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Detection of fungi from rice black bug *Paraeucosmetus pallicornis* Dallas (Hemiptera: Lygaeidae) and inhibition with crude extract of *Calatropis gigantea* (Asclepiadaceae)

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Abstract. Rice black bug (*P. palicornis*) is one of the pests that attack the rice plants in the generative phase that causes the rice easily destroyed when milled and tasted bitter after cooking hence reduces the quality and quantity of rice. The bitter taste in rice may be due to the fungus associated with rice black bug. The aimed of this research was to detect the associated fungi with rice black bug *P. pallicornis* using some sterilization methods and inhibition with of leaf crude extract of *C. gigatea*. Detection of fungi from *P. pallicornis* was conducted using three sterilization methods and control (without sterilization) namely: (1) sterilization with aquades + alcohol 70% (5, 10, 15 and 20 times dipping) + aquades; (2) aquades + alcohol 70% (10 and 20 times dipping); (3) Aquades + alcohol 90 %+ NaCl 0.5 % +alcohol 90 % + aquades . Inhibition of fungi from *P. pallicornis* with crude extract of *C. gigantea* obtained by maceration method and then made some concentration to see the effect of its inhibition on the fungi associated with the *P. pallicornis*. The results showed that without sterilization, four microbe were obtained: *Gliocladium* sp., *Aspergillus* sp., black hyphae fungus and white hyphae fungus, sterilization method of Aquades + alcohol 70% with 5 times dipping in alcohol obtained *Gliocladium* sp., 10 and 20 times dipping found *Aspergillus* sp. and *Gliocladium* sp and 15 times dipping found *Aspergillus* sp. Sterilization with 10 and 20 times dipping in alcohol 70% then washing 2 times with aquades found *Gliogladium* sp. and *Aspergillus* sp. Sterilization with Aquades + alcohol 90 % + NaCl 0.5% + alcohol 90% + aquades found *Gliocladium* sp. Crude extract of *C. gigantea* had the potential to inhibit fungi *Aspergillus* sp. and *Gliocladium* sp. from rice black bug.

1. Introduction

Rice black bug *P. pallicornis* (Dallas) (Hemiptera: Lygaeidae) is a new pest in rice plants belonging to quarantine pest with A2 categories and its currently a major problem for farmers and has spread in several rice production centers in South Sulawesi and West Sulawesi with the intensity moderate to severe thereby decreasing production. In the vegetative phase the pest attacks the rice by sucking the stem liquid with symptoms of leaves of yellowish-colored rice and generally occur in young leaves and the generative phase the pest attacks by sucking the base of the stem and the rice grain and causing bitter taste in rice due to the association with the fungus [1] this affecting the quality of the rice. The results of the laboratory studies show that on the grain and rice that has been attacked by *Aspergillus sp.* And *Gliocladium sp*. The fungus contained in rice plants attacked by *P. pallicornis* is pathogenic because it

causes death in young rice leaves [2]. The previous results also showed that by sterilization method on the surface of the body of *P. pallicornis* was found many fungi [3]. Therefore, to reassure the assumption of an association between fungus and *P. pallicornis*, it is necessary to detect this by using different sterilization method from previous sterilization. Alleged existence of this association which says that the fungal origin of *P. pallicornis* can be pathogenic to rice crops then the necessity is also to a control method which can only control *P. pallicornis* but aslo can inhibit the development of the fungus associated with *P. pallicornis* by using plant extract *C. gigantea*. The antifungal activity of *C. gigantea* was also reported in some studies and it provides an important option for the biological control a plant pathogenic fungus [4] and can be used for the remedy of infectious diseases caused by pathogenic bacteria and fungi as well as for the control of insect [5]. Extract of Calatropis are harmless and nonphytotoxic, inhibitory effects on germination and on the viability of fungal spores as well and moderate to good activity against *A. niger* as it is a saprophyte in soil causing black mould of onion, garlic and shallot, stem rot of Dracaena, root stalk rot of Sansevieria, and boll rot of cotton; spoilage of cashew kernels, dates, figs, vanilla pods and dried prune [6]

