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In vitro Biological Control of *Marasmiellus* sp. The Causal of Stem Rot of Banana Grown in Jaffna Peninsula, Sri Lanka

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Abstract: Stem rot of banana caused by Basidiomycetes fungus, *Marasmiellus* sp. (Agaricales: Tricholomataceae) is new and confined to Valikamam division of Jaffna peninsula, Sri Lanka. Symptoms of stem rot were rotted patches on rhizome and pseudo stem, gradual wilting of leaves from lower area to upper part of plant canopy, stunted growth, abnormal leaves and bunches, toppling of crown and fruiting body adhere on pseudo stem. A banana field at Thirunelvely, recorded disease incidence as high as 44.44%. The disease severity was 96.3% in left out portion of pseudostem, which yielded bunch already, 62.63% in mature plants and 23.02% in suckers. An antagonistic fungus, *Trichoderma* spp. showed suppressive effect on *Marasmiellus* sp. grown on PDA by using dual culture technique. *Trichoderma harzianum* and *T. viride* inhibited 80.6% and 92% mycelia growth of *Marasmiellus* sp, respectively. Poison food method revealed that maximum inhibition was with *Azadirachta indica* (79.19%) and *Ocimum sanctum* (59.96%) than *Lantana camara, Zingeber officinale* and *Curcuma longa* under *in-vitro* conditions. This information will help to create awareness among extension workers and growers about *Marasmiellus* sp. causing stem rot disease on banana in Jaffna peninsula and also helpful to restrict its spread in newer areas through infected suckers and other means.

Key words: Banana · Bio control · *Marasmiellus* sp. · Plant extract · Stem rot · *Trichoderma* spp

INTRODUCTION

Banana (*Musa* sp.) is the most widely cultivated and consumed 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]. Cultivation of banana is popular among farmers in Jaffna peninsula due to its higher demand and suitability to dry zone. It is a widely adopted crop and grown both as a stable food and cash crop mainly for the local markets. The total area of banana cultivation in Jaffna peninsula is 823 ha with the average annual production is 30Mt per ha [2]. Cultivation of banana is the main income source to 4000 farm families in Jaffna peninsula and the average monthly income is ranging from Rs. 18,500 to 21,000 [1]. Now a days cultivation of banana in Jaffna peninsula is in forward direction both in extent and production as in 2005 the extent of banana cultivation was 584 ha but it was increased to 823 ha in 2010 as with production of 24690 Mt [2]. He further reported that Urumpiray, Uduvil and Puthoor divisions contribute 58% of the production of Jaffna peninsula and the rest from other divisions. In addition it is grown in all home gardens to provide leaf and the fruits. Among major local banana cultivars, *Kathali* is cultivated as higher percentage as (59%) after that *Itharai* (34%) and others (*Kappal* and *Monthan*). Even though Jaffna peninsula banana farmers faced several problems of which pests and diseases are the major, hence diagnoses and management to disease in a proper manner is important and to sustain its production [2].

Marasmiellus sp. was first time recorded in banana in Jaffna peninsula, Sri Lanka. The new occurrence of stem rot disease caused 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 such as soil borne as well as air borne fungi which is capable to attacking many monocot plants, especially banana, coconut orchid and rice etc. [3]. It is also reported to cause seedling blights, root rots and wilt on millets and

