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MORPHOLOGICAL IDENTIFICATION OF WEEVIL AND FUNGAL PATHOGEN ASSOCIATED WITH SWEET POTATO TUBER DURING STORAGE

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ABSTRACT

Sweet potato is a perishable food crop that is prone to weevil and fungal infection. Development of diseases due to fungal infection will lower its quality, reduce marketable price and sometimes the tubers are unable to sell. Crop losses due to diseases not only have bad implication to farmers, but also give threat to global food production and food security. This study was conducted to identify and document the insect and fungal pathogens associated with sweet potato diseases. For this purpose, infected tubers were collected, incubated and isolated before conducting the pathogenicity test. Ninety sweet potato weevils, *Cylas formicarius* had emerged and caused extensive damaged to the tuber. Following the sweet potato weevil infestation, four fungal isolates were successfully identified as *Fusarium oxysporum* (one isolate), *Penicillium* sp. (one isolate) and *Aspergillus* sp. (two isolates). All these isolates were pathogenic to sweet potato tubers with different level of diseases severity, ranged from 33.33% to 42.59%. Identification of weevil and fungal pathogen associated with sweet potato diseases is important to help in control strategy to avoid epidemic diseases that may cause loss of economic return. Besides that, farmers should applied integrated pest management control for continuous production of good quality sweet potato tuber.

Key words: Weevil, fungal pathogen, sweet potato, disease

INTRODUCTION

Sweet potato (*Ipomoea batatas* (L.) Lam) is a common tuber crop in Malaysia and ranked as seventh in global food production (Clark *et al*., 2013). It is a creeping and dicotyledonous plant from a family Convolvulacea. The tuber is large, starchy, highly nutritious and widely consumed in human diet due to its good taste (USDA, 2009). The crop has been commercialized in many ways for economic growth development and as a cheaper source of food energy for many undeveloped countries. Nowadays, many farmers planted sweet potato because it is easily adapt in a wide range of climatic conditions. Although the tuber has physiologically tough appearance, it is perishable and prone to many weevil infestations which consequently cause fungal infection. During pre-harvest stage, weevil feeding can cause damage to both foliage and tubers which later served as an entry to many fungal infections. Pathogenic contamination through natural opening or wound will predispose the tuber to decay thus reduce the post-harvest quality (Udo *et al*., 2000). Conducive environmental factors in the tropical country such as high moisture content, fluctuated temperature and humidity assist with mechanical injuries during harvesting, storage, packaging and transportation may facilitate the entry of many fungal pathogens (Amienyo & Ataga, 2006). According to Booth (1976), the severe postharvest losses recorded in sweet potato ranged between 25 to 60% which lead to major decrease in economic growth of many countries. Besides that, sweet potato is prevalent to

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many diseases after harvest because the tubers undergo a dormancy stage. Dormancy stage will determine the storage period whereas the entire cell undergoes physiological changes such as changes in carbohydrate status, increase in respiration rate, additional weight loss and begin of sprouting (Johansen *et al*., 2008; Salimi *et al*., 2010). During this stage, the tubers are easily infected by pests and development of diseases will reduce its nutritive value, unappealing appearance, shortened the storage life which later reduce its commercial value (Oyewale, 2006). As a consequence, detrimental qualities of tubers become a major problem to many farmers because it gives limitation in the market price.

Generally, external appearance of sweet potato tuber will interpret its quality which also determines its acceptability to the potential consumer. However, damage of tuber during harvesting process usually is not obvious until development of symptoms on the infected tissue. Deterioration of tuber's quality will be higher during handling process which will restrict the distance of transportation and lead to lack of market integration and market size. Currently, various chemical pesticides have been used to overcome this issue and become a practical way to control diseases in sweet potato. To ensure marketable quality of tubers, some farmers applied overdose chemical during pre-harvest stage. Overuse of chemical pesticides will pose a major drawback to environment, human and animal health. In addition, a relatively lack of research has been conducted on post-harvest damage on sweet potato tuber compare to other crops. Therefore, this study is conducted to identify and document the weevil and fungal pathogens associated with deterioration of sweet potato after harvest. This knowledge is important to provide data for crop protection management of pests and maintain the quality of sweet potato during storage.

