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MARASMIELLUS PALMIVORUS AS A NEW CAUSAL AGENT OF REED WILT DISEASE IN IRAQ

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ABSTRACT

Background: Reed plants are widely distributed in Iraq and responsible to invade irrigation channels and drainage systems. Aim: The present study was designed to identify and characterize the causal agent of reed wilt disease. **Methods:** Disease symptoms, characteristics and growth pattern of causal agent were investigated.
Results: Reed wilt disease has been observed during the survey which was held at the end of October 2016. The percent of natural infection of this disease was found in the range of 7-18%, the causal agent was identified as *Marasmiellus palmivorus* based on macro and microscopic characteristics. Microscopic observation of *M. palmivorus* revealed the engendered white cottony mycelia turned to creamy with clamp connection. Radial growth of this fungus was greatly varied according to the type of additives supplemented in the growth media and results were as following: *M. oleifera* (7.8 cm) > wheat (7.7cm) > Reed (7.4 cm) > millet (6.8 cm) > Caladium (6.7 cm) > PDA (5.86 cm) > onion (2.94 cm).
Conclusion: The results of present study concluded that *M. palmivorus* was recorded as the causal agent of wilt disease on reed plant for the first time in Iraq and possibly for other countries as far as we know. Also, this fungus could be act as bio-herbicidal against reed plants, but more attention should be paid to this point.

Keywords: *Marasmiellus palmivorus*, reed plant, wilt disease

INTRODUCTION

Marasmiellus is an ecumenical distributed genus, particularly in tropical and subtropical regions. It comprises of about 400 species which belongs to Basidiomycota, Agaricales, Marasmiaceae Roze & Kühner (Wilson & Desjardin 2005, Kirk et al., 2008; Antonín & Noordeloos 2010). Species of marasmioid fungi have been recognized as being both saprotrophic and parasitic (Wilson and Desjardin 2005). In South America, *Marasmius equicrinis* fungus was observed to grow on dead decaying materials of tea, cocoa, coffee and other crops and considered as a common lignicolous saprophytic fungus (Kilaru and Hasenstein, 2005; Adedji, 2006; Miller et al., 2010; Molina et al, 2010). Depending upon the moisture and salt-tolerant accessibility in the environment, species of *Marasmiellus* show variability in their living behavior. Therefore, this genus is often available in coastal climates, dune soil or another littoral environment (Pérez-De-Gregorio et al., 2011). Whereas, *Marasmiellus ciesanus* was collected from an open dune in the Atlantic Islands of Galicia. It was identified on the basis of morphological features and referred as a new species from Spain (Blanco-Dios, 2015).

Although most of *Marasmiellus* spp. are found to confine to a particular living host, but many species of Marasmiellus and Marasmius are frequently mentioned in literatures as pathogenic to many plant species in particular, monocotyledonous such as sugar cane, palms, taro (Colocasia esculenta), maize and turf grass (seashore paspalum) (Kilaru and Hasenstein, 2005; Adedji, 2006; Miller *et al*., 2010). In other study, Pong *et al*., (2012) reported for first time that *M. palmivorus* as causative agent of Bunch rot disease of oil palm trees in Malaysia.

Almaliky *et al*., 2012, have examined the pathogenicity of *M. palmivorus* to cause bunch rot disease in oil palm. Besides this, banana stem rot disease has also been kenned to cause by *Marasmiellus* sp. (Thiruchchelvan *et al*., 2013). Other researchers demonstrated the severity of white thread blight (*Marasmiellus scandens*) in cocoa was form six regions of Ghana (Amoako-Atta *et al*., 2016).

Phragmites australis (common reed), naturally, grows in a variety of habitat including wetlands, freshwater conditions, marsh communities, river and lake sides. It found to grow almost in every continent except Antarctica. *Phragmites australis* has been well characterized for their capability to persist, grow and modulate according to the environment conditions (Kobbing et al., 2013; Uddin et al. 2014; Meyerson et al., 2016). *P. australis* can modify its habitats by altering marshland hydrology; reducing salinity in salty wetlands. Further their existence can rise the possibilities of fire and threaten the wildlife (Rebecca et al., 2016).

