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Efficacy of Thiophanate Methyl against Red Rot of Sugarcane

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The control of sugarcane red rot was studied in model experiments with carbendazim and thiophanate methyl. In axenic cultures carbendazim inhibited the pathogen more efficiently than thiophanate methyl (MIC 1 and 100 mgL^{-1}, respectively). However, under greenhouse conditions, the reverse picture was revealed. Carbendazim, the metabolite of thiophanate methyl, exhibited lower efficacy against red rot disease than its precursor. Fungicides applied before infection reduced the disease incidence and improved both germination of setts and plant survival. Soaking of sugarcane setts in a 0.25% suspension of fungicides for 24 h before planting was found to be more effective in controlling debris-borne infection than soaking for 1 h period at elevated doses. The persistence of effects both on disease incidence and on promoting plant growth can be observed up to 60 days after planting (DAP).

Keywords: Sugarcane, thiophanate methyl, red rot, chemical control.

Sugarcane production meets heavy losses in India (Viswanathan and Samiyappan, 1999), caused by the red rot pathogen, *Colletotrichum falcatum* Went: teleomorph: *Glomerella tucumanensis* [(Speg.) Arx and Muller]. The pathogen usually survives in the leftover debris in the form of chlamydospores, conidia and thick walled mycelia for several years, which can initiate primary infection at different developmental stages of sugarcane (Singh and Singh, 1989). Such inoculum can, however, cause endemic havoc in early stages of plant growth. Therefore, the preventive control at the germination phase is essential to maintain good crop stand and to achieve higher productivity. The pest management integrating the use of resistant varieties and application of fungicides in early stages of vegetation to control red rot disease assures the high yield of good quality.

The efficacy of fungicides against *C. falcatum* and protecting setts and canes from red rot in the field has been reported (Pandey and Agnihorti, 1996; Rao and Sathyanarayana, 1997; Sengupta et al., 1998). Thiophanate methyl is a broad-spectrum systemic fungicide used for preventive and curative treatments to control various diseases that affect legumes and vegetable crops (Sobti, 1993). The aim of the studies presented here was the evaluation of preventive value of thiophanate methyl and different application strategies against red rot disease of sugarcane.

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Materials and Methods

Chemicals

Commercial grade of thiophanate methyl (Roko® 70% WP), Wockhart, Mumbai and carbendazim (Bavistin 50% WP, BASF, Mumbai) were used throughout the experiments. Oat meal agar and oat meal broth were prepared in the laboratory and used without any further amendments.

Test organisms

The red rot susceptible sugarcane (*Saccharum officinarum* L. cv CoC 671) used in experiments was maintained in the plant pathology farm and for the experiments setts were obtained from 6 to 8-months-old canes.

The pathogen *C. falcatum* was isolated from red rot infected stalk tissue of CoC 671 and maintained oats agar slants at 20 °C. A piece of mycelial mat was transferred into Petri dishes containing the same medium to maintain colonies for further inoculations.

Biological activity studies

Responses of the pathogen and reactions of the host plant to chemicals were studied both *in vitro* and *in vivo.*

Responses of the pathogen

The fungicides tested were incorporated into the medium at 1, 5, 10 and 25 mgL^{-1} final concentrations after autoclaving. The agar plates were inoculated with 9 mm mycelial discs of *C. falcatum* and incubated at 28 ± 2 °C. The colony diameters were measured 7 days after. Similarly oat meal broths in flasks were inoculated with mycelial discs and incubated for 15 days. After which the mycelial mats were harvested, dried and expressed in mg. The inhibition rate was calculated as percentage to untreated control.

Responses of the host plant

The fungicides were tested in different doses as sett treatment, soil drenching and foliar spray against red rot incidence. During which the responses of the plant was observed visually in terms of improved plant growth or any other adverse effects like phytotoxicity.

Disease inhibition studies

The efficacy of fungicides was evaluated in soil cultures (21 × 10.5 × 18" pots) where infected stalk pieces from naturally infected canes have been incorporated prior to sowing (Viswanathan and Samiyappan, 2000). The time course of treatments depended on the mode of application: sett treatment, soil drenching (0 and 45 DAP), foliar spray (30 and

60 DAP), sett treatment + soil drench (45 DAP) and sett treatment + foliar spray (45 DAP). Germination and plant survival were recorded up to 90 DAP at regular intervals deferring to applications. Survival and disease incidence were expressed as a percentage related to untreated control.

