Evaluation and Selection of the Most Cost Effective Media for *In Vitro* Cultivation of Banana Stem Rot Fungus, *Marasmiellus* sp

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Abstract

Marasmiellus stem rot is a Basidiomycetes fungal disease first recorded in banana (*Musa* sp.) fields in Jaffna, Sri Lanka. Stem rot causes damage directly on pseudo-stem and indirectly on banana leaves as well as its fruits. A research was carried out to select the best low cost media for the *in-vitro* studies to observe the bionomics and colony morphology of the *Marasmiellus* sp. to preserve the cultures for long time without loosing its vigour, spore viability and infection capability and to determine effective control measures under laboratory conditions. Initially, the fungal colony was cottony white and later turned to creamy colour on the standard potato dextrose agar (PDA), however it failed to produce reproductive structures in culture. Other locally available cost effective media such as King Yam (*Dioscorea sp*) (KY) and Elephant foot Yam *(Amorphophallus paeoniifolius* (EFY), Sago (SG), Filter paper (FP) were taken as treatments with the control PDA medium. The results revealed that KY and EFY were cost effective excellent substrate for the production of cultures of *Marasmiellus* sp under *in-vitro* studies compare to sago (SG), filter paper (FP) with some recommended standard culture media which are water agar (WA) and nutrient agar (NA). The mean colony diameter after four days was about 90mm on KY, PDA, EFY and significantly on par with NA (69.15mm), SG (55.45mm) WA (31.15mm) and FP (22.95mm). This study confirms KY and EFY are the most suitable cost effective media over the other tested substrates for *in-vitro* studies of Marasmiellus sp compared to high cost PDA.

Key words: *Marasmiellus*, Stem rot, Banana, Cost effective media

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 450,000t. More and more rice farmers are switching to banana cultivation due to the high profit margin (Anonymous 2008). 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 (Sivakumar 2011). Recently, Basidiomycetes pathogenic fungus *Marasmiellus* sp causing stem rot is 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. Jackson (1999) reported the biology and epidemiology of this fungus on coconut. Nelson (2001) 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 fairly high therefore there is a necessity to formulate new media with easily available low cost substances for substituting PDA medium. A research work was carried out to select best 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) were taken 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 (Kapilan and Thavaranjit, 2008). It is easily available in the local market at reasonable price, in addition to that its solidification property also helpful in media preparation too Filter paper has been used as media by Fong (2003).

Materials and Methods

Pathogenic fungus was isolated by surface sterilization method described by Kinkel and Andrews (1988). The samples with fungal infection were collected in the effected fields at Kopay and Thirunelvely in Jaffna, Sri Lanka at November 2010. From selected Sample, 5mmx 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 moisture chamber to facilitate the fungal growth. Following 4-5 days of incubation, actively growing hyphae was 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°C for 5 days. After incubation, pure culture was subjected to sub-culture and stored in the refrigerator at 4°C for further study.

Selection of low cost media for *Marasmiellus*

Different substrates such as King Yam (*Dioscorea sp*) (KY) and Elephant foot Yam (*Amorphophallus paeoniifolius* (EFY), sago (SG), filter paper (FP) were taken as compared PDA medium. Priority is given in the selection for a better mycelial growth on low cost material.

Method of media preparation and its composition

A milliliter of distilled water was sprayed over the filter paper by using syringe under aseptic condition (filter paper medium), 10 g of pure sago was boiled with 100 ml of distilled water; the contents were transferred in to a conical flask, plugged with cotton wool and wet sterilized (Sago medium), King yam was cut in to small pieces and sun dried for 6 hours. Then it sample was dried in an oven at 105°C for 12 hours to remove any trace of moisture if present. Yam flour of 10 g was dissolved in 100 ml of distilled water and transferred in to a conical flask and sterilized in an autoclave (King yam medium). Elephant foot yam medium-The same procedure was carried out as king yam medium. Every medium described above are used as a treatment T_1- Filter paper (FP), T_2- Sago (SG), T_3- Nutrient agar (NA), T_4- PDA, T5- Water agar (WA), T6- King Yam (KY) and T_7- Elephant foot yam (EFY). Mycelial slug of *Marasmiellus* sp (4mm diameter) was transferred in to the culture plates and incubated at 30±3°C; the measurement of mean colony diameter was taken daily. Each treatment was replicated four times with a complete randomized design it was statistically analyzed by using SAS package. This experiment was repeated twice.

Table 1: Colony diameter of Marasmiellus sp in different culture media (n=4)

Media	Mean colony diameter* (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)	40.1^{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}

***Means in each column followed by the same letter(s) are not significantly different according to the least significant difference (LSD), p>0.05**

Results and Discussion

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 25-35°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 (2011), reported that fruiting body of Marasmiellus sp was white cap with size of 1-3 cm, stripe base was pinkish orange. Fong et al. (2003) 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 temperatures conducive for radial growth varied from 24-28°C.

Selection of low cost media for culturing of *Marasmiellus sp*

Table 1 show 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 (2009) those for the growth of evidence mushroom culture. Fong (2000) also reported that the modified filter paper technique to be used to long term preservation at 19°C and 75 % *M.inoderma* 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 (Kapilan and Thavaranjit 2008). 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.

Conclusion

King Yam and Elephant Foot Yam were found as good substitutes for the PDA for growing pure culture of this fungus.

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