International Journal of Agricultural Technology 2017 Vol. 13(7.3): 2609-2614
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

Morphological Characterization and Bacteriostatic Activity of Entomopathogenic Fungi Isolated from Short-horned Grasshopper *(Oxya hyla intrica)*

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Sogelio Jr., R.C., Natura, J.M.N. Jacob, J.K.S and Paltiyan, J.C. (2017). Morphological Characterization and Bacteriostatic Activity of Entomopathogenic Fungi Isolated from Short-horned Grasshopper (*Oxya hyla intrica*). International Journal of Agricultural Technology 13(7.3): 2609-2614.

Entomopathogenic fungi are known to be part of the rich ecosystem in the tropics playing an important role as biocontrol agent. The isolation and morphological identification of fungi from were performed to identify fungal strain present in short-horned grasshopper (*Oxya hyla intrica*) known as large group of insect that causes economic damage to forage and crops. Short-horned grasshoppers were collected in the field and were placed in an insect jar and subjected to standard classification and naming. Collected insects were paralyzed by placing on a sterilized microwavable plastic with ethyl alcohol and were incubated for five (5) days. After the incubation, insects were inoculated in potato-dextrose agar (PDA) medium for detection of fungi present in the short-horned grasshopper. Classification and identification of fungi present on the insects were based on its morphological characterization and taxonomic guidelines. *Fusarium verticillioides* and *Curvularia lunata* were the two fungal strains identified. The bacteriostatic activities of the two (2) fungal strains were performed using the immobilized disc method. Results revealed that immobilized disc of *F. verticilliodes* exuded 43.06mm zone of inhibition against *E.coli* while *C. lunata* exhibited 49.74mm. On the other hand, *F. verticilliodes* produced a mean of 33.56mm zone of inhibition while *C. lunata* exhibited 36.71mm against *S. aureus.*

Keyword: Fungi, Grasshopper, Isolation, Morphological

Introduction

Grasshoppers are one of the important agricultural pests as it is responsible for the economic damage to forage and variety of crops. Several species under the genus *Oxya* have adapted to the aquatic environment and lay their eggs on rice leaves. Moreover, both nymphs and adults feed on large sections of leaf blades (Lange *et al*., 2005; Lomer *et al*., 2001).

Chemical insecticides are common way to protect the crops and to control insect population. However, it has been reported that over 500 species

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of arthropods show resistance to various chemical substances such as contained in insecticides (Mota-Sanchez *et al*., 2002). Therefore, there is a continuous search for other way to control these pests that is safer to the environment.

One of the possible alternatives is the use of natural control agents including entomopathogenic fungi. These fungi spread fungal disease in most insect species by causing starvation to toxin production (Holder *et al*, 2005). Hence, this study was carried out to isolate and identify entomopathogenic fungi from short-horned grasshopper.

Materials and Methods

Collection and Isolation of Samples

The insects used in the study were collected from Isabela State University, Isabela. Samples were collected using insect net and placed in a killing jar prior to incubation for five (5) days to determine fungi that are present in the sample. Daily checks were performed during the first five (5) days. The insect are inoculated in previously prepared Petri plates containing potato-dextrose agar (PDA) to grow the fungal strains present in the sample. The filamentous fungi emerging from the dead individuals were transferred to Petri dishes containing PDA supplemented with antibiotics, and incubated.

Morphological characterization and Identification of Sample

The fungal species isolated from *O. hyla intricata* were identified through direct examination of the fungal isolates using agar block technique and on the basis of the macromorphological appearance of the colonies such as color, diameter, mycelial texture and their micromorphological characteristics were observed under microscope. Text book and taxonomic guidelines are used as reference to identify the fungal species.

Bacteriostatic Assay using Immobilized Mycelial Disc

Fully colonized Petri plates containing *F. verticilliodes* and *C. lunata* were subjected to immobilization using Ultraviolet lights for ten minutes. Immobilized fungal isolates were punched using 10mm cork borer.

Preparation of Culture Media and Pour Plating

Thirty-eight grams (38g) of prepared Mueller-Hinton Agar (MHA) was mixed with one (1) liter of distilled water. It was heated until a homogenous mixture was obtained. Approximately 300 ml of the prepared medium was dispensed onto clean Erlenmeyer Flasks, plugged with sterile cotton and wrapped with paper and sealed with a rubber band. The medium was sterilized for 15

minutes at 15lbs/in2, 121 ℃ using autoclave. After sterilization, the media was allowed to cool for several minutes until ready for pour plating. Approximately 15mL sterile MHA was aseptically dispensed into sterile Petri plates then allowed to cool and solidified prior to inoculation of bacterial samples.

