Original Research Article

Fungi associated with *Paraeucosmetus pallicornis* causing apparent symptoms of toxicity in rice grains and rice seedlings

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A B S T R A C T

Infestation of rice grains in the field by *Paraeucosmetus pallicornis* (Dallas) (Hemiptera: Lygaeidae) causes the rice to become broken and bitter, and the bran to become unpalatable for consumption by poultry. This article offers the hypothesis that the insect can carry fungi producing toxic substances, which in turn cause these symptoms. On adult insects collected in four regencies in South Sulawesi Indonesia we identified *Aspergillus* sp and on two samples, we also found *Gliocladium* sp. Both the *Aspergillus* sp. and *Gliocladium* sp. isolates from different regions showed different cultural characteristics, suggesting different species in each genus associate with the insect. *Aspergillus sp.* isolated from rice grains was similar to that isolated from the insect, providing evidence that the *Aspergillus* sp. was transferred from insect to rice grains. Inoculation of these two genera into seeds by immersion and seedlings by spraying with concentration of 10^2, 10^4, and 10^6 spores/ml showed that only *Aspergillus* sp. had an impact on the incidence of damage to seedlings which consisted of symptoms of apparent toxicity. Comparison of treatments with the Bone isolate and Bantaeng isolate of *Aspergillus* sp. indicated that the incidence of symptoms indicative of toxicity in seedlings infected by *Aspergillus* through seed treatment was 7.4% per one Log spores concentration for Bone isolate and 2.9% per one Log spores concentration for Bantaeng isolate. The incidence in seedling inoculated by *Aspergillus* sp. through spraying was 6.9% per one Log spores concentrationfor Bone isolate and 13.5% per one Log spores concentration for Bantaeng isolate. This research confirmed the occurence of an association between *Aspergillus* fungus and insect pest of *P. Pallicornis* in causing toxiciy symptoms in rice

Introduction

Paraeucosmetus pallicornis (Dallas) (Hemiptera: Lygaeidae) known locally as black bug is considered as a new pest on rice. Its distribution in Indonesia is limited notably to Sulawesi, but some reports also indicate that this pest exists in

East Kalimantan and East Nusa Tenggara (Rauf and Lanya, 2009). Since it was reported for the first time in 1989, the occurrences of *P. pallicornis* in North Sulawesi have been observed in almost all Regencies in South Sulawesi (Wibowo, 2009; Ala *et al.*, 2010). The life cycle of this pest including egg, five phases of nymph, and adult stages is in the range of 38 days for males and 45 days for females (Rauf and Lanya, 2009).

The nymph of the first and second instars feed on the base of the stem, while the third, fourth, and fifth instars and adults feed on developing rice grains causing black spots on the surface of the grains (Pelealu, 1991). The rice grains becomes broken and bitter and the bran unpalatable for consumption by poultry feed.Therefore, this damage leads to substantial economic impact due to decrease of rice quality. On other hand, the occurence of *P. pallicornis* on rice seedlings causes chlorosis symptoms or drying of leaves (BPTPH South Sulawesi, not published). On the basis of the finding that rice grains becomes bitter and the appearance of chlorosis symptoms on leaves, we offer the hypothesis that the insect can carry fungi producing toxic substances. Fungi such as *Aspergillus niger, A. fumigatus, Trichoderma harzianum, T. koningii, Ulocladium consortiale, Fusarium* sp. and *Mucor* sp. can be carried by some groups of insect like Hymenoptera, Lepidoptera, and Diptera (Pereira *et al.*, 2009; Braide *et al.*, 2011).

In this research, fungi associating both with *P. pallicornis* and rice grain infested by *P. pallicornis* were isolated and characterized and their impact on development of rice seedling was evaluated. It is hoped that the result of this research can provide a contribution to the overall picture concerning the interaction between fungi-insects and that it can be used as a basis for controlling this *P. pallicornis*.

Materials and Methods

Isolation and identification of fungi

Samples of adult insects of the black bug were obtained from Bantaeng, Gowa, Soppeng, and Bone Regencies in South Sulawesi. The samples of rice damaged by the insect were collected from Soppeng and Bone Regencies. The insects were kept in a cage containing rice seedlings before isolating the fungi.

In order to isolate the fungi, adult insects and rice grains were put down with 70% ethanol. The insects and rice grains were then surface sterilized by dipping in 70% ethanol for 1 minute. They were then washed with sterile water. The sterilized insects and rice grains were then put in Petri dishes containing about 20 ml of PDA medium. After three days growing fungi were selected and purified by moving to new PDA medium identified by characterizing the morphology of the mycelia and spores. Isolates were also differentiated by colony pattern, density, color, and zonation.