MATERIALS AND METHODS

Collection of sweet potato tuber, incubation and weevil identification

Sample collection was conducted during cropping season from June until October 2016 on small scale sweet potato farms at Kampung Pengkalan Kubor, Tembila, Besut (5°44'09.1"N, 102°38'02.6" E). A total of 10 kg symptomatic tubers with sign of weevil damage like external feeding and ovipositional puncture were sorted, placed into clean polythene plastic bag and transported to Laboratory for Agri-Food Pest and Disease Management (LAPDiM), Universiti Malaysia Terengganu. Fifteen of infested tubers were randomly selected and placed into three different insect cages (five tubers per cage) with dimension 30cm x 30cm x 30cm at 27±2ºC, and incubated until emergence of sweet potato weevil (SPW) adults. Emerged SPW adults were killed by freezing in -20ºC and samples were preserved in vials containing 70% alcohol for further identification under stereomicroscope (Olympus SZX16). Adult SPW were morphologically identified based on taxonomic keys developed by Wolfe (1991). The experiment was terminated when no more weevils emerged from the sweet potatoes.

Isolation of fungal pathogen

At first day emergence of adult SPW, one infested tuber was collected from each insect cage for isolation of fungal pathogens. The symptomatic tissues of tuber from the inside and on the skin surface were cut at the margin, washed and surface sterilized with 3% sodium hypochlorite before blotted dry on Whatman filter paper. Then, the infected tissues were placed onto potato dextrose agar (PDA), incubated at 27±2ºC for several days until growing of mycelia. Growing of different fungal colonies was subjected to single colony isolation to obtain a pure culture. All fungal isolates obtained during isolation process were identified based on morphological characteristics.

Pathogenicity test

All fungal isolates obtained in this study were used in pathogenicity test. For this purpose, fifteen healthy sweet potatoes tubers (three replicates for each isolate including control) were washed under running tap water before surface sterilized using 3% sodium hypochlorite. All the tubers were rinsed with sterile distilled water and air-dried before ready for inoculation process. Then, the tubers were wounded using sterile blade before inoculated with agar plug of 7-days old fungal isolates. For control, healthy tubers were inoculated with PDA plug only. All inoculated tubers were arranged in sterile containers and covered according to method suggested by Balogun *et al.* (2007). The tubers were incubated at 27±2ºC for 30-days. Development of lesion on inoculated area was evaluated every 6-days interval and scored as 0=no symptom, 1=1 to 10%, 2=11 to 20%, 3=21 to 30%, 4=31 to 50% and 5=51 to 100%. Re-isolation process was done to determine the pathogenic isolate and was carried out according to Agrios (2005). Disease severity was calculated using the following formula:

Percentage of disease severity =

Σ (n x score of disease scale) x 100/ (dmax x Σ n)

where,
n = number of replicates
dmax = maximum scale

All the data obtained in this study were analyzed using one-way ANOVA, using SPSS version 20.

RESULTS AND DISCUSSION

Sweet potato weevil (SPW) from order Coleoptera is well-known as destructive pest to many plants under family Convolvulaceae. In this study, a total of 90 SPWs emerged after 33 days of incubation period and identified as *Cylas formicarius*. From these, 47 SPWs are males and another 43 are females. The sexes can be distinguished by the filiform shape of distal antennal in males and club-like distal antenna in females (Wolfe, 1991). A life cycle of *C. formicarius* starts when the adult female lay eggs in excavated cavities of tubers. Eggs hatched and developed into larvae, pupae and adults. During oviposition stage, the female will deposit a single egg per time and sealed it with faecal plug to hide the eggs. An active female can produce 75 to 90 eggs during their life span of 33 days (Korada, 2010). All weevils undergo four stages in life cycle (Lebot, 2009) and the duration for each stage is greatly depend on the temperature and climate of the place (Capinera, 2012).