Spread Plating and Inoculation of the Test Organism

E. coli and *S. aureus* were aseptically inoculated onto previously prepared sterile MHA plates using sterile cotton swabs all around the surface of each plate. Each plates containing bacterial inoculum were inoculated with approximately 10 mm immobilized mycelial disc. Plates were incubated on room temperature and growth was measured using Vernier caliper every 8 hours in a 24-hour period.

Results and Discussion

Morphological Characterization of Fungi

The isolated *Fusarium verticillioides* and *Curvularia lunata* were subjected to agar block technique and were identified morphologically with published literature such as Quimio and Hanlin (1999) and Dugan (2005).

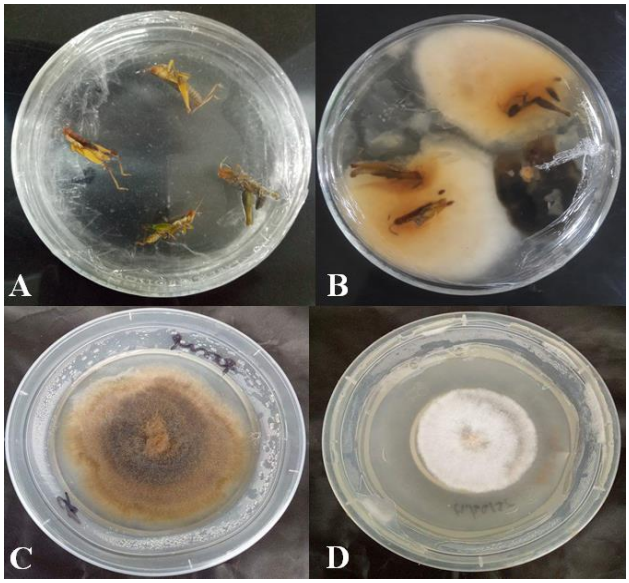

Figure 1. (A) Remains of *O. hyla intrica* as source of entomopathogenic fungi (B) Fungal colonies observed after 5 days of incubation (C) *Curvularia lunata* isolates from the body remains of *O. hyla intrica* (D) *Fusarium verticilliodes* isolates from *O. hyla intrica*

The genus *Fusarium* comprises a large group of species of filamentous fungi that are widely distributed in soil and are usually associated with plant. Most species are saprotrophic and are relatively abundant members of the soil microbiota according to Leslie and Summerell (2006). On the other hand, *Curvularia lunata* belongs to the family of *Pleosporacae* under the *Dothidiomycetes* fungi and it is also filamentous fungus that has *Cochliobolus* teleomorphic stage according to Kuzai *et al*. (2015). Morphologically, *Curvularia* is characterized by the production of sympodial conidiophores with tretic, terminal and intercalary conidiogenous cells and elongate, transversely septate conidia with a dark basal scar. Conidia are often curved at an asymmetrically swollen intermediate cell, but species with straight conidia also have been described (Sivanesan, 1987).

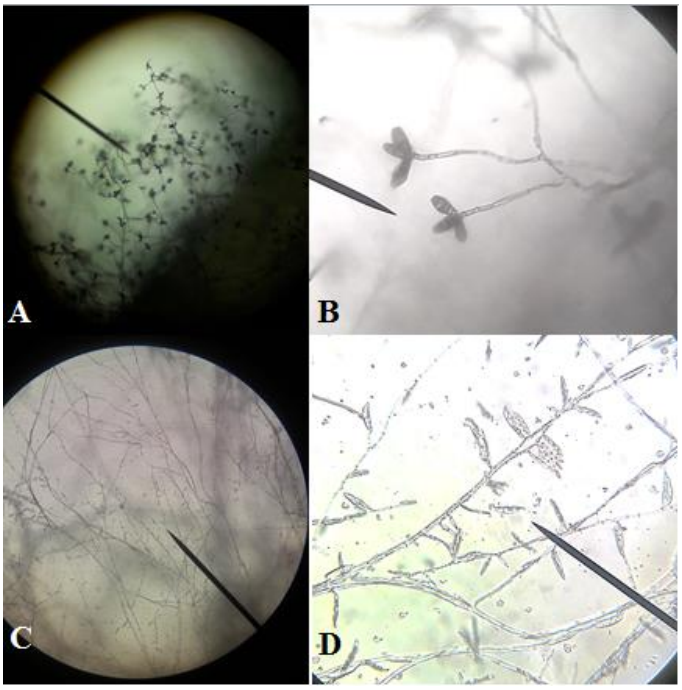

Figure 2. (A, B) *C. lunata* showing conidiophores and conidia that are strongly curved; conidia with 3 predominant septa. (C, D) *F. verticillioides* showing conidiophores and macroconidium.