To determine the effective dose and requested time for exposure of fungicides doses at 0.1, 0.25, 0.5, 0.75 and 1.0% concentrations and two periods of exposure (1 h and 24 h) were applied. The setts subjected to the above treatments were planted in pots with priorly infected soil. The sett germination, plant growth and red rot incidence were recorded regularly up to 90 DAP. Results were expressed as a percentage related to untreated control.

The *persistence of fungicidal effects* was studied on plants treated before settling (setts soaked 24 h in 0.1% suspension of fungicides) and grown up in pathogen free soil (21 × 10.5 × 18" pots). One month after planting the shoots were inoculated with the pathogen on 30 and 45 days after planting to assess the persistence of fungicidal effects. The pathogen was inoculated by the whorl method, in which one ml of inoculum (10^6 conidia/ml) was dripped in the whorl of growing sugarcane plants using a Pasteur Pipette. Then the pots were transferred to a disease testing chamber (30 ± 2 °C, 90% humidity) (Mohanraj et al., 1997). The development of symptoms (yellowish orange discolouration and drying of leaves followed by drying of entire settings) was assessed 15 days after inoculation.

Results

Development of disease syndrome

Addition of debris-borne inoculum on soil produces symptoms in different stages. The pathogen causes necrosis of root-eyes and buds which results in pre-germination death of germinating setts. After sprout emergence the pathogen causes necrotic lesions of various sizes on settlings leading to death of germinated settings. After cane formation (45 DAP) the pathogen affects the base of the clump which results in progressive yellowing of foliage and finally drying of plants. If pathogen is inoculated in whorl region it initially causes necrotic lesions on young leaves/midribs. Later the pathogen moves towards bottom from whorl and this process result in drying of canes partially or completely. In case of partial drying, pathogen reaches the matured cane stalks traversing through the stalk.

Effect of fungicides on the pathogen

Results showed that TM required a minimum of 29.2 and 73.0 mM in solid and liquid media for 100% inhibition of pathogen growth, while carbendazim required only 5.2 and 52.0 mM in the same media. In this poisoned food technique, mass accumulation of fungal growth in liquid broth was found to be higher than the apical growth on solid medium. Hence results of fungicidal effect on mycelial dry weight presented as dose response lines where the inhibition rate was given in probits versus log concentration of active substances *(Fig. 1).*

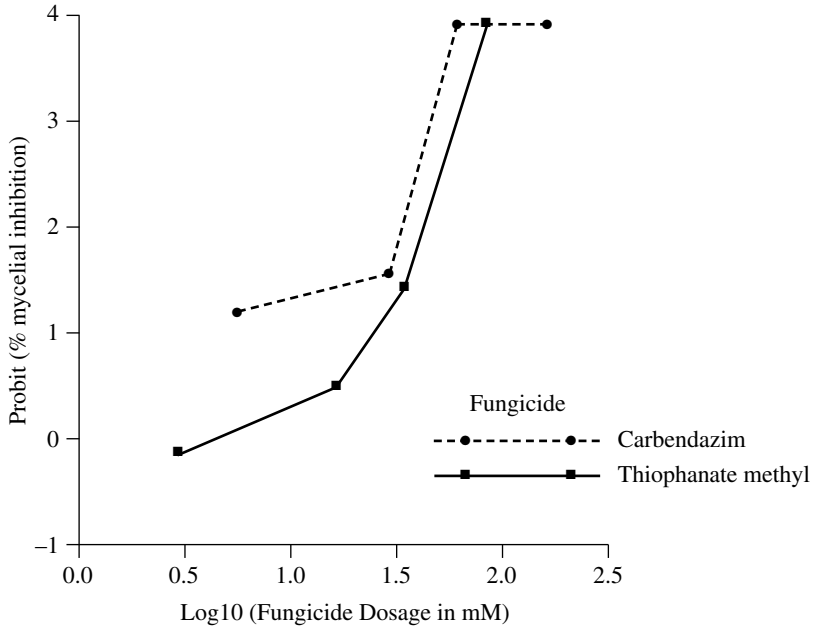

Fig. 1. Inhibitory effect of fungicides against *C. falcatum in vitro*

Effect of fungicides on the host plant

Observations were made on the effect of fungicides on sett germination, sprout emergence and settling emergence etc. The studies indicated that fungicidal treatment at different concentrations/durations favourably increased sett germination. Interestingly early emergence of settlings was recorded in treated pots. At 30 DAP fungicide treated pots showed vigorous settlings than the control. At later stages also fungicide treatment did not show any adverse effect on sugarcane growth.

