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CHEMICAL FEATURES OF FOREWING SCALES OF *Leptosia nina* Fabricius, 1793 (Lepidoptera: Pieridae)

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ABSTRACT

The functional groups have a vital role in insect physiology, behaviour, and adaptation to the environment and are crucial in biomimetics to replicate and adapt biological functions for diverse use ranging from medicine to material science and energy technologies. A study was conducted on *Leptosia nina* forewing scales to assess its chemical composition spectra using Fourier Transform Infrared Spectroscopic (FTIR) analysis. The identified functional groups from dorsal white, dorsal black spot, ventral white and ventral black spot areas of *L. nina* forewing composed of alcohols, phenols, aldehydes, aliphatic amine, alkanes, alkenes, alkyl halides, alkynes, aromatic groups, carboxylic acid groups, nitro compounds, amines, ester and ether with various stretches. The spectra showed minor variations in peaks in the four regions studied. The study is the first record revealing the functional groups of *L. nina* forewing scales.

Keywords: Chemical composition spectra, forewing, FTIR spectroscopy, functional groups. *Leptosia nina*, scales

INTRODUCTION

Butterflies are ravishing insects and have become a study model due to their artistic wing colouration (Kunte *et al.*, 2020; Aarti and Arya, 2021). The powdery stuff encasing the wings are the scales that constitute the pigmentary, structural and androconial scales which play a major role in camouflage, mate attraction, and eliciting a positive attitude in children (Das *et al.,* 2017). Wing colouration relies on pigments and the optical activity of scales; and have become the topic of choice in studies on evolution and development (Ffrench-Constant, 2012). *Leptosia nina* of family Pieridae is a small butterfly with a black spot on a white background that flies low over the grass, bobbing up and down with a weak and erratic flight pattern.

The chemical traits of wing colour are vital for exploring the chemical nature of scale pigments. Numerous biomimetic applications are inspired by the characteristics of functional groups. The ability of gecko feet to adhere to the surfaces is linked to the existence of distinctive functional groups. The researchers have developed gecko's feet-inspired self-peeling switchable dry/wet adhesive (Zhang *et al*., 2021). The shark skin possesses specific functional groups that discourage the attachment of biofouling organisms. This mechanism was bio-inspired for the production of antifouling Fe-based amorphous coating via killing-resisting dual surface modifications (Li *et al*., 2022).

The spectrochemical analysis of small molecules to supramolecular structures is performed by FTIR analysis. FTIR spectroscopy is employed for sample fingerprinting by examining the absorption peaks corresponding to the frequencies of vibrations between the bonds of atoms constituting the materials. Every substance has a different infrared spectrum; and structural features can be analysed by using FTIR which is based on the inter- and intra-molecular interactions of functional groups present in that molecule in all aggregation states (Kannan *et al.,* 2020; Kamnev *et al.*, 2021). Diagnosis and forensic purposes depend upon the biochemical information revealed by FTIR. Many studies using FTIR have been done on biological samples such as live cells, tissue samples, body fluids (Baker *et al*., 2014), and also on tissues of certain disorders like myopathies and brain tumours (Petibois *et al*., 2009) and body fluid traces (Mistek and Lenev, 2018). Meagre FTIR studies have been done on arthropods like honeybees, dragonflies, etc. (Machovic *et al*., 2017). Scanty reports are available on FTIR analysis in butterflies (Tian *et al.,* 2015, Krishna *et al*., 2020). Since FTIR is a good indicator to discover different form of materials in wing scales of *L. nina,* in which the peaks of spectrum are immediate indication of the substance present. Hence, the present FTIR analysis was done to uncover the chemical characteristics of *L. nina* forewing.

MATERIALS AND METHODS

Leptosia nina was collected from the botanical garden of Kariavattom Campus, University of Kerala, Thiruvananthapuram (8°32', 8° 34'N and 76° 52', 76° 54'E) using a handheld insect net and preserved in an insectbox for further study. Forewings were plucked out carefully using clean forceps and investigation of functional group was done by using Fourier Transform Infrared Spectroscopy (Smart Orbit™ Thermoscientific Nicolet iS50) by Attenuated Total Reflection (ATR) method (Larkin, 2014) that allows the direct measurement of wing. The spectrum was obtained using 50% transmittance on OMNIC^{TM} software (*https://www.thermofisher.com/order/catalog/product/INQSOF018*). FTIR spectrum of four regions namely dorsal white, dorsal black spot, ventral white and ventral black spot area were studied. The recorded readings were plotted in a graph using OriginPro 2023b software (*https://www.originlab.com/2023b)*. Characteristics IR absorption frequencies of organic functional groups observed were compared using FTIR frequency chart.