To the best of our knowledge there is no scientific report available to reed wilt disease caused by *Marasmiellus* genus. Therefore, the present study was conducted for identification and characterizations of causal agent of reed wilt disease (RWD).

MATERIALS AND METHODS

Field observations and disease assessment: During the month of November 2016, the natural infection of wilt disease was observed in reed plants. Observations of various symptoms were recorded in the grown reed plants. Disease incidence (DI) was assessed in five agricultural regions (Hilla, Kifil, Al Hamzah, Hashimiah, Al-Mahawil), of Babylon Province, Iraq that include an open drainage network. From each region 100 reed plants were visually observed alongside the line of drain channels. The numbers of reed plants that exhibited wilt disease symptoms were calculated. The disease incidence (DI) was assessed according to the equation given below:

$$DI = \frac{\textbf{Number of plants showing symptoms}}{\textbf{Total number of inspected plants}} \times 100$$

Isolation and identification: The diseased samples from stems and rhizomes of reed plants were collected and transferred to the laboratory. The infected plant samples were cut into small segments (3-5mm) followed by surface sterilization by using the solution of sodium hypochlorite (3%). Then samples were rinsed thoroughly by sterilized distilled water and dried on sterilized filter paper. The dried samples were inoculated on Petri-plates containing autoclaved PDA medium supplemented with 250 mg/l of chloramphenicol. Then the Petriplates were incubated at 26°C ± 2 for 7 days. The mycelium was sub cultured at regular interval of time on sterilized PDA plates. Fungal isolate was identified according to the morphological and taxonomic features such as characteristics of colony and mycelium clamp connections using 40-X Microscopic Objective Lens (Desjardin et al., 1993; Leslie and Summerell 2006; Kirk et al., 2008; Mishra and Mishra, 2012).

Growth and maintenance of fungal isolates: Reed wilt disease fungus (RWDF) isolate was inoculated in Petri dishes containing PDA (pH 7.0) followed by incubation for 5 days at 26 ± 2ºC. For making the slants, 20 ml of PDA was poured in glass tubes and left until solidified. The slants were inoculated by streaking the culture from newly formed colonies of each fungal isolate followed by incubation at 26 ± 2ºC for 6 days. The slants were stored at 5^0C in refrigerator followed by sub culturing at regular interval (30 days) of time (Obaid *et al*., 2017).

Preparation of fungal inoculum: The inoculum of RWDF was prepared according to the procedure described by Dewan and Sivasithamparam, (1989). Briefly, millet seeds (*Panicum miliaceum* L.) were taken from Hilla Local Market and filled separately in 250ml of conical flasks (as 100gm each), followed by washing with tap water and boiled gently in distilled water for 30 min. Millet seeds were rewashed again with distilled water and autoclaved twice at 15 psi for 1hr. After cooling, each flask was inoculated with three discs (5 mm) of newly formed colony of RWDF followed by incubation for 3 weeks at 26°C ± 2. The inoculated flaks of millet seeds were shaken at regular intervals of 2 days to obtain uniform fungal growth and to break the mycelial mat. Inoculum of RWDF was kept in refrigerator at 4°C in conical flasks (Alnuaimy *et al*., 2017).

Pathogenicity: 20 healthy, uniform reed plants (one-year-old), were selected from drain field and planted in plastic pots of 20*30 cm sizes with autoclaved sandy clay soil under greenhouse conditions. The plantation process was carried out in the mid of September month, 2016. Reed plants were left to grow for two months under controlled conditions. After that pots were inoculated individually with disease causal agent by mixing the previously prepared inoculum in the soil of pots at a rate of 5% each. 10 pots without inoculation served as control. Disease symptoms of reed wilt disease (RWD) were recorded after 15 days of inoculation.

