A STUDY TO DEVELOP A NATIVE HERBAL REPELLENT AND ISOLATION OF GUT MICROBIOME IN PESTICIDAL RESISTANCE Spodoptera Frugiperda - AN AGRICULTURAL CROP PEST IN KARNATAKA

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COLLEGE : REVA UNIVERSITY, BENGALURU

GUIDE (S) : Dr. NEETHU B K

SUDENT (S) : Ms. YAMINI T S
Ms. MONISHA N
Ms. KRISHNAVENI R
Ms. KEERTHI S

1. Keywords

Herbal plant, Insect repellent, *Spodoptera frugiperda*, microbiome isolation.

2. Introduction / background (with specific reference to the project, work done earlier, etc)

In the last few years, Indian economy has been growing at the slower than the expected rate. Lower growth of the agriculture sector may also be one of the important factors for this (Bedi, 2008, World Banka,b). Various crops such as cereals, oilseeds, pulses, vegetables, and fruits are severely damaged by various diseases, insects, and pests. Farmers are forced to use large quantities of chemical pesticides to protect their crops. The Indian agriculture is currently suffering a loss of about INR 8,63,884 million annually due to insects and pests alone (Dhaliwal et. al., 2010). Accounting for15% of the total global vegetable production (over 90 MT), India stands at the second position in the world after China. The area under vegetable cultivation is about 6.2 million hectares, which is 3% of the total area under cultivation in the country. Major vegetable crops grown in the country are tomato, onion, brinjal, cabbage, cauliflower, okra and peas. Vegetable plants are known to suffer heavily due to infestation of sucking pests, shoot and fruit borers and chewing pest. Aphids (Aphis gossypii Glover),Jassids (Amrasca biguttulabi guttula Ishida) and white flies (Bemisia tabaci Gennadius) suck the cell sap and prohibit normal crop growth. Besides direct damage, these sucking pests also act as vectors for various viral diseases. Amrasca biguttula biguttula (Ishida) (Homoptera: Cicadellidae) is an important sucking pest that attacks most vegetable crops (Munde et al. 2011) and lays maximum number of eggs on vegetables, which are suitable places for their survival and feeding (Bernardo and Taylo, 1990; Sharma and Singh, 2002).

Insect pests cause harm to humans, farm animals, and crops. They are responsible for one-fifth of the world's total crop production each year. Significant agricultural product losses impose a significant burden on people's daily lives. There is an urgent need to shift towards natural products as insecticides because they have fewer negative effects on human health and the

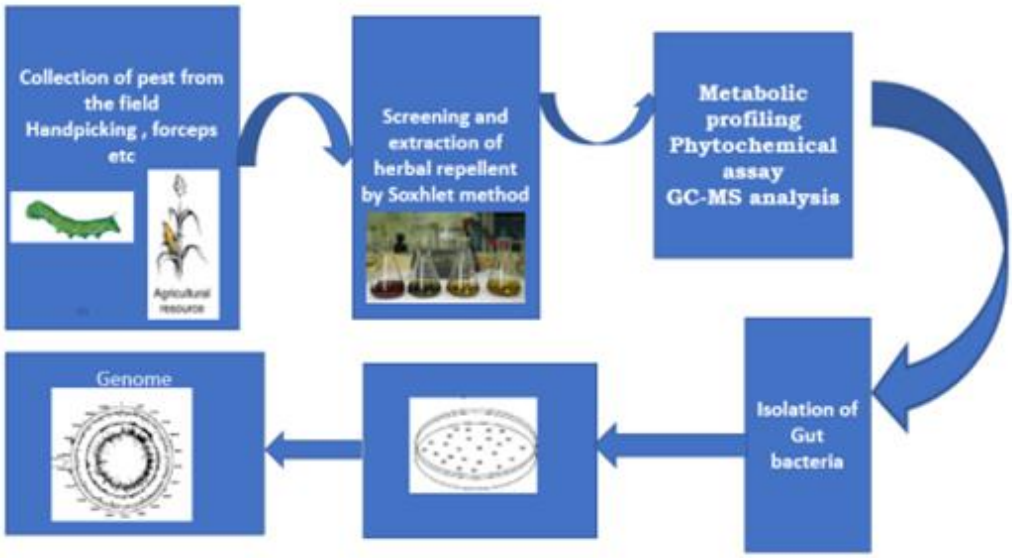
environment, as crop pests are invading the crop like Sorghum, jowar and causing major loss in the crop yield because of developing the resistance to the pesticide sprayed it is necessary to sustain the soil fertility and ecosystem from spraying such harsh chemicals, which in turn is polluting the ecosystem.

The present investigation is to develop effective herbal plant insect repellent against pesticidal resistant crop pests and saving the environment and ecosystem from the anthropogenic agents which can cause harm to the nature and ecosystem. The yearly increase in the cost of pesticides has gone out of the reach of a common farmer. Further, the irrational use of chemical pesticides has caused serious health hazards and environmental problems in various countries, including India. Vegetables picked at short intervals are more prone to have toxic residues.