Adult *C. formicarius* has a shiny reddish brown thorax, black head and bluish black elytra (Fig. 1, a-c). According to Ames (1997), *C. formicarius* is an Asian species which is widely found in tropical regions. The larvae will mine the vines or tubers of sweet potato throughout the crack soil which later produces lesions that can be observed as darken, malformation, thickening and sometimes crack or collapse (Cockerham *et al*., 1954). Due to its size, adult's *C. formicarius* feed only the tubers and created numerous small holes that can be noted as round feeding punctures at the outer surface and hollow tunnel in cross section of sweet potatoes tubers (Fig. 1, d-e). Mild damage on the tissue due to mining activity of *C. formicarius* can induce production of terpene which result in a bitter taste especially tubers under storage (Uritani *et al*., 1975; Ray & Ravi, 2005). Uncontrolled conditions of infected tubers give major problems to many farmers because the tubers might be inedible, reduce the marketable value and sometimes has no price at all. Present of *C. formicarius* population in the sampling area shows that the species are highly tolerate with high temperature and the area provide favorable ecological niches for the species. According to Okonya and Kroschel (2013), SPW population will increased in dry condition because it induce soil cracking which assist the weevil to infest the root and laying eggs. In addition, cryptic nature of larvae and nocturnal activity of *C. formicarius* adults lead to difficulty to control the population even though with application of chemical pesticides (Reddy *et al*., 2014). Once initial infestation by SPW occurred, it can be spread throughout the farms especially through the cuttings used for planting (Sutherland, 1986).

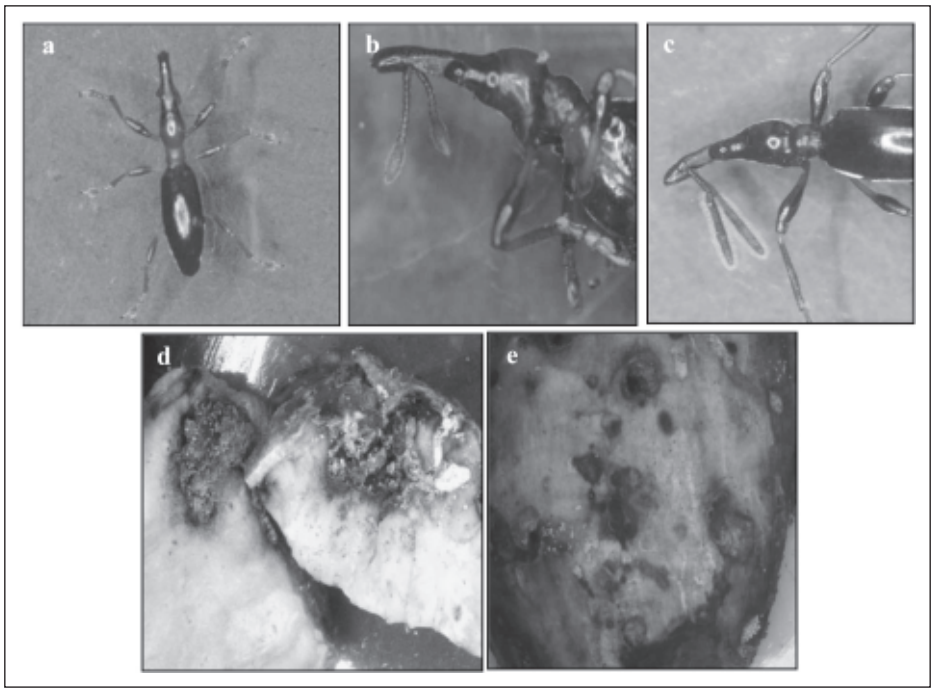

Fig. 1. *Cylas formicarius* and cross section of infested sweet potato. Adult *C. formicarius* in (a), male of *C. formicarius* in (b), female of *C. formicarius* in (c), surface of sweet potato infested by *C. formicarius* in (d), and hollow tunnel in cross section of sweet potatoes tuber in (e).

