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Study of the soil properties and bacterial community of mud dauber nest

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Abstract
Traditionally, extracts of mud dauber nest are used to treat digestive problem and various ailments. The current study deals with the analysis of physico-chemical composition of the nest soil and isolation of bacteria that have potential biochemical and therapeutic importance. Four nest samples of black and yellow mud dauber wasp were collected. The physico-chemical analysis yields the following results- pH 6.64, electrical conductivity 0.05 dS/m and organic carbon 0.59%. Among the macronutrients and micronutrients, higher content of potassium (430.08 kg/ha) and manganese (1251.67 ppm) were found respectively. Bacterial populations were isolated by serial dilution. Out of seven colonies, three well-defined colonies (IK1, IK2, and IK3) were sub-cultured and characterized. Biochemical analysis showed IK1 were positive for catalase, citrate and starch hydrolysis test, IK2 were positive for catalase and motility test while IK3 showed positive result for macconkey agar, catalase and motility test. The antibiotic sensitivity test showed that IK1 were sensitive to tetracycline and kanamycin while IK2 and IK3 were sensitive to kanamycin. In order to identify the antimicrobial factor present in these bacterial isolates further studies are needed.

Keywords: Mud-dauber nest, organic carbon, macronutrient, bacteria, antibiotic sensitivity

Introduction
Mud daubers (Mud wasps or dirt daubers) belong from either the family of Sphecidae or Crabronidae. Generally, these wasps are solitary insects that hunt spiders and build nests with mud for their offspring [1, 2]. In India, these wasps are broadly seen in the North-Eastern part and in Assam they are locally known as "Kumaroni". These insects are known to contribute to the overall health of the ecosystem. The soils used by mud daubers to construct the nests are silty and extremely hard and stiff. However, the organic content of the nest soils vary by location i.e, it is highly affected by the organic contents of the surrounding soil near nest location and the salivary secretions by mud daubers [3]. The nest material is enriched with nutrients and the clay minerals present in the soil serves as cementing agents [4]. In this era, the increase in multidrug- resistance pathogens have made it essential to discover new antibiotics. Various species of insects produce certain natural products and by-products which are known as the key source for medically used antibiotics. Most of the antibiotics are derived from *Actinomycetes* and fungi. Hence, scientists are exploring these unrecognized uncharacterized habitats to isolate microbes that are important in pharmaceutical interest [5]. *Actinomycetes* isolated from the nest material showed broad spectrum against Gram-positive, Gram-negative, acid-fast bacilli and *Candida* species [6]. Moreover, the mud extracts itself showed antimicrobial efficacy [5]. Therefore, the present study deals to gain an insight into the physiochemical properties and the microbial diversity present in the soil of mud dauber nest.

Materials and Methods
Nests sample collection
Mature, inactive nests of wasps were selected for collection. Four mud-dauber nest samples were collected from the walls of various department buildings at The Assam Kaziranga University, Jorhat using sterile technique. Nest sample collection was done during February 2023- April 2023. In absence of wasp, mud nests were gently detached from the walls using sterile forceps and scalpel. The collected nest samples were then stored in a sealable plastic bag at 4ºC in the laboratory of Biotechnology. The nests are in solid-form with rectangular or trapezoidal shape. The size of the collected nest samples ranged from 4-12 cm (length), 3-8 cm (width), and 1-5 cm (height). The nest soil colors include light brown and brown.

Physico-chemical analysis of mud nest soil

Determination of pH

For the pH determination, 200 ml beaker was taken where 20.0 g of powdered mud sample along with 100 ml distilled water was added. The obtained mixture was agitated for about 30min and then filtered on a Whatman filter paper. The pH of the obtained filtrate was determined by a digital pH meter.

Determination of Electrical conductivity

For the determination of electrical conductivity, in a 200 ml beaker 20gm of powdered mud sample was taken to which 100ml of distilled water was added. The obtained mixture was agitated for about 15min and kept for 30min to settle down. Electrical conductivity was determined by a digital EC meter. The conductivity cell was dipped in the supernatant without disturbing the sediment and the reading was taken.

Determination of Organic Carbon in the nest soil

The quantity of organic carbon in the mud sample was determined by wet digestion method, which is also known as Walkley and Black's titration method [7].

Estimation of macronutrients and micronutrients

The amount of nitrogen available in the nest sample was determined by alkaline permanganate method [8]. Similarly, phosphorus content in the nest sample was determined by Bray's I method developed by Bray and Kurtz (1945) [9]. Available potassium content in the nest sample was estimated by flame photometer, the method was developed by Toth and Prince 1949 and the method is also known as Flame Photometric method [10]. Available calcium and magnesium content were determined by Complexometric titration method (EDTA method) [11]. Available soluble zinc, iron, copper and manganese content were determined by using DTPA extract which is measured by atomic absorption spectrophotometer [12].

