



Colony structure and nest chemical profiling of selected species of wasps (Hymenoptera: Vespidae), in Dir, Pakistan

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Abstract

Wasps are found throughout the world, having great economic importance. Though they are playing a positive role in the ecosystem by promoting pollination, act as a predator for various insect's pest, they also negative impact upon honey bee farming, local ecosystem and residential. The present study was conducted from April, 2015 to November, 2017 during which a total of 57 nest samples were collected in the study area. Various aspects of the colony structure were examined for the collected nest and chemical profiling of the nest was made by XRD. The solitary wasp's (Euminanae) nests were made of mud having spindle shaped cells with size of 7.9 mm to 29.2 mm, while the nest of social wasps were made of paper material. The Vespine's nest was globular to spindle in shape and were found either areal or underground, having the cell size 9 mm to 28 mm. The Polistinae's nest was mostly found areal either on trees or buildings, with cell size varying from 9.2 mm to 19.6 mm. The X-ray diffraction (XRD) results revealed that the nest of Vespinae contain different compound like Ammonium nitrate (NH_4NO_3), 9-amidonylon6oligomer($\text{C}_{59}\text{H}_{11}\text{N}_9\text{O}_9$), Methanamine ($\text{C}_6\text{H}_{12}\text{N}_4$), 4,4` Bis (pethoxybenzylideneamino)biphenyl ($\text{C}_{29}\text{H}_{26}\text{N}_2\text{O}_2$) while that of Polistinae contained Methylene oxalate $\text{C}(\text{HCO}_2)_2$, Calcium silicate hydrate $\text{Ca}_3\text{Si}_3\text{O}_9 \cdot \text{H}_2\text{O}$, Adenine maleic acid $\text{C}_9\text{H}_9\text{N}_5\text{O}_4$, Nomifansine $\text{C}_{16}\text{H}_{18}\text{N}_2$, L-Thermospine $\text{C}_{15}\text{H}_{20}\text{N}_2\text{O}$ and Calcium nitride Ca_3N_2 . Similarly the nest of Euminanae contained calcium, silicon and aluminum in their respective phases like Calcium carbonate CaCO_3 , Silicon oxide SiO_2 , Calcium magnesiumsilicatehydroide $\text{Ca}_2\text{Mg}_5\text{Si}_8\text{O}_{22}$, aluminum Silicate hydroxide $\text{Ca}_4\text{Si}_5\text{O}_{13}(\text{OH})_2$. Further studies is required to explore the antimicrobial activities of these nest extracts as some chemicals found in the nest of Polistis species have close structural resemblance with some antibiotics.

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Introduction

Wasps of the family Vespidae show significant role in terrestrial biomes and they are considered as a good biological control agents, as their larvae feed on maggots and other insects. They also serve as pollinators of various vegetable, fruit and crops (Junaid *et al.*, 2015). Despite from being useful wasps are harmful for the human too, because they act as a pest for certain fruit, sting the human and also make nests in houses (Spradbery, 1973). The Vespidae is a cosmopolitan but chiefly tropical family having six subfamilies; Eumeninae, Eupragiinae, Masarinae, Stenogastrinae, Polistinae, and Vespinae.

The subfamily Vespinae and Polistinae are comprised exclusively of extremely eusocial species, while Eumeninae, Euparagiinae and Masarinae are all solitary; Stenogastrinae are basically eusocial wasps. Thirty-six genera of societal vespids are present in the world, mostly spread in the tropical parts of the world, excluding *Dolicho vespula* and *Vespula* (Khalid *et al.*, 2012).

Colony is an aggregate of the wasps that are living together in the same nest. They defend actively their colony by stinging and other aggressive behavior (Smith *et al.*, 2008) In societal wasp colony, there is caste system and division of labor, usually having 3 castes, there is a single queen per colony which is a fertile female, majority are workers which are sterile females and perform the functions of defense and nectar collection, and the rest few individual are fertile haploid males known as drone. The Social wasps usually build nest's shells of a tough paper like material obtained from grinding wood fibers (Khan *et al.*, 2018). The present study is aimed to examine the colony structure, nest design and chemical profiling of Vespidae wasps in Dir Pakistan.

Materials and methods

Nest collection

Nests belonging to the family Vespidae were actively collected from different sites of the study area. First the wasps were killed by spraying insecticide, then nests were collected. Further investigations of nests

were made such as diameter, circumference, presence of eggs, larvae, adults and the number and size of cells were also noted.