Inoculation of fungi on rice

Fungi isolated from insects originating from Bantaeng (*Aspergillus* sp.), Bone (*Aspergillus* sp.), and Soppeng (*Gliocladium* sp.) regencies growing on PDA medium of 5 days was used for inoculation of rice. Two methods were used for inoculation: seed treatment and application to seedlings. The seed treatment was done by submerging seeds

in spore suspensions for 24 hours. These seeds were kept on humid tissue for 48 hours and then were planted in small 250 ml pots. Inoculation of seedlings was conducted by spraying 12 day-old seedlings which were planted in small pots as above. The spore concentrations used for seed treatment or seedling application were 10^2, 10^4, and 10^6 spores per milliliter. Each treatment was replicated four times and each replication consisted of one pot containing five seedlings.

Observation and data analysis

Observations of toxicity symptoms were conducted on seedling of 33 days old. The incidence of toxicity symptoms was calculated using the formula of I = a/b x 100%, where I is the incidence of toxicity symptom, a is the number of seedlings with toxicity symptoms and b is the total number of seedlings observed. Toxicity symptom consisted of delay of growth, stunting, and chlorosis or blight on leaves of seedlings treated by fungi through seed treatment . On seedlings treated by spraying of fungi, the toxicity was just indicated by chlorosis or blight of leaves.
The rates of toxicity symptom incidence by fungi in each treatment were analyzed by a regression equation (Toxicity symptom incidence in ordinate and Log of spores concentration in abscissa). For evaluating significant differences between the regression coefficients (rates of toxicity symptom incidence) of each treatment, these regression coefficients were analyzed by the T-test.

Results and Discussion

Two genera of fungi, *Aspergillus* and *Gliocladium* were isolated from adult insects of *P. pallicornis*. *Aspergillus* produced septate and branched hyphae and macronematous, mononematous, erect, and simple conidiophores with a terminal vesicle bearing short branches or phialides radiating from the entire surface. Conidia were phialosporic, globose, unicellular and hyaline arising together at the end of terminal branches, but colored in mass disposed in long basipetal chain. While, *Gliocladium* possessed conidiophores macronematous and mononematous, erect, septate and branched, ending in a branched system of phialides. Conidia were phialosporic, unicellular, hyaline, ovoid and aggregated in conidial masses.

Aspergillus was found in all *P. pallicornis* samples collected in Soppeng, Bone, Bantaeng, and Gowa, but each isolate showed different cultural characteristics. This difference was notably found in color and zonation, where Soppeng isolate was white with yellow spores and without any zonation, while the Bone isolate was white with green spore on concentric zone, the Bantaeng isolate was white with greenish spores and no zonation , and the Gowa isolate was white with black spores and light radial furrowed on (Table1).

Aspergillus sp. isolated from rice grains of Soppeng and Bone origin possessed similar cultural characteristics to *Aspergillus* sp. isolated from adult insects of Soppeng and Bone origin respectively.

Gliocladium sp. was just isolated from adult *P. pallicornis* of Soppeng and Gowa origin, while none was obtained from insects of Bone and Bantaeng origin and also none from rice grains. As shown with *Aspergillus* sp. the two isolates of *Gliocladium* sp.had different cultural characteristics on PDA media.

The treatment of *Aspergillus* sp. Isolates from Bone and Bantaeng isolate through

seed treatment caused symptoms such as retarded growth, stunting and chlorosis or blight of young leaves, while the treatment through foliar spraying caused chlorosis followed by drying off starting from tip of young leaves. These symptoms were similar toxicity symptoms caused by copper on crops including rice (McCauley, 2009; Mostofa and Fujita, 2013; Sulistyono and Rokhmah, 2012). The higher the concentration of spores applied, the greater the severity of symptoms occurred in the rice seedlings. The incidences rate of toxicity symptoms in seedlings infected by *Aspergillus* sp. through seed treatment were 7.4% per one unit Log of spores concentration for Bone isolate and 2.9% per one unit Log of spores concentration for Bantaeng isolate and these two rates were significantly different (P ≤ 0.05) (Figure 1). The incidences rate of toxicity in seedling inoculated by Aspergillus sp through spraying were 6.9% per one unit Log of spores concentration for Bone isolate and 13.5% per one unit Log of spores concentration for Bantaeng isolate and these two rates were also significantly different (P ≤ 0.05) (Figure 2). On other hand, treatment by *Gliocladium* sp. resulted in a smaller incidence of toxicity symptoms on rice seedlings and did not showed any linearity in regression, the highest was 10% when this fungus applied through seed treatment and 9.4% through spraying.

Aspergillus sp. was found from all samples of insect collected from four regencies in South Sulawesi, this indicated that this genus is important in the *Aspergillus- Paraeucosmetus pallicornis* association. Meanwhile, these *Aspergillus sp.*showed different cultural characteristics indicating a possible difference in species. It appears that the insects could acquire the fungi from their environment, but that this is probably specific for the genus, but not specific for species.

