

First Report of *Rhizoctonia solani* Kuhn. Isolated from Parthenium Weed (*Parthenium hysterophorus* L.) in Malaysia

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ABSTRACT

Fungal pathogens are useful in biocontrol of parthenium weed (*Parthenium hysterophorus* L.) for efficient weed management. Although parthenium is a major problem in Malaysia, no initiative for developing biocontrol method has been taken yet. Therefore, a field study was conducted at Kg. Durian Tunggal, Melaka (Latitude: 2°20'55"N, Longitude: 102°17'33" E), Malaysia in August 2017 to isolate disease-causing pathogens of parthenium weed. Diseased parthenium leaves with symptoms of yellowish-brown leaf blight and the parthenium stem with reddish-brown cankers on the basal part were collected and examined after they were cultured in potato dextrose agar (PDA) medium. Isolated fungus was identified based on morphological and microscopy analyses. Two isolates, UMKRSPL1 and UMKRSPS1, were isolated qualitatively from the infected leaves and stem respectively, that yielded dark-brown colonies of sclerotia on PDA. Under the microscopic study, the mycelia with multi-nucleolus hyphal cells were noted, which were septate and hyaline.

The hyphae branched at right and acute angles to the primary hypha. There was no conidium. Pathogenicity of the fungus proved to cause similar symptoms on new, fresh parthenium leaf. Based on cultural and morphological characteristics, the pathogen was identified as *Rhizoctonia solani* Kuhn. There is no published study on *R. solani* isolated from *P. hysterophorus* in Malaysia. This is the first research on the species found on parthenium weed in the country. The identification of pathogens from parthenium

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weed would help develop bioherbicide by extracting the toxins produced by the fungus in the media broth for effective control of *P. hysterophorus* in Malaysia.

Keywords: Biocontrol, mycoherbicide, parthenium weed, *Rhizoctonia solani*, soil-borne pathogen

INTRODUCTION

Parthenium weed (locally known as Rumpai Miang Mexico) is an invasive alien species (IAS) found in Malaysia. Crop yields suffer as a result by at least 40%. This species replaces native flora and lead to allergic reaction in o humans and compromises animal health through releasing toxins, which leads to serious socio-economic losses to the people (Adkins & Shabbir, 2014; Karim, Norhafizah, & Maszura, 2017a). Chemical analysis of parthenium plants confirms all plant parts, including trichrome and pollens, contain bitter glycoside parthenin, a significant sesquiterpene lactone which is responsible for causing allergic reactions in humans and animals (Patel, 2011).

The concerned authority of the country reported that the parthenium weed had affected more than 70 hectares of land in 10 states in Malaysia (DOA, 2015; Karim, 2015). Controlling this IAS is important for any country (Dilipkumar, 2016). Although the above-ground parthenium infestation has been reduced to some extent (Tashny, 2016) a vast amount of weed seedbank has remained in the soil (Karim, Nurzafirah, & Norhafizah,

2017b), indicating parthenium hazards have not been eradicated. Therefore, particular emphasis should be given by the concerned researchers to find ways to control the soil seedbank.

A few weed scientists at Universiti Malaysia Kelantan (UMK) identified this IAS in Malaysia in 2013 (Karim, 2013, 2014) and since then, the researchers of the Parthenium Weed Research Group (PWRG) of the university have been researching on the best method to control this invasive alien weed (IAW). Parthenium weed infestation can be prevented or managed by manual, chemical or biological methods. The manual method is suitable when the weed is still young. This is by removing the plants before they flower and subsequently burning them. However, it is an expensive method of control, and it not possible to completely eradicate them. The chemical control can suppress the IAS efficiently, but it leads to environmental pollution and can create herbicide resistance in the weed. Biological control using insect or pathogen, on the other hand, is the most eco-friendly and sustainable method of control (Singh & Srivastava, 2009). Biocontrol using mycotoxins from parthenium fungi is an eco-friendly and sustainable approach, and with current concerns related to biosafety and bioterrorism, the use of mycotoxins as weapons to control parthenium hazards cannot be ignored. It is well-documented that the fungal species produces

phytotoxic metabolites (mycotoxins) which induces symptoms similar to those of the pathogens themselves. Some of the metabolites of *Colletotrichum* sp. have been shown to play a significant role in pathogenesis creating large necrotic lesions on the leaves and stem of the host plant. Singh, Quereshi, Banerjee and Pandey (2010) observed the herbicidal potential of cell free culture filtrate of *Phoma herbarum* (FGCCPH#27) against parthenium weed.