Growth of RWDF in different nutrient sources: Solid media were prepared from different sources such as A: PDA, B: Leaves of *M. oleifera*, C: Millet seed, D: Wheat seed, E: Reed stem, F: Caladium leaves, G: Outer leaves of red onion. Plant parts/organs of *M. oleifera* leaves, reed stem, Caladium leaves and outer leaves of red onion were cut manually into 3-5mm thickness. All samples including wheat and millet seeds were exposed to complete dry in oven (80ºC) followed by grinding in electronic blender until they become fine powder. The prepared samples were stored in sterilized containers till further usage (Umechuruba and Elenwo, 1999).

To prepare growth media, 2g of each nutrient source was dissolved in 100 ml of distilled water using magnetic stirrer. The dextrose and agar were added to the growth medium at the same rate as in PDA and mixed thoroughly followed by autoclaving at 15 psi for 15 minutes. Then 20 ml of the autoclaved medium was poured into each of the sterile Petri dishes. On solidifications, the plates were inoculated with a disk (0.5 cm) of fungal tissues from the edge of RWDF colony (4 days old). Three replicates for each source were taken and incubated for 6 days at 26±2 ^{0}C.

Radial diameter (in cm) of RWDF was calculated from two intersecting lines from the center of the dish at regular interval of 48 h on various culture media.

Statistical analysis: Present experiments were arranged in a randomized complete block design. The quantitative data obtained were analyzed by the analysis of variance, the level of significance was determined by LSD comparisons at the 5% probability level.

RESULTS

Disease symptoms: The percent of natural infection of RWD (Table 1) was found to be in the range of 7-18%. The higher infection rate was reported from Al Hamzah district (18%) whereas the lowest infection rate was observed in Al-Mahawil district (7%). The disease symptoms of infection were found all over the plant body as depicts from Figure 1A. The symptoms were initially started as brown to black rot lesions on the base of lower section of stem (Figure 1B). The signs further appeared as white cottony growth of fungal mycelium, and colonized the lower parts of stem, just above the soil and shown progressive growth up to 21 days which finally lead to death of plant. The fungus infection was found to occur fairly faster during the wet conditions (Figure 1C).

Table 1: Prevalence of reed wilt infection in different regions of Babylon Province.

No.	District	Disease incidence
1	Hilla	12
2	Kifil	15
3	Al Hamzah	18
4	Hashimiah	13
5	Al-Mahawil	7

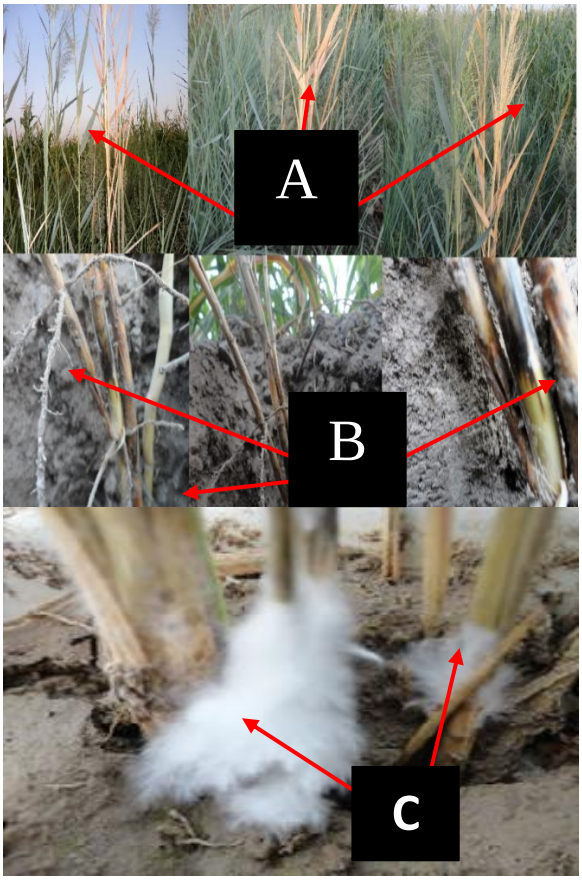

Figure: 1: The symptoms of RWD on naturally infected plants in field drain systems, (A): Reed plants completely died as a result of infection, B: Brown to black rot lesions on the base of lower section of stem, C: Mycelial growth colonized the lower parts of stem above the soil level.