RESULTS AND DISCUSSION

FTIR analysis on functional groups of dorsal white area of *L. nina* forewing (Fig. 1) showed the peaks at 3279.79, 2918.03, 2849.54, 1650.92, 1541.10, 1451.02, 1376.10, 1158.57, 1073.99 and 458.65 cm^{-1} (Table 1). These peaks showed the presence of carbonyl and hydroxyl groups with O-H linkage with broad and weak stretching and alkane group with C-H stretch. The presence of aldehyde was noted with =C-H bond with weak bending. Alkene C=C was observed with narrow and strong stretching. Nitro N-H stretch was observed with medium stretching. Other groups noted were C-N amine, C-F alkyl halide and S-S aryl disulfide with asymmetric weak bending. On dorsal black spot area, the peaks obtained were 3270.65, 2919.66, 1631.90, 1540.84, 1444.45, 1374.99, 1255.38, 1158.16, 1072.37, 1027.78, 519.05 and 459.99 cm^{-1}. The O-H (hydroxyl) group linkage was noted with normal

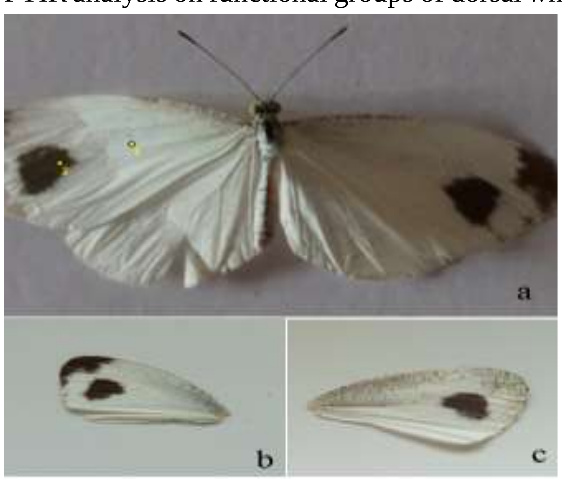

Fig. 1: *Leptosia nina* forewing regions a) (dorsal); b) dorsal forewing c) ventral forewing

broad and weak stretch. Alkane C-H stretch was noted with weak bending. Alkene C=C stretched with medium bending. Aromatic C=C stretched with medium bending, alkyl halide C-F stretch, Acid C-O stretch and ester C-O stretch was observed. Alkyl halide C-F group and C-Br group, aryl disulfide (S-S) stretch and ester C-O bond was noted in this area.

The ventral white area exhibited peaks at 3726.71, 3703.89, 3624.69, 3268.78, 2919.31, 2161.27, 2142.28, 1978.69, 1629.71, 1512.72, 1443.34, 1376.15, 1255.30, 1155.80, 1070.28, 1026.44, 953.20, 892.32, 482.32 and 459.57 cm^{-1}. The characteristic groups included O-H stretch with weak bend, symmetric C- H alkane stretch, -C triple bond C variable stretch, Alkenyl C=C stretch, secondary amine N-H bend, aromatic C=C broad and medium stretch, alkane -C-H bending, methyl C-H asymmetric weak bend, primary amine C-N stretch, aliphatic fluoro compounds C-F stretch, tertiary amine C-N stretch, cyclohexane ring vibrations and methane. Methylene, vinylidene, polysulfides and aryl disulfides were also noted.

Table 1: FTIR spectra (hydrocarbons and functional groups) of *Leptosia nina* forewing

	Spectral peak values (cm^{-1})			
Hydrocarbons and functionalgroups	Dorsal White	Dorsal black spot area	Ventral White	Ventral black spot area
Alcohols & phenols	3279.79	3270.65	3726.71, 3703.89, 3624.69, 3268.78	3726.57, 3703.53, 3270.35, 1073.03
Aldehydes	2849.54	Not detected (ND)	ND	ND
Alkanes	2918.03, 1451.02, 1376.10	2919.66	2919.31, 1443.34, 1376.15	2919.22, 1446.12, 1374.72
Alkenes	1650.92	1631.90	1629.71, 953.20, 892.32	1632.41
Alkyl halides	1073.99	1374.99, 519.05	1255.30, 1070.28, 1026.44	1253.80, 1158.82, 1073.03, 1026.97, 534.08
Alkynes	ND	ND	2161.27, 2142.28, 953.20	2161.27
Aromatic groups	ND	1540.84, 1444.45	1512.72	1515.82
Methylene	ND	ND	1026.44	2919.22
Carbonyl groups	ND	ND	ND	2161.27, 1374.72
Carboxylic acid	3279.79	1255.38	ND	ND
Nitro compounds	1541.10	ND	ND	1979.51
Primary, secondary & tertiary amines	1158.57	ND	1155.80	1253.80, 1158.82
Ester	ND	1158.16, 072.37, 1027.78	ND	ND
Ether	ND	ND	ND	1158.82
Polysulfides	ND	ND	482.32	ND
Aryl disulfides	458.65	459.99	459.57	460.49

Ventral black spot area showed the peaks at 3726.57, 3703.53, 3270.35, 2919.22, 2161.27, 1979.51, 1632.41, 1515.82, 1446.12, 1374.72, 1253.80, 1158.82, 1073.03, 1026.97, 534.08 and 460.49 cm^{-1}. Functional groups identified were alcoholic weak O-H stretch, methylene symmetric C-H stretch, symmetric carbonyl compounds, presence of transition metal carbonyls with symmetric stretch, N multiple-NCS isothiocyanate symmetric stretch, alkenyl C=C stretch, olefinic alkene, secondary amine N-H bend, open chain imino and azo group, aromatic C=C medium stretch, carboxylate C-H alkane bending, phosphorus oxy compounds P=O stretch, aliphatic nitro compounds, alkyl halide C=F stretch, amine C-N medium stretch, ether C-O weak stretch and aryl disulfide S-S symmetric stretch.