In the integrated weed management approach, the use of mycoherbicide is beneficial (Kaur, Aggarwal, Yadav, & Gupta, 2016). No information regarding the control of parthenium weed using bioherbicide is available in Malaysia. Therefore, this research was carried out to isolate and identify the fungal pathogens that affect the growth of parthenium weed, so that it can be used to develop a bioherbicide for controlling the IAW, especially to control weed growth along the roadsides, residential areas and fallow lands in the country.

MATERIALS AND METHODS

Plant Materials

Five diseased leaves and one infected stem of parthenium weed were collected from a parthenium weed infested area in Melaka (Kg. Durian Tunggal; Latitude: 2°20'55" N, Longitude: 102°17'33" E), using zippered plastic bags and placing them in a cool box. The collected samples were carried back to the UMK laboratory for investigation.

Methods

Potato Dextrose Agar (PDA) was used for isolation of fungal pathogen. The PDA was prepared by mixing 19 g of commercial PDA premix with 500 ml of distilled water. The mixture was stirred on a hot plate for a few minutes to dissolve the powder completely (Kaur & Aggarwal, 2015a). The solution was then transferred into a sterilised media bottle for autoclaving at 121°C at a pressure of 15 psi for 15 minutes. 5 ml of streptomycin sulphate was added to the media after autoclaving to exclude the growth of any unwanted bacteria or other micro-organisms. The autoclaved PDA solution at the rate of 10 ml per plate was transferred to Petri dishes and placed in the laboratory for solidification. The PDA culture was ready after three hours (Aggarwal, Kaur, Kumar, & Saini, 2014).

The infected leaves and stems were cut into 6 mm sizes and were sterilised in 1% Sodium hypochlorite solution. They were washed in sterilised distilled water for four times. The sample plant parts were then placed on PDA medium of Petri dishes. The samples were incubated at 25°C in dark condition for seven days during which the fungi grew well on the PDA medium. In order to obtain a pure culture, the isolated fungi were aseptically transferred to new PDA plates and the cultures were incubated for seven days under the conditions mentioned earlier. The pure culture was maintained on PDA slants for further investigation (Kaur et al., 2016).

The identification of fungal isolate was done by preparing lactophenol cotton blue mounts from moist plate culture. Morphological characteristics of the fungal pathogens, such as the development of hyphae colour and septum of hyphae, conidia, conidiophores (if any), number of transverse and longitudinal septa and the size of the beak, etc. were recorded at different stages for identification of the pathogens. With the help of light stereomicroscope at 10×, 40× and 100× using micrometry, the size and shape of conidia (asexual spores) or conidiophores if present, type of hypha, number of the beak were observed.

The pathogenicity test was done following the technique of Koch's Postulate with slight modification in which the fresh susceptible parthenium leaves, one leaf per plate replicated three times, were placed on the newly prepared PDA culture of isolated fungus based on the procedure adapted from Kaur and Aggarwal (2015b) and kept for seven days under similar climatic conditions as described earlier. The microbial fungal mass, including hyphae, was the pathogenic unit since no conidia were found. The specimens were regularly observed for the appearance of symptoms after three days of incubation (Aggarwal et al., 2014; Aneja, Khan, & Kaushal, 2000).

The pathogen was identified based on basic morphological characteristics as discussed in the literature (Chen et al., 2014; Tredway & Burpee, 2006; Whitman et al., 2012). However, the molecular identification of the pathogen is in progress.

RESULTS AND DISCUSSION

The fungi were identified by their essential characteristics and the early symptoms that appeared on the parthenium leaf and stem. Symptoms of the disease, the colonial morphology that grew on PDA media, mycelia, and hyphal characteristics were observed under microscope are shown in Table 1.

Symptoms of Leaf and Stem of Parthenium Weed

The presence of the pathogen led to light-yellow coloured spots and blights on the leaf of parthenium weed. The fungus formed colonies of blackish sclerotia on the surface of PDA cultures after two weeks, which were irregularly shaped, >1 mm in width (Figures 1A and 1B). In the stem specimen, a concentric ring of sclerotia was also observed (Figure 2H).

Observation under the microscope showed the mycelia of the fungus was initially colourless, which turned brownish with maturity. No conidium or conidiophore was observed. It produced hyphae branches at right and acute angles to the main hypha. The branch hypha was slightly constricted at the branch origin, and there was a septum near the branch origin. The hyphae were observed with more than two nuclei.

Pathogenicity was proved on a detached fresh leaf of parthenium (Figure 3). The symptoms appeared on the fifth day after artificial inoculation which showed a water-soaked lesion. Later the light-yellowish lesions grew which later covered larger areas and the leaf tips became rotten.

