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Research Paper

ANTIBACTERIAL AND ANTIFUNGAL POTENTIALITIES OF LOCAL EARTHWORMS AND CASTS

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Abstract

A study was carried out on the antibacterial and antifungal properties of four earthworms (*Pontoscolex corethrurus, Megascolex konkanensis, Drawida ghatensis and Metaphire houletti)* and their casts against selected pathogens like *Vibrio cholera, Vibrio parahaemoliticus, Bacillus subtilis, Staphylococcus aureus, Salmonella typhi, Escherichia coli, Candida albicans and Aspergilus niger.* Analysis was also done on the antibacterial and antifungal potentialities of actinomycetes present in earthworm casts. The actinomycetes samples were isolated from earthworm casts and six strains were used for the study. The result revealed the resistance of earthworm powder against selected pathogens like *Vibrio cholera, Vibrio parahaemoliticus and Salmonella typhi.* Antibacterial properties were exhibited by both exotic and native species. The casts did not show any resistance against bacterial strains. The earthworm powders showed antifungal resistance against both *Candida albicans and Aspergilus niger*. The antifungal potentialities were seen confined to exotic species. The *Pontoscolex* casts also showed resistance against the fungus *Candida albicans*. The isolated two actinomycetes strains showed resistance against bacterial strains and one exhibited resistance against fungal strains.

Key words: Bacteria, Fungi, Actinomycetes.

INTRODUCTION

As early as 300BC, since the time of Aristotle, earthworms have been recognized as potential organisms in creating fertile soils and referred to them as 'intestines of earth'. These oligochaete annelid worms represent a key component in the biological strategies of nutrient cycling in soils and the structure of their communities gives a clear indication of the type of soil system they inhabit [4] Earthworm activity aerates and mixed the soil, and is conducive to mineralization of nutrients.

Studies have shown that worm castings added to the soil at low rates may result in improved disease resistance because plants are found to be healthier and stronger when grown in worm castings, as worm castings provide beneficial microbes that compete with disease causing organisms [7]

The use of earthworms in a medicine was documented at very early date, in 1340 AD[6].Moreover, in the folk medicine (North American Indians, doctors in East Asia) the earthworms have been used for the treatment of various diseases [1]. Traditional Chinese medicine has also widely used the earthworms for a long time. The research on the pharmaceutical effects of earthworms has been initiated along with the development of biochemical technologies. Many bioactive molecules which can be considered as potential drug have been detected in the earthworms. These molecules exhibited different activities, such as fibrinolytic, anticoagulative, anticancer, antimicrobial and thus may be exploited for the treatment of a variety of diseases[1].

Actinomycetes: Actinomycetes are a group of gram positive bacteria. Some of these bacteria are pathogenic for human and animals and produce unique bioactive compound which include antibiotics, enzymes and vitamins. The actinobacteria might be useful as antibiotics against human disease [8].

OBJECTIVES

To determine the antibacterial and antifungal effect of earthworm powder of selected local earthworm species (endogeic and epigeic). To evaluate the antibacterial and antifungal potentialities of actinomycetes present in the earthworm casts. To ascertain the antibacterial and antifungal properties of earthworm casts of selected earthworms.

MATERIALS AND METHODS

The study mainly focused on four species of earthworms both exotic and native such as *Pontoscolex corethrurus* (Muller, 1856), *Megascolex konkanensis* (Fedarb,1898), *Drawida ghatensis* (Michaelsen,1910), and *Metaphire houlleti* (Perrier ,1872).

A bed was prepared for culturing earthworms and for collecting casts. The four species were kept in separate vermibeds prepared following the method given by Ismail (1997). Soil and litter were used for preparing vermibed. The worms were released gently to the surface of vermibed. Plastic net was used for covering the tray to avoid predators. Vermibed was maintained in the laboratory condition.

Earthworm casts were collected twice in a month using binocular lens. The earthworm castings were carefully taken with spatula and kept in sterile polythene bags. The collected castings were used for the detection of antibacterial and antifungal potentialities and for the isolation of actinomycetes.