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Sudan grass [4]. It has a wide host range including *Cocos nucifera*, *Musa* spp., taro and rice [5], *Cocos nucifera, Colocasia esculenta, Commelia* spp., *Musa* spp., *Synedrella nodiflora, Zea mays, Oryza sativa, Pennisetum glaucum, Sorghum sudanense* [3], millets [4] and *Oncidium goldiana* [6]. Also cause root rot in *Zea mays* and *Saccharum officinalis* [7] and Alfalfa and Khell. Its worldwide distribution found in Brazil, Bolivia and Malaysia [8], Singapore [6], Pohnlangas [5], South America, Egypt, Australia, French Polynesia [3] and Egypt [7]. It is well distributed in marginal soil (soil with the poor nutrition or physical structure and low in organic matter high in clay) poorly drained and wet areas [9]. The severity is high when the rainfall in higher than 5000mm [10]. The disease is significant on commercial banana production when the annual rain fall is higher [11]. The symptoms of banana pseudostem rot caused by *Marasmiellus inoderma* were poor growth, wilting and a brownish-black to maroon coloured internal pseudo stem necrosis, plant stunting and plant death. The internal stem necrosis was confined primarily to the outer layers of the banana pseudostem [5]. Signs of *M. inoderma* are white mycelial growth within the external layers of the pseudostem (xylem) and at the base of psuedostems and white to cream-colored fungal fruiting bodies adhering to outer layers of banana pseudo stem [5]. The fungus is able to penetrate leaves, pseudo stems, or roots to cause the following symptoms, decay and withering of outer leaf sheaths/blades; leaf stunting; cracked pseudo stems, slow plant growth, plant stunting and death and narrow pseudo stems. White mushrooms often appear along the cracks in the affected pseudo stems [9]. Outer leaf sheaths and leaf bases wither and decay; leaves are slow to emerge and are stunted. White or pink fungal growth commonly occurs between the leaf sheaths and in wet weather carpophores develop on the pseudo stem and on debris on the soil. The roots may also be attacked [3]. In most cases, there was no discolouration in the corm or true stem, as most symptoms are in the leaf sheaths. Occasionally the growing point was killed and rot entered the corm from the edges. In some cases, fruiting bodies were present on the outside of the pseudo stem while White mycelium was present on brown lesions when the dried outer leaf sheaths were stripped away [11]. *Marasmiellus inoderma* has white colour mycelium and produced white to cream coloured fungal fruiting bodies [5]. In culture medium it grew vigorously and produced dense white (Snow-white) cottony mycelium with feathery edges on all solid substrate tested [12], produced white colonies, often with thick, ropy, mycelial strands and all hyphae were septate, binucleate, hyaline with clamp concentration, no spores were observed. Basidiocarps developed in moist chamber cultures after 60 days as mushroom [13]. According to these details this study was carried out toward its management by using biorationals.

MATERIALS AND METHODS

Investigations were carried out in both laboratory of Department of Agricultural Biology, Faculty of Agriculture, University of Jaffna and farmers' banana fields in Jaffna. The details of materials used and methodology adopted in these investigations are described as follows.

Distribution of *Marasmiellus* sp. Disease on Banana in Jaffna: Survey of Banana Stem rot was carried out in different cultivation area throughout Jaffna Peninsula, Sri Lanka. Prevalence of *Marasmiellus* sp. stem rot disease on banana was found in Valikamam area and did not expose the disease symptoms in other area such as Thenmaradchy, island and Vadamaradchy areas. Valikamam, Kopay, Thirunelvely, Nilavarai, Karanthan, Kondavil, Kokuvil and Chankanai areas were recorded as disease intense areas. Among this Karanthan, Kopay and Thirunelvely area had the highest disease intensity of 50-60% but in remaining areas disease intensity were ranged from 1-30%. Disease intensity was recorded using the following formula.

$$\text{Disease intensity} = \frac{\text{No of diseased plant}}{\text{No of plants randomly selected in a field}} \text{ X } 100$$

Isolation of the Pathogen: Pathogenic fungus was isolated as described by Kinkel and Andrews [14]. The samples were collected in the areas of Kopay and Thirunelvely in Jaffna, Sri Lanka. Confirmation was carried out by inoculating fungal mycelium into the healthy banana plants using both soil drench and streak methods. Symptoms and signs were confirmed, on the basis of Koch's postulate. Various symptoms of *Marasmiellus* rot on banana and development and survival rate at various temperatures and humidity range in Jaffna peninsula was recorded. Since, *Marasmiellus* stem rot disease is newly recorded in banana grown in Jaffna district, prevalence and distribution of the disease were studied. Within the identified field's percentage of diseased plants, suckers, death plants, environment and cropping pattern were recorded periodically.

