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Banana stem rot disease; causal agent (*Marasmiellus* sp.), host range and selection of low cost media for its cultivation

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ABSTRACT

A Basidiomycetes fungus Marasmiellus sp. (Agaricales: Tricholomataceae) caused pseudostem rot on banana (Musa sp.), is found in Jaffna, Sri Lanka. Since it is latest to this region, this study was carried out to understand its disease cycle, symptoms, host range, occurrence and intensity of disease and tend to be selecting the cost effective media for its growth. On potato dextrose agar (PDA) fungus produced cottony white colony, later turned to cream and branched mycelium under light microscope. White fruiting body; with Pileus 3±2 cm in diameter, strip length 3.5±1.5 cm but small in size under adverse climatic circumstances. Rotted patches on rhizome and pseudo stem, gradual wilting of leaves from lower area to upper part, diminutive growth, strange foliage and bunches, toppling of crown, fruiting body adhere on pseudo-stem are the major syndromes of disease. Incidence of stem rot was confined only to Valikamam division of Jaffna. Amongst foremost banana cultivars grown in Jaffna; Kathali, Itharai and Monthan exhibited stem rot excluding Kappal. Under in-vitro condition PDA, King yam (KY) and Elephant foot yam (EFY) media recorded superior (90 mm) mean colony diameter following that nutrient agar (NA) (69.15 mm), sago (S) (55.45 mm) and the lowest growth was recorded on the water agar (WA) and Filter paper(FP) media as 31.15 mm and 22.95 mm, respectively. KY and EFY were found as excellent substrate for Marasmiellus sp. and the fungus was grown well and substituted the PDA for growing pure culture as well as in-vitro experiments.

Key words: Stem rot, *Marasmiellus*, low cost media, banana, host range

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INTRODUCTION

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 [2]. Local banana cultivars such as *Kappal*, *Kathali* and *Itharai* are grown for the fruits and *Monthan* cultivar is mainly cultivated for the cooking purpose, *Then kathali, Senik kathali, Pulik kathali* are the sub cultivars of *Kathali* , *Kolikkoodu, Anaikodan (Kappal), Itharai; Colombo Itharai, Local itharai* and *Monthan; Sampal Monthan,Vellai Monthan,Abishaka monthan,* in addition that *Sevvaazhai, Poovillaathavaazai, Malaivaazhai, Maruththuvavaazhai, Yaanaivaazhai, Neththiraa, Scavandish, Panrivaazhai* and *Pachchainaadaan* also cultivated in Jaffna for various purposes other than fruit consumption [10].

However, Jaffna banana farmers are facing several pests and diseases problems. Proper detection as well as diagnose of pest and disease is incredibly important to implement effective control measures to sustain the production as well as to amplify its production [10]. Recently, Basidiomycetes pathogenic fungus *Marasmiellus* sp. causing stem rot is recorded on banana in Jaffna, Sri Lanka. This new incidence 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 anticipated to become a serious problem in forthcoming periods due to its mode of dispersal. Hence, it is crucial to understand the life cycle and epidemiology of *Marasmiellus* sp. [5] reported the biology and epidemiology of this fungus on coconut. [8] Described the symptoms of this disease on banana however the biology and epidemiology was not studied in banana until now.

Potato dextrose agar (PDA) is universally used as a general purpose medium for the culturing of broad range of fungi. Cost of this commercially available medium is comparatively high thus there is a necessity to invent new media with easily on hand low cost substances for substituting PDA medium. A research work was carried out to choose superlative low cost media for the *in-vitro* studies to observe the bionomics and colony morphology of the fungi to preserve the cultures for long time without loss of vigour and maintain spore viability and infection capability of the pathogen as well as under take effective control measures in laboratory conditions. The locally available such as King Yam (*Dioscorea* sp) (KY) and Elephant foot Yam (*Amorphophallus paeoniifolius* (EFY), sago (SG), filter paper (FP) as treatments for the study with the control of PDA because of the Sago (*Metraxylon sagu*), king yam and elephant foot yam contains considerable amount of starch and small amount of reducing sugars and it is not much used as a stable food in Sri Lanka [6]. It is easily available in the local market at reasonable price, in addition to that its solidification property also helpful in media preparation too and filter paper has been used as media by Fong [4].

