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Extraction of crimson pigment and Biofuel (Insect oil) with Antimicrobial activity from *Dysdercus koenigi* (cotton stainer)

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Abstract

Dysdercus koenigi is an hemipteran insect which is a common pest of the cotton crop. This is cosmopolitan in distribution and has been declared as a pest globally. Apart from infesting the cotton crops, it is also known to infect other plants belonging to Malvaceae family. The insect is often found as male and female in copulatory position. They reproduce quickly and hence their population is found in large numbers in any kind of terrain. Majority of the times, it is eliminated using effective pesticides or insecticides. Literature review on other insects revealed, that certain species of insects and larvae which when processed appropriately yielded oil from crude extracts, and those oils had anti microbial activity. These studies and observations, prompted me to study and explore the untapped potential of this insect pest, *Dysdercus koenigi*. In the present study, approximately 100-200 numbers of *Dysdercus koenigi* were handpicked from Chennai terrain from university of madras, Guindy campus. The insects were processed as per the standard protocol. Three different commercial products have been extracted, isolated and analyzed. A light colored crimson, dye was extracted using aqueous extraction method. Insect lipid fraction was extracted in the form of oil, using Hexane as the solvent. From 7 grams of insect weight, 7 ml of oil was extracted. Further, this oil was tested if it could be used as a fuel to light a lamp. A lamp was lighted using this oil, which burnt successfully for 10 mints, suggesting that *Dysdercus* oil can be used as biodiesel. In addition to this, it was intended to test if the oil possessed any antibacterial activity. Therefore the oil, which was extracted, was tested *in vitro*, for anti bacterial activity using well diffusion method. The oil was found to be effective against the following bacterial species, *viz* ***Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Enterococccus*, *Pseudomonas aeruginosa*,** confirming its antibacterial properties. The minimum inhibitory concentration (MIC) was found to be 150µL. This study was successful in unleashing the hidden and unknown potential of *Dysdercus koenigi* as most economically valuable insect rather than a pest insect.

Key words : *Dysdercus koenigi,* biofuel, antibacterial activity, crimson colored dye, cotton pest.

D*ysdercus koenigi*, is an hempterian insect belonging to the family *Pyrrhocoridae*. This is a common pest attacking the cotton bolls, as it causes red colored spots on the pods and hence commonly called as cotton stainer. *Dysdercus koenigi* grows to a length of 12 to 18 mm in length. The adults and the nymphs of this insect cause staining of the cotton bolls, because of the fungi they carry and cause extensive damage to the cotton crop. It is also reported to cause damage to crops belonging to Malvaceae, solanaceae and cucurbitaceae[9]. Majority of the studies and available scientific literature about *Dysdercus koenigi*, suggests that it is a serious pest of majority of crops[6]. A detailed review of literature suggests that *Dysdercus* either as nymph or as an adult is known to infest vegetable crops, ornamental crops, oil seeds, cereals and also citrus fruits.[7,8,10,11]. As these insects are frequently found in large numbers, it suggests that they are quite successful in evolution, owing to their high adaptive ability, which may be due to their unusual gut micro biota or may be because of high reproductive capacity. When an extensive survey about different insect pests and insect larvae was performed, it revealed that there are instances, where certain insects and their larvae were exploited to extract valuable oil which had anti microbial activity. Insects such as *Aspongopus vidiuatus* (melon bug) and *Agonoscelis pubescens* (sorghum bug) were used to extract oil and these oils had some valuable properties. Similarly, insect species of *Hermetia illucens Larvae and Bombyx mori were* also used to extract oil and were tested for their anti microbial activity[12]. These studies generated an interest and prompted me to take up this study. about *Dysdercus* Based upon these observations, it was hypothesized that, *Dysdercus koenigi*, which is a cotton pest, and a hardy insect may seem to possess certain valuable constituents which may be extracted and exploited commercially.

Aqueous Extraction of colored dye from dried insects (Dysdercus koenigi) :

Approximately 10 gms of insects were collected from the local terrain *i.e.* from **University of Madras Guindy campus Chennai.** Most of the time both male and female insects were seen joined or were found attached together as illustrated in fig I and II. After being collected the insects were thoroughly washed in clean water were killed using hot water. The insects were homogenized using distilled water using motor and pestle. The light color pigment from the insect body was extracted. This homogenized extract was filtered using **What man** filter paper. (grade-I) The pigment obtained was light crimson in color and was collected in to a bottle. The yield of the pigment was approximately 5ml.