Different Dosage of *Trichoderma viride* Tested Against *Marasmiellus* sp *In vitro* Conditions: Different dosage of *T. viride* and *T. harzianum* were evaluated against *Marasmiellus* sp. using dual culture technique described by Rangeswaran and Prasad [15]. A mycelial disc of 4 mm diameter from 5 days old culture was obtained from both species. *Trichoderma* species and targeted fungal pathogen were dually placed on freshly prepared PDA medium with equal distance of 3cm from the periphery [16]. Following ratios as treatments were carried out under *in-vitro* conditions.

T_1 (M: T= 1:1) – *Marasmiellus: T.viride* at the ratio of 1:1 mycelia dick
T_2 (M: T= 1:2) - *Marasmiellus*: *T.viride* at the ratio of 1:2 mycelia dick
T_3 (M: T= 1:4) - *Marasmiellus*: *T.viride* at the ratio of 1:4 mycelia dick
T_4 (M: T= 4:1) - *Marasmiellus*: *T.viride* at the ratio of 4.1 mycelia dick
T_5 (M: T=1:0) –*Marasmiellus* with out *T. viride* as control

Then inoculated plates were incubated at 30±3°C for 5 days. At the end of incubation period, the radial growth and the antagonistic zones were measured. Inhibition percentage of mycelial growth for the tested pathogen in relation to the growth of control was calculated using the formula I = [100 (C-T)]/C as described by Kulkarni and Lingappa [17].

where
I = Inhibition of mycelial growth.
C = Mycelial growth in control
T = Mycelial growth in treated with *Trichoderma* sp.

Each treatment was replicated four times. This experiment was repeated thrice to confirm the results. Same set of experiment was designed for the other species *T. harzianum*.

Efficacy of Plant Extracts Formulations to Suppress the *Marasmiellus* sp. Under *In vitro* Condition: Five plants species were used in this study to develop extract formulation, namely *Azadirecta indica* (leaf) [18], *Lantana camara* (Leaf) [19], *Zingeber officinale* (rhizome) [20], *Curcuma longa* (Processed rhizome), [21] and *Ocimum sanctum* (Leaf) [18] have been proven to possess antifungal activity. Plant parts were chopped off into small pieces and air-dried for two days and then grounded in to fine particles and extracted with distilled water. The extracts were filtered using No 2 Whatman filter paper. Five different plant extracts were formulated using the same procedure described above five grams of each finely ground sample as dissolved in 50ml of distilled water and allowed for two hours and then a filtered. Each extract of 40ml were collected in a separate conical flask and plugged with cotton wool and paper and sterilized.

Two different concentrations of 3ml (20% v/v) and 5ml (30% v/v) of each plant formulation were amended into 15 ml of melted PDA medium. Nearly about 2-5 drops of chloromphenicol were added to each plate before the PDA medium become solidifying, a mycelial agar slug (4 mm diam.) of *Marasmiellus* sp. taken from the edge of a 5-day old culture was put in the center of a Petri dish and incubated at room temperature for 5 days at dark. The diameter of fungal growth was measured until any petri dish was fully covered by mycelial growth. This took five days and the inhibitory activity was calculated by the data of colony diameter at the fifth day, according to the formula as follows:

Inhibitory activity (%) = [100(A – B)]/A

where
A - Colony diameter on non-amended control
B - Colony diameter on amended medium [22].

Each treatment was replicated thrice. This experiment was repeated twice.

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.

RESULTS AND DISCUSSION

Being a new disease of banana in Jaffna, Sri Lanka, it is vital to understand the life cycle, host range and mode of spread of the pathogen. Jackson [3] reported the biology and epidemiology of this fungus on coconut. Nelson and Javier [5] described the symptoms of this disease on banana however the biology and epidemiology was not studied in banana. Hence, this research was conducted to understand the, symptoms associated, disease establishment within plants, prevalence of disease in Jaffna and management of the disease using different materials.

Character and Structure 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 30±5°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°C) 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 [23], reported that fruiting body of *Marasmiellus* sp. was white cap with size of 1-3 cm, stripe base was pinkish orange. Fong and them [24] reported that on orchid basidiospore liberation was optimum at 24°C, germination was at 24-28°C and spores were the source of primary infection of *Marasmiellus* sp. Temperature does affect the growth of *Marasmiellus* sp. and temperature was conducive for radial growth varied from 24-28°C.