MATERIALS AND METHODS

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

Isolation and Characterization of pathogen

Pathogenic fungus was isolated by surface sterilization method described by [7]. The fungal pathogen was isolated from the diseased banana rubbish. Sample size of 5 mm X 5 mm pseudo-stem cuttings were selected and subjected to the surface sterilization method, includes the procedure of washing with 70% ethanol for one minute and rinsed with distilled water for 8-10 times. And they were transferred to the moisture chamber for growth of fungus, after four to five days of actively growing mycelial tip was transferred to the Petri dishes containing PDA medium supplemented with few drops of Chloromphenicol (5%). Then the Petri dishes were incubated in room temperature of 30 ± 3^0C for five days. After incubation, produced pure culture subjected to sub-culture and stored in the refrigerator at 4^0C for further study. Confirmation test was carried out by the inoculating *Marasmiellus* sp. mycelium into the healthy banana plants using both soil drench and streak methods. Symptoms and signs were confirmed, on the basis of technique of Koch's postulate. Culture morphology and growth pattern of mycelium on PDA medium and straw bed was used for formation of fruiting body. Identification of the Characters and structures of *Marasmiellus* sp. based on the mycelial colour, odor, appearance, characteristics of spores, colony growth pattern and growth rate in culture, formation of the fruiting body, basidiocarp and its characteristics such as colour, size, gills number and length of strip.

Field observation for the study of symptoms and sings

The chosen banana fields of Thirunelvely and Kopay areas were selected for study. Observation of various symptoms of *Marasmiellus* rot on banana and development and survival rate at various temperature and humidity range in Jaffna was recorded. All symptoms of *Marasmiellus* in various parts of banana were observed and recorded.

Alternate hosts of *Marasmiellus* sp.

The identified alternative hosts such as paddy, maize, ornamental banana, coconut, arecanut, dwarf leaf were grown on pots at the Department of Agricultural Biology, University of Jaffna. *Marasmiellus* sp. was inoculated by soil drench and streak methods.

Susceptibility of banana varieties to *Marasmiellus* sp.
Susceptibility check was carried out in mother plant conservation field, District Agricultural Training Center, Department of Agriculture at Thirunelvely, Jaffna. The visual inspection including the characters such as mycelial growth and development of fruiting body on banana pseudo-stem were used for this experiment during the period of May 2011. The examination was carried out to screen 19 major and sub-cultivars found in Jaffna peninsula. A total number of 19 samples with 3 replicates were used for the observation.

Growth rate of *Marasmiellus* sp. Vs *Trichoderma viride*
Sterilized PDA medium was poured in to the petri plates and a drop of chloromphenicol was added and solidified. Followed by, 4 mm diameter of mycelial agar slug was directly placed at the center of the PDA plates under laminar flow condition. The inoculated plates were incubated in room temperature (30 ± 3^0C). The measurement of average colony diameter (mm) was taken daily. This experiment was repeated three times with three replicates.

Selection of low cost media for *Marasmiellus* sp.
Different types of materials such as filter paper, king yam (*Dioscorea* sp), sago, water agar and elephant foot yam (*Amorphophallus paeoniifolius*), nutrient agar were selected and the growth of mycelium in different substrates was compared PDA medium. Priority is given in the selection for a better mycelial growth on low cost material.