Preparation of Insect extract for antibacterial activity :

2.25 g of the insect sample was soaked in 50 ml of hexane for overnight 7-8 hours. The extract was filtered into a pre weighed beaker and concentrated over a water bath using rotatory evaporation under reduced pressure (100 mbar) and reduced temperature 55°C. The obtained extract is used for further antibacterial activity.

Preparation of Mueller-Hinton Agar (For bacteria) :

Mueller-Hinton agar preparation

includes the following steps.

1. Mueller-Hinton agar should be prepared from a commercially available dehydrated base according to the manufacturer instructions. Immediately after autoclaving, allow it to cool in a 45 to 50°C water bath and Pour the freshly prepared and cooled medium into glass or plastic, flat-bottomed petridish on a level, horizontal surface to give a uniform depth of approximately 4 mm. This corresponds to 25 to 30 ml for plates with a diameter of 100 mm. The agar medium should be allowed to cool to room temperature and, unless the plate is used the same day, stored in a refrigerator (2 to 8° C) and a representative sample of each batch of plates should be examined for sterility by incubating at 30 to 35°C for 24 hours or longer.

Preparation of inocula :

To standardize the inoculum density for a susceptibility test, a BaSO 4 turbidity standard, equivalent to a 0.5 McFarland standard or its optical equivalent (*e.g.*, latex particle suspension), should be used. Then the inocula of bacteria were prepared from the 12 hrs broth cultures and standardized to 10 8 Cfu/ml. The infective dose for most organism are 10 5 Cfu/ml.

Moisture :

If, just before use, excess surface moisture is present, the plates should be placed in an incubator(35°C) or a laminar flow hood at room temperature with lids ajar until excess surface moisture is lost by evaporation (usually 10 to 30 minutes). The surface should be moist, but no droplets of moisture should be apparent on the surface of the medium or on the petridish covers when the plates are inoculated.

Agar well diffusion method :

The antibacterial studies of the Animal extract were carried out by Agar well diffusion method.

The crude insect extract was dissolved in Hexane to get a refined extract :

Cefepime CPM 30 mcg were used as a standard drug (Bauer- Kirby method) for this test. The antibacterial activity was evaluated by employing 12-18 hrs cultures, of *Staphylococcus aureus*, *Klebsiella pneumoniae, Escherichia coli*, *Entero cocccus*, *Pseudomonas aeruginosa* using Muller - Hinton agar medium. Wells (6mm) are made into the agar plate with sterile well borer. 25µl, 50µl, 75µl, 100µl of the test sample and 25µl of the standard solution were transferred to the wells in the microorganism inoculated plates aseptically and labeled accordingly. Sterile distilled water act as a negative control. Then the plates were maintained at room temperature for 2 hrs enabling the diffusion of the solutions into the medium. The petriplates were incubated 37°C for 24 hrs for antibacterial screening. The diameters of the zone of inhibitions were measured by measuring scale in millimeter (mm). At least three replicates were carried out for the extract against each of the test with the organisms.

Extraction of oil from insect, Dysdercus koenigi :

7gm of the insect sample was soaked in 155.5 ml of hexane for overnight 7- 8 hours. The extract was filtered into a pre weighed beaker and concentrated over a water bath using rotatory evaporation under reduced pressure

Fig. 1. (Male and Female insects)

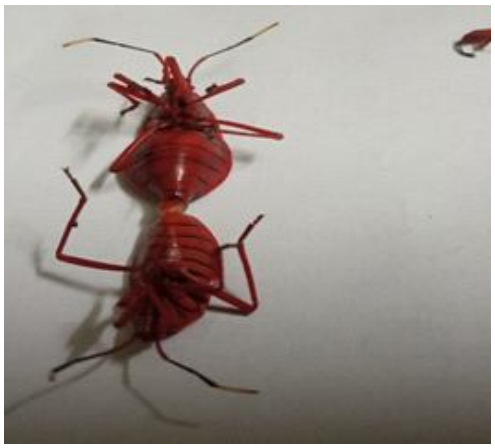

Fig. 2. (Male and female joined on the ventral side)

Fig. 3. (Crimson Dye / pigment)

Fig. 4. (Crimson Dye on the fabric)

Table-1. Anti bacterial activity of the Insect extract inhibitory activity in mm with standard

