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In vitro assessment of fungicides against banana stem rot fungus, *Marasmiellus sp.*

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ABSTRACT

Stem rot on banana is the latest and economic important disease to Jaffna; Sri Lanka, because of this infection, immediate control measure need through chemical application that's why this research study was carried out as in-vitro condition against the fungal pathogen. Five broad spectrum contact fungicides at two levels of concentrations as manufacture recommended level and half of the recommended level were evaluated, using poison food technique, radial growth of mycelia of Basidiomycetes fungus Marasmiellus sp. (Agaricales: Tricholomataceae) isolated from Banana. It was observed that the fungicides Coblite (Copper 50% (w/w) WP as copper oxychloride), Pomarsol forte WP 80% (thiram 80%), Captan (Captan 50% (w/w) WP), Ridoaxyl (Metalaxyl 8 % (w/w) +Mancozeb 64% (w/w) WP), Max (Chlorothalonil 75% (w/w) WP)) at all the concentrations tested inhibited mycelial growth of the fungus. All fungicides significantly differed from the control except coblite. The highest percentage inhibition was recorded as 86.76% (11.92 mm Mean Colony Diameter (MCD)) from the manufacture recommended level of concentration (0.5g/100ml) of Metalaxyl 8% (w/w) + Mancozeb 64% (w/w) WP indicating combined effect of two compatible fungicides, followed by Chlorothalonil at both concentrations 0.15g/100ml and 0.075g/100ml were inhibited 64.09% (32.32 mm MCD) and 61.02% (35.08 mm MCD) of colony growth respectively, This investigation revealed that, Mancozeb and Metalaxyl in combination and Chlorothalonil based fungicides can be the best option for effective control of Marasmiellus sp.

Key words: Metalaxyl, Chlorothalonil, Mancozeb, Banana, Stem rot, *Marasmiellus* control

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INTRODUCTION

Banana (*Musa* sp.) is one of the widely cultivating and consuming fruit in Sri Lanka. It is also an attractive perennial fruit crop for small holders due to its high economic gains throughout the year. In Sri Lanka banana is cultivated more than 50,000 ha and annual production is about 450,000 Mt. More and more rice farmers are switching to banana cultivation due to the high profit margin [1]. The banana cultivation is popular among Jaffna farmers because of its suitability to dry zone and its higher demand. It is cultivated for fresh consumption and to make some sweet foods. The total area of banana cultivation in Jaffna district is 823 ha and the average annual production is 30Mt per ha [10]. Banana cultivation is considered as main source of income to 4000 farm families in Jaffna and the average monthly income of Rs. 18,500 to 21,000 [1]. However, Jaffna banana farmers are facing several pests and diseases problems. Proper identification as well as diagnose of pest and disease is very important to implement effective control measures to sustain the production as well as to increase its production [10]. Recently, Basidiomycetes pathogenic fungus *Marasmiellus* sp. causing stem rot was first time recorded on banana in Jaffna, Sri Lanka. This new occurrence of stem rot disease initiates infection and damage directly on pseudo-stem and indirectly on banana leaves as well as its fruits production. It is expected to become a serious problem in forthcoming periods due to its mode of dispersal. Hence, it is vital to understand the life cycle and epidemiology of *Marasmiellus* sp. [2] Jackson reported the biology and epidemiology of this fungus on coconut. [9] Nelson and

Javier described the symptoms of this disease on banana however the biology and epidemiology was not studied in banana until now. However many *in-vitro* studies have demonstrated that some fungicides restrict or prevent the growth of fungal pathogens [6] and [3]. In the literature there are only few or no reports about the influence of fungicides on mycelial growth of *Marasmiellus* sp. from banana plant. In view of the importance of the crop and the effect of fungal diseases on the yield, there is a need to identify management options for disease associated with this important crop. The aim of the study was to determine *in vitro* effects of selected fungicides on the mycelial growth of *Marasmiellus* sp. isolated from banana.