Actinomycetes present in the earthworm casts were isolated for analyzing the antibacterial and antifungal potentialities by selective method.1gm of cast was dissolved in 9ml sterile distilled water and serially diluted and spread on the Kustors agar plate. After 4 days the actinomycetes were separated from the plate and streaked on another plate for the full growth of individual actinomycetes.

The isolated actinomycetes were inoculated in the prepared broth and kept in a room temperature for 5-6 days. After 6 days this was pipetted into the well.

Earthworms were powdered and pasted in four ways: (1)Earthworms were soaked in distilled water for 6hrs, 12hrs and 24hrs; allow the soil in the digestive track to be excreted. They were then placed in a petridish and placed in an incubator for 6hrs, 12hrs and 24hrs at a temperature of 35°C, 55°C and 60°C. Then the earthworms were taken and powdered using motor and pistil.(2)In the second method earthworms were

soaked in distilled water for 30mins, 3hrs, 6hrs,12hrs and 24hrs allowed the soil in the digestive track to be excreted. They were washed thoroughly and cleaned using brushes to remove the external fluids. Then the worms were taken and made to a paste using motor and pestle. (3)The earthworms were directly prepared to a paste using distilled water.(4)The worms were placed in a Polythene bag and placed in a freezer for 6 hrs and the paste was prepared using distilled water.

Antibacterial potentialities using well diffusion method
Earthworm powder and casts
The young culture of selected pathogens *Vibrio cholera (A), Vibrio parahaemoliticus(B), Bacillus subtilis(C), Staphylococcus aureus(D), Salmonella typhi(E)* and *E.coli(F*) were prepared in nutrient broth(1.3gm in 100ml and inoculated in 10ml) and lawn culture of different pathogens were prepared by swabbing young culture (16-18hrs) in Muller Hinton 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 earthworm solution and their casts of all the species were pipetted into the well and plates were incubated for 24hrs in an incubator. Zone of inhibition around the wells were recorded in mm.

Actinomycetes: The young culture of selected pathogens (*Vibrio cholera, Vibrio parahaemoliticus, Bacillus subtilis, Staphylococcus aureus ,Salmonella typhi* and *E.coli*) were prepared in Kustors broth and lawn culture of different pathogens were prepared by swabbing young culture (16-18hrs) in glycerol yeast agar (Kustors 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 four day old culture of all the isolated actinomycetes strains(6 strains) in appropriate broth was pipetted into the well and plates were incubated for 24hrs at room temperature. Zone of inhibition around the wells were recorded in mm.

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 powder, casts and actinomycetes
The young cultures of selected pathogens (*Candida albicans and Aspergilus niger*) were prepared in broth and lawn culture of different pathogens 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 actinomycetes strains in appropriate broth 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.

RESULTS AND DISCUSSION

The dried earthworm powders and casts incubated at 35°C, 55°C and 60°C did not demonstrate any resistance to the selected six bacteria. The earthworm paste made from earthworms put in distilled water for 1hrs, 3hrs, 6hrs, 12hrs and 24hrs failed to show resistance to the selected six bacteria.

The earthworms put in distilled water for 30min and dissolved in distilled water exhibited marked resistance to the selected pathogens. It was found that only *M.konkanensis* showed resistance to the *S.typhi and V.parahaemoliticus*. The earthworm powder of *M. konkanensis* showed maximum diameter of zone of inhibition against *S.typhi* (16mm) followed by *V. parahaemoliticus* (7mm).(Fig:1)

B E

Fig:1 Sensitivity of *Megascolex konkanensis* against *Vibrio parahaemoliticus* (B) *and Salmonella typhi* (E) (shown as diameter of zone of inhibition)

The worm powder of *M.konkanensis* showed maximum diameter of zone of inhibition against *S.typhi* (17mm) followed by *V.parahaemoliticus* (14mm) and worm powder of *M.houletti* exhibited maximum diameter of zone of inhibition against *S.typhi* (24mm) followed by *V. parahaemoliticus* (13mm). The remaining earthworm powder and earthworm casts did not exhibit any resistance (Fig:2).