Following infestation by *C. formicarius*, four fungal isolates have been successfully isolated and morphologically identified as *Aspergillus* sp. (two isolates), *Penicillium* sp. (one isolate) and *Fusarium oxysporum* (one isolate) (Table 1). On the PDA, *Aspergillus* sp. has colony color as typical blue-green with powdery appearance, and yellowish pigmentation (Figure 2, a-b). Under microscopic observation, the conidiophore is enlarged at the tip, forming a swollen vesicle which is completely or partially covered with round conidia (2–5 mμ) (Fig. 2, c-d). Fig. 2 (e-f) shows isolate of *Penicillium* sp. that has dark green colony color and pale yellow pigmentation on PDA. The colony appearance is powdery with a rapid growth. Unlike *Aspergillus* sp., the conidiophores are simple or branched that support the phialides in brush-like clusters known as penicilli (Fig. 2, g-h). The conidia are in chain, unicellular, round to ovoid shape and have rough or smooth wall. *Fusarium oxysporum* in this study showed dense cottony colony while colony color and pigmentation ranged from white to violet and white to pale violet, respectively (Fig. 2, i-j). Under *in situ* observation, *F. oxysporum* has short monophialides (8–14 μm) (Fig. 2, k) extended from aerial mycelium with abundance of chlamydospores. Microconidia produced are non-septate, ellipsoidal and are straight or slightly curved in shape (Fig. 2, l). Macroconidia are fusiform and have a slightly pointed apical tip with a basal 'foot' cell (pedicellate) at the opposite end that make it appears as sickle-shaped or canoe-shaped.

Table 1. Morphological identification of fungal isolates associated with sweet potato weevil infestation

Morphological identified species	Colony color	Pigmentation	Colony appearance
Aspergillus sp. (UMTSP 1)	blue-green	yellowish	powdery
Aspergillus sp. (UMTSP 2)	blue-green	yellowish	powdery
Penicillium sp. (UMTSP 3)	dark-green	pale yellow	powdery
F. oxysporum (UMTSP 4)	white to violet	white to pale violet	cottony

