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ORIGINAL ARTICLE

Extraction and Investigation of the Coloring Pigments Present on the Wing of Butterflies for its Development as a Potential Dye

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ABSTRACT

Dyes and pigments are the main forms of natural colorants. Dyes are soluble while pigments are insoluble and are suspended in a medium or binder. Dyes and pigments are present on the body of insects or wings of the butterflies maybe a natural good source of colorants which may be used in histological studies in the stream of life science as well as a potent natural food color in the stream of food technology as these coloring pigments are extracted frombutterflies. Therefore, most probably these color or dies would be used as a safe and almost nontoxic food color for ourselves as it is carbohydrate in nature, usually chitin. The dyes can be solved in a solution, whereas the pigments are insoluble in a medium or binder, it results into the formation of suspension rather than a solution. This is due to the differences in particle size of dyes and pigments.

Keywords: *Lepidopetera, appendage, scales and bristles (macrocheates) pigments, suspension, carmine, synthetic pigments, natural stain*

INTRODUCTION

Lepidoptera is derived from ancient Greek word (lepidos) 'scale' and (ptera) 'wings' as anorder of insects that includes butterflies and moths. In ancient era butterflies and moths are known to be a good source of natural colors and dyes (Vukusic *et al*. 2000). These insects play a key role in the maintenance of environment as food in the food chain as well as pollinators in plant reproduction. Many moths and butterflies species are of extremely economic interest due to their role as a source of textile thread and natural stain and dyes (Sandberg 1997), as for example, Various stains and dyes are produced from insects such as carmine from *Dactylopius coccus* (Stathopoulou *et al*. 2013) which is used in cytology for the study of chromosomes, apart from these, various stain and dyes are used in other histopathological studies such as kermes dyes produced from an scale insect *Kermes vermilio* that thrives on the sap of *kermes oak*, *Quercus coccifera*.

The carmine dye was used in North America for coloring fabrics and became an important export item in textile industries during the colonial period after the invention and evolution of synthetic pigments and dyes such as alizarin in the late 19th century, natural dye production gradually diminished. In recent era health fears over artificial food colors and additives, however, renewed the popularity of insect dyes, and the increased demand has made cultivation of insect profitable again as butterflies get their wing color due to the presence of pigments on their wing structures. The ordinary color comes from chemical pigments that absorb certain wavelengths of light and reflects other as butterflies that appears green absorb the red and blue wavelengths of light and reflects the green, that's why when the light reflects back it seems to be green. Considering the above facts it seems to be very important to investigate the probability of extraction of food color from the butterflies.

MATERIALS AND METHODS

Butterflies (specimens) of different colors (3) were collected from natural colorful vegetation area having multiple flowering plant population by the help of aerial nets (butterfly net). The collected butterflies were separated into three separate groups viz. Black (*Papillio polytes*) Group I, Brownish orange (*Junonia almana*) Group II, mustard yellow (*Eurema smilax*) Group III, and kept into a transparent plastic container with emerging site. The above mentioned each group of butterfly container were mildly fumigated with chloroform to anaesthetize the insect. The anesthetized insect were kept out from the container and placed on the glass top of work table separately at a distance of approximately 1feet (ft) to avoid the intermingling of pigments present on the wings of different groups ofthe butterflies.

Group I Group II Group III

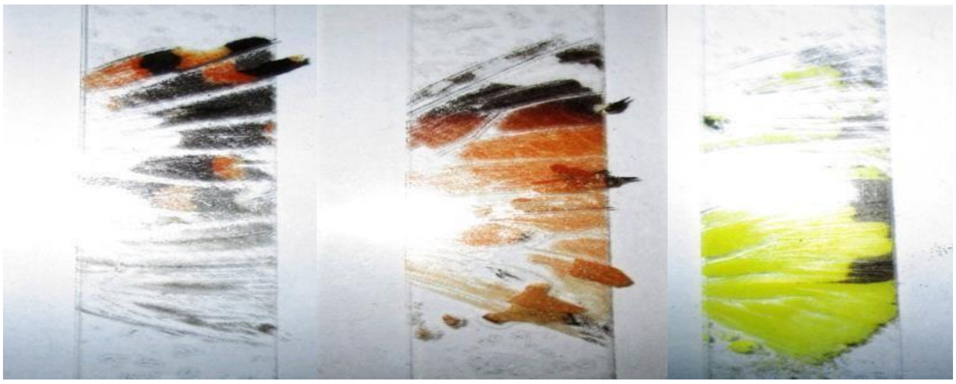
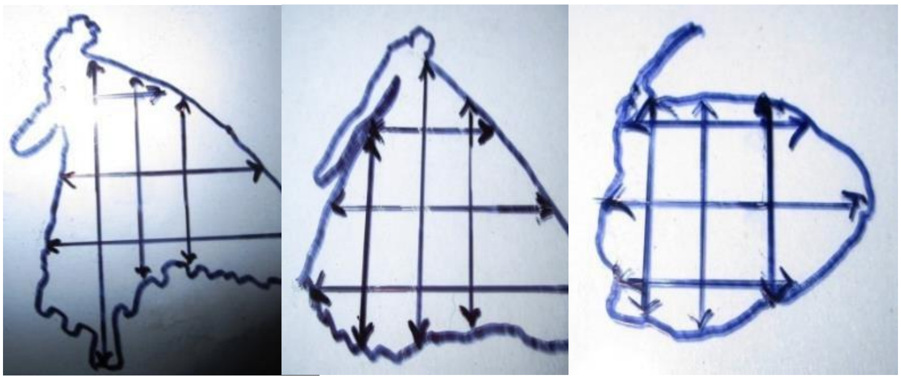