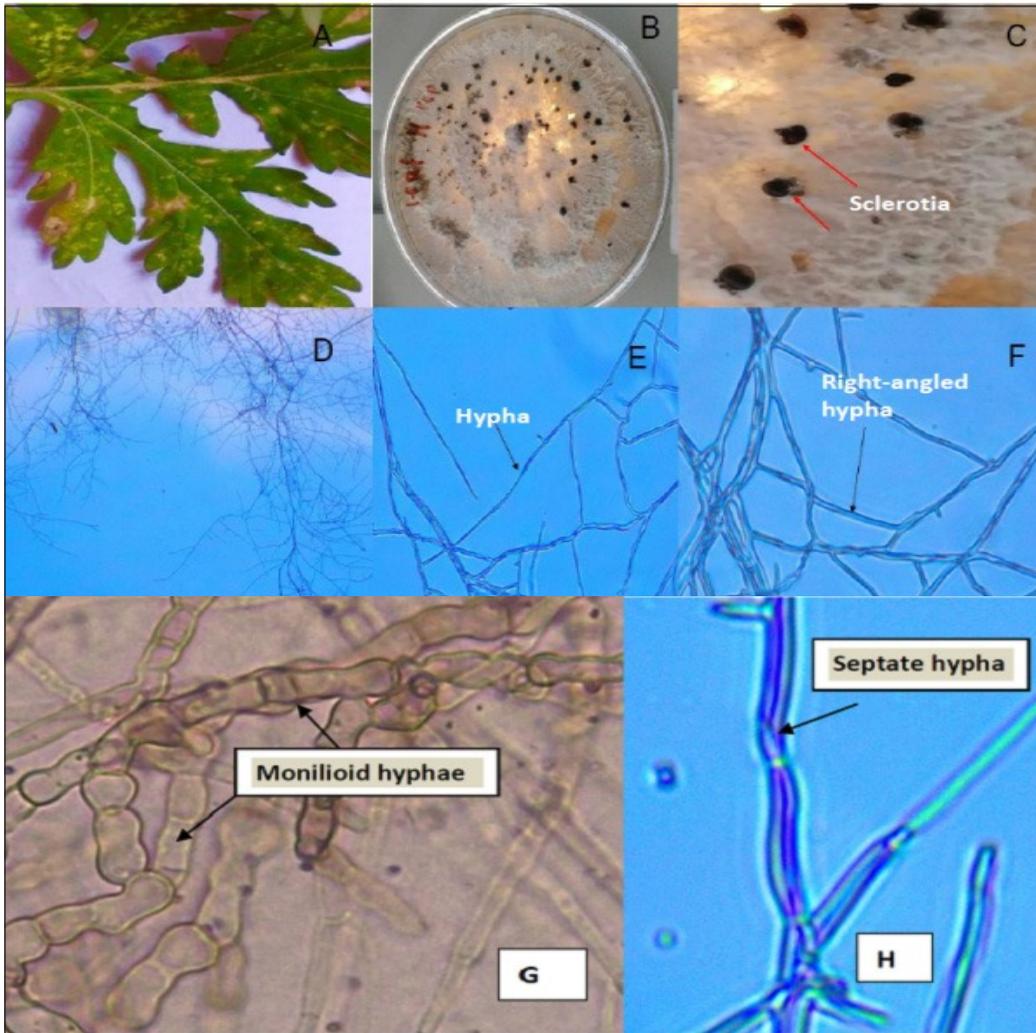


Figure 1. Isolated fungus from infected parthenium leaf. (A) Leaf blight disease on parthenium leaf; (B) Full colonial growth of the fungus with blackish sclerotia developed on the surface of PDA; (C) Magnified sclerotia (red arrow); (D) Mass of mycelia under microscope with 10× magnifying; (E) Fungal hyphae under microscope with 40× magnifying (black arrow); (F) Right angled hyphae of the fungus under microscope with 100× magnifying (black arrow); (G) Monilioid hyphae; and (H) Septate hyphae of the isolated fungus

Two weeks later, light brown mycelia and sclerotia of the pathogen were observed. The symptoms of the disease found under *in vitro* conditions were similar to that found in the field conditions. Kumar, Jayaraj and Muthukrishnan (1979) reported the wilting sign of parthenium weed caused by *R. solani* in India.