Symptoms of *Marasmiellus* sp. Stem Rot on Banana: Infected plant showed disease symptoms from root to terminal part of the leaves, majority of rotting was observed near the soil line. In the rotted rhizomes, necrosis was observed internally and externally. A layer of white mycelium was developed between the tissues. This finding is in line with Jackson [3] who stated that *M. inoderma* is soil, air as well as seed borne. Subsequently, in the pseudo-stem near the soil line the fungus continued to grow numerously and rotting developed at the external sheath of the pseudo-stem. As a result rotting the surface characterized by black colour patches. Infected surfaces covered with white mycelium and the peripheral region of the patches becomes pink and gradually expanded. Prior to infection the sheath appeared as water soaked. During invasion into next sheath, it gradually lost water and started to tear. Finally it was in a fully dried condition. A large area of fully covered creamy mycelial layer was observed between the sheaths. Mycelial colony continued to invade up to 3-4 layers from the external part of the pseudo-stem. Initial infection was observed at bottom, middle and neck regions of the pseudo-stem. White or cream colour fruiting body formed in the pseudo-stem at low temperature and higher relative humidity environment. At early infection 2-3 months old banana starts to wilt, leaves become small and unfurl, burnt margin appeared in the folded leaves and finally died. Infection at the middle stage of growth the stem failed to expand in size and this lead to zero productivity. Such plants failed to produce bunches and those produced small bunches. Later stage of growth of banana and during expansion of bunches, infection occurred in the neck region affected the production of bunches. The infected plant toppled at the crown even when mild windblown. At any stage of its growth, severe infection was resulted wilting leaves of except central leaves. Leaves wilted characteristically from lower areas to upper region. Symptoms of *M. inoderma* stem rot on banana was previously reported by Nelson *et al*. [9], Nelson and Javier [5] and Jackson, [3] as withering and decay of outer leaf sheath and leaf base, slow emergence of leaves and stunted growth of white or pink mycelial growth between the leaf sheath.

Disease percentage by visual observation of *Marasmiellus* sp. rot on banana at Thirunelvely banana field is given in Table 1.

Disease percentage was calculated using the following formula:

$$\text{Disease percentage DI} = \frac{\text{No of diseased plants}}{\text{No of plants in field}} \text{X } 100$$

Data presented in Table 2, show that disease incidents at Thirunelvely field was 44.44%, among them higher percentage of disease was observed in after harvested plants as 96.30%. Mature plants have shown 62.63 % of disease and the lowest disease percentage was recorded in younger plants (23.02 %). These observations showed that the plant debris having the potential inoculants for further spreading of *Marasmiellus* sp. so that, sanitation of field is very important to prevent further disease spreading. Occurrence of disease was high at the center of field.

Figure 1 shows rotting on pseudo stem distributed at bottom 51%, rotting of whole plant as 39%. In mature banana rotting was observed at bottom 57 %, whole pseudo stem (24 %), middle to bottom (13 %) and middle (6 %) pseudo stem.

Table 1: Treatments with different plant extract formulation

Plants	Concentration 20 % v/v	Concentration 30 % v/v
Lantana camara	T1	T7
Ocimum sanctum	T2	T8
Curcuma longa	T3	T9
Zingeber officinale	T4	T10
Azadirecta indica	T5	T11
Control	T6	T12

Table 2: Diseased percentage and symptom distribution at Thirunelvely banana field

	Diseased percentage (%)	No of bottom rotting	No of middle rotting	No of rottingon full plant	Rotting on Bottom -Middle
Mature plants	62.63	35	4	15	8
Suckers	23.02	26	0	6	0
Harvested plants	96.30	0	0	26	0
Total plants	44.44	61	4	47	8

Table 3: Different dosage of *Trichoderma harzianum* against *Marasmiellus* sp under *in vitro* conditions