3. Objectives

- Collection of crop pest from the agriculture field and study of Lifecycle.
- Screening and extraction of native herbal plant repellent and evaluating the LC25 and LC50.
- Metabolic profiling of the extract i.e., phytochemical assay and (GC-MS analysis).
- Isolation/Screening of Gut microbiome in pre adult stages of *Spodptura frugiperda*.
- Isolated pesticidal resistance and repellent bacteria activity by sequencing.

4. Methodology (about 30 lines) (materials, methods, details of work carried out, including drawings, diagrams etc)



Pest collection:

Pest larvae collection of the *Spodoptera frugiperda* from cabbage/Sorgum fields in karnataka. Mix all collections into one stock colony because of no geographic differentiation in this species from these sites. We reared all individuals on cabbage leaves spread evenly across five screen cages (35 × 35 × 15 cm) in growth chambers at constant temperature (25 ± 1 °C) with 15-h light:9-h dark photoperiod, and relative humidity set at 60 ± 10%. To further enlarge the population for our experiments, we reared these insects in the artificial diet in plastic boxes at 25 ± 1 °C. Developed into 3rd or 4th instar larvae, pupae or adults, they were exposed to 10 °C for 24 h (to simulate gradually reduced temperatures in late autumn and allow a thermal acclimation) just before they were placed to the low-temperature regimes for overwintering tests.

Collection of Plants:

A survey of relevant literature on plants used as repellents against crop pests. On screening assay were able to finalise with four different plant species. Basil (*Ocimum basilicum*), Dill (Anethum graveolens), Eucalyptus(*Eucalyptus teriticornis)* and peppermint (*Mentha × piperita)*

Preparation of Plant Extracts

The leaves were collected, for each extraction procedure, 500 g of powdered plant material was extracted with methanol universal solvent. The solvent from the extracts were removed using a rotary vacuum evaporator to collect the crude extract. Standard stock solutions were prepared at 1% by dissolving the residues in acetone. After maceration, the results are filtered, and then the macerate is concentrated at 400^{o}C - 500^{o}C in a vacuum evaporator so that 100 grams of concentrated extract of leaves are produced with a concentration of 100%. Then the concentrated extract was diluted with distilled water for different concentration.

Phytochemical assay

The methanol extracts of Basil, Marigold and Leucas aspera were submitted to the qualitative phytochemical screening tests to identify some anti-insect phytochemical compounds including alkaloids, flavonoids, saponins, tannins, polyphenols, and terpenoids which, according to the literature, possess an insecticidal property. The methods performed by Prashant et al. were performed to determine these phytocomponents.

GC MS Analysis

GC–MS analysis of the crude extracts of whole plants was carried out on Skanda technologies which comprised of an auto sampler and gas chromatography interfaced to a mass spectrometer (GC–MS) instrument employing the following condition: capillary column – 624 ms (30 m × 0.32 mm × 1.8 m) operating in an electron mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1.491 ml/min and injection volume of 1.0 ml, injector temperature of 140 °C; ion source temperature of 200 °C. The oven temperature was programmed for 45 °C. Mass spectra were taken at 70 eV.

Screening of Gut microbiome:

Scanning of plate TLC plate was dried in flowing air at room temperature. Densitometric scanning was carried out using Camag TLC Scanner III (Camag, Muttenz, Switzerland) between wavelength of 200-450 nm with a slit dimension of 6.00 × 0.30 mm, with scanning speed of 20 mm/s, and data resolution was at 100 µm/ step. The source lamps for radiation were deuterium and tungsten lamps. All remaining measurement parameters were left at default settings. The chromatograms were integrated and regression analysis and statistical data were generated using Win CATS evaluation software (Version 1.4).

Expected Results

Results from previous work on herbal repellents, on crop pests has indicated a significant control in the pest attack. The herbal pesticide higher concentration exhibited reduction in the larval population (77%) on crop pests as compared to the untreated control. Per cent damage in the crop pest's treatment was at par with the chemical control. It was found to be effective in controlling the pest (sucking and fruit borer). Results of trials conducted on tomato plants showed that there was a significant reduction in the population of insects after spray. The herbal pesticide was found to have deterrent action against white flies (Bemisia tabaci) and other pests, which reduced damage to the tomato plants and the fruits. This resulted in the higher yield of tomato as compared to untreated control plots. There was an increase of more than 30% in the yield of tomato as compared to untreated control plots.

Conclusions: The irrational use of chemical pesticides has caused serious health hazards and environmental problems in developing countries including India. The herbal repellent developed to prove efficacy in controlling crop pest such as bollworms. The value-added herbal repellent provides an affordable and sustainable environment friendly solution for enhancing productivity of the small farmers. Excessive use of chemical pesticide creates many serious threats such as elimination of beneficial predators and insects, depletion in soil microbial diversity, resistance among the pests and diseases, deposition of toxic residues, etc. They found that the chemical pesticide results in the destruction of various beneficial microbes, flora & fauna and causes serious diseases in human. Therefore, there is a great need to develop green and cheaper alternatives for handling important pests.

Scope for future work

This process of developing a value-added product (based on herbal plant repellent innovations) is an unique example of land-to-lab-to-land approach. There is a great need to develop and promote such approach and green formulations at a larger scale.