Morphological characteristics of RWDF: RWDF isolate was identified as *Marasmiellus palmivorus* from its colony morphology and microscopic examination. This fungal isolate produces dense, cottony mycelia with soft edges which later on turn to creamy. It covers the plates within 4 to 6 days after incubation (Figure 2 A, B, C).

Microscopic observation revealed branched hairy mycelium with clamp connections (Figure 2D). Septation was not obvious in the fungal mycelium. Spores and other reproductive parts were not observed under the light microscopy examination.

Figure 2: Growth of *M. palmivorus* on PDA at 26 ±2 °C.

i. Morphological features (colonies) after 4 days, upper side (A), reverse side (B) and 20 days (upper side)- (C) of incubation
ii. Lactophenol cotton blue mount of slide culture showing clamp connections under the light compound microscope after 10 days of incubation (40X).

Pathogenicity: Pathogenicity was evaluated by inoculating fungal inoculum of *M. palmivorus* into the healthy reed plants. Symptoms and signs were confirmed according to of Koch's postulates. Various symptoms of RWD were recorded and found to be similar with observed symptoms during the survey. In control experimentation, no disease symptoms were appeared.

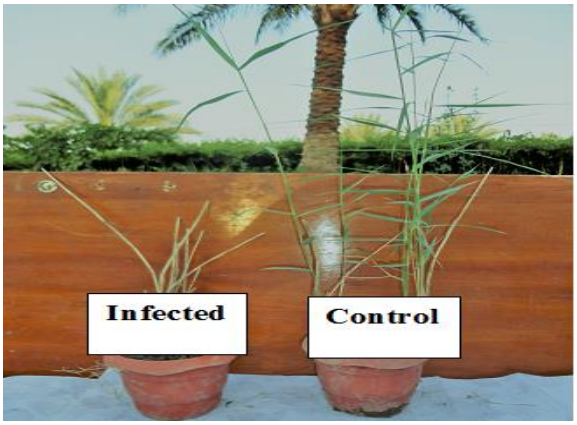

Figure 3. **Left**: Symptoms of wilt disease caused by *M. palmivorus* on reed plant after 15 days of inoculation and **right**: Healthy reed plant (control).

Growth of *Marasmiellus palmivorus* in different nutrient sources: The results of this experiment revealed that the growth of *M. palmivorus* was greatly varied according to the type of media used. The growth of this fungus was most profound with *M. oleifera*, wheat and reed stem supplemented powdered materials in the medium (Figure 3). Hence, growth diameter was found to be substantially affected by source of nutrient added. After 2 days of incubation, the growth of tested fungus was fairly similar (2.5 cm) in *M. oleifera*, millet, wheat and caladium supplemented medium but after 4days, the average of growth reached to 4.5, 4.3, 4.3, 4.0, 3.7 and 1.93cm in *M. oleifera*, Reed, Caladium, millet, wheat, PDA and Onion supplemented medium respectively. After 6 days the tendency of fungal growth in various supplemented medium was recorded as following: *M. oleifera* (7.8 cm) > wheat (7.7cm) > Reed (7.4 cm) > millet (6.8 cm)> Caladium (6.7cm)> PDA (5.86cm)> onion (2.94cm). Therefore, fungal growth proportional was increased with time in all the formulated media (Figure 4).