MATERIALS AND METHODS

Isolation of pathogen

Pathogenic fungus was isolated by surface sterilization method described by Kinkel and Andrews [5]**.** The samples with fungal infection were collected from the effected fields at Kopay and Thirunelvely in Jaffna, Sri Lanka during November 2010. From selected sample, 5 x 5mm size of pseudo stem cuttings were selected for easy handling and subjected to the surface sterilization by using 70% ethanol for one minute and rinsed with distilled water for 8-10 times. Thereafter, sterilized samples were transferred to the moist chamber to facilitate the fungal growth. Following 4-5 days of incubation, actively growing hyphae were transferred to the petri dishes containing PDA medium supplemented with 2-4 drops of Chloromphenicol to get pure culture of *Marasmiellus* sp. Petri dishes were incubated in room temperature of 27-33^{0}C for 5 days. Pure culture was of the pathogen was made through sub-culture and stored in the refrigerator at 4^{0}C for further study.

Evaluation of fungicides

Five broad spectrum contact fungicides viz. Coblite (Copper 50% (w/w) WP as copper oxychloride), Pomarsol forte WP 80% (thiram 80%), Captan (Captan 50% (w/w) WP), Ridoaxyl (Metalaxyl 8 % (w/w) +Mancozeb 64% (w/w) WP), Max (Chlorothalonil 75% (w/w) WP) were evaluated *in vitro* against *Marasmiellus* sp., by poison food technique described by [7]. The efficacy of the fungicides was evaluated on PDA at two concentrations (Table 1). Required quantity of each fungicide for each concentration was weighed and dissolved in 100 ml distilled water and freshly prepared PDA and allowed to cool to a pouring temperature of 40-45 ^{o}C added two to four drops of Chloromphenicol.

Fifteen milliliter of PDA was amended with different fungicide rate as a milliliter was poured into 90 mm diameter Petri dish. Each plate including the control (without fungicide or an ml of distilled water) was inoculated with even size as 4mm in diameter agar slug with five days old *Marasmiellus* sp. culture. The Petri plates were incubated in room temperature at 30±3 ^{0}C and observed daily for mycelial growth. Replicated five times and a Petri dish represented a replicate as described by Mamza [11]. Radial growth was recorded after five days of inoculation, that mean control plates reached the maximum radial growth of fungus in Petri dish as 90 mm in colony diameter.

Statistical analysis

All the experiments were designed according to the complete randomized design (CRD) and obtained data were statistically analyzed using SAS package and the significance among the treatments were determined according to the least significant difference (LSD) test at 95% of confidence interval.

Table 1: Treatments according to the concentrations

Treatment	X g/ 100ml	Concentration (1X g/ 100ml)	Concentration (0.5 X g/ 100ml)
Coblite	0.4	T_1	T_2
Pomarsol forte WP 80%	0.15	T_3	T_4
Captan	0.15	T_5	T_6
Redoaxyl	0.5	T_7	T_8
Max	0.15	T_9	T_{10}
Control	-	T_{11}	-

X- Recommended level (The experiment was repeated twice with five replicates)

RESULTS AND DISCUSSION

Cultural characters of *Marasmiellus* sp.

Regular cottony growth and rounded shaped white colony was initially developed and turned to creamy with smooth, branched and hyaline mycelium in the culture. Spores or any other reproductive parts were not observed under the light microscope examination, with a temperature range of 25-35^{0}C. However macroscopic fruiting body of *Marasmiellus* sp. was white and the pileus measured was 3±2 cm diameter, length of stripe was 3.5±1.5cm and it produced in high humidity and low temperature environment. It is growing commonly in rainy season but in low

humidity and higher aerial temperature (30^0C) as dry season pileus was small (2±1.5 cm in diameter), small stripe (2±1cm), creamy to pale yellow in colour. Number of gills varied 30±5 in number with size of fruiting body and lamella arrangement was in two layers. One is from the center of pileus and other layer started in between the centre and periphery. Supported these results Andre [4] reported that fruiting body of *Marasmiellus* sp. was white cap with size of 1-3 cm, stripe base was pinkish orange. Fong [12] reported that on orchid basidiospore liberation was optimum at 24^{o}C, germination was at 24-28^{o}C and spores were the source of primary infection of *Marasmiellus* sp. Temperature does affect the growth of *Marasmiellus* sp. and temperatures conducive for radial growth varied from 24-28^{o}C.