Treatments	Mean colony diameter (mm)*	Growth inhibition (%)
T1 - *Marasmiellus* sp: *T. harzianum* at the ratio of 1:1	43.92b	51.20
T2 - *Marasmiellus* sp: *T. harzianum* at the ratio of 1:2	22.13d	75.41
T3 - *Marasmiellus* sp: *T. harzianum* at the ratio of 1:4	17.46e	80.60
T4 - *Marasmiellus* sp: *T. harzianum* at the ratio of 4:1	38.66c	57.05
T5 - *Marasmiellus* sp: *T. viride*: *T. harzianum* at ratio of 1:1:1	23.67d	73.70
T6 - Control - *Marasmiellus* sp without *T. harzianum*	90.00 a	0.00

* All the value with the means of four replicates

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

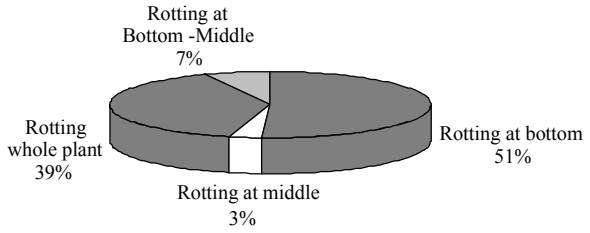

Fig. 1: Distribution of rotting in banana at Thirunelvely banana field

The Antagonistic Effect of *Trichoderma spp.* Under *In vitro* Conditions

Trichoderma Harzianum: Highest percentage of fungal inhibition was observed in T_3 – (*Marasmiellus* sp (M): *T. harzianum* (Th) ratio at 1:4) treatment as 80.6% (17.46 mm MCD) followed by 75.41% (22.13mm MCD) inhibition from T_2 (M: Th= 1: 2), 73.70% (23.67mm MCD) from T_5 (M: Th: Tv = 1:1:1, then T_4 (M: Th= 4:1) and T_2 (M: Th= 1:1) showed the inhibition as 57.50% (38.66 mm MCD) and 51.20% (43.92 mm MCD), respectively. Therefore *Marasmiellus* sp: *T. harzianum* at a ratio of 1: 4 was highly significant combination of best antagonism than other treatments and control (Table 3).

Trichoderma Viride: Data presented in Table 4 revealed that T_2 (M: Tv= 1:2) and T_3 (M: Tv= 1:4) were shown the highest inhibition as 92.29% (6.94 mm MCD) and 92.04% (7.17 mm MCD). Next best T_4 (M: Tv= 4:1) had the 70.04% (26.97 mm MCD) inhibition and lowest inhibition as 53.51% (41.85 mm MCD) was measured in treatment T_1(M: Tv= 1:1).

Efficacy of Plant Extracts Formulations to Suppress *Marasmiellus* sp.: In the present investigation dried leaves and rhizome extracts of five different plants showed varying antifungal property against *Marasmiellus* sp. All the treatments consisting five planting materials and their concentrations were inhibit the fungal radial growth ranging from 1.33% (88.80 mm MCD) to 79.19% (18.73mm MCD). Higher inhibition was observed in *Azadirachta indica* (79.19%) with concentration of 30 % (v/v), followed by *Ocimum sanctum* 30% (58.96%), *Lantana camara* 30 % (43.19). In very lowest inhibition percentage or no significance difference from the control treatment were recorded from *Gingiber officinale* 20 % (v/v) and *O.sanctum* 20% (v/v), as 01.33 % (88.80 mm MCD) and 03.78% (86.60 mm MCD).

