Isolation, Identification and Biological Activities of Fungal Species from Ant House of Bilaspur District Chhattisgarh, India

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Abstract-*Ants are arthropods that can constantly be found in urban environments, thus becoming synanthropic animals, which can facilitate the transmission of infectious agents to man and domestic animals, for example the fungus that causes mycoses. The aim of this study is to evaluate fungi carried by ants in home environments. The samples passed through the steeping process and then were immersed in broth and incubated at 37° C for 24 hours. After the growth period, the material was inoculated on Sabouraud agar plates and incubated. The fungi isolates were transferred to slides by imprinting technique, stained with lactophenol cotton blue and identified microscopy, observing their morphological characteristics. Ant-fungus symbiosis relationship is important characteristics. they play an important role in our survival as well. They are an important component of our food chain and suppose if they perish human species may not be able to generate resources and clear debris from earth and may perish. It is really unfortunate in present scenario that much emphasis has been laid on those natural resources which are of direct importance to humans and to look for alternative resources in the wake of exhaustion of natural resources. And human intellect authoritatively terms it "The Development"*

Keywords- Ant house, Fungus, Sabouraud agar medium, Bilaspur, Imprinting technique

I. INTRODUCTION

There are more species of ants in a square kilometer of Brazilian forest than all the species of primates in the world, more workers in a single colony of driver ants than all the lions and elephants in Africa" (Wilson, 1975).

"An ant-hill is a pile of earth, sand, or clay or a composite of these and other materials that build up at the entrances of the subterranean dwellings of ant colonies". Each colony consists of series of underground chambers, connected to each other and surface of earth by small tunnels. There are rooms for nurseries, for food storage and for mating. The colony is built and maintained by a large group of worker ants. During excavating process of colony, these ants carry tiny bits of dirt in their mandibles and deposit them near the exit of the colony to form an ant-hill.(Stone, 1985). Different ant species create nests varying in structure and size due to the difference in their feeding strategies. From pedological point of view, ants can build two types of nests, that is, type I nest and type II nest (Paton et al., 1995). The ant-hill protects the nest opening from water inflow during rain and also enhances wind driven nest ventilation. The colonies are closed and reopened by ants with seasonal periodicity, which in turn leads to respective disappearance and reappearance of ant-hills. (Cosarinsky and Roces, 2007).

Ants are social insects of the Family Formicidae, belonging to Order Hymenoptera of the Class Insecta. They are found in all the continents except Antarctica and few remote islands like Greenland and Hawaiian island. Ants vary in colour, mostly red or black, but few are green and grey also. Out of the estimated total of 22,000 species of ants, about 600 species have been found in India.(Fernandez, 2003) Besides ants, a number of other organisms like bacteria, fungi, actinomycetes, microarthopod, centipedes and millipedes are found in ant nests (Sleptzovaa and Reznikovab, 2006).

Ants thrive in most of the ecosystems and form 15-20% of the terrestrial animal biomass, which exceeds that of the vertebrates (Schultz, 2000).

Ants influence soil properties through the construction of their nests due to their burrowing habit and their ability to change physical characteristics, such as infiltration, water retaining capability, etc., of their hills/mounds. There is considerable information available on the effects of ants on soil conditions like bulk density, organic matter content and porosity within the nest area (Cammeraat et al., 2002; Dashtban et al., 2009).

According to Shakesby et al. (2003), water infiltration rate in ant-hill soil and that of surrounding area is accelerated by ants, which form large macropores [biopores] and mix organic matter with mineral soil during nest building.

According to Lobry de Bruyn and Conacher (1994), the cortex covering the ant-hills likely plays an important role

in absorbing the impact of the rain drops and in improving the water infiltration inside the ant-hills. This impact of ants on water infiltration and erosion could be more important in agricultural soils, where heavy machinery and herbicide use have reduced soil porosity and organic matter (Cerda A and Jurgensen, 2008).

Sharma and Sumbali (2013) studied the correlation of fungal diversity with soil pH of ant-hills and found that with the increase in the alkalinity of the ant-hill soil, there is corresponding increase in number of fungal species and their respective colonies.