Preparation of different types of media
Filter paper medium: Whatman filter paper (No. 1) of 90 mm diameter was positioned in sterilized Petri dish and1ml of distilled water was sprayed by using syringe under aseptic condition. Sago medium: 10g of pure sago was boiled with 100ml of distilled water; the contents were transferred in to a conical flask, plugged with cotton wool and wet sterilized. King yam and Elephant foot yam media: The Yam was cut in to small pieces and sun dried for 6 hours. Subsequently it was dried in an oven at 105^0C for 12 hours to remove any trace of moisture if present. The moisture free sample was finely grounded using a grinder. Yam flour of 10g was dissolved in 100ml of distilled water and transferred in to a conical flask and sterilized in an autoclave and moreover some standard media used (Nutrient agar, Potato dextrose agar (PDA), Water agar). Every medium described above are used as a treatment; T_1- Filter paper, T_2- Sago, T_3- Nutrient agar,T_4- PDA, T_5- Water agar, T_6- King Yam and T_7- Elephant foot yam. Each treatment was replicated four times. This experiment was repeated twice. 4 mm diameter of mycelial disc *Marasmiellus* sp. was transferred in to the culture plates and incubated at 30 ± 3^0C; the measurement of mean colony diameter was taken daily.

Statistical analysis
Above mentioned experiment was 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

Isolation and confirmation of pathogen
White colour fungal colony was isolated and the pure culture used to re-inoculate on banana plant. Plate: 1 (A) Show the same diseased symptom that was observed in the field. Fruiting body formed on the paddy straw bed plate: 1(B). These confirmed the isolation of pathogen from the field successfully done.

Characters and structures of *Marasmiellus* sp.
Regular cottony growth and rounded shaped white colony was initially developed (plate: 1(C) 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 ± 5^0C. However macroscopic fruiting body of *Marasmiellus* sp. was white and the pileus measured was 3±2 cm diameter, length of stripe was 3.5±1.5 cm 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; small pileus (2±1.5 cm in diameter), small stripe (2±1 cm), creamy to pale yellow in colour. Number of gills varied as 30±5 in number with size of fruiting body and lamella arrangement in two layers, one is from the center of pileus and other layer started in between the centre and periphery. Supported these results [1], reported that fruiting body of *Marasmiellus* sp. was white cap with size of 2±1cm, stripe base was pinkish orange. [9] Reported that on orchid basidiospore liberation was optimum at 24^oC, germination was at 26 ± 2^oC 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 26±2°C.

Plate: 1:- The Fungal pathogen of *Marasmiellus* sp. (A) Re-inoculated banana show the rotten symptom (B) Fruiting bodies on straw bed (C) Pure colony on PDA (D) Adaxial side of fruiting body

Symptoms and epidemiology of *Marasmiellus* sp. 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. 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 [5] who stated that *Marasmiellus* sp. is soil, air as well as seed born. 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 in to 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. At the 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. In addition those plates 2 and 3 show the some typical symptoms and signs of *Marasmiellus* sp. on banana in field as well as in laboratory conditions in Jaffna.

Symptoms of *M. inoderma* stem rot on banana was previously reported by [9],[8] and[5] 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.

Distribution of *Marasmiellus* sp. disease on banana in Jaffna

Prevalence of *Marasmiellus* sp. stem rot disease on banana was found in Valikamam area and did not expose the disease symptoms in other areas; Thenmaradchy, Island and Vadamaradchy. In Valikamam; Kopay, Thirunelvely, Nilavarai, Karanthan, Kondavil, Kokuvil and Chankanai were recorded as disease intense areas. Among this Karanthan, Kopay and Thirunelvely areas had the highest disease intensity around 50- 60% but other area disease intensity were ranged from 1- 30%. Disease intensity was recorded by using formula as followed.

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

Survey of *Marasmiellus* sp. rot on banana at Thirunelvely banana field via visual observation of *Marasmiellus* sp. rot on banana pseudo-stem results of the survey are given shows 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. Disease percentage was calculated using the following formula;

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

Rotting on pseudo-stem distributed in mature banana at bottom 57 %, whole pseudo-stem 24 %, middle to bottom 13 % and middle 6 %.