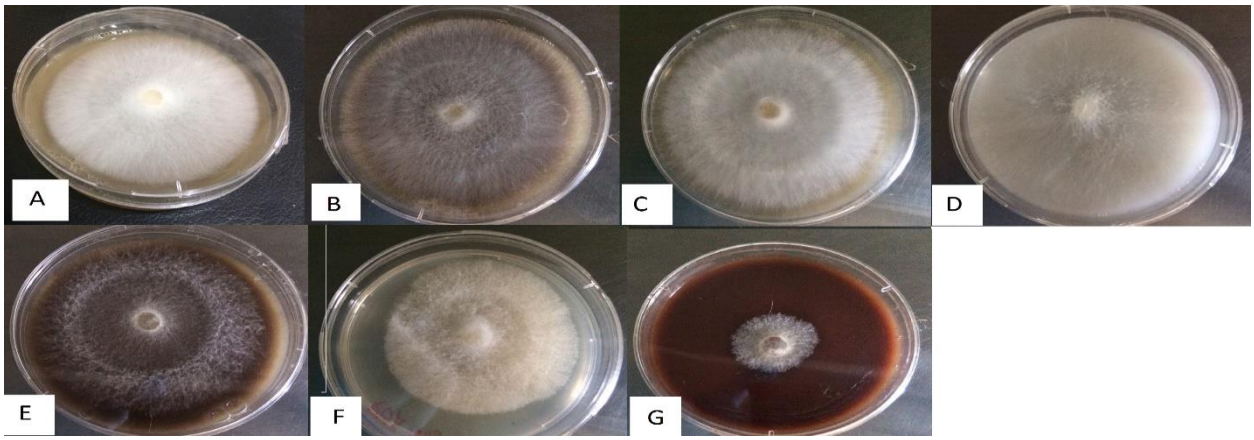

Figure 4: Growth of *M. palmivorus* in different substrates (A: PDA, B: *M. oleifera*, C: Millet seeds, D: Wheat flour, E: Reed stems, F: Caladium, G: Outer leaves of onion after six days of incubation at 28± 2 °C.

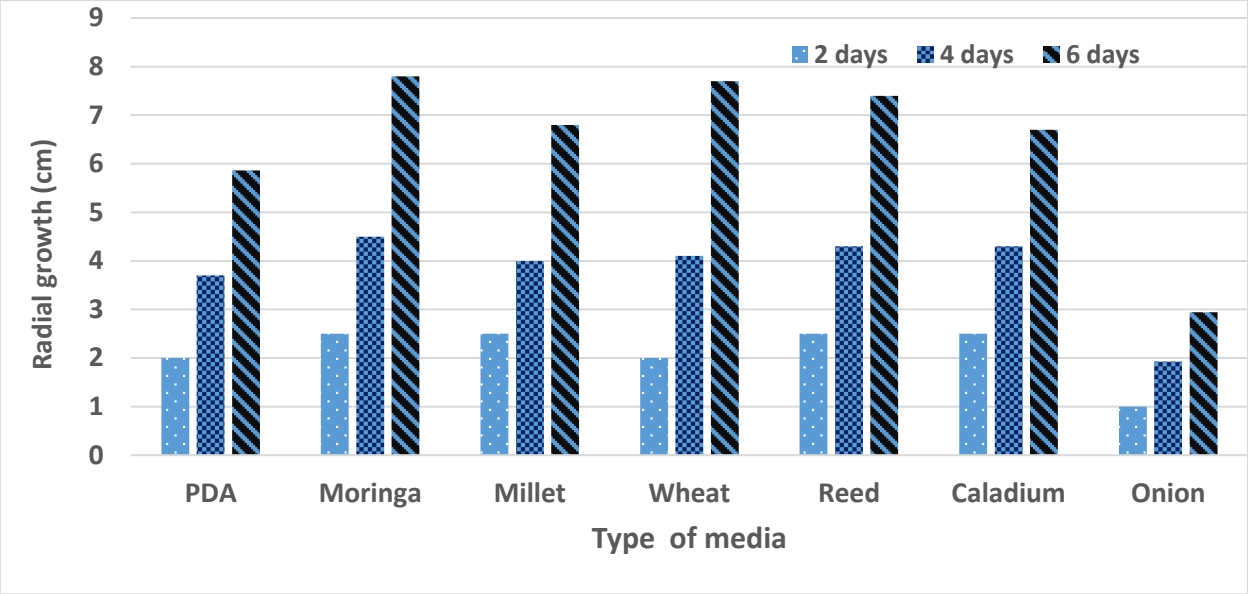

Figure 5: Growth of *M. palmivorus* in different types of media (PDA, *M. oleifera* leaves, C: Millet seeds, D: Wheat flour, E: Reed stems powder, F: Caladium powder, G: Outer leaves of onion, after six days of incubation at 26± 2 °C. (L.S.D. $_{0.05}$= 0.19 for growth media, 0.12 for time and 0.33 for interaction)