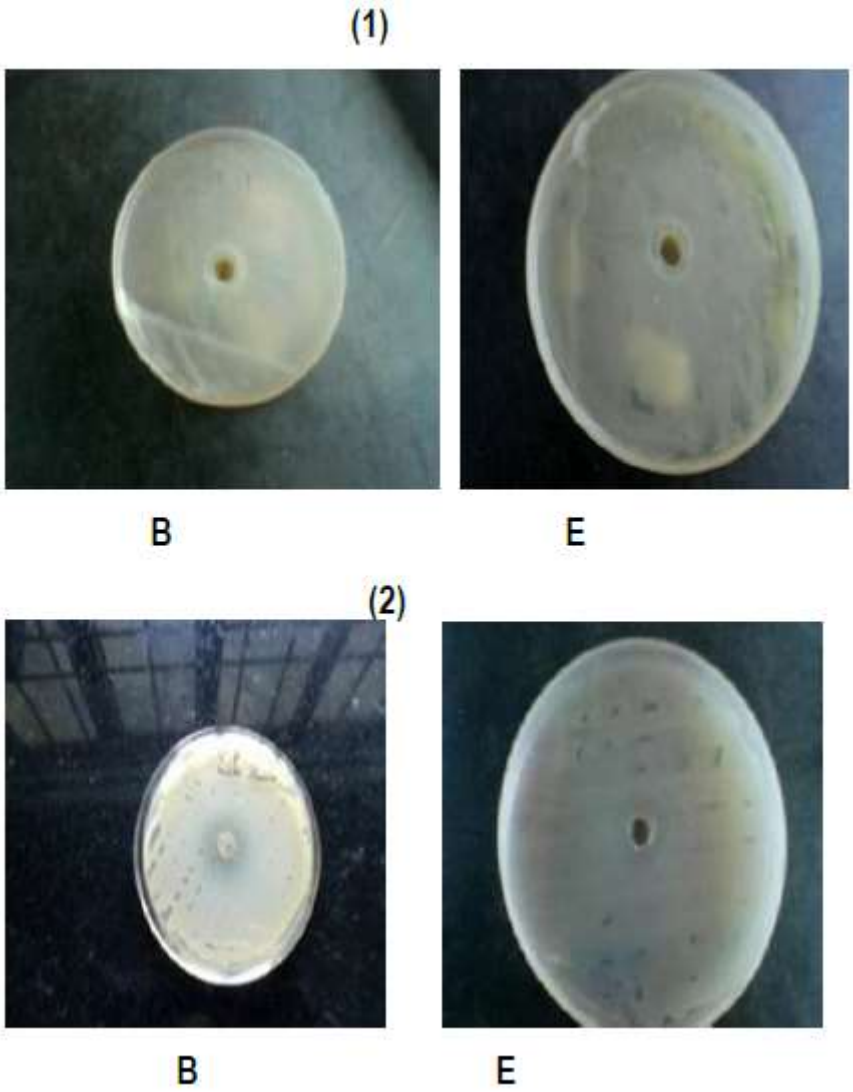


Fig:2 (1).Sensitivity of *Megascolex konkanensis* against *Vibrio parahaemoliticus (B) and Salmonella typhi (E)* and (2).Sensitivity of *Metaphire houletti* against *Vibrio parahaemoliticus(B) and Salmonella typhi(E)*

In the case of earthworm powder obtained from freezed earthworms, resistance pattern was found differential. *M.konkanensis* showed maximum diameter of zone of inhibition against *S.typhi* (16mm) followed by *V. parahaemoliticus* (12mm). Earthworm powder of *M.houletti* showed larger diameter of zone of inhibition against *S.typhi* (15mm) and lesser against *V.parahaemoliticus* (14mm).