Efficacy against red rot disease

Treating setts or drenching soil with TM at the time of planting protected setts from debris-borne pathogen as indicated by the improved germination and reduced post emergence mortality *(Table 1).* The treatments improved sett germination from 29 to 37% over control at 20 DAP and reduced disease incidence from 67 to 88% at 90 DAP.

Persistence of fungicidal effect

In the fungicide treated plants when inoculated 30 DAP restricted lesion development was noticed and marked variation was observed among treatments as compared to control *(Table 2).* However inoculation on 45 DAP resulted in symptom development as in control. Results of this study showed that TM considerably reduced pathogen infection and improved plant growth as compared to carbendazim.

Table 1

Efficacy of thiophanate methyl (0.1%) against soil borne inoculum of sugarcane red rot

Treatments	Per cent germination (20 DAP)	Per cent infection (90 DAP)
Sett treatment	90.0a	10.0ab
Soil drench at planting	95.0a	15.0b
Soil drench (60 DAP)	70.9b	35.0c
Sett treatment + soil drench at planting	95.0a	5.0a
Sett treatment + soil drench (60 DAP)	90.0a	10.0ab
Control	70.9b	45.0d

*DAP: days after planting; Data were transformed (arcsine) before comparison was made. In a column means followed by same letters are not significantly different at 5% by DMRT.

Table 2

Effect of sett treatment with fungicides on red rot incidence and plant growth of sugarcane (60 DAT)

No. of pots*	Infection rating			Plant height (cm)		
	TM	Car	Control	TM	Car	Control
1.	+	+++	+++++	14.0	10.0	6.5
2.	–	+++	++++	15.0	9.4	8.7
3.	+	++++	++++	11.7	10.8	12.0
4.	++	++	+++	9.4	11.0	10.4
5.	+	+++	++++	12.0	8.7	11.3

*Three plants from each pot was used for the inoculation. DAT: Days after sett treatment; TM: Thiophanate methyl; Car: Carbendazim.
+++++: Plant completely killed and dried; ++++: all leaves (5) dried/4–5 freshly infected and 2–3 dried leaves; +++: 2–3 freshly infected and 2–3 dried leaves; ++: 1 freshly infected leaf/lesion at the point of inoculation and 1–2 dried leaves; +: 1 dried leaf; –: no infection.

Standardization of dose and time course of treatment

All concentrations of both fungicides protected the setts from debris-borne inoculum and improved germination by 25 to 60% over the control *(Table 3)* without further reference to the time course of treatments. Plant survival ranged from 25 to 100% in various treatments opposing to 16.7% in control at 90 DAP. Irrespective of dose and period of treatment plant survival has been improved till 60 DAP. However, in later stages, i.e. 60 DAP, 0.25% concentration showed maximum effect followed by 0.1%. At these concentrations TM was more effective than carbendazim irrespectively on the tract of time of soaking and the stages of plant development.

Mode of application of fungicides

Studies on the mode of application of fungicides showed that sett treatment followed by either soil drenching or foliar stray were found to be more effective than sett treatment alone *(Table 4).* Sett treatment + soil drenching (45 DAP) proved to be the most effective

Table 3

Efficacy of different fungicidal concentrations and periods of sett treatment against red rot in sugarcane

