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Nesting Habits Are Aspects in the Development of Antimicrobial Defences in Paper Waspsagainst various Pathogens

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ABSTRACT

In the present study, paper wasp nest and social behaviour were carried out of *Polistes flavus*. Nest of paper wasp nest were found among house rope wall, bunches of leaves in the tree with 1-5 flat steps layers containing hundreds of hexagonal cells in one sided hanging to downward.To test this,thepaper wasp nest extracted from wasp species of varying community complexity and nesting habits and assayed their antimicrobial compounds against cultures of *Bacillus subtilis, Lactobacillus acidophilus, Pseudomonas aeruginosa, Streptococcus mutans, Streptococcus faecalis*and *Candida albicans*. Waspnest extract showed antimicrobial activity. The paper wasp nest samples are used with solvents that is ethanol and acetone for preparing their extractions.By agar disc diffusion technique, the diameter of inhibition zones in mm^2 in presence of paper wasp nest at a concentration of µg/ml.The paper wasp nest extract sample shows the maximum zone of inhibition with solvent ethanol and acetone against *Bacillus subtilus*(148.365 mm^2), and *Streptococcus mutans*(462.37 mm^2).From antimicrobial susceptibility tests it was found that more active against the test bacteria and fungi. These could substitute highly toxic antibiotics which can resolve the problem of medicine resistance. P value of solvents (Acetone and Ethanol)andMicrobial strainsin the given group of Anova report is0.361711869and 0.121667804respectively.

Key words: **Antibiogram, *Bacillus subtilis, Lactobacillus acidophilus, Pseudomonas aeruginosa, Streptococcus mutans, Streptococcus faecalis* and *Candida albicans*.**

1. INTRODUCTION

Hymenopteran's insects mainly wasps, honey bees, hornets inflict venom to maintain self-defense for protection of territory [1].Papery wasp nest toxins have been evolved to capture prey and make defense against predators and/or microorganisms. Generally, wasp envenomation happens after little trouble occurred in near locality of their hive. Chemically, the venom is a mixture of biologically active materials of high, medium, and small molecular weight with a variation of physiological function [2].Papery wasps (Hymenoptera, Vespidae, Eumeninae) are mostly solitary hunters, with some species having basicallycommunitybehaviour [3].The paper wasp, *Polistes flavus* is the most common form of wasp which is diverse throughout the world and mostly made their nest in human houses and trees. It is also the single major genus within the family Vespidae, with over 300 known species and subspecies. Their characteristic preferences for nestbuilding sites lead them to usually build nests on human habitation, where they can be very undesirable; while generally non-aggressive, they can be reactive into defending their nests. but the nature of the biochemical cues underlying this finding mechanism remains unknown. Chemical analyses have revealed that the paper nest of several species of Polistes is enclosed with the same HC mixture as on the wasp shell [4,5,6,7,8].

Certain the diversity of wasp taxa we predicted that we would detect a large amount of difference in the relative strength of antimicrobial defences among species, based on life history behaviours. We extracted paper wasp nest antimicrobial compounds from the wasp species which span a range of nesting habits and social complexity. Using an established bioassay, the activity of these compounds was measured and compared. Based on previous studies we expected those species with the highest group sizes to possess the strongest antimicrobial compounds[9].

The dilution method is used to determine the minimal inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC) which are, respectively, the lowest concentrations that inhibit visible bacterial growth and the lowest concentration that kills bacteria (10,11).In this study we investigated whole nest to provide an initial valuation of the microbiota associated to a papery wasp nest against *Bacillus subtilis, Lactobacillus acidophilus, Pseudomonas aeruginosa, Streptococcus mutans, Streptococcus faecalis* and *Candida albicans*, a standard microbialexamine.