Microorganisms Isolation

For microbe isolation, standard serial dilution and steak-plate technique was employed. The stock solution was prepared by mixing 1 gm of mud sample with 10ml of sterile water and vortexed properly. After an hour, the suspension was serially diluted (10^{-1} to 10^{-8}). From the serially diluted mud sample, 100 µl was spread on Nutrient Agar (NA) (HiMedia) plate and incubated for 24 hour at 37 °C. The plate obtained contains mixed bacterial colonies. Well-defined colonies with differential colony morphology were pure-cultured for further identification.

Morphological characterization of the Bacterial isolates

The colony morphology such as colony color, colony texture, elevation, margin, colony form of all the bacterial isolates was evaluated [13]. Gram staining technique, with 100X optical microscopy visualization was used to ascertain the shape, arrangement and classification of bacteria.

Biochemical characterization of the Bacterial isolates

A series of biochemical test was done for the identification of bacteria using standard techniques as mentioned in Bergey's Manual of Determinative Bacteriology [14, 15]. The biochemical test include MacConkey Agar test, Motility test, Catalase test, Indole test, Citrate test, Urease Hydrolysis test, Starch Hydrolysis test.

Antibiotic Susceptibility Profile

Antibiotic susceptibility test was carried out qualitatively by the Kirby- Bauer disc diffusion method on Muller- Hinton agar (MHA) (HiMedia) plates using three antibiotics - ampicillin, kanamycin and tetracycline (HiMedia). The bacteria isolated from the mud sample were grown in Nutrient Broth (HiMedia) for 24 h at 37 °C. After incubation, 50 µl bacterial culture was spread in MHA plate and air-dried. Sterile filter-paper disk dipped on each antibiotic were placed gently on the respective plates and incubated for 24 hrs at 37 °C and susceptibility was determined by the zone diameter. The diameter of zone of inhibition was measured in millimeters and results were interpreted as described in Kirby Bauer chart as susceptible, intermediate and resistant [16].

Result and Discussion

For the present study, the wasp species selected from the University campus was Sc*eliphron caementarium* (black and yellow mud dauber). The mud nests were identified according to the literature described by Dvorak *et al.* 2010; Kim *et al.* 2014 [17, 18]. Nests samples were collected from various departmental buildings of The Assam Kaziranga University, Jorhat. Parts of the mud sample collected were finely grounded for physico-chemical analysis.

Physiochemical analysis of the mud nest soil

The physiochemical composition of the mud sample was investigated and the results are tabulated in Table 1. The pH was found to be 6.64 which imply that the soil is slightly acidic in nature. Such pH value in the soil improves the availability of the nutrient. The electrical conductivity of the mud sample was 0.05 dS/m. The organic carbon of the nest material was 0.59%. This indicates that the organic matter of nest soil is highly affected by the total organic matter content of the surrounding soil near nest location.

The macronutrients - Nitrogen, Phosphorus, Potassium, Calcium and Magnesium were found to be 267.12 kg/ha, 25.47 g/ha, 430.08 kg/ha, 5.0 meq/100, and 10 meq/100, respectively, and the micronutrients zinc, iron, manganese and copper were 242.02 ppm, 887.12 ppm, 1251.67 ppm, and 198.06 ppm, respectively. From this result, it can be deduced that the soil used by mud dauber for nest construction contains mixture of both macro- and micro-nutrients and this chemical composition is responsible for the strength, lightness and adhesive properties of nest. Here, out of the macro- and micronutrients high amount of potassium and manganese are found respectively, however, the chemical composition varies from location to location.

Table 1: Physiochemical analysis of the mud nest soil

Parameter	Observation
pH	6.64
Electrical Conductivity	0.05 dS/m
Organic Carbon	0.59%
Nitrogen	267.12 kg/ha
Phosphorus	25.47 g/ha
Potassium	430.08 kg/ha
Copper	198.06 ppm
Calcium	5.0 meq/100
Magnesium	10.0 meq/100
Zinc	242.02 ppm
Iron	887.12 ppm
Manganese	1251.67 ppm

Characteristics of bacterial colonies isolated from the mud sample

Bacterial colonies were identified based on their morphology and biochemical reaction. Three bacteria were selected from the master plate (Fig.1) for purification which was labeled as IK1, IK2 and IK3. Fig.2 & Table.2 shows the morphological characteristics of all the three isolates. All the isolated colonies were moderate in size, entire in margin, convex like elevation, round and smooth surface with opaque opacity. The colour of the colonies of IK1, IK2 and IK3 were pale orange, white and light yellow respectively. In the microscopic observation, IK1 and IK2 were identified as Gram positive while IK3 was Gram negative in reaction and all the three isolates were rod-shapers in morphology.

