EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 3
587-591
Year: 2018

ANTIFUNGAL POTENTIALITIES OF COELOMIC FLUID OF LOCAL EARTHWORMS AGAINST *ASPERGILLUS* SPECIES

Sethulakshmi K. C.*[1] and Ranilakshmi[2]

[1]*School of Environmental Sciences, Mahatma Gandhi University, Priyadarshini Hills PO, Kottayam, Kerala 686560.
[2]Kerala Veterinary and Animal Sciences University, College of Veterinary and Animal Sciences, Pookode, Wayanad. Lakkidi P.O. 673576.

***Corresponding Author: Sethulakshmi K. C.**
School of Environmental Sciences, Mahatma Gandhi University, Priyadarshini Hills PO, Kottayam, Kerala 686560.

Article Received on 08/01/2018 **Article Revised on 28/01/2018** **Article Accepted on 18/02/2018**

ABSTRACT
The study aims to findout the antifungal potentialities of coelomic fluid of selected earthworms against eight pathogenic *Aspergillus Sps* using well diffusion methods. The *Aspergillus niger, Aspergillus flavus Aspergillus steyni, Aspergillus nidulans, Aspergillus terreus, Aspergillus penicillioides, Aspergillus fumigates* and *Aspergillus ochraceus* fungi were used as test organisms. The fungal strains were maintained on Sabouraud Dextrose Agar slant at 4°C. The coelomic fluid released due to cold shock and gets collected at the lower side of the petriplate. Antifungal activity of coelomic fluid was done by Well diffusion method. The *P.corethrurus* shows high antifungal activity against *A.steyni* (18mm), *A.niger* (14mm), *A.terreus* (9mm*) and A.fumigates* (8mm) simultaneously. The *M. konkanensis* shows high antifungal activity against *A.steyni* (20mm), follows *A.niger* (18mm), *A.flavus* (16mm) and *A.terreus* (16mm). The *D.ghatensis* shows high antifungal activity against *A.terreus* (20mm), *A.flavus* (11mm) and *A.penicillioides* (8mm). The *M. konkanensis* shows more antifungal activity against different fungus selected for the study. The antifungal activity was very less in *D.ghatensis*. The zone of inhibition is absent against *A.ochraceus* and *A.nidulans* in both the earthworms.

KEYWORDS: Antifungal, Coelomic fluid, Earthworms and *Aspergillus sps.*

INTRODUCTION
Earthworms are metamerically segmented Oligochaeta belongs to the phylum Annelida. They are negatively phototactic, soft bodied, true coelomates, saprophytic creatures of agro-ecosystem and major macro fauna of soil biota. Earthworms have also been living with the aid of their defensive mechanism since long time during the course of evolution and always face the invasion of microorganisms in their environment.[3] Earthworm also prevails coelomocytes located in the coelomic fluid is responsible for both innate cellular immune functions such as phagocytosis and encapsulation against pathogenic microorganisms[2] and humoral components includes lectin, pre-forming proteins, phenoloxidases and proteases nullifies antigenic material by agglutination and cytotoxicity etc.[6]

The coelomic cavity is filled with fluid containing free wandering coelomocytes derived from mesenchymal lining. The transport of coelomic fluid between neighbouring segments is ensured by channels comprised of sphincters within the septa. Each segment of the coelomic cavity is opened to the outer environment by paired nephridia and by one dorsal pore through which soluble metabolites and corpuscular materials respectively can be expelled out.[1,10]

The coelomic fluid is generally secreted by the earthworms for maintaining moisture to help their physiological activities such as respiration and burrowing activities. It consists of watery matrix, the plasma and a large number of coelomocytes. These coelomocytes play a very important role in building innate immunity of earthworms, are differentiated into four different types of immune cells such as amoebocytes, mucocytes, circular cells and chloragogen cells, which have different shape, size and have wide variety of functions.

A fungus is any member of the group of eukaryotic organisms that includes microorganisms such as yeasts and molds. These organisms are classified as a kingdom, Fungi, which is separate from the other eukaryotic life kingdoms of plants and animals. *Aspergillus* species are highly aerobic and are found in almost all oxygen-rich environments, where they commonly grow as molds on the surface of a substrate, as a result of the high oxygen tension. Commonly, fungi grow on carbon-rich substrates like monosaccharides (such as glucose) and

polysaccharides (such as amylose). *Aspergillus* species are common contaminants of starchy foods (such as bread and potatoes) and grow in or on many plants and trees. Literature revealed that many aspects with respect to ecology, physiology, behaviour of earthworms have been studied much but very little has been known regarding composition of coelomic fluid, its importance and usage with respect to medicinal value.[8]

OBJECTIVE

The main objective of the study is to find out the antifungal potentialities of coelomic fluid of selected earthworms against eight pathogenic *Aspergillus Sps* using well diffusion methods.