2. Material and methods

2.1. Isolation and Identification of Fungi Emerged from P. pallicornis

Samples of *P. pallicornis* were obtained from Gowa Regencies at South Sulawesi. The adults were kept in a cage containing rice seedlings during the trip to the laboratory. *P. pallicornis* was randomly selected and stored in an airtight glass container which placed in a freezer with a temperature of ± -5°C for 1 hour before the fungus isolation process. Isolation of fungi associated with the separated insects done in three successive stages as follows: detection of fungi from *P. pallicornis* with three method sterilization was modified based on [7] and without sterilization. The process detection of fungi from *P. pallicornis*: (1). Adults were washed with aquades and Sterilized with alcohol 70% (5, 10, 15 and 20 times dipping) and washed again with aquades, (2). The adults were washed with Aquades and strelized with alcohol 70% (10 and 20 times dipping), (3). The adults were washed with Aquades and sterilized with alcohol 90% and subsequently with NaCl 0.5% + 90% alcohol and the finally washed with aquades. The sterilized insects were put in petri dish containing of 13 ml PDA medium. The dishes were checked for fungal growth after 7 days. The emerged fungi were picked-up and transferred to new PDA medium and identified by characterizing the morphology were described according to their macroscopic features such as colour, shape and growth of cultured colonies, as well as microscopic characteristics like structure of hyphae, conidia and conidiophores. Obtained data then compared with the descriptions of fungi species present in the literature [8].

2.2. Preparation of Calotropis gigantea Extract

Fresh leaves of *C. gigantea* were collected from areas in and around Takalar Regency of South Sulawesi, Indonesia and shade-dried for 2–3 days, macerated with methanol solvent for 7 days and filtered through filter paper. The solvent was removed by rotary-evaporator for 6–8 h to obtain crude extract in the form of paste. It was then placed in a reagent bottle, and stored in the refrigerator as a stock solution prior to its use. The extract were prepared 3 concentration that is 1%, 1.5%, and 3% by diluting the stock solution with acetone and then suspended with 1% ml of tween-80 for each concentration.

2.3. Preparation Liquid Media for Inhibition Extract to Fungi from P. pallicornis

The extract potato containing sugar 20 grams and 1 Chloromphenicol grain were homogenized in the erlenmeyer. Furthermore, the erlenmeyer was wrapped with aluminum foil and plastic wrapping and then sterilized in an autoclave for 2 hours at 121ºC.

2.4. Method of Treatment

The liquid media was prepared as much as 100 ml and add the extract in accordance with concentration and then grow fungi from Rice black bug (*Aspergillu*s sp. and *Gliocladium* sp.). Observations were

made 2 times each at the age of fungi 7 HST and 14 HST were to calculate the weight of dried mycelium of fungi after being treated with *C. gigantea* leaf extract by filtering the fungus until no water dripped from the sieve. Then the dried mycelium is weighed using a digital scale.

3. Results and Discussion

The assessment of fungal detection in *P. pallicornis* revealed that the presence of *Aspergilus* sp. dan *Gliocladium* sp. was the most frequent with the process where adults were washed with aquades and sterilized with alcohol 70% with 5, 10, 15 and 20 times dipping and washed again with aquades. In NaCl treatment only presence Gliocladium. *Aspergilus sp.*, *Gliocladium sp.*, white fungi and black fungi was detected in not sterilized as control. Macroscopic and microscopic observations of *Gliocladium* sp and *Aspergilus* are shown in table 1 and table 2.

These results show that *Aspergillus* sp. and *Gliocladium* sp. were consistently found in various sterilization methods. Interpretation of these data that the fungi are associated with *P. pallicornis*. The results also show that the presence of Aspergilus is not only found in adult insects but also on the nymph of *P. pallicornis* from rearing in the laboratory which is F1. From the results of previous tests showed that fungus was found in the left side body of the *P. pallicornis.* This may be because these fungi accumulate in the left gastrointestinal tract. The similarity between *Aspergillus* sp. isolated from insect and from rice grain, suggested that *Aspergillus* sp. from rice grain may come from *P. pallicornis* insect and that the fungi probably reside in the digestive tract including the mouth parts. From these sites, the fungi could be transferred to the rice grain through their stylets when they feed [1].

Association between *Aspergillus* sp. and insect attacking plant have been reported that *A. flavus* to be associated grew from spiracle, recta and mouth parts on surface insects, internal organs of Lygus and stink bug and colonies of *A. flavus* developed from intestinal tissue [9].

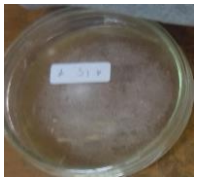
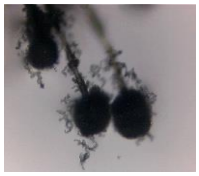
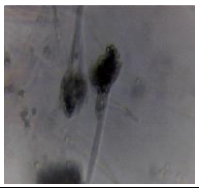
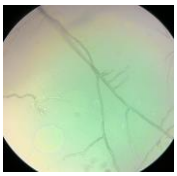
Table 1. Characterization of fungi in adults *P. pallicornis*

No.	Genera	Macroscopis	Microscopis
1	*Aspergillus* sp.	Colonies on agar culture are spread with pale dark brown colour. Growth with irregular shape.	Conidiophores, simple, bearing conidial head.
2	*Gliocladium* sp.	Colonies on agar culture are brownish green colony color. Growth with circular thick shape and entire margin.	Conidiophores hyaline, erect, simple or branched oppositely or verticillately especially in metula bearing spore masses at phialides on the apical branches.
3	White hyphae fungi	Colonies on agar culture are white. Growth like cotton with circular thick shape and entire margin.	Have no conidiophore and unbranched.
4	Black hyphae fungi	Colonies on agar culture are dark cotton with white edge. Growth with circular thick shape and entire margin.	Have no conidiophore and branched.