Photo 1: Photographs of the wing slides (Group I, Group II and Group III)

Thereafter the pigments were picked up in the form of color bands with the help of a piece of transparent stick films by holding the ends with the help of thumb and index finger of both the hands and sticking it gently on the wingand the pigment bands are fixed on a glass slide by fixing the transparent stick films on the glass slide. In this way color bands for three different groups of butterflies were prepared for the sake of further experimental analysis.

DETERMINATION ON SURFACE AREA OF THE WING

Further the different group of wings are covered with a glass slide separately which measure about 5”× 3.5” (inches) and wing area margins were traced with the help of a glass marker, thereafter each traced wing samples were measured longitudinally and vertically (average length and breadth).



Group I Group II Group III

Photo 2: Photographs of the traced slides (L=Longitudinal and V=Vertical)

The surface area of wing for each sample groups was calculated as following:-

Group I: **Average Length (L)= L_1 +L_2 +L_3 = 4.7+ 3.2+ 2.7 =10.6/ 3 = 3.53**
Average Breadth (B)= B_1+ B_2+ B_3= 2.2+ 3.3+ 4.1 =9.6/ 3 =3.2
Total surface area of the wing= L × B =3.53 × 3.2= 11.296

Group II: **Average Length (L)= L_1+ L_2+ L_3 =2+ 2.9+ 2.3 =7.2/ 3=2.4**
Average Breadth (B)= B_1+ B_2+ B_3 =2+ 3.3+ 4.1 =9.4/ 3 = 3.1
Total surface area of the wing=L× B =2.4×3.1 = 7.44

Group III: **Average Length (L)= L_1+ L_2 + L_3 = 1.7+ 1.8+ 1.6 =5.1/ 3= 1.7**
Average Breadth (B)= B_1+ B_2+ B_3 =1.7+ 2.2+ 1.5 = 5.4/3 =1.8
Total surface area of the wing= L× B =1.7×1.8 = 3.06

Isolation and extraction of pigments selected through pigment bands: The selected wing specimens i.e. Black, Brownish orange and Mustard yellow were separately placed on a glass top and then selected pigmented area of different wing specimens were carved out from a fine pointed scissors from each three groups of samples. Thereafter these pigmented wing parts of different colors were separately placed in glass vials (approximately 5cm in length and 2 cm in diameter), containing acetowater (D.W and Glacial acetic acid 9:11) and agitated on a vortex shaker for a while, two to three times. The above mentioned vials containing wing pigments were kept for one weekat room temperature after that i.e. after one week each vial were again agitated on a vortex shaker and kept for a while to settle the wing particle.

Thereafter the solution containing pigments of separate color i.e. Group I (sample 1st Black), Group II (sample 2nd Brownish orange) and Group III (sample 3rd Mustard yellow) were decanted in respective properly labeled new glass vials with the help of a micropipette. The remaining wing particles were drawn out from respective vials and dried separately in between the folds of filter paper and then weighed again.

The vials containing pigment solution were subjected to direct heat over the flame till boiling and change in color and then stored away from the light and heat for further processing to use it as a stain.

RESULTS AND DISCUSSION

The extracted butterfly wing's pigments were quantified in the present work and the qualitative biochemical characteristics would be done further. The quantitative analysis was listed in Table 1.

Table 1: Quantitative analysis of butterfly extracted pigment

Specimens	Net weight of wings	Wing weight after extraction	Difference	Weight of pigment
SI^B (Black)	10 mg	0.003 mg	9.997 mg	9.997 mg
SII^B (Brown orange)	10 mg	0.002 mg	9.998 mg	9.98 mg
$SIII^Y$ (Yellow)	0.05 mg	0.002 mg	0.048 mg	0.048 mg

The difference in weight is actually the pigments isolated from the wing is depicted in difference column in the above quantization table. Staining technique is variously used to enhance contrast in specimens, normally at the microscopic level. Stains and dyes are frequently used in the study of biological tissues and cells as well as biomedical fields, such as histopathology, hematology and cytopathology, which is extremely helpful in diagnosis of the diseases. Apart from these in the field of biochemical studies, it is also very helpful in the visualization of DNA, Proteins, Lipids, which are classspecific stain, dye to a substrate for qualitative or quantitative analysis.

As the stains are also used for dying living tissues in vivo for medical diagnostics, what we call vital staining or intra-vital staining are also very useful. The preparatory steps of stain or dyes depending upon the type of planned analysis and some of them are chemical agent which extenuate the stains or dyes and have the power to stain the materials. This work is an effort to isolate and extract three pigments to evolve it as a potent food color which is non-toxic for living tissues.

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