Identification

The identification of collected wasps and their nests were made by different keys like Dvorak and Carpenter (2010) new records of Vespidae wasps from Yemen with synonymy in *Belonogaster* (Hymenoptera: Vespidae: Polistinae and Eumeninae); Carpenter *et al* (2009) a catalogue of the Eumeninae (Hymenoptera: Vespidae) of the Ethiopian region Ebrahimi and Carpenter (2008); like Das and Gupta (1989) social wasps of India and adjacent countries; Carpenter and Kojima (1997) Vespinae check list of the world.

Chemical analysis

A portion of the selected nest was taken and was grinded into fine powder with the help of pestle and mortar. The powder samples were taken in sterile tubes and were properly labelled. And then shifted to CRL (Centralized Resources Laboratory) University of Peshawar for XRD analysis to find out the chemical composition.

Results

Fifty seven nests belonging to the various species of family Vespidae were collected from different spots of the study area. Collection was made from April 2015 to November 2017. The nests belonging to the subfamily Polistinae were easily accessible as they were located either inside houses or nearby trees that is why they were collected in greater number as compared to those of Eumeninae or Vespinae. The smallest nest recorded was for Eumeninae (*Antipipona deflenda*) having 7.5 mm cell size as shown in the Fig. 1, while the largest one was recorded for Vespinae (*Vespa velutina*) with a length of about 23.5 inches, 9.6 inches diameter with 11 combs. This nest was also covered by an envelope all around possessing a single opening for entry and exit. The nests of Eumeninae were made of mud that is why they are called potter wasps while those of Vespinae and Polistinae were made of wooden materials. Eumeninae nests.

Table 1. Composition of *Vespa velutina* colonies.

S.No	Length	Diameter	Combs	Cells	Eggs	Larvae	Pupae	Adults
1	23.5'	9.6'	11	9055	25	30	70	20
2	20'	9.2'	9	6244	14	04	19	24
3	11'	9.1'	4	1542	02	09	17	6
4	21	9.5	9	5781	05	16	28	19

About 6 nests were collected belonging to the two species of Euminanae such as *Antipipona deflenda* and *Delta dimidiatopenne*. These nests were built of mud material Fig.1.

Polistinae nests

About 40 nests were collected belonging to the

subfamily, Polistinae. Most of the nests were those of *Polistes flavus*. While very few nests were collected for the *P. rothneyi*, as they built their nests in trees.

All these nests were constructed of paper like material having a single comb and no protective envelope as shown in (Fig. 2).

Table 2. Shows composition of nests of four different species of Polistinae.

Colony	Length	Diameter	Combs	Cells	Eggs	Larvae	Pupae	Adults
<i>Polistes stigma</i>	70mm	50mm	01	257	05	20	8	40
<i>P. olivaceous</i>	95mm	85mm	1	324	01	03	08	45
<i>R. cythiformis</i>	variable	variable	variable	3-10	00	01	00	5
<i>Polistes rothneyi</i>	165mm	70mm	1	241	08	97	45	55

Vespine nests

A total of eleven nests of the subfamily were collected, of which nine were of *Vespa velutina* and only two of the *Vespa flaviceps*. As the nests of *Vespa velutina* were made high up in tree and in buildings and contained thousands of hot tempered individuals, so all of these were removed during winter when these were abandoned and no individual were present.

Vespa velutina build huge aerial nests with many combs. However the first time an underground nest was reported for *V. velutina*. The combs are protected by a cover all around only leaving a hole for the

entrance/exit. All the cells are hexagonal of 19 mm in diameter and 28 mm length. The nests of *Vespa flaviceps* were underground about 26 cm deep in the soil.

It is also having many combs with hexagonal cells but smaller in size. Unfortunately the nests could not be got completely as they were damaged during digging.

Chemical analysis

The elements like C, H, O, N, Ca, Si and Al were present in greater amount having different phases (Table3).

Table 3. Chemical composition of the wasps nests by XRD analysis.