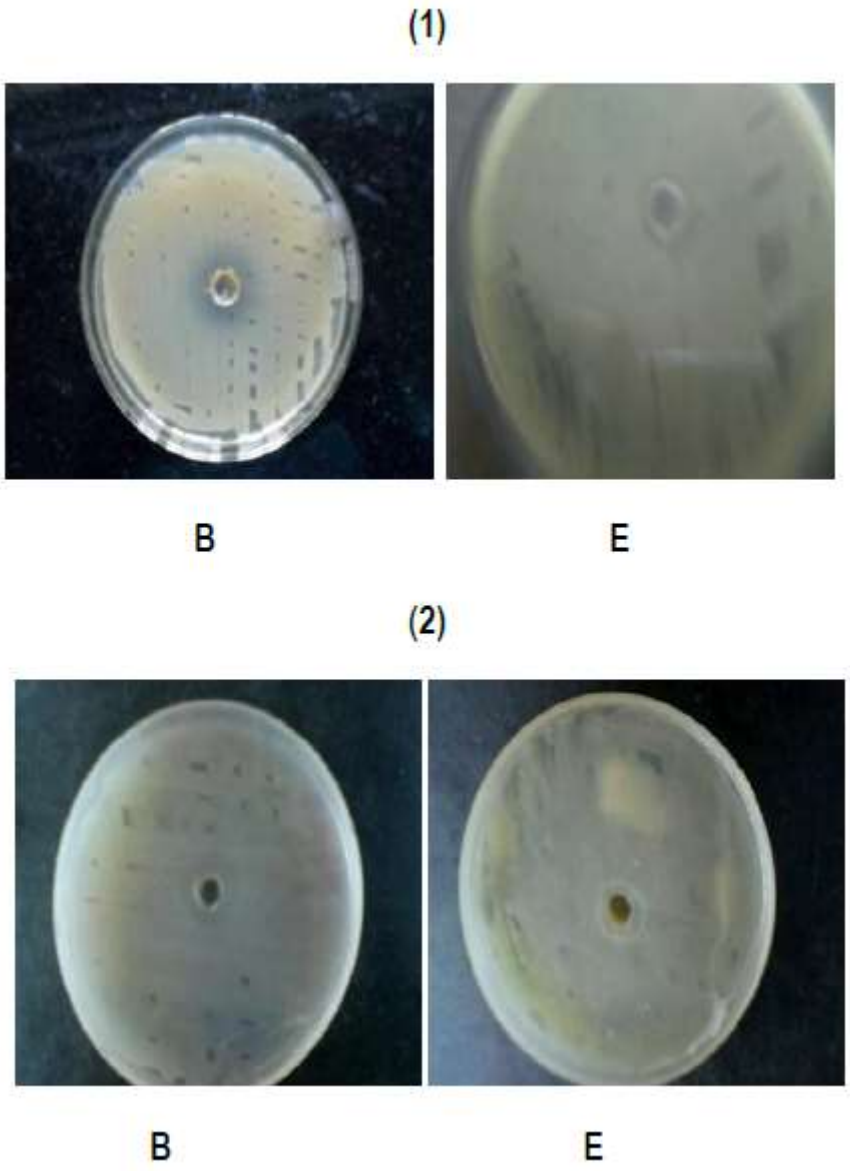


Fig.3 (1)Sensitivity of freezed *M.konkanensis* against *Vibrio parahaemoliticus (B) and Salmonella typhi (E)* and (2)Sensitivity of *Metaphire houletti* against *Vibrio parahaemoliticus (B) and Salmonella typhi (E).*

ANTIBACTERIAL ACTIVITY BY WELL DIFFUSION METHOD IN ACTINOMYCETES

Six actinomycetes strains (isolates) were used for the detection of antibacterial activity and the two strains showed resistance against the selected six pathogens. Sample B (*Pediococcus acidilactici*) and E (*Bacillus acidicola*) are identified samples.

The sample B *Pediococcus acidilactici* possessed maximum diameter of zone of inhibition against *S.aureus* (24mm) followed by *B.subtilis* (12.4mm). Sample E *Bacillus acidicola* possessed maximum diameter of zone of inhibition against *S.typhi* (17mm). The remaining strains did not show any resistance against other pathogens.