C-H stretching and bending is the property of alkanes and is the most predominant hydrocarbon present in butterflies. The structure of alkanes acts as a barrier to water and is well suited for hydrophobic property (Drijfhout *et al.*, 2009). In present study the observed absorbance spectra bands at 2918.03, 1451.02, 1376.10 (dorsal white), 2919.66 (dorsal black spot area), 2919.31, 1443.34, 1376.15 (ventral white), 2919.22, 1446.12, 1374.72 cm^{-1} (ventral black spot area) were assigned to be alkanes (Table 1). Alkanes are embedded to alkenes in the outer surface layer of cuticle. The length of long hydrocarbon chain on the either sides of double bond and the angle formed by the double bonds in alkenes renders the molecule more specific (Rundel *et al.*, 2005). Here, the spectral bands 1650.92 (dorsal white), 1631.90 (dorsal black spot area), 1629.71, 953.20, 892.32 (ventral white), 1632.41 cm^{-1} (ventral blackspot area) are assigned to alkenes (Table 1). Absorbance spectra band at 2161.27, 2142.28, 2161.27 cm^{-1} represented alkynes. Alkynes are observed in ventral region only. A lkanes, alkenes and alkynes are important for nest, mate and egg recognition (Martin and Drijifhout, 2009). The second predominant compound observed were alkyl halides, alcohols and phenols useful for insect communication and gender recognition (Schlick- Steiner *et al.*, 2006). Here, the spectra band at 1073.99 (dorsal white), 1374.99, 519.05 (dorsal black spot area), 1255.30, 1070.28, 1026.44 (ventral white), 1253.80,1158.82,1073.03,1026.97,534.08 (ventral black spot area) cm^{-1} belonged to alkyl halides (Table 1). Alkyl halides are compounds that carbon group banded with halogen, and also they function as an anti-aphrodisiac agent (Chung and Carroll, 2015). O-H stretch represent alcohols and phenols. Here the spectra band obtained for alcohols and phenols are 3279.79 (dorsal white), 3270.65 (dorsal black spot area), 3726.71, 3703.89,3624.69, 3268.78 (ventral white), 3726.57,3703.53, 3270.35, 1073.03 (ventral black spot area) cm^{-1}. These components are involved in communication, nest-mate recognition, task-specific cues, dominance and fertility cues, chemical mimicry, mate selection and kin recognition in butterflies (Rundel *et al.*, 2005). By controlling the trans-cuticular water flux hydrocarbons in cuticle gives protection against desiccation (Martin *et al.*, 2004).

Carboxylic acids in cuticle mainly include amino acids and fatty acids (Archana *et al.*, 2022). The carboxylic acids in insect's cuticle act as anti-desiccation agent and water proofing agent (Chung and Carroll, 2015). The spectra band obtained for carboxylic acid were 3279.79 and 1255.38 cm^{-1}. Nitro compounds showed spectral bands 1541.10 (dorsal white) and 1979.51 (ventral black spot area) cm^{-1}. Amines were observed with bands 1158.57 (dorsal white), 1155.80 (ventral white), 1253.80, 1158.82 (ventral black spot area) cm^{-1}. The spectra bands 1158.16, 1072.37 and 1027.78 cm^{-1} were assigned to ester, found on the dorsal black spot area only. The spectrum 1158.82 cm^{-1} was assigned to ether, found only on the ventral black spot area (Table 1). These hydrocarbons exhibit natural variation in insects and are used as a taxonomic tool. Species can be discriminated by their hydrocarbon profiles which are even morphologically indistinguishable (Schlick-Steiner *et al.*, 2006).

Conclusion: The FTIR has a major role in exploring the chemical nature of *L. nina* forewing. The study revealed the presence of important functional groups. The stretches and bends of molecules determine the increasing and decreasing of bond length and angle between the molecule, respectively, which indicates the nature of particular group present in the area. Alkanes, alcohols and alkyl halides contribute as major groups. Other groups identified are aldehydes, amines, polysulfides, aryl disulfides ester and ether. The present study is the first to describe the chemical composition of *L. nina* forewing scales. There is considerable potential for the creation of biomimetic materials inspired by the functional groups found within butterfly wings, emphasizing their colouration property.

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Ethical statement: The butterfly used in this study is not endangered or protected. No permission is necessary to study these butterflies in India.

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Conflict of interest: Authors declare that there is no conflict of Interest.

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