According to Kristiansen and Amelung (2001), ant nests are associated with high levels of nutrients and organic matter. In addition, the amount of soil porosity in ant-hill soil is also significantly higher than the normal soil.

Moisture content was lower but soil pH was significantly higher in ant-hill soils. In addition to this, plant species associated with ant nests usually differ from species growing in adjacent area (Folgarait, 1998).

II. MATERIAL AND METHOD

Sample-Soil sample is collected from the ant house of the mangla chowk area, sipat road area and Gandhi chowk area.

Sabouraud agar medium-Sabouraud agar medium is a semi-synthetic medium is that in which the chemical constituents are partially known.

Fungus stain-The fungal propagules either are hyaline(colourless) or of different colours. The hyaline mycelia/spores/conidia, etc and their cytoplasm can be stained by using lactophenol and cotton blue. Cotton blue stains cytoplasm and result in light blue background. Lactophenolacts as cleaning agents, whereas phenol as fungicide. For routine work of fungal identification the commomly used stain is mounting fluid i.e. lactophenol plus cotton blue.

III. METHOD

(1) SAMPLE COLLECTION- Sample is collected from the ant house of the mangla chowk area, sipat road area and gandhi chowk area.

(2) SOIL TESTING- Soil testing is often performed by commercial labs that offer a variety of tests, targeting groups of compounds and minerals. The advantages associated with local lab is that they are familiar with the chemistry of the soil in the area where the sample was taken. This enables technicians to recommend the tests that are most likely to reveal useful information.

(3) SERIAL DILUTION-

1. 3 test tubes are taken ,transferring 9 ml distilled water in each test tube, plugging them properly, labelling 1-3 and autoclaving at 15lb/inch2 for 30 minutes.
2. Small amounts of the soil is collected from 3 desired fields.
3. 1g of soil of the 1 desired fields is weighted and it is transferred into test tube 1 containing 9ml sterilised water. It gives the dilusion 1:10(i.e. 10-1)
4. Test tube is shaked gently for 10 mintes by using magnetic stirrer to get homogeneous suspension.
5. 1ml suspension is transferred from 10-1 dilusion into test tube 2 containing 9ml sterilised water to get dilusion 10-2. Mix the suspension gently.
6. Similarly, serially 1ml suspension is transferred from 10-2 dilusion to test tube 3 containing 9ml water to get the final dilusion of 10-3. Mix the suspension gently.
7. Now, the soil sample 1 is ready for inoculation.
8. The steps is repeated with the soil sample of desired fields 2 and 3.

(4) MEDIA PREPARATION-

1. Accurately chemical ingredients of the sabourauds agar media is weighted and transferring them into a beaker containing 500ml distilled water. It is gently heated and shaked to dissolve the ingredients.
2. Finally this medium is transferred into a measuring cylinder of 1 litre capacity and more distilled water is added to make the volume to 1 litre.
3. pH of the broth is measured by using a pH paper and adjusted to 5.6 by using 1N HCL or NaOH drop-wise.
4. Medium is poured into one or more conical flasks, Cotton plug is putted, covered the plug with aluminium foil/paper and autoclaved at 121℃ for 20 minutes.
5. Medium was poured in sterile glass plates in a laminar flow hood.

(5) SPREAD PLATE METHOD –

1. 3 sabouraud agar plates are taken and label them 1,2 and 3 respectively.
2. Aseptically, plates are inoculated with 10-1,10-2 and 10-3 suspension.

3. The bent L-form glass rod is sterilised by putting it first in ethanol(95%) in a beaker, then on the flame of Bunsen burner and cooled the rod for 30 seconds.
4. L-form of glass tube is touched very gently on the surface of agar of plate 1 and moved it.
5. L-shaped glass rod is sterilised and followed the steps with plate 2 and 3.
6. All inoculation steps are repeated with soil sample 2 and 3.
7. All the plates are incubated at 25℃ for 2-3 days.