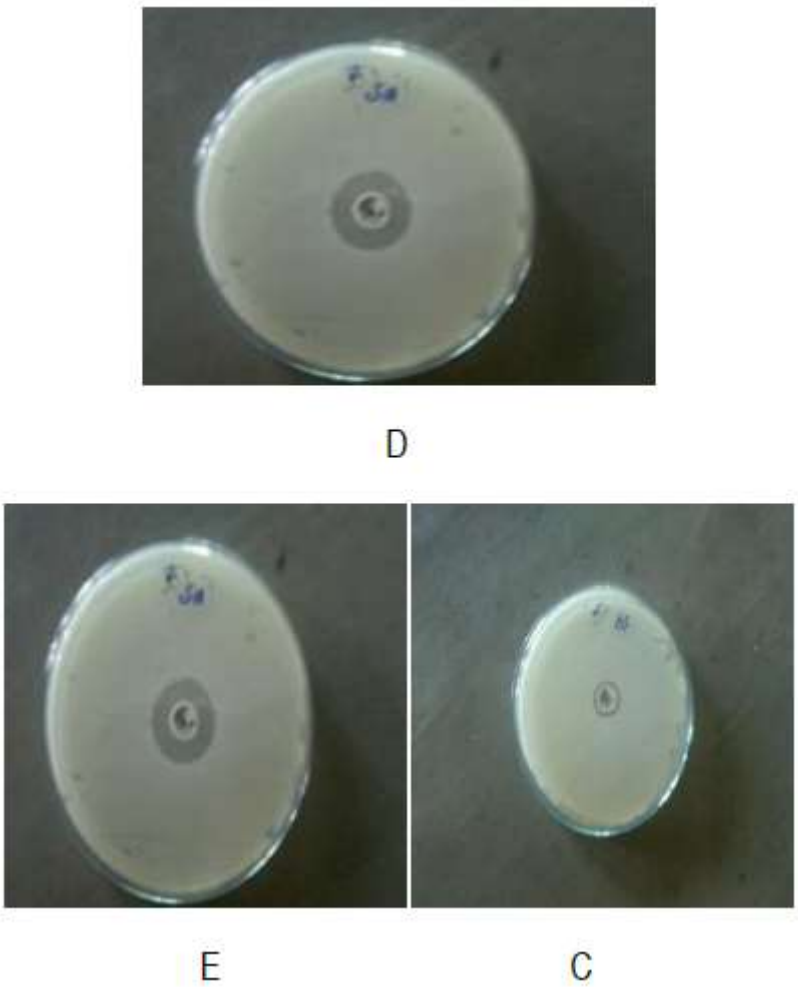


Fig.4 Sensitivity of Actinomycetes *Pediococcus acidilactici* against *Staphylococcus aureus (D) Bacillus acidicola against Salmonella typhi (E), Pediococcus acidilactici against Bacillus subtilis (C).*

DETERMINATION OF ANTIFUNGAL ACTIVITY BY WELL DIFFUSION METHOD IN EARTHWORM POWDER AND CASTS

The antifungal properties of earthworm powder and cast showed sensitive results in two worms (*P.corethrurus and M.houletti*) against fungus *Candida albicans* (G) and *Aspergilus niger (H).*

The earthworms dipped in distilled water for 30min showed resistance to the selected fungi. The *C. albicans* was prepared in sabourauds dextrose agar and *A. niger* spread in potato dextrose agar. The *M.houletti* was sensitive to *C.albicans* (zone of inhibition at 15mm diameter) and followed by *P.corethrurus* (14mm) and *M.houletti* sensitive to *A.niger* (21mm).