2. MATERIAL AND METHODS

Paper wasp nest were collected from a residential buildingin Ranchi, Jharkhand. The wasp nest was taken to laboratory and washed thoroughly using distilled water to remove dust and other unnecessary materials. The washed nest was allowed to air dry at room temperature and kept in air tight container until further use. The wasp nest extractions are prepared by using the organic solvent. The common structure of organic solvents (at least 1 carbon and 1 hydrogen atom), low molecular weight, volatility and lipophilicity and they occur in liquid firm at room temperature.On the time of extraction solvents diffuse into the powder ofwasp nest and solubilize compound with similar polarity. The two solvents were used for wasp nest extraction, i.e., Ethanol, and Acetone.The wasp nest sample was grinded through mortar-pestle to make powder form. The grind samples individually mixed with different solvents in 1:10 ratio. After mixing, keep the solution in dark place for 72 hrs. After 72 hrs. the sample was filtered through filter paper in a clean and air-dried beaker. The obtained filtrate was kept in room temperature for complete dry. The weight of beaker having the solid filtrate was then measured in order to calculate the difference.

Pathogens Used: The analysis of Antibacterial activity of paper wasp nest extract was performed against sixmicrobial pathogens which are given below- *Bacillus subtilis, Lactobacillus acidophilus, Pseudomonas aeruginosa, Streptococcus mutans, Streptococcus faecalis* and *Candida albicans*.

The agar disc diffusion is best methods to analyze the anti-microbial against numerous microorganisms. The zone of inhibition measures the diameter in mm [12]. Procedure: The Nutrient media and sabouraud dextrose media are prepared for bacteria and fungi. The culture media and petri dish were sterilizing in autoclaved. After autoclaving, they were place in Laminar air flow. In Laminar air flow, the media were carefully poured into the Petri dish and allowed to get solid form. After that paper disc was prepared and sterilize. The wasp nest extract (Ethanol and Acetone) was loaded into the disc and the plates was incubated at 37℃and 28℃Temperature for 24 hrs. After that analyzed the result in growth plate and measure the diameter in mm of zone of inhibition and calculate the value.

3. RESULT AND DISCUSSION

The papery wasp nest sample was collected from the Ranchi, Jharkhand.

Figure 1: Paper wasp nest sample.

Figure 2: Powder form of wasp nest samples

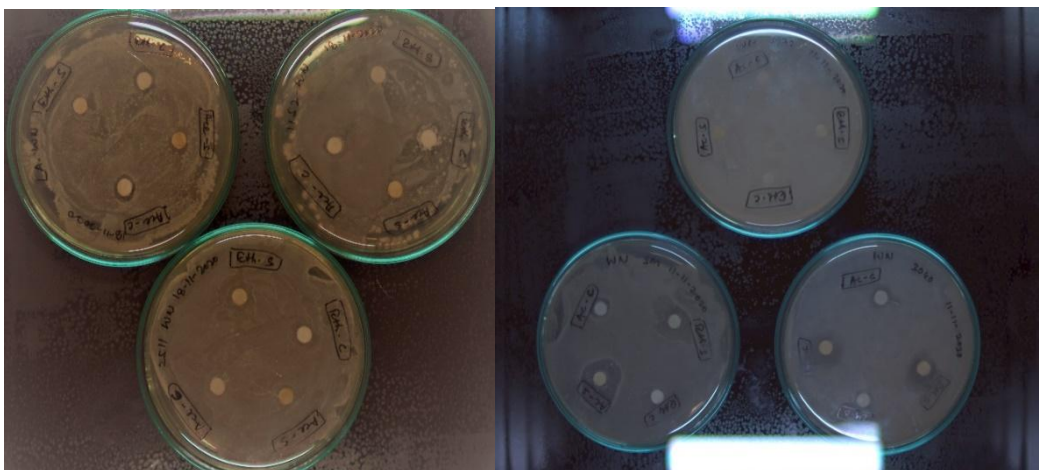

Figure 1: Show the antimicrobial activity of ethanolic and acetone solvents test results