MATERIALS AND METHODS

Collection of Earthworm

Hand sorting method was used for collecting earthworms. This method is widely used for sampling earthworms in India (Edward and Lofty, 1977; Reynold, 1977). The quadrate is provided on 20*20*30cm2 and are gently broken and the worms are hand sorted (5). The collected earthworms were identified with the help of standard manual and experts. The study mainly focused on four species of earthworms both exotic and native such as *Pontoscolex corethrurus* (Muller, 1856), *Megascolex konkanensis* (Fedarb, 1898) and *Drawida ghatensis* (Michaelsen, 1910).

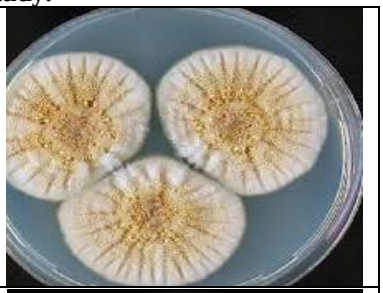
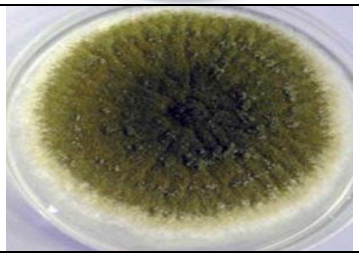
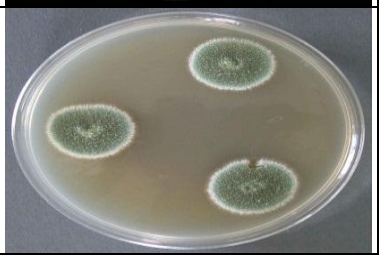
Pontoscolex corethrurus ***Megascolex konkanensis*** ***Drawida ghatensis***

Figure 1: Earthworms selected for the study.

Micro-Organisms for Antifungal Studies

For the antifungal screening, eight species of aspergillus isolates were selected. The fungal strains were obtained from Tropical Institute of Ecological Sciences, Kottayam. *Aspergillus niger*, *Aspergillus flavus Aspergillus steyni*, *Aspergillus nidulans*, *Aspergillus terreus*, *Aspergillus penicillioides*, *Aspergillus fumigates* and *Aspergillus ochraceus* fungi were used as test organisms. The fungal strains were maintained on Sabouraud Dextrose Agar slant at 4°C. The fresh fungi were grown at 28°C for 48 hrs.

Table 1: The fungal isolates selected for the study.

A.niger		***A.steyni***	
A.fumigates		***A.nidulans***	
A.ochraceus		***A.terreus***	
A.flavus		***A.penicillioides***	

Coelomic Fluid Collection Method

The selected local earthworms were washed in distilled water and they were placed on ordinary wet filter paper in plastic tough which is covered by alluminium foil with fine pin holes. After 48 hrs, the gut was cleared of organic matter as they feed on filter paper. Coelomic fluid was collected by placing the earthworms in petri plates held in a slanting position in the palm. Their body surface was rubbed with wet finger and later with ice cubes taken in a beaker. The coelomic fluid released due to cold shock and gets collected at the lower side of the petriplate. The released fluid from their body was collected by pasteur pipette.

Figure 2: Coelomic fluid.

Antifungal Potentialities Using Well Diffusion Method

Preparation of Inoculums

Suspension of fungus was prepared as per Mac –ferland Nephlometer standard. A 24hr old culture was used for the preparation of fungus suspension. A suspension of fungus was made in distilled water and the turbidity was adjusted such that it contained approximately 1.5*10 cells / ml. The inoculum was prepared by mixing dextrose, peptose and distilled water.

Earthworm Coelomic Fluid

The young cultures of selected pathogens were prepared in broth and lawn culture of eight *Aspergillus sps* were made by swabbing young culture in 5-6 days old in potato dextrose agar and Sabourauds dextrose agar and waited for 15 minutes to absorb the culture to the medium. Agar wells (3mm) in diameter were punched in the plates using a sterile gel puncture. 30µL of a five day old culture of all the selected fungal strains in appropriate broth and Coelomic fluid were pipetted into the well and plates and incubated for 4-5 days at room temperature. Zone of inhibition around the wells were recorded in mm.