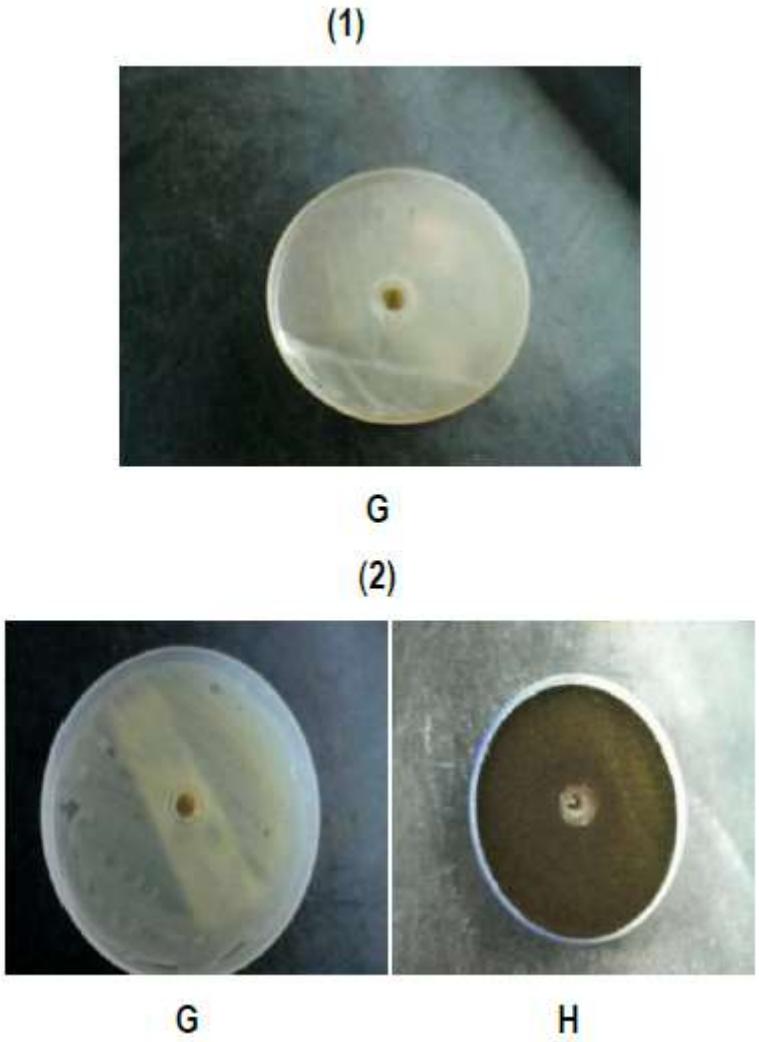


Fig.5 (1) Sensitivity of *Pontoscolex corethrurus* against *Candida albicans(G)* (2) Sensitivity of *Metaphire houletti against Candida albicans (G)* and Aspergilus niger (H).

The selected earthworms are directly used for the experiment. The *C.albicans* was prepared in Sabourauds dextrose agar and *A.niger* spread in Potato dextrose agar. The earthworm *M.houletti* against *A.niger* showed resistance (diameter 20mm). *P.corethrurus* has shown sensitivity against *C.albicans* and the zone of inhibition noted was 13.5mm. From the analysis it was found that *M.houletti* showed more resistance to *A.niger* compared to *P.corethrurus* sensitivity against *C.albicans*.

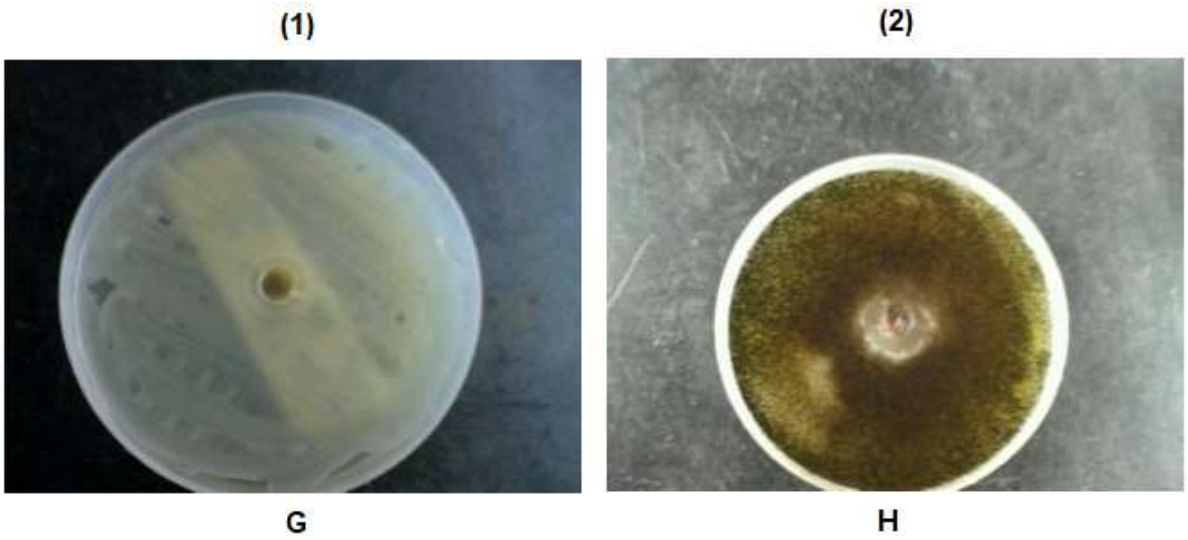


Fig.6 (1)Sensitivity of *P.corethrurus* against *Candida albicans* (G) and (2)Sensitivity of *Metaphire houletti* against *Aspergilus niger (H)*