Ceresini (2011) reported the symptoms of the disease caused by *R. solani* depended on the host plant and the strain of the fungus. Usually, the symptoms are wilting, black necrotic collar rot of the seedling, and the blight on leave area. The symptoms appear at the lower and older leaves as a small brown spot with a circular ring. Dry

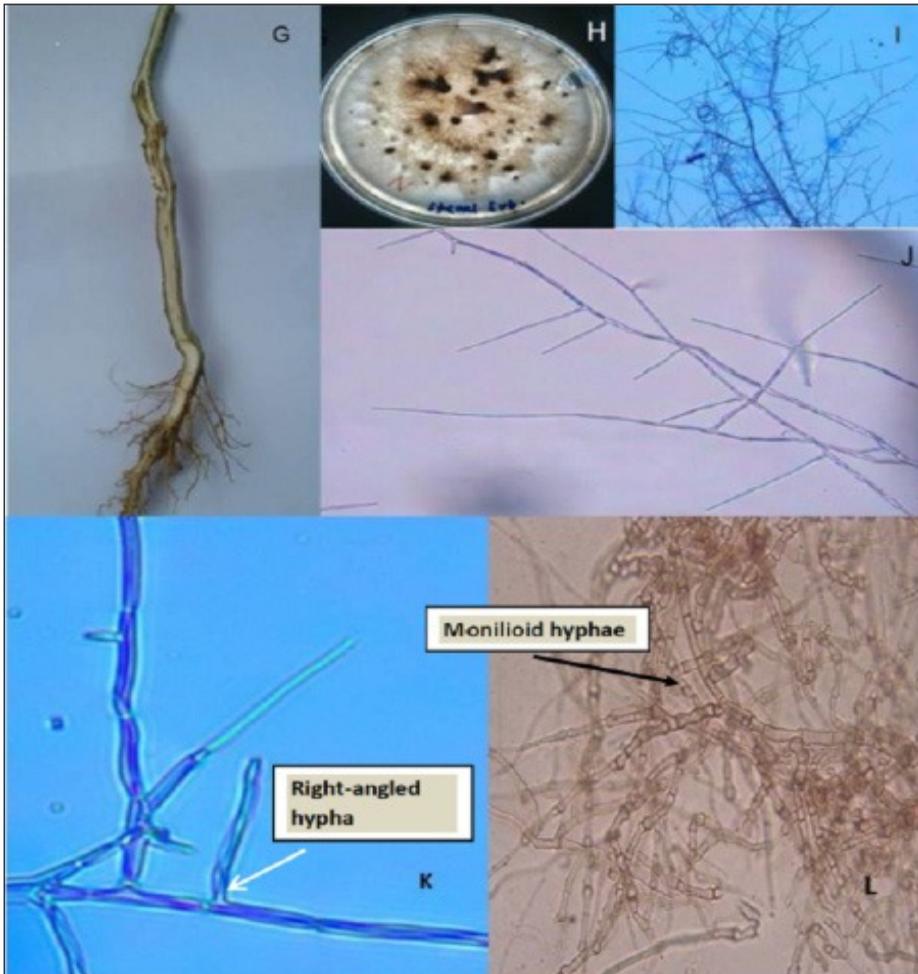


Figure 2. Isolated fungus from infected parthenium stem. (G) Cankers on parthenium stem; (H) Fungal colony and sclerotia; (I) Fungal mycelia under the microscope with 10× magnifying; (J) Acute angle hyphae of under microscope with 40×; (K) Right angle hyphae (white arrow) under the microscope with 100×; (L) Monilioid hyphae

sunken, rusty-brown on stem and root that are near to soil line are also the symptoms of infection by *R. solani*. This disease leads to the stunting of the older plants and seedlings, wilting and yellowing. However, the illness sometimes does not appear as apparent damage to the hosts; it can girdle the stem causing the plant to become stunted and resulting its eventual death.

As per statement of Whitman et al. (2012), *R. solani* had septa that formed in the branch near constriction. As the hyphae mature, it becomes more rigid and uniform. The branches rise at 90° and also at acute angles of 45° from the primary hypha. Typically, most of the hyphal cells produce new arms near to the end of the principal hyphae. In the primary hyphae near the



Figure 3. Pathogenicity test. (a) Fresh parthenium leaf after two days of inoculation; (b) Symptoms developed after five days of inoculation (after infection with the fungus the leaf turned to light yellowish color and tips are rotten)

branches, sometimes there is a single or no septum. Secondary septa are formed in older hyphae and with thinner or similar thickness as cell wall, while the primary septum is thicker at the junction with the cell wall. The young mycelia initially present as a white colour, but they turn to brown later.