STATISTICAL ANALYSIS

Statistical analysis was carried out using SPSS software for plotting the graph based on the zone of inhibition.

RESULTS AND DISCUSSION

The coelomic fluid of selected earthworms *Pontoscolex corethrurus, Megascolex konkanensis* and *Drawida ghatensis* was collected by cold shock method were tested for antifungal activity against eight pathogenic fungal isolates using well diffusion method. The antifungal property of coelomic fluid against selected eight strains was found that *M. konkanensis* and *D.ghatensis* shows high zone of inhibition against *A.terreus* and *A.steyni*.

The *P.corethrurus* shown that high antifungal activity against *A.steyni* (18mm), *A.niger* (14mm), *A.terreus* (9mm) and *A.fumigates* (8mm) simultaneously. The *M. konkanensis* shown high antifungal activity against *A.steyni* (20mm), follows *A.niger* (18mm), *A.flavus* (16mm) and *A.terreus* (16mm). The *D.ghatensis* shown high antifungal activity against *A.terreus* (20mm), *A.flavus* (11mm) and *A.penicillioides* (8mm). The *M. konkanensis* shows more antifungal activity against different fungus selected for the study. The antifungal activity was very less in *D.ghatensis*. The zone of inhibition is absent against *A.ochraceus* and *A.nidulans* in both the earthworms. The *A.terreus* shows antifungal activity against all the three earthworms. The antifungal activity of *A.niger, A.flavus* and *A.steyni* shows resistance against earthworms.

From the above experiment it is indicated that the coelomic fluid of selected earthworms have antifungal properties against *Aspergillus sps* used in this study. While coelomic fluid of *P. corethrurus, M.konkanensis* and *D.ghatensis* was uneffective against *A.nidulans* and *A.ochraceus*. Thus it indicates that all worms producing their coelomic fluid are not effective against all fungus. The coelomic fluid contains several bioactive compounds such as proteins, exhibits a variety of biological functions such as antibacterial, anti-cancer, haemolytic, cytotoxic, hemagglutinating and proteolytic activities etc.

Table 2: Zone of inhibition of antifungal activity of coelomic fluid of local earthworms.

Fungal isolates	*P. corethrurus(mm)*	*M. konkanensis(mm)*	*D. ghatensis(mm)*
A.niger	14	18	0
A.fumigates	8	0	0
A.ochraceus	0	0	0
A.flavus	0	16	11
A.steyni	18	20	0
A.nidulans	0	0	0
A.terreus	9	16	20
A.penicillioides	0	0	8

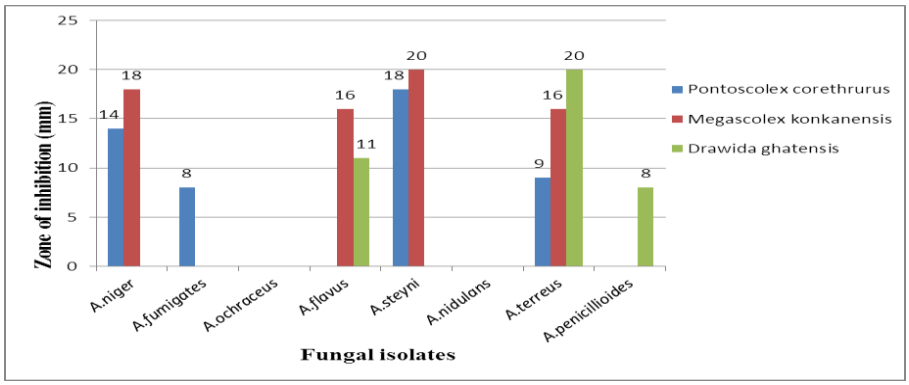


Figure 3: Antifungal activity of earthworms against selected fungal strains.

CONCLUSION

From the present work, conclude that the coelomic fluid of selected earthworms shows anti fungal properties against *A.niger*, *A.fumigates*, *A.flavus*, *A.steyni*, *A.terreus* and *A.penicillioides.* The antifungal activity is absent in *A.ochraceus* and *A.nidulans* in both the earthworms. The *M.konkanensis* shows high resistance against almost all the species of fungal isolates. So this work suggest some of the coelomic fluid components might be useful for pharmaceutical applications and have high medicinal value.

