. Close examinations revealed that certain seedbeds have been wiped out entirely due to the destruction of the hypocotyls of the germinating seeds. This results in damping off and foot rot of seedling. Surviving plants had a net work of silvery mycelium with brownish sclerotia on leaves, petioles and stems. Later this disease was reported from seedling nurseries and the most conspicuous symptom was the presence of translucent irregular patches on young leaves during prolonged humid periods. However, though the die-back of seedlings were noticed the mortality rate was extremely low in seedling nurseries. The causal organism was identified as *Thanatephorus cucumeris* (Frank) Donk (IMI 343000, anamorph: *Rhizoctonia solani* Kuhn) with the collaboration of International Mycological Institute, UK and pathogenecity was confirmed by fulfilling Koch's postulates (Jayasinghe, 1993).

Though this is the first detailed account of the occurrence of *T. cucumeris* on *Hevea* in Sri Lanka, *T. cucumeris* is known to cause target leaf spot disease, a serious leaf disease of *Hevea* in the states of Amazonas and Para in Brazil since 1950's (Stevenson & Carpenter, 1950; Langford, 1953). The other countries where this disease occurs on rubber are Bolivia (Stevenson & Carpenter, 1950), Costa Rica (Carpenter & Langford, 1950), Ivory Coast (Boisson, 1966), Papua New Guinea (Anon. 1960) and Peru (Lorenz, 1948).

Since, *T. cucumeris* and it's anamorph cause many types of diseases on over 200 host plants under diverse environmental conditions (Baker, 1970; Mordue, 1974) numerous reports are available on evaluation of fungicides against the fungus. The fungicides which have being reported to be effective against various isolates are benodanil (Cole & Cole, 1978; Hiepko, 1982; Kataria *et al.*, 1993), benomyl (Cole & Cole, 1978; Cotterill, 1989; Sinha, 1992), carbendazim (Sinha, 1992), carboxin (Anon, 1981), furmecyclox (Kataria *et al.*, 1993), iprodione (Anon, 1981; Kataria *et al.*, 1993), pencycuron (Kataria *et al.*, 1993), quintozene (Mordue, 1974), thiabendazole (Cotterill *et al.*, 1989) and tolclofos - methyl (Kataria *et al.*, 1993). With regard to the management of *T. cucumeris* on *Hevea*, copper based fungicides and triadimephon have been recommended in the South American region (Anon, 1994). Apart from this recommendation for the control of target leaf spot disease, no investigations have been carried out on the management of *T. cucumeris* on *Hevea* in seed germination beds.

The present study addresses this issue and reports the effectiveness of a range of fungicides against the mycelial growth of á rubber isolate of *Thanatephorus cucumeris* on agar and in soil and against *Thanatephorus* root rot of *Hevea* seedlings in pots with special emphasis on managing the disease in *Hevea* seed germination beds.

MATERIALS AND METHODS

Organism

The fungus *Thanatephorus cucumeris* (IMI identification No 343000) isolated from infected *Hevea* seedlings was used through out the study. Cultures were maintained on Potato Dextrose Agar (PDA) at room temperature (28 ± 2°C).

Fungicides

The fungicides used were benomyl (Benlate, 50% *a.i.*, Du Pont), captan (Captan, 50% *a.i.*, Chevron), chlorothalonil (Daconil, 75% *a.i.*, SDS-Biotec), copper oxychloride (Cobox, 50% *a.i.*, BASF) mancozeb (Dithane M-45, 80% *a.i.*, Rohm & Haas), metalaxyl 8% + mancozeb 64% (Ridomil MZ, 72% *a.i.*, Ciba-Geigy), oxadixyl 10% + mancozeb 56% (Sandofan M, 66% *a.i.*, Sandoz), pencycuron (Monceren, 25% *a.i.*, Bayer), propineb (Antracol, 70% *a.i.*, Bayer), propineb 56% + oxadixyl 10% (Fruvit, 66% *a.i.*, Bayer), sulphur (Thiovit, 80% *a.i.*, BASF), tebuconazole (Folicur, 250 EC, Bayer), thiram (Pomarsol Forte 80% *a.i.*, Bayer), triadimefon (Bayleton, 25% *a.i.*, Bayer), triadimenol (Bayfidan, 250 EC, Bayer) and tridemorph (Calixin, 750 EC, BASF).