***In vitro* evaluation of fungicides**

Evaluation of five broad spectrum contact fungicides with two different levels of concentrations were tested there inhibitory effect on the growth of *Marasmiellus* sp. under *in-vitro* condition. Radial growth of *Marasmiellus* sp. In presence of the fungicides has been presented in the plate 1 and table 2.

Plate 1: Effects of fungicides on *Marasmiellus* sp.

A-1X g/100ml concentration of fungicides against *Marasmiellus* B- 0.5X g/100ml concentration of fungicides against *Marasmiellus* sp.

Plate A and B: upper line (L-R) -control, coblite (X=0.4), pomarsol forte wp 80% (X= 0.15),
Lower line- captan (X=0.15), redoaxyl (X=0.5) and max (0.15) fungicides

A B

Table2: Evaluation of fungicides against *Marasmiellus* sp. under the *in-vitro* condition

Treatments (X g/100ml)	1X concentration		0.5 X concentration	
	Mean of colony diameter (mm)*	Mean inhibition (%)	Mean of colony diameter (mm)*	Mean inhibition (%)
Coblite (0.4)	90.00 [a]	0.00	90.00 [a]	0.00
Pomarsol forte WP 80% (0.15)	42.40 [c]	52.89	56.64 [c]	37.07
Captan (0.15)	73.16 [b]	18.71	73.84 [b]	17.96
Redoaxyl (0.5)	11.92 [f]	86.76	42.52 [c]	52.76
Max (0.15)	32.32 [e]	64.09	35.08 [de]	61.02
Control	90.00 a	0.00	90.00 a	0.00

** Values are mean of five replicates*

Figures having same letter in a column indicate the values are not significantly different according the LSD at 0.05 α and 95% confidence interval.

The treatments expressed ranging (0-86%) inhibitory effect. Coblite (copper 50% (w/w) WP as copper oxychloride) at two levels of concentration did not inhibit the fungal growth. All other fungicides significantly differed from the control. Highest percentage of inhibition of the *Marasmiellus* sp. was recorded has 86.76% (11.92 mm MCD) from the 1X g/100ml concentration level of redoaxyl (Metalaxyl 8% (w/w) + Mancozeb 64% (w/w) WP), followed by max (Chlorothalonil 75% (w/w) WP) both recommended and half of recommended levels (HRL) 64.09% (32.32 mm MCD) and 61.02% (35.08 mm MCD) respectively and captan had little effect on growth of *Marasmiellus* sp.
The results of *Marasmiellus* sp. against two levels of fungicides are shown in the figure 1. Redoaxyl, max and pormarsol forte 80% WP were inhibited *Marasmiellus* sp. under *in-vitro* condition and these could be recommended to control *Marasmiellus* sp. [12]. evaluated of fungicides such as mancozeb, triadimefon, maneb, propineb, etridiazole and maneb+carbendazim and reported that all inhibited the growth of *Marasmiellus* sp. therefore it could be concluded that carbamate group of fungicides have more effect on *Marasmiellus* sp. in orchid both *in-vitro* and *in-vivo* conditions. Among the fungicides, Triadimefon and mancozeb are the best to inhibit *Marasmiellus* sp. as these fungicides successfully controlled the formation of basidiocarp on infected tissues as well as germination of the basidiospores

CONCLUSION

Mancozeb, Chlorothalonil based fungicides can be recommended for effective control.

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