(6) POUR PLATE METHOD FOR PURE CULTURE-

1. After the growth of the fungus culture, small amount of fungus culture is taken by the inoculating loop.
2. This fungus culture is inoculated Sabouraud agar medium.
3. Inoculation steps are repeated with soil sample 2 and 3.
4. All the plates are incubated at 25℃ for 2-3 days in inverted position in an incubator.
5. Evaluate the plates for obtaining pure culture.

(7) STAING OF FUNGUS-

1. 1.Young fungal cultures(5-7 days old) growing on culture medium are procured .
2. A drop of mounting fluid is putted in the centre of a glass slide.
3. A portion of mycelia mat from fungal colony transferred into the drop of mounting fluid with the help of flamed and cooled needle.
4. With the help of two needles gently spreaded the fungal propagules so that the mycelia should be mixed with stain.
5. The process is repeated for all the fungal colonies.

IV. RESULT

SOIL TESTING- Table no 1 shown the result of the soil testing and comparative account between the selected ant house and normal soil.

Table 1- Result Of The Soil Testing And Difference Between Selected Ant House Soil And Normal Soil

No	Property	Ant house soil	Normal soil
1.	Soil porosity	Higher	Lower
2.	Melting point	Low	High
3.	Boiling point	Low	High
4.	Solubility in water	No(most)	Yes
5.	Solubility in non polar liquids	Yes	No
6.	Covalent	Ionic	
7.	Molecules	Ions	
8.	Types of bonding	Many	Few
9.	Reaction	Complex	Simple
10.	occurs	No	Yes
11.	between	Living/ Non living things	Non living things
12.	Atoms per molecules		
13.	Structure	High	Low
14.	Electrolyte	Usually	Non-combustible
15.	Source	combustible	
		High	Less
16.	Refractive index	Carbon, Hydrogen,	All the known elements.
17.	Combustibility	Oxygen,	
18.	Stability	Nitrogen,	
	Composition	Sulfur, Phosphorus.	Yes
		No	Non-volatile
	Making salts	High	High
	Volatile	volatile	
	Reaction rate	Slow	

Table no 2 shown the result of the soil testing of the ant house from selected places. sand soil is present in the mangla chowk area(Soil sample 1), Silt soil is present in the sipat chowk area(Soil sample 2) and Clay soil is present in the Gandhi chowk area(Soil sample 3).

Table 2- Result Of The Soil Testing Of Selected Ant House Soil

No	Area	Sample	Soil type
1	Mangla chowk area	Soil sample 1	Sand soil
2	Sipat road area	Soil sample 2	Silt soil
3	Gandhi chowk area	Soil sample 3	Clay soil

Table no 3 shown the properties of the ant house soil from the selected places

Table 3- Result Of The Soil Testing Of Selected Ant House Soil And Their Properties

Property/behavior	Sand	Silt	Clay
Water-holding capacity	Low	Medium to high	High
Aeration	Good	Medium	Poor
Drainage rate	High	Slow to medium	Very slow
Soil organic matter level	Low	Medium to high	High to medium
Decomposition of organic matter	Rapid	Medium	Slow
Warm-up in spring	Rapid	Moderate	Slow
Compactability	Low	Medium	High
Susceptibility to wind erosion	Moderate (High if fine sand)	High	Low
Susceptibility to water erosion	Low (unless fine sand)	High	Low if aggregated, otherwise high
Shrink/Swell Potential	Very Low	Low	Moderate to very high
Sealing of ponds, dams, and landfills	Poor	Poor	Good
Suitability for tillage after rain	Good	Medium	Poor
Pollutant leaching potential	High	Medium	Low (unless cracked)
Ability to store plant nutrients	Poor	Medium to High	High
Resistance to pH change	Low	Medium	High

Soil test is very important test in soil microbiology. After the soil testing of the ant house, we can concluded that different types of the soil is present in different places and their quality, texture and the presence of the microorganisms is also differ.

Table no 4-5 shown the growth of fungus on the Sabouraud agar medium according to the serial dilusion. The result provides good growth of the fungus on the Sabouraud agar plates.