S.No	Sub Family	Selected Species	Compound Detected
1	Euminane	<i>Delta dimidiatopenne</i>	Calcium carbonate CaCO_3 Silicon oxide SiO_2
		<i>Antipipona deflenda</i>	Calcium magnesiumsilicatehydride $\text{Ca}_2\text{Mg}_5\text{Si}_8\text{O}_{22}$ Aluminum Silicate hydroxide $\text{Ca}_4\text{Si}_5\text{O}_{13}(\text{OH})_2$
2	Polistinae	<i>Polistes rothneyi</i>	Methylene oxalate $\text{C}(\text{HCO}_2)_2$ Calcium silicate hydrate $\text{Ca}_3\text{Si}_3\text{O}_9 \cdot \text{H}_2\text{O}$ Adenine maleic acid $\text{C}_9\text{H}_9\text{N}_5\text{O}_4$
		<i>P. stigma</i>	Nomifansine $\text{C}_{16}\text{H}_{18}\text{N}_2$ L-Thermospine $\text{C}_{15}\text{H}_{20}\text{N}_{20}$ Calcium nitride Ca_3N_2
		<i>Vespa velutina</i>	Ammonium nitrate (NH_4NO_3) 9-amide nylon 6-oligomer $(\text{C}_{59}\text{H}_{111}\text{N}_9\text{O}_9)$ Methanamine $(\text{C}_6\text{H}_{12}\text{N}_4)$ 4,4' Bis(pethoxybenzylideneamino)biphenyl $(\text{C}_{29}\text{H}_{26}\text{N}_2\text{O}_2)$

Discussion

In the present study, a total of fifty seven nests belonging to the Vespidae family were collected from May 2016 to November 2017. Almost all the specimen were collected from the rural areas in District Dir.

About 116 nests belonging to the European wasps, including 46 from urban and 70 from rural areas, were collected but there was no difference in the structure and composition of rural and urban nests (ward *et al.*, 2002).



Fig. 1. Nest of *Antipipona deflenda*.



Fig. 2. An active Nests of *Polistes olivaceous*.

In the present study, it was found that the nest of Vespinae contain different compound like Ammonium nitrate (NH_4NO_3), 9-amidonylon6oligomer($\text{C}_{59}\text{H}_{111}\text{N}_9\text{O}_9$),Methanamine($\text{C}_6\text{H}_{12}\text{N}_4$),4,4` Bis(pethoxybenzylideneamino)biphenyl ($\text{C}_{29}\text{H}_{26}\text{N}_2\text{O}_2$) while that of Polistinae contained Methylene oxalate $\text{C}(\text{HCO}_2)_2$, Calcium silicate hydrate $\text{Ca}_3\text{Si}_3\text{O}_9 \cdot \text{H}_2\text{O}$, Adenine maleic acid $\text{C}_9\text{H}_9\text{N}_5\text{O}_4$,

Nomifansine $\text{C}_{16}\text{H}_{18}\text{N}_2$, L-Thermospine $\text{C}_{15}\text{H}_{20}\text{N}_2\text{O}$ and Calcium nitride Ca_3N_2 . Similarly the nest of Euminanae contained calcium, silicon and aluminum in their respective phases like Calcium carbonate CaCO_3 , Silicon oxide SiO_2 , Calcium magnesiumsilicatehydride $\text{Ca}_2\text{Mg}_5\text{Si}_8\text{O}_{22}$, Aluminum Silicate hydroxide $\text{Ca}_4\text{Si}_5\text{O}_{13}(\text{OH})_2$. These results can be compared with the results obtained by Parveen

and Shah, 2013 who found that the nest of *Vespa velutina* contained elements like Ca, Mg, Si, K and Al while that of *Polistes flavus* contained Ca, Al, Mg and Silicon. So they reported no such differences in the nests of the two mentioned species.

However, our findings are a little bit different as the

nests of polistinae and euminae contain a greater amount of Ca⁺⁺, Si and Mg present in the form of calcium silicate hydrate, Graphite silicate, Calcium nitride, Silicon oxide, Calcium carbonate, Calcium aluminum silicate, Calcium magnesium silicate hydride.



Fig. 3. Nest of *Vespa velutina*, the envelope is partially removed to visualize the combs.

While the nests belonging to the subfamily Vespinae were having somewhat different chemical composition and mostly contained amines as a major constituent.

In the recent study the biggest nest documented was of *V. velutina* which was about 23.5 inches in length with a diameter of 9.6 inches and contained 11 combs as shown in the figure 1.

Whereas the smallest nest was of *A. deflenda* having 7.3 mm size, shown in the figure 2. Macdonald and Mathew 1976, had recorded the biggest nest of *V. vidua* with 6 combs and 2477 cells while the smallest one was having 3 combs with 944 cells.

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