The earthworm casts were analyzed against both fungi and found *M.houletti* casts showed resistance to *C.albicans* and *A.niger*. The *M.houletti* showed larger diameter of zone of inhibition against *C.albicans* (14mm) than against *A.niger* (13.2).

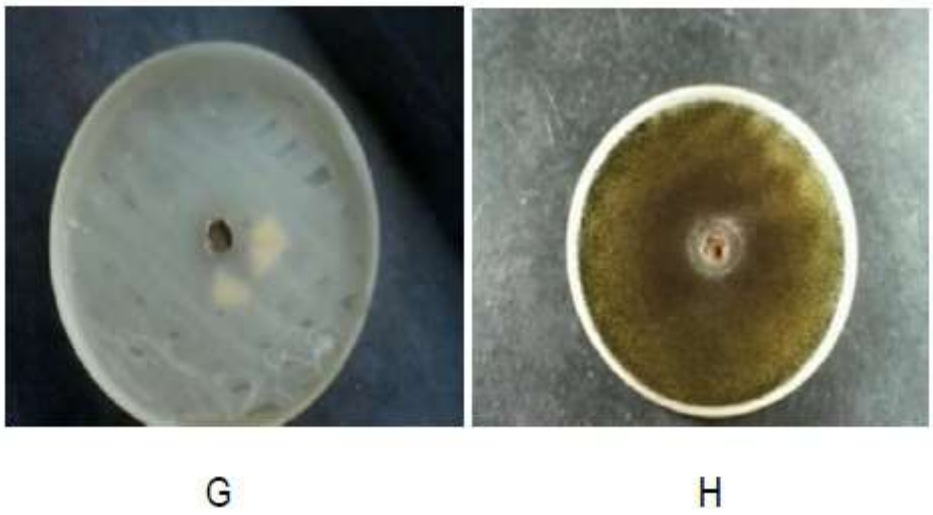
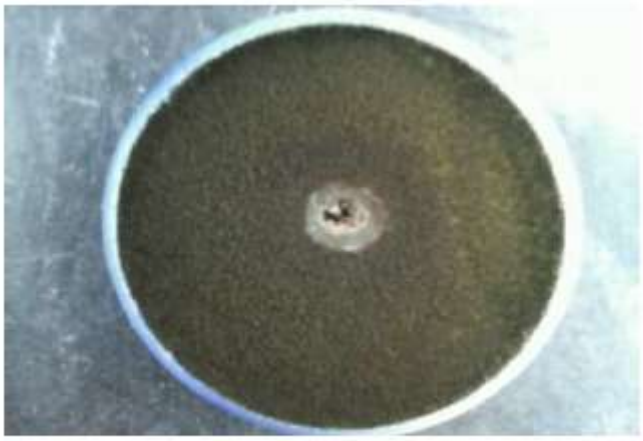


Fig.7 Sensitivity of M*etaphire houletti* cast against *Candida albicans* (G) and *Aspergilus niger (*H*)*

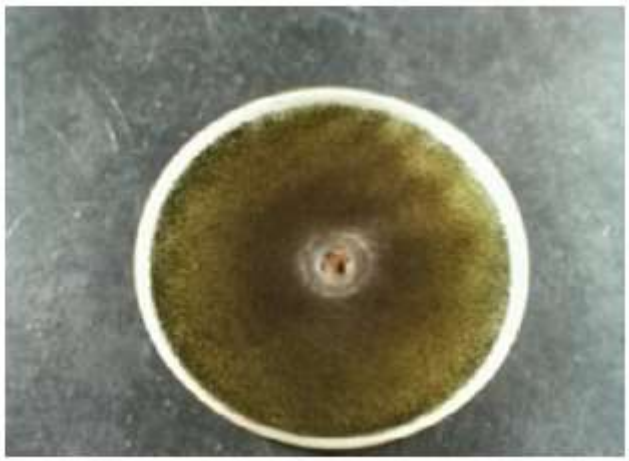
In the case of earthworm powder obtained from freezed earthworm *M.houletti* showed resistance against *A.niger* and the zone of inhibition is 22mm.