Effect of plant material with different concentration has shown suppressive mycelial growth effect against *Marasmiellus* sp. on PDA medium and is shown in Table 5. However it is recorded first that the extract of plant materials inhibited the banana stem rot causing

Table 4: Different dosage *Trichoderma viride* against *Marasmiellus* sp under *in vitro* conditions

Treatments	Mean colony diameter (mm)*	Growth inhibition (%)
T1 - *Marasmiellus* sp: *T. viride* at the ratio of 1:1	41.85b	53.51
T2 - *Marasmiellus* sp: *T. viride* at the ratio of 1:2	6.94d	92.29
T3 - *Marasmiellus* sp: *T. viride* at the ratio of 1:4	7.17d	92.04
T4 - *Marasmiellus* sp: *T. viride* at the ratio of 4.1	26.97c	70.04
T5 - Control - *Marasmiellus* sp without *T. viride*	90.00a	0.00

*All the value with the means of four replicates

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

Table 5: Inhibitory activities of plant extracts on the growth of *Marasmiellus* sp on PDA medium

	Concentration 20 % v/v		Concentration 30% v/v	
Plant extracts	Mean colony diameter (mm)*	Mean inhibition (%)	Mean colony diameter (mm)*	Mean inhibition (%)
Lantana (*Lantana camera*)	68.13dc	24.30	51.13e	43.19
Holy basil (*Ocimum sanctum*)	86.60a	03.78	36.93f	58.96
Turmeric (*Curcuma longa*)	76.87b	14.59	70.67c	21.48
Ginger (*Gingiber officinale*)	88.80a	01.33	80.27b	10.81
Neem (*Azadirachta indica*)	63.87d	29.04	18.73g	79.19
Control	90.00a	0.00	90a	0.00

* All the values are means of three replicates

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

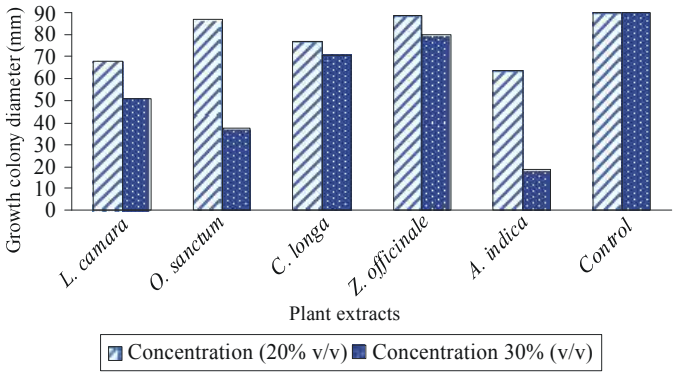

Fig. 2: Efficacy of plant extracts against *Marasmiellus* sp.

fungus *Marasmiellus* sp. under *in vitro*. Hence formulations developed from their plant materials can be used to control the stem rot disease on banana caused by *Marasmiellus* sp.

Neem has insecticidal, antibacterial and anti fungal activity due to the presence of chemicals such as Azdirechtin, salannin, nimbin [25]. Joseph *et al.* [18] reported that whole plant of *Ocimum* sp. was used as medicine for various diseases. Leaves are containing a bright yellow volatile oil, have antibacterial properties and act as an insecticide. It contain carotones is responsible for fungicidal property.

Deepika and Padma [19] reported that *Lantana* had fungicidal activity against plant pathogenic fungi such as *Alternaria* sp. Turmeric had constituents such as flavonoid, curcumin (diferuloylmethane) and various volatile oils, including tumerone, atlantone and zingiberone [26] but it had a little effect on *Marasmiellus* sp., *G. officinale* contains several chemicals such as zingerone, shogaols, gingeroles [27, 28] however it does not inhibit the growth of *Marasmiellus* sp. as there is a no significant differences from the control.

CONCLUSION

Marasmiellus stem rot on banana is the first record in Sri Lanka. Prevalence of this disease is confined to the banana grown in Valikamam division in Jaffna Peninsula. Rotting as 51% at the bottom portion of the pseudostem is indicating that pathogen mostly soil borne in nature. Rotting and drying of pseudo stem, stunted growth of plants, toppling of crown, wilting of the leaves and abnormal bunches were the major symptoms of *Marasmiellus* stem rot on banana. It caused moderate to

heavy yield loss of banana. Biorationals had good response in suppressing the fungus; *Marasmiellus* sp. *Trichoderma* spp. had significant responses up to 92% growth inhibition of *Marasmiellus* sp. under *in vitro* conditions. Leaf extract of *A. indica* yielded the highest inhibition of *Marasmiellus* sp. (79.19%).

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