Original Research Article

Identification of Cuticular Pheromonal Compounds from Leaf Beetle, *Alticini sp*.

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ABSTRACT

The development of a new technique aside from insecticide spraying was necessary to control the insect pest population of agricultural crops. Insecticides are the cornerstones upon which pest management practices are based in the current civilization of farmers and are likely to remain so as long as effective and inexpensive chemicals are available. However, many insect-pest management techniques using Non-lethal mechanisms for insect pest control have been successful. Therefore the study on the feasibility of disrupting communication using synthetic sex pheromone is the need of the hour. These techniques include the use of pheromones to disrupt mating. Hence the present study is focused to identify the pheromonalcuticular compounds using GC-MS, SDS-PAGE and MALDI-TOF matrix analysis. In the GC-MS analysis we found 39 compounds and the compounds such as octadecane, heptadecane, undecane, dodecane and heptacosane were reported as pheromone carrier protein in earlier studies.

Key words: Insecticides, Pest population, Agricultural crops, Sex pheromone, Cuticular compounds.

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INTRODUCTION

Flea beetles are common pests throughout the world that attack plants families Brassicaceae (e.g., broccoli, kale, cabbage, collards) and Solanaceae (e.g., potatoes, tomatoes, eggplant, peppers). Both larval and adult stages have chewing mouthparts, which they use effectively for the destruction of plant parts. Below- and above-ground feeding damage can kill seedlings and small transplants. In addition, scars on tubers from below-ground feeding can reduce marketability, while scars on a variety of foliage from above-ground feeding may render produce unmarketable. Flea beetle feeding damage can sometimes lead to total crop loss. Flea beetles are highly mobile, which makes control difficult.[1]

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Rapid population growth, together with a high emphasis on achieving food grain self-sufficiency has compelled Indian farmers to resort to the substantial use of pesticides. It is estimated that more than 100,000 tons of DDTs has been applied in India alone, primarily for agricultural use and malaria eradication programs, due to their low cost and broad spectrum toxicity, making them effective in the control of pests and diseases.[2-5] The use of pesticides has increased tremendously since the time when they were successfully deployed in strategies to increase crop productivity. The quantity of pesticides sold worldwide to the agricultural sector had reached over 1.3 million metric tons of active ingredients by 1995 (FAO). Of this amount, 295 thousand metric tons (about 23% of the 1995 total sales) was attributable to insecticides. The use of chemical pesticides has provided a valuable aid to agricultural production, increasing crop protection and yield. However, the discovery of pesticidal residues in various sections of the environment has raised serious concerns regarding their use; concerns which well outweigh the overall benefits derived from them.[6,7]

Pheromones are species-specific chemicals that affect insect behavior, but are not toxic to insects. They are active (e.g. attractive) in extremely low doses (one millionth of an ounce) and are used to bait traps or confuse a mating population of insects. Pheromones can play an important role in integrated pest management for structural, landscape, agricultural or forest pest problems.[8]

The term sex pheromone is used to include all chemicals that are released from one organism and that induce responses, such as orientation, precopulatory behavior and mating, in another individual of the same species.[9] According to Mason and Jansson[10] stated that confused mating behavior of males by the synthetic sex pheromone is effective for controlling pest population in different fields. They determined that mass trapping with the pheromone is an effective method to control pest in the field. Jones *et al.*[11] studied the sex attractant produced by the virgin female pink bollworm moth by isolating it in pure form and identifying it as 10-propyl-trans- 5, 9- tridecadienyl acetate. They later successfully synthesized the compound by confirming the structure of the compound and makes possible practical use to control the destructive pest of cottonin the common name "propylure".

MATERIALS AND METHODS

Sample collection

The insects were collected from paddy field of Perumacherry, Virudhunagar District, Tamil Nadu. Female insects were identified by seeing the presence of anal cercus and anala style in the IXth and Xth tergum segment. Collected insects were stored in a plastic container. The collected insects were maintained in a clean plastic container. They are cleaned with detergent and 70% ethanol. For the rearing of insect, honey and water was provided in cotton at the corners of the container.

Extraction of cuticular pheromonal compounds

For the extraction of cuticular pheromonal compound using GC-MS 30 insect were taken in a 10ml screw cap test tube containing 3ml of hexane (100µl/insect).Then the tube was stored in the freezer for one day. After one day of incubation in the freezer, 1.5ml of sample was taken from the tube for analysis (Leonhardt *et al.*)[12]

Protein estimation by Bradford

The sample was prepared by dipping the insect in the Phosphate Buffered Saline (PBS) and stored in the refrigerator. Then the sample was taken from the above mixture and the protein was determined by adapting the method of Bradford using Bovine Serum Albumin (BSA) as standard.

SDS-PAGE analysis

For the protein separation 30 insects were taken in a screw cap bottle. To the screw cap bottle add 2ml of phosphate buffer saline and stored in refrigerator at 4°C for further studies. To determine the protein profile of the insect cuticular pheromones on the basis of molecular weight, the SDSPAGE (12%) was carried out according to the methods of Laemmli.[13] The separated bands with low kilo Daltons were excised from the gel and given for MALDI-TOF analysis.