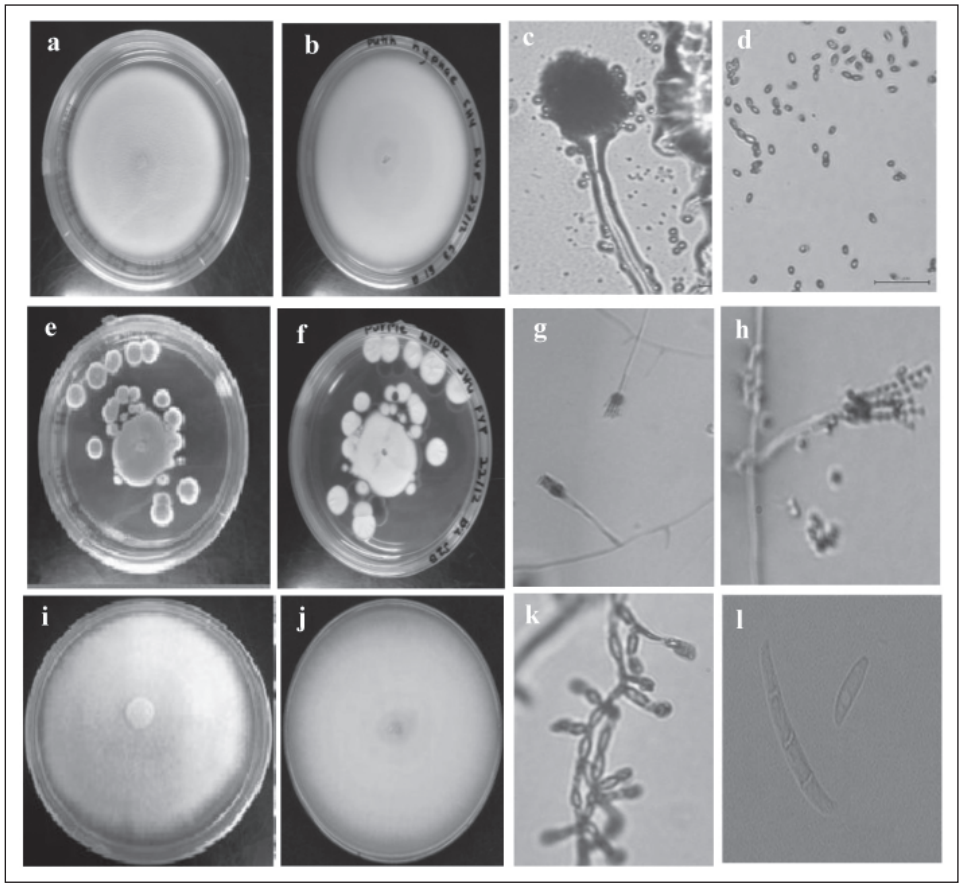

Fig. 2. Top colony color (a), pigmentation (b), swollen vesicle (c) and conidia (d) of *Aspergillus* sp., top colony color (e), pigmentation (f), brush-like cluster, penicillin (g-h) of *Penicillium* sp., top colony color (i), pigmentation (j), short monophialide (k) and macro- and microconidia of *F. oxysporum* (l).

Under proper storage system, sweet potato tubers can be stored up to six month and still retain their good quality. However, long-term storage is a challenging process not only to farmers, but also to many food industries in terms of food security and its marketing (Van Oirschot *et al*., 2003). Vulnerable of sweet potato tubers to weevil infestation during pre-harvest stage can provide an assess way for many microbial attacks. Hue & Low (2015) reported that *C. formicarius* are major contributes to the damage of tubers which later reduce its storage life and crop loss. During storage, damage of tubers can continuously occurred due to weevil infestation, mechanical injuries or microbial decay. Example of fungal diseases are black rot, dry rot, Fusarium rot, stem rot, soft rot and blue mold rot which can be enhanced under conducive environmental condition. In addition, high nutrient and water contents in the tubers make it easily susceptible to many diseases which contribute to crop losses during storage (Oladoye *et al*., 2016).

From pathogenicity test, all fungal isolates obtained during isolation process were pathogenic to healthy sweet potatoes. The symptoms appear as dark brown color of lesion around the inoculated area. After re-isolation, the similar pathogen was obtained, thus Koch's postulate were fulfilled. According to Suleiman and Falaiye (2013), some pathogens will produce extracellular enzymes to break down the cell wall before start the degenerative process in the infected cell. As a result, lesion may occur on the infected skin tissues which give an opportunity for spreading of fungus. Among the fungal isolates, *F. oxysporum* (UMTSP 4) showed highest disease severity (D.S), 42.59% followed by *Penicillium* sp. (UMTSP 3), D.S=40.74%; *Aspergillus* sp. (UMTSP 1), D.S=38.89% and *Aspergillus* sp. (UMTSP 2), D.S=33.33% (Fig. 3). All isolates were significantly pathogenic with the control ($p<0.05$). These results indicated that different fungi isolate induce different level of disease severity on sweet potato tuber. *Penicillium* sp., *Ceratocystis fimbriata, A. niger*, *A. fumigates*, *F. solani*, *Rhizopus stolonifer* are common pathogens associated with diseases in sweet potato (Agu *et al*., 2015). Study reported by Ray & Misra (1995) showed that *F. oxysporum* was pathogenic to sweet potato while *Aspergillus* spp. were found as secondary invaders. In addition, Ameinyo & Ataga (2006) reported that *F. oxysporum, R. stolonifer, Erwinia chrysentheli* and *C. fimbriata* are responsible for sweet potato diseases especially during storage. Following this, Olaitan (2012) found that *F. oxysporum, A. niger, Penicillum* sp., *R. stolonifer* and *Botryodiplodia theobroma* were pathogenic to the sweet potato tuber.