Table 1: Showing the zone of inhibition against pathogen microbial strains

Wasp nest inhibition on *Candida albicans* in different solvents					
Solvents	**r_1 (mm)**	**Area of disc (mm^2)**	**r_2 (mm)**	**Area of inhibition (mm^2)**	**Zone of inhibition (mm^2)**
Acetone (sample)	3	28.26	4.5	63.585	35.325
Acetone (control)	3	28.26	0	0	No inhibition
Ethanol (sample)	3	28.26	4.75	70.84625	42.58625
Ethanol (control)	3	28.26	5	78.5	50.24
Wasp nest inhibition on *Pseudomonas aeruginosa* in different solvents					
Solvents	**r_1 (mm)**	**Area of disc (mm^2)**	**r_2 (mm)**	**Area of inhibition (mm^2)**	**Zone of inhibition (mm^2)**
Acetone (sample)	3	28.26	0	0	No inhibition
Acetone (control)	3	28.26	0	0	No inhibition
Ethanol (sample)	3	28.26	0	0	No inhibition
Ethanol (control)	3	28.26	0	0	No inhibition
Wasp nest inhibition on *Enterococcus faecalis* in different solvents					
Solvents	**r_1 (mm)**	**Area of disc (mm^2)**	**r_2 (mm)**	**Area of inhibition (mm^2)**	**Zone of inhibition (mm^2)**
Acetone (sample)	3	28.26	7.75	188.60	160.34
Acetone (control)	3	28.26	4.5	63.59	35.33
Ethanol (sample)	3	28.26	6	113.04	84.78
Ethanol (control)	3	28.26	4.25	56.72	28.46
Wasp nest inhibition on *Streptococcus mutans* in different solvents					
Solvents	**r_1 (mm)**	**Area of disc (mm^2)**	**r_2 (mm)**	**Area of inhibition (mm^2)**	**Zone of inhibition (mm^2)**
Acetone (sample)	3	28.26	12.5	490.63	462.37
Acetone (control)	3	28.26	4.75	70.85	42.59
Ethanol (sample)	3	28.26	0	0	No inhibition
Ethanol (control)	3	28.26	5.25	86.55	58.29
Wasp nest inhibition on *Bacillus subtilis* in different solvents					
Solvents	**r_1 (mm)**	**Area of disc (mm^2)**	**r_2 (mm)**	**Area of inhibition (mm^2)**	**Zone of inhibition (mm^2)**
Acetone (sample)	3	28.26	5.5	94.985	66.725
Acetone (control)	3	28.26	5.25	86.55	58.29
Ethanol (sample)	3	28.26	7.5	176.625	148.365
Ethanol (control)	3	28.26	5.75	103.816	75.556
Wasp nest inhibition on *Lactobacillus acidophilus* in different solvents					
Solvents	**r_1 (mm)**	**Area of disc (mm^2)**	**r_2 (mm)**	**Area of inhibition (mm^2)**	**Zone of inhibition (mm^2)**
Acetone (sample)	3	28.26	5	78.5	50.24
Acetone (control)	3	28.26	4.75	70.84625	42.58625
Ethanol (sample)	3	28.26	5.75	103.81625	75.55625
Ethanol (control)	3	28.26	4.75	70.84625	42.58625

The papery wasp nest samples were collected from Ranchi area, Jharkhand than performing the antibiogram test, the overall result show the increased activity of wasp nest extract sample. The wasp nest samples are used with solvents that is ethanol and acetone for preparing their extracts. By agar disc diffusion method, the diameter of inhibition zones in mm^2 in presence of yellow wasp nest at a concentration of μg/ml.The highest zone of inhibitions was recorded in the case of wasp nest extract with solvent acetone against *Streptococcus mutans*(462.37 mm^2),*Enterococcus faecalis*

(160.34 mm^2), *Bacillus subtilus* (66.725 mm^2), *Lactobacillus acidophilus* (50.24 mm^2), *Candida albicans* (35.325 mm^2) and *Pseudomonas aeruginosa*are not found zone of inhibition respectively.With solvent ethanol against *Bacillus subtilus*(148.365 mm^2),*Enterococcus faecalis* (84.78 mm^2),*Lactobacillus acidophilus* (75.55625 mm^2), *Candida albicans* (42.58625 mm^2), *Pseudomonas aeruginosa* and *Streptococcus mutans* both strains are not found zone of inhibitions. The paper wasp nest extract sample shows the maximum zone of inhibition with solvent ethanol and acetone against *Bacillus subtilus*(148.365 mm^2), and *Streptococcus mutans*(462.37 mm^2) respectively (Table 1).