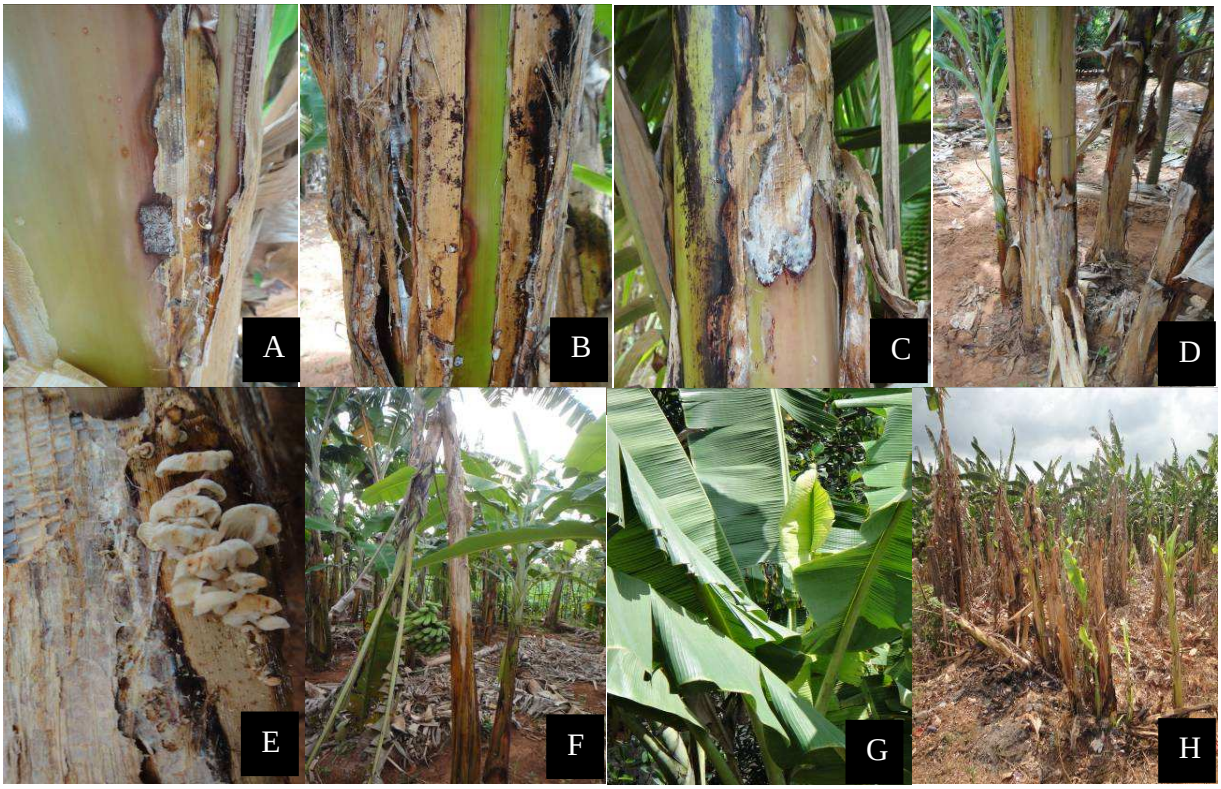

Plate: 2:- Various symptoms of *Marasmiellus* sp. stem rot disease on banana (A) Rotting of pseudo-stem sheath, (B) Dried and cracked outer sheath (C) Typical rotting on middle part pseudo-stem (D) Rotting adjacent of soil line (E) Mycelial growth and fruiting body on banana pseudo-stem (F) Toppling of banana crown (G) Small leaf symptom (H) Infected banana field at Jaffna

Alternate hosts for *Marasmiellus* sp.

The investigation was carried at the mean temperature of 30±3^{0}C. The table 1 revealed that paddy, maize and arecanut were shown disease symptoms. Jackson [5] and Nelson and Javier [8] reported that here mentioned plants served as alternate hosts for *Marasmiellus* sp. except arecanut. However this study showed that arecanut also a host but coconut, dwarf copperleaf and ornamental banana are not host to *Marasmiellus* sp. in Jaffna.