Poisoned food technique (PFT)

Mycelial growth inhibition was evaluated by the poisoned food technique (Schmitz, 1930). Six dosage rates of 50, 100, 200, 400, 800 and 1600 μg/ml of each of the test fungicides were evaluated. Measured quantities of fungicides were added into autoclaved PDA media at 45 ± 2°C to get the required concentration. Ten ml of each amended medium was then poured into a Petri dish using a parrot. Each plate was thereafter inoculated at the centre with a mycelial disc, 5mm in diameter, taken from the periphery of actively growing 3-day old cultures, and incubated at 28 ± 2°C for 3 days. Four replicates were used for each concentration. In the control experiment no fungicide was added to the medium. The colony diameter was measured on the 3rd day and the percent inhibition of growth in each treatment was calculated with respect to the growth of the control by the equation given by Vincent (1927):

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

where I = percent inhibition of mycelial growth with respect to control, C = growth in control and T = growth in treatment.

Soil fungicide screening technique (SFST)

The soil fungicide screening technique described by Zentmeyer (1955), Corden and Young (1962), and Sharma and Mohanan (1991) was modified slightly and used to test the lethal effect of the fungicides on mycelial growth in the presence of soil. Air-dried sieved (25 mesh cm^{-2}) soil of pH 4.6 was used throughout the study. Ten grams of soil was autoclaved (2 h at 15 psi) and placed in sterile tubes (20 mm diameter and 140 mm long). A mycelial disc (8 mm diameter), obtained from close to the centre of a 8-day-old culture of the fungus on PDA at 28 ± 2°C was transferred to the soil surface in the tubes. Another 10 g of sterile soil was then placed over the mycelial disc. Afterwards, 10 ml of the desired aqueous fungicide solution (concentrations used were 50, 100, 200, 400, 800, 1600 or 3200 μg/ml) was gently poured over the soil surface. Each concentration had four replicates and in the control experiment 10 ml of sterile distilled water was used instead of the fungicide. The mouths of the tubes were then covered with aluminium foil, and incubated for 24 h at 28±2°C. At the end of this period tubes were emptied and the mycelial discs were washed with sterile distilled water to remove any adhering soil particles. The discs were then placed with the mycelial surface down, on the surface of 10 ml PDA in a Petri dish, and incubated for three days at 28 ± 2°C. Colony diameter was measured at the end of the incubation period. Percent inhibition of the mycelial growth in each treatment was calculated using the equation given for the poisoned food technique.

Pot culture trial

Only five fungicides *viz*. benomyl (150 μg/ml), copper oxychloride (1500 μg/ml), mancozeb (3200 μg/ml), pencycuron (250 μg/ml) and tebuconazole (150 μg/ml) were screend using the manufacturers recommended concentrations under pot culture trials. Copper oxychloride and mancozeb were included in the pot trials as they are freely available in the rubber growing districts. The method described by Papavizas & Lewis (1986) was employed with slight modifications.

To bulk up mycelium for use as inoculum, two agar plugs, 12-mm in diameter, were removed from 3-day-old cultures of the isolate of *T. cucumeris* growing on PDA medium and mixed with 150 g cornmeal sand medium (Coarse sand 96%, Cornmeal 4%, sufficient water to moisten) in 500 ml conical flasks. After incubation at 28 ± 2°C for 16 days, the contents of the flask along with 5 g decayed leaves were added to a 22-cm-diameter earthenware pot containing 4 kg of soil.

Fifteen rubber seeds of the clone PB 86, which were just about to germinate were sown in each inoculated pot. Soil drenching was done with test fungicides in water suspension at the rate of 200-ml per pot. Drenching was repeated after 4-days. There were four pots per each treatment. In the control, only water was used.

In addition to the testing of fungicides, traditional burning of the soil was also included as a treatment. A mixture of paddy husk and straw was used for the burning of inoculated soil and the seeds were introduced 24-h after burning.

RESULTS AND DISCUSSION

***In vitro* screening**

It was observed that the efficacy of fungicides varied with the concentrations used and type of screening technique employed. In laboratory screening trials EC 100 (100% inhibition of fungal growth) was considered as the effective dosage of a fungicide because *Thanatephorus* infections in Sri Lanka reach epidemic levels mostly during prolonged humid periods where rapid leaching of applied fungicides is evident. Further, a fungicide was considered to be effective against the fungus only if it achieved EC 100 in both PFT and SFST as it has been shown that it is essential to test soil drenching fungicides in the presence of soil (Zentmeyer, 1955; Corden & Young, 1962).