RESULTS

From the preliminary quantitative analysis of sample, it was estimated that the amount of protein was 6.083±0.06µg/ml in cuticular extract of experimental animal. From the result it was found that 39 compounds were found to be present in the extracted sample, the protein bands separated between 17- 22 kDa were excised from the gel and subjected to trypsinolysis for peptide mass fingerprinting (Figure 1). The mass spectrum of the 17 and 25 kDa proteins were obtained by MALDI-TOF and the mono isotopic number of mass spectra were scored and analyzed with MASCOT search. In GC-MS analysis of the sample extract it was revealed the presence of 39 compounds, such as, octadecane, heptadecane, undecane, dodecane and heptacosane. In the analysis it was found that the presence of chemosensory protein in the first hit and 8 matches were found with coverage of 10%. Polypeptides share a small size (12–18 kDa), very high solubility and a capacity of reversibly binding small molecules, such as odorants and pheromones (Plate 1). However, they are structurally very different.

DISCUSSION

The GC-MS results of our study were supported by Sugie *et al.*[14] whom isolated and identified sex pheromone of the peach leafminer moth as 14-methyl-1-octadecane. Similarly Ho and Millar[15] identified that the compounds present in meta-thoracic glands of adults and dorsal abdominal glands of nymphs of the stink bugs and the revealed the presence of hexenal, oxo-2-hexenal, octenal, octenyl acetate, oxo-2-octenal, decenal, undecane, dodecane, tetradecane and pentadecane. Gries *et al.*[16] (2002) identified the sex pheromone of *Lymantria lucescens* and *Lymantria serva* in pheromone gland extract as 2-methyl-(Z)-7-octadecene. Bierl *et al.*[17] 2014 also identified the presence of cis-7,8- epoxy-2-methylocta-

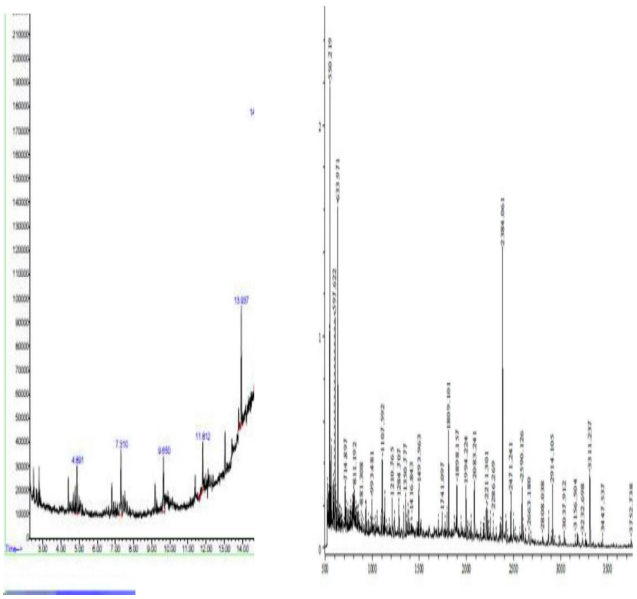

Figure 1: GCMS and MALDI-TOF peaks of sample.

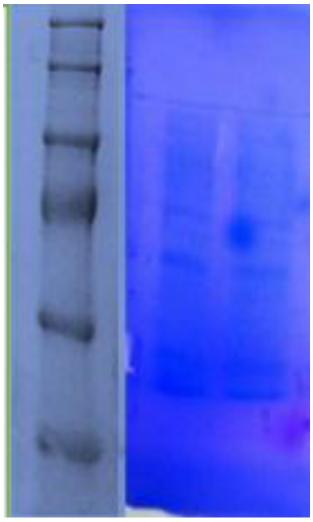

Plate 1: Electrophoresis analysis of protein bands from the sample. L1 – Molecular Marker; L2 –Sample.

decane in abdominal tips of female moth. The above studies support that hexa and octadecanal compounds were responsible for pheromone carrying lipoprotein. In MASCOT MATRIX analysis it was revealed that the Chemosensory proteins (CSPs) are believed to be involved in chemical communication and perception. A number of such proteins, of molecular mass C13 kDa, has been matched with the different sensory organs of a wide range of insect species in the online tool in the present analysis. Similarly in the present study the peptides were matched with the different sequence of CSPs of antennae and proboscis of the moth *Mamestra brassicae*.[18]

CONCLUSION

It is conclude that identifying the phermonal compounds of an species helps to attract the opposite sex of the pest to the prey and thereby reducing chance of mating in an ecosystem.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

GC-MS: Gas Chromatography Mass Spectrophotometry , **SDS-PAGE:** Sodium Dodecyl Sulphate - Poly Acrylamide Gel Electrophoresis, **MALDI-TOF:** Matrix Assisted Laser Desorption/Ionization-Time of Flight

SUMMARY

The efficacy of this research work has to be tested at field level in years to come during the days of our research destiny.

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