Table 2: Anova test report of Solvents

Anova: Single Factor calculation of solvents						
SUMMARY						
Groups	***Count***	***Sum***	***Average***	***Variance***		
Acetone	6	775	129.1666667	29522.98094		
Ethanol	6	351.2875	58.54791667	3231.00119		
ANOVA						
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	14961.02355	1	14961.02355	0.913539215	0.361711869	4.964603
Within Groups	163769.9106	10	16376.99106			
Total	178730.9342	11				

Table 3: Anova test report of Microbial strains

Anova: Single Factor calculation of Microbial strains						
SUMMARY						
Groups	***Count***	***Sum***	***Average***	***Variance***		
Streptococcus mutans	2	610.735	305.3675	49299.57001		
Enterococcus faecalis	2	235.89625	117.948125	3594.142132		
Bacillus subtilus	2	151.505	75.7525	162.9915125		
Lactobacillus acidophilus	2	92.82625	46.413125	29.28994453		
Candida albicans	2	35.325	17.6625	623.9278125		
Pseudomonas aeruginosa	2	0	0	0		
ANOVA						
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	125021.0128	5	25004.20256	2.793249578	0.121667804	4.387374
Within Groups	53709.92141	6	8951.653569			
Total	178730.9342	11				

Our Anova examines (Table 2 and 3) show that as group size and within-paper waspnest extract relatedness increased monotonically, so did antimicrobial strength (Spearman's rank correlation $p < 0.05$ forMIC100).P value = 0.000 (calculated) is less than .05 (confidence interval).As we have strong evidence to support that we can reject null hypothesis.There is a significant difference between the sixmicrobial strains groups.So, it can be concluded that at least one of the means is different in the given two groups. P value of solvents (Acetone and Ethanol)andMicrobial strainsin the given group of Anova report is0.361711869and 0.121667804respectively.

Polistes flavus, the heavily sclerotized sting shaft ensured the mechanical diffusion into the object, while the highly specialized venom gland carries the powerful venomous secretion. For this purpose, an imposing muscular supply enclosed the glands reservoir [13]. Through the present study, the aggressive behaviour of the wasp was clearly found. The motive for such behaviour was to protect themselves from external environmental factors, such as interference of humans, removal of their nests from the houses and during cutting the tree branches where they made their nests [14]. The wasps collected their food from fields of different crops like rice, cotton, wheat etc. The raw materials from cotton field were used for structure of their nests. It was observed that the wasps collected their food from different fruits markets. The fruits were also found as a source of food. Such resources and foods were easily available from the

environments of the wasp nests [15]. Wasps have well developed managing system for protection of their nests. One can maintain that due to such behaviour wasps were used their old nests for the next season [16]. Observed pseudo attack in *Polistes flavus*[17]. Threatening behaviours of the wasps have been described [18]. It was observed that the drone wasps flew nearby around their nests, but not toward the object where nest was found. Therefore, these wasps built their nests in the homes and trees because they are familiar with people. Studied the wasp hunting behaviour [19]. Wasps hunted their prey in two steps: in the first step, wasps explored the environment until they located and visited a potential prey, and in the second one, they attacked and tried to capture the prey. One can argue that was used hunting prey according to their food requirements. Examined the wasps' foraging behaviour [20]. It was observed that Hymenoptera modified their flight when they perceived a black spot on a light object. They involved on small elements that has a high contrast with its surroundings. In these situations, the spiders' retreated, which were made of white silk can be an efficient visual and mechanical protection. Reported that *Polistes flavus*showed variances in the nests structure and architectural patterns from other wasp species with nest construction methods [21].

CONCLUSION

In the present study, antimicrobial activity of purified paper wasp nest was determined in vitro.By agar disc diffusion method, the diameter of inhibition zones in mm^2 in presence of paper wasp nest at a concentration of µg/ml.The paper wasp nest extract sample shows the maximum zone of inhibition with solvent ethanol and acetone against *Bacillus subtilus*(148.365 mm^2), and *Streptococcus mutans*(462.37 mm^2).P value of solvents (Acetone and Ethanol)andMicrobial strainsin the given group of Anova report is0.361711869and 0.121667804respectively. From antimicrobial susceptibility tests it was found that more active against the test bacteria and fungi. These could replace highly toxic antibiotics which can solve the problem of drug resistance.

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