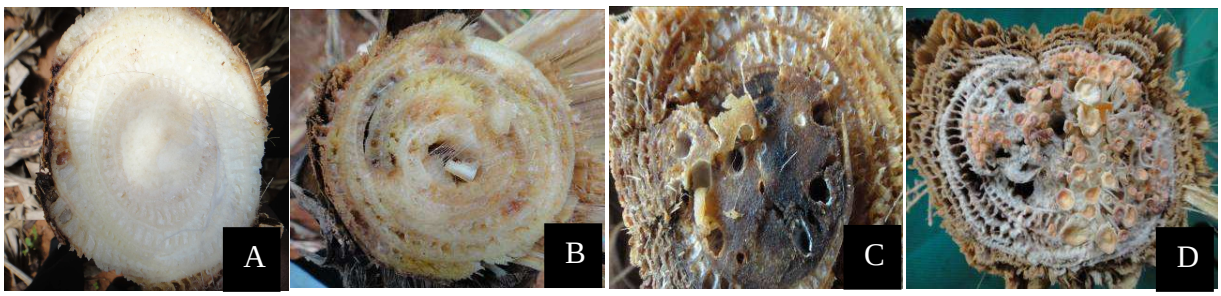

Plate: 3:- Infected banana pseudo-stem (cross section) by *Marasmiellus* sp. (A) Necrosis on pseudo-stem early stage B Necrosis in later stage of infection (C) mycelial growth to reach the center of pseudo-stem (Combination of *Marasmiellus* sp. infection and pseudo-stem weevil) (D) Fruiting body formed after 15- 25 days in laboratory

Table 1: Alternate hosts for *Marasmiellus* sp.

Botanical name	Common name	Symptom observed
Oryza sativa	Paddy	++
Zea mays	Maize	++
Cocos nucifera	Coconut	--
Areca catechu	Arecanut	++
Alternanthara sessilis	Dwarf copperleaf	--
----------	Ornamental banana	--

++ Disease observed, -- Disease not observed

Plate 4: *Marasmiellus* sp. infected different tested hosts (A) water soaked paddy grains (B) comparison of infected and non infected paddy grains (C) infection on arecanut

Susceptibility of banana cultivars to *Marasmiellus* sp.

Kappal, Kathali, Monthan and *Itharai* are the major cultivars in Jaffna, among them *Kappal* cultivar was not a host to *Marasmiellus* sp. but it was found on other cultivars and sub-cultivars. In addition, *Sevvaazhai, Poovillaathavaazai, Malaivaazhai* had *Marasmiellus* rot disease. But *Maruththuvavaazhai, Yaanaivaazhai, Neththiraa, Scavandish, Panrivaazhai* and *Pachchainaadaan* cultivars not expressed disease symptoms in Jaffna.

Growth rate of *Marasmiellus* sp.

Growth of both *Marasmiellus* sp. and *Trichoderma viride* on PDA medium has shown in figure 1. *T. viride* grew after four days of inoculation with diameter of 90 mm but *Marasmiellus* sp. required five days to grow completely. Figure 1 clearly shows the differences on second day onward after inoculation. On second day, *Marasmiellus* sp. and *T.viride* mean colony diameters were 30.17 mm and 53.42 mm respectively. These results were obtained under the mean room temperature of 28 ± 2^0C.