Kaur et al. (2016) reported the pathogens with the production capability of lignin-degrading enzymes could play an essential role in the control of parthenium weed. Lignin is present in middle lamella, the secondary cell wall of xylem vessel and fibres that strengthen the plants. Only a small group of microorganisms are capable of degrading this lignin since the lignin is more resistant to enzymatic degradation than other plant substances. Wibberg et al. (2014) also recorded *R. solani* as an efficient producer of lignin-degrading enzymes. O'Briert and Zamani (2003) noted the production of pectic enzymes (polygalacturonase and pectin lyase) by the phytopathogenic *R. solani* AG-8 (ZG-1). These pectinases play a role in breaking the plant cell walls as

well. The authors stated in their reports that plant cell walls are primarily polysaccharide in composition. In case of host-pathogen interactions, degradation of cell walls involves actions of polysaccharides secreted by the pathogens. Most of the degradative enzymes are glycoside hydrolases, which degrade the cellulose and pectate matrices by adding water to break down the glycoside bonds. The pectate network is also degraded by polysaccharide lyases, which cleave the glycosidic bonds via β -elimination mechanism (Herron, Benen, Scavetta, Visser, & Jurnak, 2000). This observation was also reported by Tredway and Burpee (2006) and Chen et al. (2014).

Based on the disease symptoms, characteristics of sclerotia on the PDA surface observed, attributes of fungal mycelia, hyphae, and hyphal branching habits (Table 1), reconfirmation through pathogenicity test, and the descriptions given by different authors, the present study confirmed the isolated fungal is *R. solani* Kuhn.

R. solani is the plant pathogenic fungus that is widespread and widely recognised species of Rhizoctonia. This fungus is commonly known as a soil-borne pathogen with a high diversity of host plant. The pathogen is a basidiomycete fungus, which does not produce any asexual spores like

conidia. Sometimes it creates sexual spore (basidiospore), unlike other basidiomycete fungi. The basidiospores are not enclosed in the fruiting body. In nature, *R. solani* reproduces asexually through vegetative mycelia and sclerotia (Uchida, 2011).

Table 1
Identified characteristic of *R. solani* from infested parthenium plant

Fungal Species	Parthenium Sample	Colony colour on PDA	Sclerotia	Spore and hyphal structure	Isolated code
<i>R. solani</i>	Leaf	Produce dense mycelia with whitish-brown colour on PDA media	Produced sclerotia with irregular shapes and dark brown colour. Sclerotia grew in loose group.	Did not produce any spore. Septa formed in the branches. Hyphae become more rigid and uniform when it matures. Main hyphae branched at 90° and acute angle of 45°. Hypha produced specialised form called monilioid cells.	UMKRSPL1* (UMK <i>R. solani</i> Parthenium leaf)
<i>R. solani</i>	Stem	Produce dense mycelia with light brownish colour on PDA.	Produced sclerotia with irregular shapes and dark brown colour. Sclerotia grew in loose groups.	Did not produce spore. Septa formed in the branches. Hyphae become more rigid and uniform when matured. Main hyphae branched at 90° and acute angle 45°. Hypha produced specialised monilioid cells.	UMKRSPS1 (UMK <i>R. solani</i> Parthenium stem)

*The number indicates that time of investigation

These soil-borne fungi can be used to make bioherbicide to control the parthenium weed, especially those growing along the roadsides, fallow lands and residential areas where there are no susceptible crops. The mycoherbicide can be applied to the soil of parthenium infested area as the mycelia

may reside in the soil, which can attack the host plants. The fungi are attracted to the host by the chemical stimuli released by the growing plants nearby and decomposing plant residue. The pathogen attaches itself to the host by direct penetration of the plant cuticle or using natural openings in the

hosts. Hyphae come in contact with the plant and attach themselves to the host producing an appressorium, which penetrates the plant cells. After making a connection, the pathogen obtains nutrients from the plant cell. The pathogens also release ligninolytic enzymes that break down the cell walls and continue to colonise and grow inside the dead tissue. In severe infections, the plants die.

CONCLUSION

The study identified *Rhizoctonia solani* as a soil-borne pathogen which has strong pathogenicity with parthenium weed. Therefore, these pathogens may be the right candidates for producing bioherbicide for controlling parthenium weed in Malaysia for application on non-cropped areas. More research is needed to increase the virulence of the pathogen so that biocontrol efficacy of the identified pathogens can be increased. In addition, more studies on molecular characterisation and identification of anastomosis groups of the collected isolates of *R. solani* will be carried out. There is no published information on the species *R. solani* isolated from *P. hysterophorus* in Malaysia and this is the first report of the species found on parthenium weed in the country.

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