Most of the fungicides tested in our study using PFT were effective in inhibiting fungal growth totally at a concentration of 50 μg/ml (benomyl, metalaxyl 8% + mancozeb 56%, thiram, tebuconazole and tridemorph). The fungicides such as captan, mancozeb, oxadixyl 10% + mancozeb 56% achieved EC 100 at 100 μg/ml. All other fungicides except copper oxychloride, sulphur and triadimefon showed EC 100 within the tested range (Table 1).

Table 1. *Effectiveness[a] of fungicides against* Thanatephorus cucumeris *as evaluated by two test procedures*

Fungicides	Test procedures	
	PFT[b]	SFST[c]
Benomyl (Benlate)	50	800
Captan (Captan)	100	NA
Chlorothalonil (Daconil)	800	NA
Mancozeb (Dithane M 45)	100	NA
Metalaxyl 8% + mancozeb 64% (Ridomil)	50	NA
Oxadixyl 10% + mancozeb 56% (Sandofan M)	100	NA
Oxadixyl 10% + propineb 56% (Fruvit)	800	NA
Pencycuron (Monceren)	400	1600
Propineb (Antracol)	400	3200
Tebuconazole (Folicur)	50	800
Thiram (Pomarsol Forte)	50	NA
Triadimenol (Bayfidan)	200	NA
Tridemorph (Calyxin)	50	NA

a Lowest concentration (μg/ml) required to inhibit 100%
b Poisoned food technique
c Soil fungicide screening test
NA, EC 100 was not achieved at the tested range. Data for copper oxychloride, sulphur and triadimefon are not included as no dosage tested gave EC 100.

The number of effective fungicides reduced markedly when SFST was employed to screen the effectivity. Only four fungicides *viz.* benomyl (800 μg/ml), pencycuron (1600 μg/ml), propineb (3200 μg/ml) and tebuconazole (800 μg/ml) reached EC 100 at the tested concentrations (Table 1). It is of interest to note that much higher concentrations of fungicides were required to obtain EC 100 in SFST. As suggested by previous workers (Sharma & Mohanan, 1991; Jayasinghe & Wijesundera, 1996) the possible reason for this is that in SFST the toxicity of the chemicals to mycelia is measured in the presence of soil whereas in PFT fungicides acts directly on the growing mycelia.

Based on the results of our *in vitro* study four fungicides, benomyl, pencycuron, tebuconazole and propineb were identified as having the potential for management of the rubber isolate of *Thanatephorus cucumeris*. These four were the only chemicals which reached EC 100 in both screening techniques employed (Table 1).

Pot culture trials

In pot culture trials only two fungicides, benomyl and pencycuron were highly effective in controlling the pathogen (Table 2). Tebuconazole was moderately effective and we are unable to comment on propineb as propineb was not included in pot trials. However, traditional burning of the soil was found to be superior to all these chemicals. Hence, it is clear that most economical way of preventing the disease is the soil burning of the seed germination beds with disease histories, prior to placing the seeds as a prophylactic measure.

Table 2. *Survival percentage of* Hevea *seedlings in the pot culture trial*

Treatment	Post-emergence survival (%)
Burning	100.0 (1.57) a
Pencycuron (Monceren)	93.7 (1.26) b
Benomyl (Benlate)	85.4 (1.11) b
Tebuconazole (Folicur)	62.5 (0.68) c
Mancozeb (Dithane M 45)	41.7 (0.43) cd
Copper oxychloride (Cobox)	25.0 (0.25) d
Control	18.7 (0.20) d

Transformed values are given in parentheses.
Means with the same letter are not significantly different at P = 0.05 according DMRT.

The results of our experiments with chemicals clearly show that pencycuron or benomyl could be used as a curative measure in management of *Thanatephorus* collar rot of *Hevea* seedlings in seed germination beds. Effectiveness of benomyl has also been shown for the management of sore shin infection of tobacco seedlings (Cole & Cole, 1978) and sclerotial disease of maize (Sinha, 1994) caused by *Rhizoctonia solani*. The fungicide pencycuron was reported to be effective against seedling root rot in *Brassica* spp. (Kataria *et al.*, 1993) caused by the same fungus.

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