Aspergillus flavus has been widely accepted occurs in the field were damaging preharvest maize. Insect pest *M. nigrivenella* collected feeding on maize ears was found to be significantly correlated to *A. flavus* infection and aflatoxin contamination causes damage before and after harvest [10]. The presence of aflatoxin content may cause damage to plants in the vegetative phase causing yellow color while in the rice grain cause bitter taste. *P. pallicornis* can carry a fungus that produces a toxic substance and *Aspergillus* sp. isolated from rice grains was similar to that isolated from the adults *P. pallicornis* and from rice seed was just isolated *Aspergillus* sp., while from adult insect Isolated *Aspergilus sp.* and *Gliocladium* sp [1]. Further, this research confirmed the occurrence of an association between *Aspergillus* fungus and insect pest of *P. Pallicornis* in causing toxicity symptoms in rice.

Gliocladium sp is an antagonist fungus used to control some plant diseases so it is likely to cause damage to very small rice. *Gliocladium sp*. isolated from *P. pallicornis* does not give effect to the damage of seeds and rice plants [11]. The existence of *Gliocladium sp* may be derived from planting because of the habit of *P. pallicornis* who likes to hide in the fractures of the soil and most species of *Gliocladium* are saprophytes in various environments and can be associated with healthy plant roots to protect them from pathogenic [12] and *Gliocladium* can be carried by adults *P. pallicornis*. Sometimes many saprophytic fungi in the soil can be found in the area of the digestive system of insects [13]. The ability of various species of the genus *Gliocladium* is to produce hydrolytic enzymes and antifungal, and antibacterial compounds [7]. In addition, some species of the genus *Gliocladium* have the ability to encapsulate foreign substances. This is what causes the application of various insecticides against *P. pallicornis* have no real effect.

Table 2. Macrosopic and microscopic identification of fungi in *P. pallicornis*

Num.	Genera	Macroscopic	Microskopic
1	*Aspergillus* sp		
2	*Gliocladium* sp		
3	White fungi		
4	Black fungi		

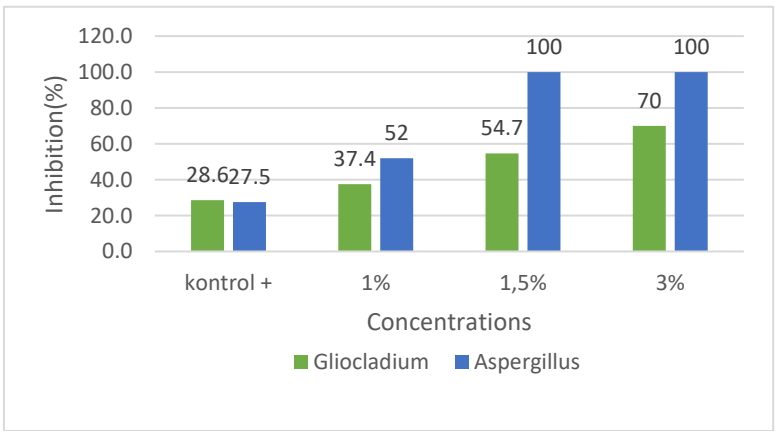


Figure 1. Percentage of inhibition fungi from *P. pallicornis* by *C. gigantea* leaf extract

Inhibition of fungi *Aspergilus* and *Gliocladium* from *P. pallicornis* with crude extract *C. gigantea* indicated that all concentrations of *C. gigantea* crude extract (1%, 1.5%, and 3%) were able to inhibit the development of *Aspergillus* sp. and *Gliocladium* sp (figure1). It can be seen that the weight of *Aspergillus* sp. and *Gliocladium* sp. after application of concentration was lower than control. This clearly shows that 3% concentrations inhibit than concentrations of 1.5% and 1%. *Calotropis* (*Asclepiadaceae*) commonly known as is a useful medicinal plant. The two species i.e. *C. gigantea* and *C. procera* are to a great extent having a very similar chemical properties like of tannins, saponin, flavonoids, terpenoids and cardiac glycosides [14, 15] cardenolide, glucosides, a non-protein, amino acid, flavonoids and steroids [16] and aqueous extracts of leaves have demonstrated strong inhibitory effect microorganisms with the mechanism of action could be by inhibition of fungal cell wall, protein and amino acid, sphingolipid biosynthesis and electron transport chain [17].

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