Previous research has demonstrated that *Aspergillus* sp. associated with insects can be detected on surface of the insect, in the intestines, mycangia, feces, insect galleries, and borer holes (Nesci and Etcheverry, 2002; Perez *et al*., 2003; Henriques *et al*., 2009). Our method of isolation of *Aspergillus* sp. could not be used to indicate precisely where the fungus resides, since the isolation was done from the entire adult insect body. 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.

Association between *Aspergillus* sp. and insects have been reported previously such as *A. ochraceus, A. flavus*, and *A. niger* with coffee berry borer, *Hypothenemus hampei* (Vega *et al*., 1999; Perez *et al*., 2003), *A. flavus* with corn ear borer *Helicoverpa zea* (Nesciand Etcheverry, 2002), and *Aspergillus* sp. with ambrosia beetle of cork oak, *Platypus cylindrus* (Henriques *et al.,* 2009). But these research studies did not give any information concerning the impact of *Aspergillus sp.* when it is applied directly to plant and/or plant parts concerned.

Previous reports indicated that Aspergillus infection on rice seedlings caused leaf spot or necrosis symptoms (Hinampas *et al*., 2013), this is due to the activity of enzymes produced by the fungus. Some species of Aspergillus such as

Table.1 The fungi isolated from adult insect of *Paraeucosmetus pallicornis* and their cultural characteristics on PDA media

| Genus and origin	Upper surface				Lower surface
	Cultural aspect	Density	Color	Zonation	
Aspergillus (Insect adult, Soppeng)	Effuse, powdery	Light	Light yellow	absent	Idem to upper surface, except the color yellowish
Aspergillus (Insect adult, Bone)	Effuse, powdery	High	Creamish with greenish spores in concentric zone	Concentric	Idem to upper surface, except the color yellow
Aspergillus (Insect Adult, Bantaeng)	Effuse, powdery	Light	White with greenish spore	Absent	Idem to upper surface except the color white
Aspergillus (Insect Adult, Gowa)	Effuse, powdery	Light	White with black spores	Slight radial furrowed	Idem to upper surface except the color white
Gliocladium (Insect adult, Soppeng)	Effuse, Powdery	High	Green	Absent	Idem to upper surface, except the color (yellow)
Gliocladium (Insect adult, Gowa)	Effuse, velvety	High	Grey	Absent	Idem to upper surface, except the color (Yellow pigmented)

A. fumigatus produces a wide range of glycosylhydrolases that have the capacity to degrade the major plant cell wall polymers (Tekaia and Latge, 2005). So far, *Aspergillus* spp. have been known as the causal agents of necrotic or rot symptoms such as crown rot of peanut, black rot of onion, tuber rot of yam, stem rot of Dracaena, kernel rot of maize, fruit rot of banana, rot of tomatoes, and rot of mango (Sharma, 2012). However, our results showed that the rice seedlings inoculated with Aspergillus expressed symptoms of retarded growth, stunting, and chlorosis on leaves of infected rice seedlings and bitterness of rice and its bran These symptoms were probably due to the action of toxins rather than the enzymes. *Aspergillus* spp. secrete a number of toxic substances such as aflatoxin, ochratoxin A,