H

Fig.8 Sensitivity of *Metaphire houletti* cast against *Aspergilus niger (H)*

DETERMINATION OF ANTIFUNGAL ACTIVITY BY WELL DIFFUSION METHOD IN ACTINOMYCETES



H

Fig.9 Sensitivity of actinomycetes *Bacillus acidicola* against *Aspergilus niger* (H)

The Sample E *B.acidicola* possessed maximum diameter of zone of inhibition against *A. niger* (17m) and *C.albicans* did not show any resistance. The remaining strains did not show any activity against fungus.

SUMMARY AND CONCLUSION

The dried earthworm powder did not exhibit the antibacterial properties and hence showed nil resistance against all six bacteria when the earthworm was dried at 60°C. The dried earthworm powders and casts incubated at 60°C also failed to show resistance to the selected six bacteria. The dried earthworm powders and casts incubated at 35°C and at 55°C were also not resistance to the selected six bacteria. The earthworms put in distilled water for 1hrs, 3hrs, 6hrs, 12hrs and 24hrs in distilled water were failed to show resistance to the selected six bacteria.

Of the earthworms put in distilled water for 30min *M.konkanensis* showed resistance to the S. *typhi* and *V.parahaemoliticus*. The remaining species did not show any resistance to the pathogens. When the raw earthworm paste was used *M.konkanensis* and

M.houletti were found sensitive to *S.typhi and V. parahaemoliticus* and casts did not show any resistance. Freezed earthworms also showed resistance in *M.konkanensis* and *M.houletti* against *S.typhi* and *V. parahaemoliticus.*

Six actinomycetes strains (isolates) were used for the detection of antibacterial activity and noted that *P.acidilactici* showed resistance against *S.aureus* and *B.subtilis* and *B.acidicola* exhibited resistance against *S.typhi*. It was found that the earthworm powder of two earthworms and casts (*P.corethrurus and M.houletti*) showed resistance to two fungi (*Candida albicans and Aspergillus niger)*.

The earthworms put in distilled water for 3hrs did not show any resistance to the selected fungus. The earthworms put in distilled water for 30min *M.houletti* showed resistance against *C.albicans* as well as *A.niger* and *P.corethrurus* showed resistance against *C.albicans*. When the selected earthworms and their casts were directly used *M.houletti* showed resistance against *A.niger* and *P.corethrurus* against *C.albicans*. The casts of *M.houletti* showed resistance against *C.albicans* and *A.niger*. The freezed earthworms (*M.houletti*) showed resistance against *A.niger.*

Of the six actinomycetes strains (isolates) used for the detection of antifungal activity, *B. acidicola* exhibited resistance against *A.niger*.

The study revealed that *P.corethrurus* and *M.houletti* are sensitive to fungus, and *M.konkanensis* and *M.houletti* sensitive to bacteria. The *M.houletti* cast showed resistance to fungus. *D.ghatensis* did not show sensitivity to the bacteria and fungi. From the study it was found that exotic species are sensitive to both bacteria and fungi and native species are sensitive to bacteria only.

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ABBREVIATIONS

P.corethrurus - Pontoscolex corethrurus,
M.konkanensis - Megascolex konkenensis ,
D.ghatensis - Drawida ghatensis
M.houletti -Metaphire houletti
V.cholera - Vibrio cholera
V.parahaemoliticus - Vibrio parahaemoliticus
B.subtilis - Bacillus subtilis
S.aures - Staphylococcus aureus
S.typhi -Salmonella typhi
E.coli -Escherichia coli
C.albicans -Candida albicans
A.niger -Aspergilus niger

ETHICAL APPROVAL:

This article does not contain any studies with human participants or animals performed by any of the authors.

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