Fig 1: Master plate obtained after serial dilution. The three isolates are shown by the arrow.

Table 2: Morphological characterization of the bacterial isolates from the nest material

Isolates	Gram Stain	Shape	Color	Margin	Texture	Form
IK1	+	Rod	Pale orange	Entire	Smooth	Round
IK2	+	Rod	White	Entire	Smooth	Round
IK3	-	Rod	Yellow	Entire	Smooth	Round

Fig 2: Streaking of Isolate A bacterial strain on nutrient agar plate

Biochemical characterization of the Bacterial isolates

The isolated bacteria might be a source of compounds for biochemical and medicinal significance. There may be many more products from soil microorganisms yet to be discovered, despite the fact that many compounds have been discovered as microbial metabolites. In this preliminary study, all the isolates were screened for the presence of enzyme catalase and whether the bacteria can hydrolyse starch, urea, citrate and tryptophan. The result (Table.3) indicates the enzyme catalase is present in all the three isolates. IK1 has the ability to hydrolyze starch while IK2 and IK3 don't. IK1 is non-motile in contrary IK2 and IK3 are motile. Bacterial isolate IK1 and IK2 do not ferment lactose in MacConkey agar as it is Gram positive on the other hand IK3 shows positive result. The results analyzed might have potential application of the bacterial isolates in industrial purpose.

Table 3: Biochemical analysis of bacterial isolates

Isolate No	Biochemical test						
MacConkey Agar		Catalase	Motility	Indole	Urease	Starch hydrolysis	Citrate
IK1	-	+	-	-	-	+	+
IK2	-	+	+	-	-	-	-
IK3	+	+	+	-	-	-	-

(+: positive result, - : negative result)

Antibiotic Susceptibility Profile

One of the most significant secondary metabolites is the antibiotic that was identified from bacteria. Currently, most of the clinically used antibiotics were developed from soil bacteria [19]. In this investigation the pattern of antibiotic sensitivity of the three isolates were examined against three different antibiotics (Ampicillin, Kanamycin and Tetracycline) by disc- diffusion method. After incubation, the zone of inhibition were measured. It was found that the isolate IK1 is sensitive to antibiotics Kanamycin and Tetracycline and showed intermediate sensitivity to ampicillin. On contrary, both Isolate IK2 and IK3 showed resistant to ampicillin, intermediate sensitiviy to tetracyclin and sensitive to Kanamycin.(Table 4, Fig. 3). To fully comprehend the molecular basis for the development of antibiotic resistance by these strains, more research is required. The necessity for the development of novel antibiotic has increased, due to the emergence of multi-drug resistant pathogen [20]. Further studies are in progress in our laboratory to identify the antimicrobial factor present in these bacterial isolates.

Table 4: Antibiotic sensitivity pattern of bacterial isolate

Isolates No	Ampicillin (mm)	Tetracycline (mm)	Kanamycin (mm)
IK1	13	24	22
IK2	8	13	20
IK3	0	14	24

Resistance=<10 mm, Intermediate=10-15 mm, Susceptible=>15 mm

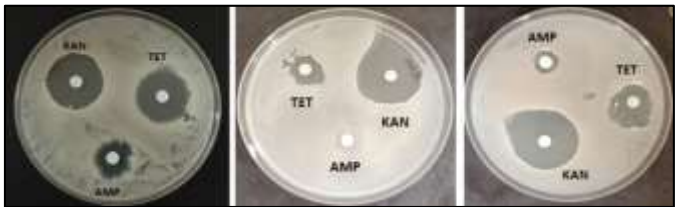

Fig 3: Zone of inhibition of antibiotic susceptibility test

Conclusion

In this study, the physiochemical analysis concludes that the soil is enriched with potassium, calcium, magnesium etc. The bacterial strains isolated from the mud nest shows sensitivity against antibiotics like Ampicillin, Kanamycin and Tetracycline. Hence, this study might lead to the development of potent antimicrobial compound. Further studies must be done to check the antimicrobial efficacy of the nest extract

along with the fungal diversity present in the mud nest soil which might be important in pharmaceutical interest.

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Conflict of interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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