ACKNOWLEDGEMENT

We would like to thank M.G.University, Kottayam providing laboratory facility to carry out work and manuscript preparation.

ABBREVATIONS

P.corethrurus - *Pontoscolex corethrurus,*
M.konkanensis - *Megascolex konkenensis*
D.ghatensis - *Drawida ghatensis*
A.niger -*Aspergillus niger*
A.fumigates - *Aspergillus fumigates*
A.ochraceus - *Aspergillus ochraceus*
A.flavus - *Aspergillus flavus*
A.steyni - *Aspergillus steyni*
A.nidulans - *Aspergillus nidulans*
A.terreus - *Aspergillus terreus*
A.penicillioides - *Aspergillus penicillioides*

REFERENCES

1. Bilej M, De Baetselier P, Beschin A. Anti-microbial defence of the earthworms. *Folia Microbiol.*, 2000; 45: 283-300.
2. Cooper, E., Balamurugan, M., Huang, C.Y., Tsao, C.R., Heredia, J., and Tommaseo-ponzetta, M., et al. 2012. Earthworms dilong: Ancient, inexpensive, noncontroversial models my help clarify approaches to integrated medicine emphasizing neuroimmuno systems. Evid. Based complement. Alternative medicine, 20(12): 152-164.
3. Engelmann P, Kiss J, Csongei V, Cooper EL, Nemeth P. Earthworm leukocytes kill HeLa, HEp-2, PC-12 and PA-317 cells in vitro. *Journal of Biochemical and Biophysical Methods.*, 2004; 61: 215-227.
4. Ismail, S.A.Vermitechnology The biology of Earthworms, Hyderabad. Orient Longman. 1997.
5. Julka, J.M. Earthworm Resource and Vermiculture. Zoological Survey of India earthworms. *Journal of microbiology residence technology.*, 1993; 47(44): 237-253.
6. Lange S, Kauschke E, Mohrıng W, Cooper EL. Biochemical characteristics of eiseniapore, a pore-forming protein in the coelomic fluid of earthworms. *European Journal of Biochemistry*, 1999; 262: 547-556.
7. Lavelle, P. Earthworm activities and the soil system. Biology of fertile soil fertile, 1988; 6: 237-251.
8. Reynolds, J.W., and Reynolds, W.M. Earthworms in medicine. Am. *Journal of Nursing*, 1972; 120(72): 1273-1283.
9. Waksman, S.A., Bugie, E. and Schatz, A. 1944. Isolation of antibiotic substances from soil microorganisms, with special reference to streptothricin and streptomycin. in: Proc Staff Meet Mayo Clin. pp: 6: 537–548.
10. Reynolds, Reynolds. Earthworms in medicine. The *Vermiculture Journal*, 1979; 2: 6-7.
11. Weidong Pan, Sianghui Liu, Feng Ge, Tao Zheng. Reconfirmation of anti-microbial activity in the coelomic fluid of the earthworm, *Eisenia fetida andrei* by colorimetric assay. *J Biosci*., 2003; 28(6): 723-731.
12. Joshi B. H. and Patel A. A., Screening of multi-trait plant growth and health promoting actinomycetes from rhizosphere soil, *J.Environ. Res. Develop.*, 2015; 10(2): 227-238.
13. Cotuk and Dales., The effect of coelomic fluid of the earthworm *E. foetida Sav.* On certain bacteria and the role of the coelomocytes in internal defence *Comparative Biochemistry and Physiology*, 1984; 78(2): 271-275.
14. Reynolds, J.W. The earthworms (Lumbricidae and Sparganophilidae) of Ontario. Life Sci. Misc. Publ., Roy. Ont. Mus., 1977; 141 pp.
15. Edwards, C. A. and Lofty, J. R. 1972. Biology of earthworms. Chapman & Hall, London. 283 pp.
16. Reynolds and Reynolds 1972. Earthworms in medicine. *Amer. J. Nursing*, 72: 1273.
17. Popovic, M., Lj. Tiska-Rudman, T. Hrzenjak Tissue extracts of earthworm Eisenia foetida (G-90) as a blood anticoagulant and fibrinolytic. *Vet. Arhiv*., 1996; 66: 161-167.
18. Lehrer R I, Rosenman M, Harwig SSL, Jackson R and Eisenhauer P. Ultrasensitive assays for endogenous antimicrobial polypeptides; *J. Immunol. Methods*., 1991; 137: 167–173.