Discussion

Reed plants are known to invade irrigation channels and field drain systems in many regions of Iraq. In the present study, percent of infection rate in reed plants was recorded in the range of 7-18%. It has been observed that the prevalence and percent of infection of *M. palmivorus* was varied according to the area or region. In a study, Thiruchchelvan et al., 2013 reported that the stem rot disease on banana was found in Valikamam area whereas the disease symptoms were not observed in other areas. They also noticed that infection rate was higher in of mature plants of banana (62.63 %) in comparison to younger plants (23.02 %). Similarly, Amoako-Attah et al. 2016, have mentioned that older cocoa trees are more susceptibility to *Marasmiellus scandens* (white thread blight disease) than younger ones. Differences in percent of reed wilt infection in different districts could be related to ambient conditions and to the availability of plant debris which possibly act as potential inoculants for spreading of fungal infection (Thiruchchelvan et al., 2012).

Based upon morphological and colony characteristics such as presence of clamp connections in the mycelium, the RBDF isolate was identified as *M. palmivorus*. furthermore, the results were found to be consistent with the descriptions given by Pong et al. 2012. This identification was also confirmed by the same authors through DNA sequence analysis of *M. palmivorus*.

Culture media is considered as one of the vital requirement for the growth and development of fungi. *M. palmivorus* was found to grow much faster in supplemented growth medium of leaves of *M. oleifera*, flour of millet seeds, powdered stems of reed plants, flour of wheat in comparison to Caladium, PDA or outer leaves of red onion. Further, *M. oleifera*, millet seeds or powdered stems of reed plants were suggested as preferable growth sources and has potential to replace the use of PDA for culturing of *M. palmivorus*.

Similarly, the alteration in growth patterns have investigated by Thiruchchelvan et al. 2013. They reported that a four days incubation of fungus on PDA, King yam and Elephant foot yam media are better for higher growth in diameter instead of filter paper, sago nutrient agar and water agar.

Conclusion

Reed wilt disease caused by *M. palmivorus* was recorded for the first time in Iraq and perhaps globally on this plant. The preliminary experimental studies have also shown the selective action of *M. palmivorus*. towards weeds without causing any damage to nearby plant seedlings. However, further studies are needed to explore its selective herbicidal activity in near future.

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REFERENCES

Adedji, A.R., Thread blight disease of tea [Camellia sinensis (L.) O. Kuntze] caused by *Marasmius pulcher* (Berk & Br.) Petch in the South Western Nigeria. African Scientist 7 (3): 107-112 (2006).

Almaliky, B.S.A., Z.A. MiorAhmad, J. Kadir, and W. Mui, Pathogenicity of *Marasmiellus palmivorus* (Sharples) Desjardin comb. Prov. on Oil Palm Elaeis guineensis. Wulfenia 19 (8): 144-160 (2012).

Alnuaimy, A.A., J.K.A. AL-Janabi, L.A.H. ALObaidi and A.F. Marjan, The expression of resistance genes in tomato induced by abiotic and biotic factors against Fusarium oxysporum f. sp. lycopersici. International Journal of Chem. Tech. Research 10(6): 838-850 (2017).

Amoako-Attah I., A.Y. Akrofi, R.B. Hakeem and E. Kumi-Asare, White thread blight disease caused by *Marasmiellus scandens* (Massee) Dennis & Reid on cocoa and its control in Ghana. African Journal of Agricultural Research 11(50): 5064-5070 (2016).

Antonín, V. and M.E. Noordeloos, A monograph of marasmioid and collybioid fungi in Europe. IHW-Verlag: Eching, Germany (2010).

Blanco-Dios J., Agaricales of the dunes of Galicia (IV): *Marasmiellus ciesanus* (Omphalotaceae), a new species found in the National MaritimeTerrestrial Park of the Atlantic Islands of Galicia (Spain). Mycosphere 6(5): 585 – 59 (201e5).

Desjardin, D.E., S.A. Gordon and R.H. Petersen, Observations on two rhizomorph-forming species of Marasmiellus. Mycol. Res. 97: 111 – 122 (1993).