TABLE 4- DETAILS OF THE FUNGAL GROWT

No	Area	Sample	Soil type	Growth	Colour
1	Mangla chowk	Soil sample 1	Sand	(++)	White
2	Sipat road area	Soil sample 2	Silt	(+)	White
3	Gandhi chowk	Soil sample3	Clay	(++)	Light yellow

Symbols- (++)- Good growth
(+) – Normal growth

Table 5- Serial Dilusion Method

No	Sample	Soil type	10-1	10-2	10-3	10-4
1	Soil sample 1	Sand	(+++)	(++)	(+)	(-)
2	Soil sample 2	Silt	(++)	(+)	(+)	(-)
3	Soil sample 3	Clay	(+++)	(++)	(+)	(-)

Symbols-(+)- Normal growth

(++)- Good growth
(+++)- Very good growth
(-)- poor growth

PURE PLATE METHOD-Pure plate method is always gives pure culture of the specific and one type of the fungal colony. The colonies of similar shape, size and colours will be visible.

Table no 6 shown the growth of fungus on the Sabouraud agar medium for pure culture. The result provides good growth of the fungus on the Sabouraud agar medium.(Soil sample 1, Sand soil)

Table 6- Pure Plate Method Of Soil Sample 1(Sand Soil)

No	Sample	Soil type	Fungus growth	Colour
1	Soil sample 1	Sand	(++)	White
2	Soil sample 1	Sand	(++)	White
3	Soil sample 1	Sand	(+++)	Light yellow

Symbols- (++)- Good growth
(+++) – Very good growth

Table no 7 shown the growth of fungus on the Sabouraud agar medium for pure culture. The result provides good growth of the fungus on the Sabouraud agar medium.(Soil sample 2, Silt soil)

TABLE 7- PURE PLATE METHOD OF SOIL SAMPLE 2(SILT SOIL)

No	Sample	Soil type	Fungus growth	Colour
1	Soil sample 2	Silt	(++)	White
2	Soil sample 2	Silt	(++)	White

Symbols- (++)- Good growth

Table no 8 shown the growth of fungus on the Sabouraud agar medium for pure culture. The result provides good growth of the fungus on the Sabouraud agar medium.(Soil sample 3, Clay soil)

Table 8- Pure Plate Method Of Soil Sample 3(Clay Soil)

No	Sample	Soil type	Fungus growth	Colour
1	Soil sample 3	Clay	(+++)	Lighe cream
2	Soil sample 3	Clay	(+++)	Light brown

Symbols- (+++)- Verygood growth

MICROSCOPIC OBSERVATION- Figure 1-3 shown the result of the microscopic observation of the pure culture of the soil sample 1. Aspergillus, Penicillium and Trichothecium species is present in the soil sample 1(Sand soil).


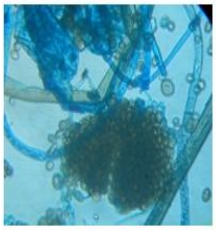


Figure 1:- Aspergillus Figure 2:- Penicillium Figure 3:- Trichothecium

Figure 4-5 shown the result of the microscopic observation of the pure culture of the soil sample 2. Trichoderma and Rhizopus species is present in the soil sample 2(Silt soil).

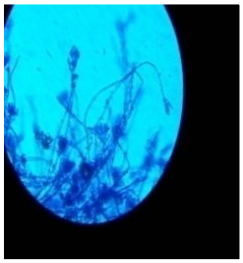

Figure 4:- Trichoderma

Figure 5:- Rhizopus

Figure 6-7 shown the result of the microscopic observation of the pure culture of the soil sample 3. Candida and Mucor species is present in the soil sample 3(Clay soil).