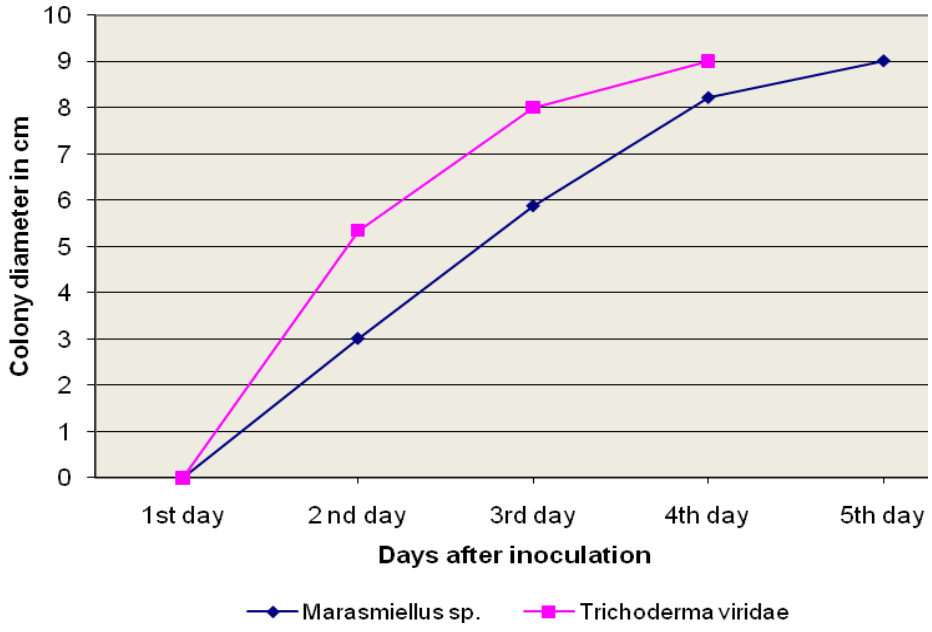

Figure 1: Growth comparison of *Marasmiellus* sp. and *Trichoderma viride* on PDA

Selection of Cost effective media for culturing of *Marasmiellus* sp.

Table 2 shows that PDA, KY, EFY media recorded higher mean colony diameter (MCD) four days after completion. Growth of *Marasmiellus* sp. was high in these media and after that NA medium (69.15 mm), SG medium (55.45.mm) and the lowest growth was recorded on the WA and FP media as 31.15mm and 22.95 mm, respectively. KY and EFY media were substitute for PDA medium. Similar result was reported by Thileepan [11] those for the growth of evidence mushroom culture. Fong [4] also reported that the modified filter paper technique to be used to long term preservation at 19^0 C temperature and 75 % relative humidity for *Marasmiellus* sp. isolate is viable after two years of storage. Sago and nutrient agar media are commonly used as bacterial media. Sago incorporated with king coconut water medium was used for culturing some soil bacteria and for substituted the nutrient agar medium [6]. However low cost media such as king yam and elephant foot yam media gave best results and it was replaced the usage of PDA medium (Plate 4).

Table 2: Mean colony growth of *Marasmiellus* sp. in different culture media

Media	Mean colony diameter in mm *		
	Second day	Third day	Fourth day
Filter paper (FP)	12.25 ^{d}	18.80 ^{e}	22.95 ^{e}
Sago (SG)	26.00 ^{c}	42.45 ^{d}	55.45 ^{c}
Nutrient agar (NA)	29.10 ^{b}	55.90 ^{c}	69.15 ^{b}
Potato dextrose agar (PDA)	40.15 ^{a}	74.90 ^{a}	90.00 ^{a}
Water agar (WA)	12.80 ^{d}	22.35 ^{e}	31.15 ^{d}
King yam (KY)	41.17 ^{a}	74.35 ^{ab}	90.00 ^{a}
Elephant foot yam (EFY)	39.65 ^{a}	70.40 ^{b}	90 .00 ^{a}

** All the values from mean of four replicates Values having same letter in same column are not significantly different according to the least significant mean separation at .05 α and 95 % confidence interval*

Plate 4: *Marasmiellus* sp. grown on different substrate: Top (L-R) King Yam medium, Potato dextrose agar, Elephant foot yam medium; Bottom (L-R) Water agar, Filter paper medium, Sago medium, Nutrient agar.

CONCLUSION

Prevalence of stem rot disease was confined and disease intensity 40% to 60% to the banana grown in Valikamam area; Jaffna. In a selected banana field show 51% rotting at the bottom of the pseudo-stem indicating that the soil borne nature of *Marasmiellus* sp. has influenced high incidence of the disease nearer to soil line. 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 was found *Kathali*, *Itharai* and *Monthan* except *Kappal*, which are the major cultivars of banana in Jaffna. Paddy, Arecanut and Maize also infected by *Marasmiellus* sp., Cost effective growing media was developed from King yam and Elephant foot yam, which readily substituted PDA medium to maintain the pure culture of *Marasmiellus* sp. at the laboratory.

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