Dewan, M.M. and K. Sivasithamparam, Efficacy of treatment with a sterile red fungus for control of take-all in wheat. New Zealand Journal of Crop and Horticultural Science 17: 333-336 (1989).

Kilaru, A. and K.H. Hasenstein, Development and pathogenicity of the fungus *Crinipellis perniciosa* on interaction with cacao leaves. Phytopathology 15: 101–7 (2005).

Kirk, P. M., P.F. Cannon, D.W. Minter and J.A. Stalpers, Dictionary of the Fungi, 10th ed. CABI, Wallingford, UK (2008)

Kobbing,J., N.Thevs and S.Zerbe, The utilisation of reed (Phragmites australis): a review. International Mire Conservation Group and International Peat Society 13, 1-14 (2013).

Meyerson, L.A., J.T. Cronin and P. Pyšek, Phragmites australis as a model organism for studying plant invasions. Biological Invasions Pp. 1-11 (2016).

Miller, G.L., D.E. Desjardin and L.P.Tredway, First Report of *Marasmiellus mesosporus* causing *Marasmiellus* Blight on Seashore Paspalum. Plant Disease 94(11): 1374 (2010)

Mishra, A. K. and V.K. Mishra, Field survey for some fungal disease on eggplant. International Multidisciplinary Research Journal 2(9): 23 (2012).

Molina, A.B., R.C. Williams, C. Suwanda, B. Komolong and P. Kokoa, Final report mitigating the threat of banana Fusarium wilt: understanding the agroecological distribution of pathogenic forms and developing disease management strategies. Australian center for International Agricultural research ACIAR, Australia (2010).

Obaid, A.J., J.K.A. Al-Janabi and W.R. Taj-Aldi, Antifungal Activity of Anise Essential Oil against Growth and Morphological Characteristics of Trichophyton Rubrum. Journal of Global Pharma Technol. 7(9):53-68 (2017).

Pérez-De-Gregorio, M.A., A. Vizzini, M. Contu, C. Roqué and E. Ercole *Marasmiellus* celebanticus (Agaricales, Omphalotaceae), a new species of *Marasmiellus* sect. Candidi collected in the Mediterranean area. Phytotaxa 25: 49 – 59 (2011).

Pong, V.M., A.M.A. Zainal, B.S.A. Almaliky, J. Kadir and M.Y. Wong, Isolation, Fruiting and Pathogenicity of *Marasmiellus palmivorus* (Sharples) Desjardin (comb. prov.) in Oil Palm Plantations in West Malaysia. Pertanika J. Trop. Agric. Sci. 35: 38–48 (2012).

Rebecca, D.D. and T.N.B. Roberts, Monitoring of Phragmites australis expansion and recruitment within the Black Swamp and lower Tookayerta Region. Conservation Council of SA and The University of Adelaide, final report (2016).

Thiruchchelvan, N., G. Thirukkumaran and G. Mikunthan, In vitro Biological Control of *Marasmiellus* sp. The Causal of Stem Rot of Banana Grown in Jaffna Peninsula, Sri Lank. Academic Journal of Plant Sciences 5(3): 94-101 (2012)

Thiruchchelvan, N., G. Thirukkumaran and G. Mikunthan, Banana stem rot disease; causal agent (*Marasmiellus* sp.), host range and sel-

ection of low cost media for its cultivation. J. Nat. Prod. Plant Resour., 3 (2):97-104 (2013)

Uddin, M.N., R.W. Robinson, D. Caridi and M. A.Y. Harun, Is phytotoxicity of *Phragmites australis* residue influenced by decomposetion condition, time and density? Marine and Freshwater Research 65: 505-16 (2014).

Umechuruba, C.F. and E.N. Elenwo, Diagnostic Techniques in Mycology. Beek Publishers, Port-Hercout. Pp. 30 – 56 (1999).

Wilson, A.W. and D.E. Desjardin, Phylogenetic relationships in the gymnopoid and marasmioid fungi (Basidiomycetes, euagarics clade). Mycologia 97(3): 667–679 (2005).