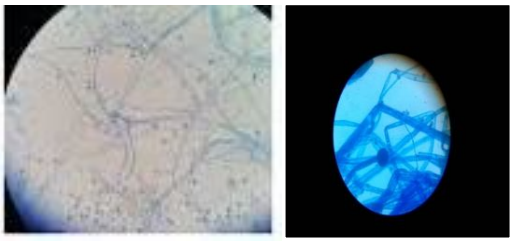

Figure 6:- Candida

Figure 7:- Mucor

Table no 9 shown the presence of the different types of the fungus from the selected ant house soil.

Table 9- Result Of The Presence Of The Different Types Of The Fungus Species From The Selected Ant House Soil

No	**Area**	**Sample**	**Soil type**	**Fungus**
1	Mangla chowk area	Soil sample 1	Sand soil	1) *Aspergillus* 2) *Penicillium* 3) *Trichothecium*
2	Sipat road area	Soil sample 2	Silt soil	1) *Trichoderma* 2) *Rhizopus*
3	Gandhi chowk area	Soil sample 3	Clay soil	1) *Candida* 2) *Mucor*

V. DISCUSSION

Ants are important components of ecosystems not only because they constitute a great part of the animal biomass but also because they act as ecosystem engineers. Ant biodiversity is incredibly high and these organisms are highly responsive to human impact, which obviously reduces its richness.

Ants are one of the most abundant insects on the earth. They influence soil properties through the construction of their nests due to their burrowing habit and their capability to change physical characteristics, of their hills. The food of ants is essentially cellulosic material and since the ants do not produce cellulose dissolving enzymes, digestion of cellulose is carried out with the aid of micro-fauna including fungi, bacteria and microarthopods which are associated with them. Ants are involved in a symbiotic relationship with fungi for the last 50 million years. Most of the workers have attributed that the symbiotic association has evolved to such a level that the ants cease to produce their own digestive enzymes as the fungus associated with them provides them with hydrolytic enzymes and easily assimilated nutrients. An ant-hill is tremendously important for studying mycodiversity because it is built by worker ants that carry tiny pieces of dirt, leaf debris, animal and agricultural wastes from a particular area and deposit them at the mouth of ant colony. Thus, fungal diversity of ant-hill represents the fungal diversity of a particular area.

Ants provide many services free of cost which ensure the survival of our race. They are the major soil turners, channelers of energy, pollinators, scavengers, biological control agents and to sum up the ants are important component of food chain. More recently they are being used as indicator organisms which provide us cues regarding our deteriorating ecosystems. In developed nations like Australia, this concept has already been used effectively. In India too, for the first time this potential of these tiny creatures is being put to practice for assessing the health of Himalayan montane systems which is deteriorating due to growing human activities. It is high time that we pay attention to organisms for our survival and follow the famous epithet "Live and Let Live".

VI. SUMMARY AND CONCLUSION

The fungi gain advantages in:

1. Access to plant material that can easily be penetrated and has an increased surface area.
2. The provision of an optimal microclimate.
3. The selective inhibition of other fungi that are competitively superior and the prevention of microbial infections by termite secretions.
4. It serves as nitrogen-rich food, which is advantageous because the dead plant material consumed by termites contains very little nitrogen.
5. symbiotic fungi provide termites with cellulase and xylanases.

Ants are everywhere, but only occasionally noticed. They run much of the terrestrial world as the premier soil turners, channelers of energy, dominatrices of the insect fauna - yet receive only passing mention in textbooks on ecology. They employ the most complex forms of chemical communication of any animals and their social organization provides an illuminating contrast to that of human beings, but not one biologist in a hundred can describe the life cycle of any species. The neglect of ants in science and natural history is a shortcoming that should be remedied, for they represent the culmination of insect evolution, in the same sense that human beings represent the summit of vertebrate evolution.

VII. CONCLUSION

The ant house soil include nucleic acids, fats, sugars, proteins, enzymes and many fuels. It is the result of activities of ants.

Ant house soil contains different types of the fungus in different places. The presence of the fungus is depends on the quality, texture and types of the ant house soil. Sand soil containing ant house, Aspergillus, Penicillium and Trichothecium species is present. Silt soil containing ant house, Trichoderma and Rhizopus species is present. Clay soil containing ant house, Candida and Mucor species is present.

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