Bacteriostatic Assay

The two isolated fungi, *F. verticillioides* and *C. lunata,* were examined for their bacteriostatic activity against test bacteria *E. coli* and *S. aureus* using mycelial block. All of the fungal strains were determined to inhibit the growth of both *E. coli* and *S. aureus*.

Table 1. Bacteriostatic assay of *F. verticillioides* and *C. lunata* against *E. coli* and *S. aureus*

Fungal Isolates	Zone of inhibition of immobilized mycelial disc (mm)		
	E. coli		
	8 hours	**16 hours**	**24 hours**
F. verticilliodes	16.69 ±2.26[a]	22.26 ±4.36[a]	43.06 ±6.73[b]
C. lunata	16.92 ±0.64[a]	25.87 ±4.93[a]	49.74 ±10.62[a]
	S. aureus		
F. verticilliodes	**8 hours**	**16 hours**	**24 hours**
C. lunata	25.41 ±1.34[a]	31.62 ±4.27[b]	33.56 ±1.81[b]
	25.79 ±0.51[a]	35.32 ±2.96[a]	36.71 ±4.20[a]

The data presented in Table 1 shows the result of bacteriostatic assay of *F. verticillioides* and *C. lunata* against *E. coli* and *S. aureus* using immobilized mycelial discs. *C. lunata* shows a bacteriostatic activity with 49.74 mm compared to *F. verticillioides* with 43.06 mm after 24 hours of observation. Moreover, *C. lunata* also exhibited higher bacteriostatic against *S. aureus* with 36.71 mm in comparison with the results exuded by *F. verticillioides* with 33.56 mm. The inhibition of *F. verticilliodes* and *C. lunata* against *E. coli* increased dramatically in every 8-hour period. The inhibition of the two fungi against *S. aureus* is relatively slower and smaller. The results of this current study is congruent with the study of Trigos *et al.* (2006) wherein *Curvularia spp.* and *Fusarium spp.*, isolated from different plants including mango, grass and chilli pepper, exhibited high bacteriostatic activity against *Pseudomonas areuginosa* and *Pectobacterium carotovorum*.

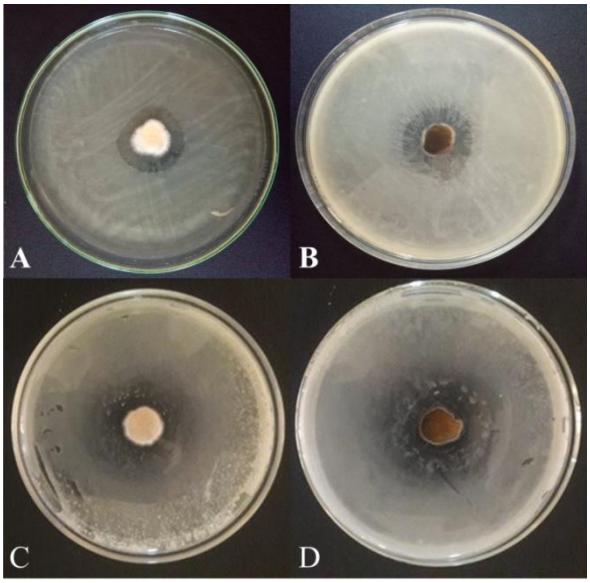

Figure 3. (A, B) Zones of inhibition of *F. verticillioides* against *E. coli* and *S. aureus.* (C, D) Zones of inhibiton of *C. lunata* against *E. coli* and *S. aureus.*

The results showed that *F. verticilloides* and *C. lunata* (Figure 3) possesses bacteriostatic activity against *E. coli* and *S. aureus*. In comparison to common commercial antibacterial, these two fungi have more effective antibacterial properties against the same test bacteria. Consequently, the entomopathogenic fungi are also detrimental to the health of their host. The results indicate that the two entomopathogenic fungi isolated from *O. hyla intrica* can be used as potential biocontrol agents.

Acknowledgement

The authors acknowledge the help and unending support and tutelage of Mr. James Kennard Sanz Jacob which helped the authors properly conduct and finish this study.

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(Received 18 October 2017; accepted 25 November 2017)