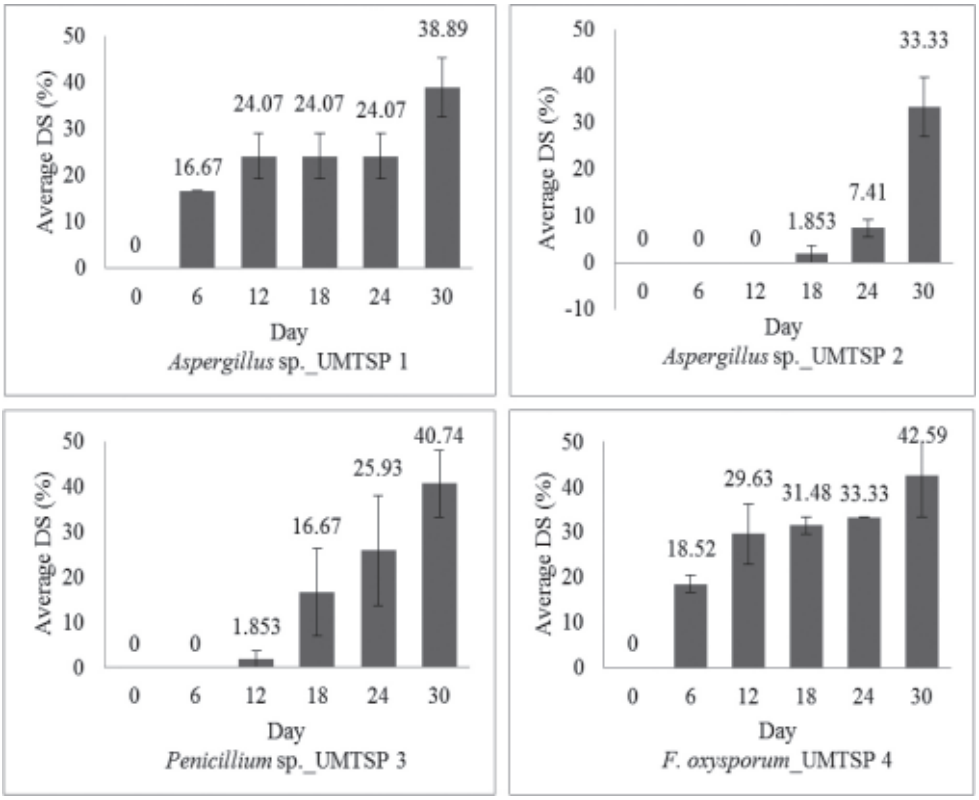

Fig. 3. Average percentage of disease severity (DS) in *Aspergillus* sp. (UMTSP 1), *Aspergillus* sp. (UMTSP 2), *Penicillium* sp. (UMTSP 3) and *F. oxysporum* (UMTSP 4).

Development of diseases on sweet potatoes after harvest is attributed to dormancy stage of the storage organ whereas all the physiological activities of the cell are decline. Different species of plant pathogenic fungi found in this study was suggested due to its worldwide distribution and their ability to adapt in local environmental condition. The favorable climatic pattern, hot and humid in the tropic can trigger fungal development in the field, after harvest and during storage. Several fungi species obtained in this study were also reported as common pathogen to many plant hosts such as banana, tomato, onion, yam, corn and mango (Sinha & Saxena, 1987; Prakash & Raoof, 1989; Narayana *et al*., 2007; Awuah & Akrasi, 2007; Adebesin *et al*., 2009; Palencia *et al*., 2010). The ability of these fungi to infect the plant crops not only reduce its quality and yield, but also can contaminate the produce with production of mycotoxins (Prelusky, 1994; Gourama & Bullerman, 1995; Webley *et al*., 1997). Accumulation of mycotoxins within the plant and produces are strictly needed to be controlled as they pose hazardous risk to human and animal health. At the meantime, changes in dispersal and growth pattern of fungi might also cause outbreak of epidemic infection (Benedict & Park, 2014). Therefore, occurrence of diseases in sweet potato tubers is a major concern among farmers and food industries because it promotes the post-harvest loss, limit the large scale investment and expose the tubers to low food security prospect.

CONCLUSION

Infestation by weevils of *C. formicarius* contributes to major problems during post-harvest storage of sweet potato tuber. Following infestation, fungal infection takes place that make the tuber prone to many diseases. Based on this study, *F. oxysporum* was highly pathogenic to healthy sweet potato tuber compared to *Penicillium* sp. and *Aspergillus* spp. Rotten tubers due to fungal infection will reduce the post-harvest quality thus reduce the selling price of the marketable produce. Therefore, study on lifecycle of *C. formicarius* can help in control strategy of this population. Identification of fungal pathogens is important to avoid epidemic diseases which cause big loss of economic return. Farmers should applied integrated pest management control to ensure continuous production of sweet potato for food supply and economic growth development.

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