Treatments	Per cent germination	Per cent plant survival (days after planting)							
		30	45	60	75	90	120	150	Mean
TM 0.1% – 1 h	100.00	91.65	66.65	66.65	66.65	58.30	41.65	33.30	54.14
TM 0.25% – 1 h	91.65	91.65	75.00	66.65	66.65	66.65	66.65	66.65	66.67
TM 0.5% – 1 h	91.65	75.00	58.35	50.00	50.00	50.00	50.00	41.70	50.01
TM 0.75% – 1 h	91.65	83.30	58.35	58.35	50.00	41.65	33.30	33.30	44.84
TM 1.0% – 1 h	91.65	91.65	83.30	75.00	66.70	66.70	33.30	33.30	56.31
Car 0.1% – 1 h	66.70	66.65	58.35	33.35	25.00	25.00	25.00	16.70	34.38
Car 0.25% – 1 h	91.65	75.00	66.70	50.00	50.00	50.00	33.35	33.35	46.89
Car 0.5% -1 h	100.00	100.00	83.35	58.30	50.00	41.70	41.70	33.35	54.18
Car 0.75% – 1 h	91.65	91.65	91.65	83.30	83.30	66.70	58.35	58.35	68.75
Car 1.0% – 1 h	91.65	91.65	75.00	75.00	50.00	50.00	33.35	16.70	56.26
TM 0.1% – 24 h	91.65	91.65	75.00	75.00	75.00	75.00	75.00	75.00	71.88
TM 0.25% – 24 h	100.00	100.00	100.00	100.00	100.00	100.00	91.65	91.65	88.54
TM 0.5% – 24 h	83.30	83.30	66.65	66.65	66.65	58.35	41.65	33.35	55.20
TM 0.75% – 24 h	83.30	83.30	75.00	75.00	75.00	75.00	41.70	41.70	60.43
TM 1.0% – 24 h	91.65	91.65	83.30	83.30	75.00	66.65	41.65	33.35	59.43
Car 0.1% – 24 h	91.65	91.65	75.00	66.65	66.65	58.35	58.35	50.00	63.54
Car 0.25% – 24 h	66.65	66.65	66.65	58.35	41.65	33.35	33.35	33.35	41.73
Car 0.5% – 24 h	75.00	75.00	66.70	66.70	66.70	66.70	50.00	41.70	54.25
Car 0.75% – 24 h	83.30	83.30	83.30	66.70	58.35	58.35	33.35	33.35	53.13
Car 1.0% – 24 h	75.00	75.00	66.65	50.00	41.65	41.65	41.65	25.00	45.83
Control + Debris	41.65	33.30	25.00	25.00	25.00	16.70	16.70	16.70	21.89
Control – Debris	91.65	91.65	91.65	91.65	91.65	83.35	83.35	83.35	88.09
Mean		82.94	72.35	65.53	60.98	56.83	46.59	21.71	56.12

TM: Thiophanate methyl; Car: Carbendazim. CD (P=0.05) – per cent germination: 28.25; per cent survival: days – 7.64, treatments – 12.67, days × treatments – 35.84.

one in inhibiting the disease incidence up to 86% as related to the control. Other combined treatments viz. sett treatment + foliar spray (45 DAP) and soil drenching (0 and 45 DAP) inhibited the disease development at 71%. Though both fungicides significantly suppressed the red rot disease the TM treatments inhibited the disease at 42–86% while carbendazim treatments at 29–71% depending on the methods of application.

Discussion

Recent studies have indicated the efficacy of fungicides against red rot disease of sugarcane. However none of them could be used in the field due to practical difficulties in fungicide application. Agnihorti (1990) reported on fungicidal effect of TM against *C. falcatum in vitro* as well as on protective effect against debris-borne infection. In our studies we also demonstrated the fungicidal effect of TM *in vitro,* however it showed only a partial control of the disease in standing canes and failed to result in complete control

Table 4

Efficacy of various fungicidal application methods against red rot

Type of treatment	Fungicides	Per cent germination (20 DAP)	Per cent infection	
			(30 DAP)	(90 DAP)
Sett	Car 0.1%	80.0	9.0	27.0
Sett	TM 0.1%	66.7	9.0	27.0
Soil (O and 45 DAP)	Car 0.1%	73.3	9.0	18.0
Soil (O and 45 DAP)	TM 0.1%	73.3	9.0	18.0
Foliar spray (30 and 60 DAP)	Car 0.1%	40.0	36.0	45.0
Foliar spray (30 and 60 DAP)	TM 0.1%	40.0	27.0	36.0
Sett + Soil (45 DAP)	Car 0.1%	73.3	9.0	18.0
Sett + Soil (45 DAP)	TM 0.1%	80.0	9.0	9.0
Sett + Foliar spray (45 DAP)	Car 0.1%	73.3	18.0	27.0
Sett + Foliar spray(45 DAP)	TM 0.1%	73.3	9.0	18.0
Control with debris	–	33.3	45.0	63.0
Control without debris	–	86.7	0.0	0.0

TM: Thiophanate methyl; Car: Carbendazim; DAP: days after planting. CD (P=0.05) – per cent germination: 9.30; per cent infection: days – 3.47; treatments – 8.49; days x treatments – 12.01.

with development of the crop. This is in accordance to the findings of Agnihorti (1983), who reported that fungicides like Bavistin, Benomyl, Vitavax, Areton, Topsin etc. have been found effective *in vitro* against *C. falcatum* but these not given satisfactory control of the disease under field conditions. Partial chemical control of red rot disease under field conditions have also been reported by Chand et al. (1974); Lewin et al. (1976); Rao and Sathyanarayana (1995).