Name of the Organism	50 µl (l00 mg ml)	l00µl (100 mg/ ml)	l50(µl (100 mg/ ml)	200µl (100 mg/ ml)	Standard Cefepime disc -30mcg	Negative control (DMSO)
Staphylococcus aureus		10mm	12mm	l5mm	2mm	
Enterococcus			13mm	15mm	16mm	
Escherichia coli			12mm	I4 mm	27mm	
Klebsiellapneuimoniae			12mm	19mm	28mm	
Pseudomonas aeruginosa		11 mm	13mm	I6 mm	27mm	

Fig. 5 (Oil)

Fig. 6 (Lamp with oil)

(100 mbar) and reduced temperature 55°C. 7 ml of oil was extracted from 7 grams of the insect extract. It was brown in color and sticky in nature.

In the present study, a light crimson dye was extracted using water extraction method. (Fig-3) The dye may be called as Chennai, dye as it was extracted from the insects of Chennai terrain. The dye was used to color the fabric and the dye was found to stable. (Fig-4) Further, in the present study, *Dysdercus* oil was extracted using hexane. This oil was deep brown in color (Fig-5) and was used for lighting a lamp (Fig-6) and also its antimicrobial properties were tested, whose results have been illustrated in the table-1.

The present study on *Dysdercus koenigi* yielded fruitful results. This insect which has gained a pest status, is often discarded or eliminated using pecsticides, but the present study, had illustrated the beneficial side of the insect. As hypothesized, I was successful in extracting the light cromson pigment, and oil from the crude insect extract. This study is first of its kind, where an insect pest, which is frequently found in huge masses, in urban landscape has been extensively reserached upon. It was quite interesting to note that the extracted oil from this insect exhibited antibacterial activity. In the present study, *Dysdercus*, extract, showed antibacterial effect upon *Staphylococcus aureus*, *Klebsiella pneumoniae, Escherichia coli*, *Entero-cocccus*, *Pseudomonas aeruginosa.* Though different concentrations, were used, 150µL was considered to be the minimum inhibitory concentration, (MIC) which was found to be effective against all the bacterial strains. These results coincide with the observations, of Saviane *et. al.*,[12], who have reported antibac-terial activity of oil extracted from *Hermetia illucens* (black soldier fly and *Bombyx mori* (domestic silkworm).Similarly the studies

of Basma *et.al.*,[2] who have reported antibacterial and anti cancerous properties of *Periplaneta* extract, (cockroach) suggest that insects can be considered as new sources of natural antibacterial agents. Based upon these evidences, it may be suggested that the insect extract of *Dysdercus cingulatus* may be considered to be used as a natural antibiotic.

Well this is one aspect about the oil extracted from *Dysdercus koenigi*, however the other facet that remains unexplored, which was not attempted in the present study, is the processing of bio fuel using this oil. An impressive study by Abdalbasit Adam Mariod[1] had reported that oil which was extracted from *Aspongopus vidiuatus* (melon bug) and *Agonoscelis pubescens* (sorghum bug) was processed to synthesize biodiesel, suggesting that insects can be looked up as a new source of oil and biodiesel. Apart from these benefits, there are instances where in insect fat and oil are also being considered for preparation of oleogels and oleogelators, which can be used in food industry[5]. In view of these studies, it may be said that further experimentation may be considered before *dysdercu*s can also be recommended to be used in food industry. Additionally in the present study, a light crimson pigment/dye also has been extracted, which was used to color the fabric, and this pigment was found to quite stable even when the fabric was washed with detergent. This observation coincides with that of Gao *et.al.*,[3] who have extracted, isolated and identified a pigment, sepiapterin, from *Bombyx mori,* which may be used for treating BH4 deficiencies. An exhaustive review by Gulsaz *et. al.,*[4], about insect pigments, illustrates the use of various insects, for extraction of different pigments, which can be used for diverse purposes. From these studies, it is quite evident that insects, which may have been declared as pests, can still be beneficial because of their attributes such as oil yielding capacity, or as food additives, or may also be used as a wealthy source of proteins. In conclusion, this study has been quite successful in exploring and unleashing some of the undiscovered traits of *Dysdercus* which are quite valuable. Products, which have been obtained from this study, such as natural crimson dye, crude oil can be used in industrial applications and also its antibacterial properties may be exploited in preparation of a medicated ointment or as a gel which in future will be attempted and scaled up for commercial purposes. An Indian patent has also been filed in this regard.

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