Fig.1 Incidence with toxicity symptoms of 33 days old rice seedling following treatments through submerging seed in three spores concentration of *Aspergillus* sp. (Bone and Bantaeng isolates). The rates of these two incidences (7.4 and 2.9) were significantly different (P 0.05)
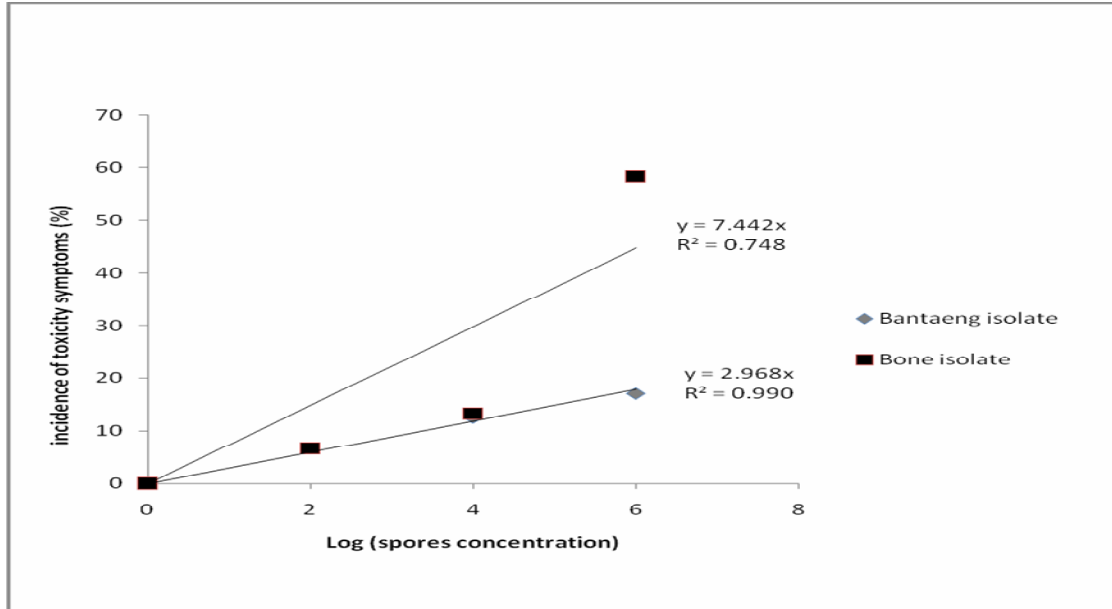
Fig.2 Incidence with toxicity symptoms of 33 days old rice seedling following treatments through spraying with three spores concentration of *Aspergillus* sp. (Bone and Bantaeng isolates). The rates of these two incidences (13.5 and 6.9) were significantly different (P 0.05)
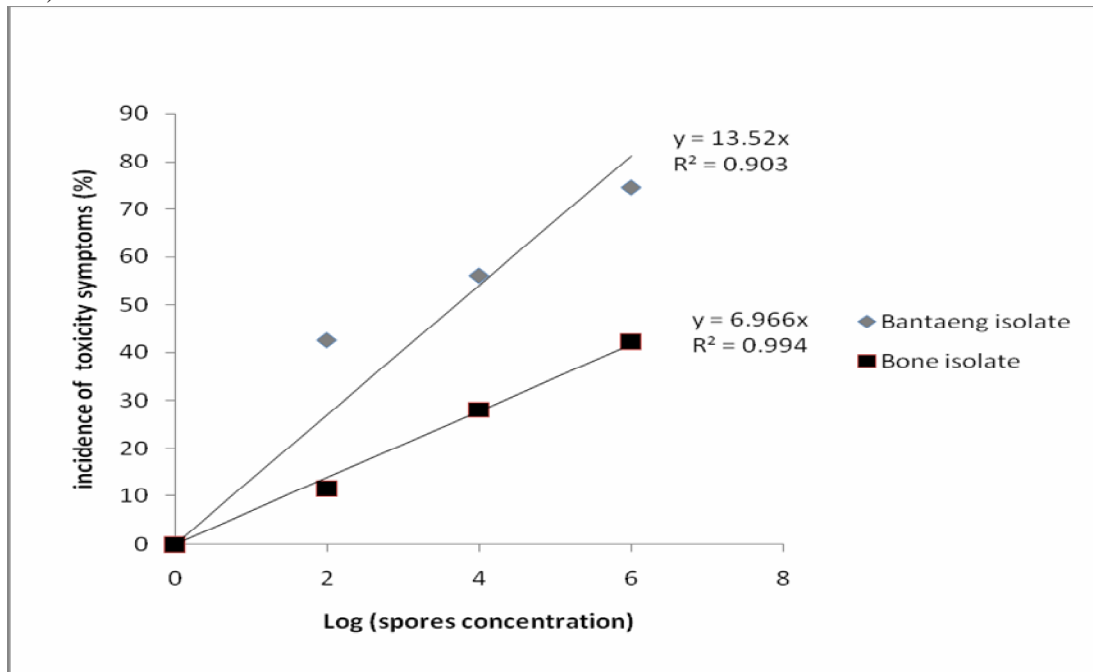
sterigmatocystin, cyclopiazonic acid, citrinin, patulin, and tremorgenic toxins (Vega *et al*., 1999; Reddy *et al*., 2009; Bhetariya *et al*., 2011). Therefore, the current study results suggested that all symptoms expressed on the inoculated plants were caused by toxin activities.

The *Aspergillus* sp. isolated from Bone caused toxicity symptom when this isolate was applied through seed treatment, while the *Aspergillus sp.* Isolate from Bantaeng resulted in a greater incidence of toxicity when this isolate was applied through foliar spray~~ing~~. The last case corresponds to the occurrence of similar symptoms when *P. pallicornis* exists at a high population density on young rice in Bantaeng rice fields. This supports the hypothesis that the two isolates above were different species with each species having a specific interaction with *P. pallicornis* and rice plants.

Our results provided new insights into the *Aspergillus- P. pallicornis*-rice plant interaction and the resulting damage to rice plants. The damage is not only caused by mechanical injury through sucking and piercing of insect stylet, but also by disturbance of metabolism through *Aspergillus* intervention both in the vegetative and generative phases of rice development. The damage in the vegetative phase is as important as that the damage to rice grains in the generative phase.

Acknowledgement

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