Results of the present study indicated that sett treatment with TM resulted in improved plant growth as compared to carbendazim treatment and untreated control. Efficacy of this fungicide in promoting plant growth has been reported in other crops either. The seedling growth, biomass production, nodulation and phenol content of *Sesbania sesban* (Siddiqui et al., 1997) were promoted. Various biochemical parameters viz., chlorophyll, protein and phenol contents of *Hibiscus esculantus* and *Capsicum annum* have also been improved (Soabiha and Siddiqui, 1997). Our results also indicated the superior effect of TM over carbendazim in protecting canes from red rot disease and improving plant growth.

Persistence study confirms the systemic nature of TM and the persistence of fungicidal efficacy up to 60 DAP in the plants. The results also indicated the superior effect of TM over carbendazim in protecting canes from red rot incidence and improving plant growth.

Soaking sugarcane setts in optimum concentration of fungicide overnight was found to be more effective compared to 1 h treatment indicating that increased duration of fungicide treatment is required for diffusion of the fungicidal compound into the setts. The lack of increase in efficacy with increased concentration might be due to saturation of the chemical at higher concentration. Kirtikar and Verma (1963) reported that dipping healthy setts in mercurial, sulphur and copper fungicides of different strength for 10 min did not provide protection against red rot infection through contaminated soil/water carrying

spores. Lewin et al. (1976) also reported that among systemic, non-systemic and antibiotics studied, dip treatment of setts in Agallal (0.5%) for 15 min gave excellent control of red rot. Chand et al. (1974) reported that 1 h pre-treatment of setts with Benomyl (0.25%) and Vitavax (0.05%) increased germination over the control by 50% and reduced the red rot incidence in sugarcane. Rao and Sathyanarayana (1995) attempted soaking of setts with fungicides from 30 min to 48 h periods and found that 24 h soaking was more effective than other durations. They also reported that none of the fungicides (Benomyl, carbendazim, Triforine, Chlobenthiozone, Triadimefon, carboxin, TM) tried offered complete control of the disease but reduction in disease incidence was observed with carbendazim and Triadimefon. However there were reports with less duration, our results indicated that for improved efficacy of fungicide at least 24 h soaking is required. However, treating the sugarcane setts with fungicides for such long duration at planting may be impractical due to the large volume of seed material and requirement of other facilities. In this regard, to exploit the fungicidal efficacy to contain red rot disease, modified fungicide treatments through high-pressure diffusion techniques may be useful. Results of the present study have also shown that additional fungicide applications through soil/foliar treatments along with sett treatment were found to be more effective than single application in protecting the canes from red rot incidence.

Comparative study indicated that carbendazim inhibited *C. falcatum* in axenial cultures more effectively than TM. Similar results were published by Rao and Sathyanarayana (1995). However, studies under field conditions revealed that TM showed better effect than carbendazim when sett treatment alone has been given at lower concentrations of 0.1 and 0.25%. In our studies both fungicides exhibited similar efficacy against red rot incidence when applied at higher concentration/repeatedly. Previous works of Yang and Seaberg (1974) and Yang and Braud (1977) have also revealed that soaking the setts in fungicide suspension before planting and subsequent spraying on three budded setts at the time of planting increased the germination. Efficacy of TM in protecting sugarcane in field plots artificially infested with *C. falcatum* or *Ceratocystis paradoxa,* the sett rot pathogen, was also reported. Pliansichai (1999) recommended stool spray application repeatedly during one to five month's stage with benomyl, thiobendazole, propiconazole, tubecanazole and thiophanate methyl in Thailand to control the disease. Since Thailand farmers have adopted whole stalk cuts for planting, the fungicides were also applied to the whole stalk cuts in bulk before planting or sprayed directly on the whole stalk cuts lying in the furrows during planting. Contradictorily, Padmanaban et al. (1990) reported that none of the chemical dips/foliar sprays (carbendazim or copper oxychloride) with various amounts of surfactants was effective in controlling this disease under field conditions.

Partial chemical control of disease under field conditions might be due to impervious nature of the rind and inability of the fungicide to reach the site of infection in the tissue (Agnihorti, 1983; Rao and Sathyanarayana, 1995). Further in most of these experiments the sugarcane setts were soaked for a limited duration, which resulted in limited uptake of fungicidal compound. In the present studies enhanced efficacy of fungicide at optimum dose (0.1–0.25%) and increased soaking duration (24 h) was noticed, indicating that these factors are essential for better chemical control of sugarcane red rot.

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