STUDIES ON INSECTICIDAL PROPERTIES OF SELECTED PLANT EXTRACTS

FINAL REPORT OF MINOR RESEARCH PROJECT

Submitted to

UNIVERSITY GRANTS COMMISSION

South Western Regional Office BANGALORE

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SUMMARY OF THE FINDINGS

The study was to evaluate the insect repellent activity of certain plants and to compare the sensitivity of certain insects towards selected essential oils, composite light and a few colors. While on the experimental arena, chemical and photo sensitivity tests were carried out using a ‘modified T-maze’ called as ‘A-B sensitivity apparatus’. Plant parts were extracted using methanol-water and ethyl acetate. Essential oil was extracted with hydro distillation method. Preliminary evaluation procedure was focused on repellent activity against Sitophilus granarius, but further studies used more insect species. Using new indices, the repellent and insect potential were compared. In Preliminary evaluation, 18 out of 19 plants showed repellent activity. Further studies on the essential oil of available plants showed that, Merremia vitifolia has the highest repellent activity than the Peperomia Pellucida and the positive control, Elettaria cardamomum. Merremia vitifolia essential oil have the lowest absolute effective surface concentration and highest repellent index. Considering the Insect properties, Sitophilus granarius is the strongest and hence had the highest Anti-Repellent Index of 5.8421 µl/gcm2 against Elettaria cardamomum essential oil. In photo sensitivity test all the insects showed more affinity towards the middle of the visible spectrum and are repelled by darkness.

Considering certain methodologies with diffusion related errors, the percentage repellent activity wasn’t directly proportional to the quantity of the repellent and also there were reduction in repellent activity with time. The A-B sensitivity apparatus and its methodologies, preserves the ideal and preliminary condition of pure diffusion but eliminate the errors due to diffusion of volatile compounds. The experiments showed that even one micro-liter of essential oil gave 100% repellent activity. The certain extracts in Preliminary evaluation test also gave respectable results of around 90%.

The results here show that the botanical insecticides are toxic to all the tested insect orders. In that Merremia vitifolia essential oil was the most effective contact toxin and

repellent. While Elletaria cardamom essential oil was a slightly better contact toxin than Peperomia pellucida essential oil. But the repellent activity potential of PP essential oil was much better than EC essential oil. It seems that a repellent, especially a spacial repellent is the most eco-friendly alternative to safe guard the natural predators, non-target insects and especially the weak beneficial insects. Even crude extracts of potential plant parts can become helpful insect repellent and/or toxin especially in pre-infestation period. Botanicals together with the natural responses to check pest population like natural predator population and host plant adaptability has to be further explored.

Elletaria cardamom, Merremia vitifolia and Peperomia pellucida Essential oils were tested against insects of 6 orders viz. Coleoptera, Diptera, Hemiptera, Hymenoptera, Isoptera and Lepidoptera. And were found to be effective contact toxins. For the test, a slightly modified Impregnated paper assay was used in which Absolute lethal surface concentration and duration of the test was recorded. Based on this and other parameters the toxicity potential and insect strength were compared using new indices. It was found that Merremia vitifolia essential oil was the most powerful contact toxin. Among tested Insects, Coleoptera pests of stored products was the strongest followed by larvae of Lepidoptera. While Pyrrhocoridae family was the weakest one. The contact toxicity of Peperomia pellucida essential oil was slightly lower than that of the positive control, Elletaria cardamom essential oil. Comparing contact toxicity with the potential of chemical sensitivity using certain indices, it was found that there was a huge increase in the insecticidal potential of the essential oils, when used as a repellent.

REPORT OF THE UGC SPONSORED MINOR PROJECT

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INTRODUCTION

Insects, which coexist with the humans constitutes about 50% of all species on earth. They are essential for a healthy and vibrant ecosystem. Beneficial or non-pest insects comprise 99.5% of the total number of the known insect species. Of the remaining 0.5%, only a few of these can be a serious menace to people. But these few can cause more damage annually destructing one fifth of the world's total crop production and transmission of 17% of all infectious diseases which cause more than 1 million deaths annually.. Hence efficient pest management is essential.

Control of insects by chemical pesticides has proven negative effect, which is catastrophic to humans and the environment. Pesticides are responsible for an estimated 200,000 acute poisoning deaths each year and destruction of the ecosystem. In contrast to chemical pesticides, the botanical pesticides have reduced toxicity to non-target organisms, reduced persistence in the environment, usable in organic agriculture, low mammalian toxicity and safe for farm workers and nearby

As nature being an almost unlimited source of bio-active natural products and these botanical repellents are even more effective in protecting the non-target organisms, insect predators and environment. Moreover, insects become resistant to an insecticide more quickly than a repellent. Consistent and injudicious applications of pesticides lead to the development of resistance in insects, destruction of beneficial organisms and increases in residual problems, thereby posing a threat to human health and its ecological partners. Insecticides can be derived from leaf, bark and seed extracts of plants. Even the compost made out of plants can contribute to insect pest control.

Plants produce secondary metabolites that may possess many biological properties including insecticidal property. Plants produce secondary metabolites that are effective against arthropod pests such as blood sucking insects. Repellents based on natural compounds from plants are widely used to prevent insect bites and are more appreciated by consumers than synthetic compounds. However, plant based insecticides and insect repellents are used only to some extent in agriculture practice.

OBJECTIVES

The objectives of current study are to find new insect repellents or attractants based on botanicals. This goal can be achieved by

1. Selection of various plants with insect repellence or insecticidal activity
2. Preparation of various extracts of these plants for scientific evaluation
3. Testing the extracts against various insect pests in laboratory conditions
4. Isolation of the active principle from the plant matter
5. Purification of the active compound from the isolated active fraction
6. Chemical characterization of the purified compound
7. Evaluation of interaction between the purified compound and a variety of insect pest

MATERIALS AND METHODS

Collection of plant materials for preliminary evaluation

19 plant species were used for the preliminary screening studies. Among them, *Podocarpous gracilior, Pithecellobium dulce, Cassalia curviflora, Dryneria quercifolia* and *Tectonia grandis* were collected from the campus of St. Thomas College, Pala. Other fourteen plants were collected from accessible barren uncultivated lands, and land near sacred groves in the Kottayam, Ernakulam and Pathanamthitta districts of Kerala. Insecticidal property and repellent activity of *Elletaria cardamom* is well known so, *Elletaria cardamomum* essential oil was used only for advanced analysis. While *Merremia vitifolia* and *Peperomia pellucida* are used for both preliminary evaluation and further studies. Both seeds and leafs of *Hydnocarpus laurifolia* and *Tabernaemontana alternifolia* were separately analysed. Plants used for preliminary evaluation and further studies are *Averrhoa bilimbi* Linn., *Cosmos sulphureus* ,

Chassalia curviflora , *Cinnamomum malabatrum, Corypha umbraculifera* , *Cuscuta reflexa* Roxb, *Drynaria quercifolia* (L.) , *Hydnocarpus laurifolia* (Dennst.) ,*Dendrophthoe falcata* (L.f.) ,*Merremia vitifolia* , *Mikania scandens* B.L.Rob. , *Mimosa diplotricha* ,, *Pajanelia longifolia* , *Peperomia pellucida* Kunth. , *Pithecellobium dulce* (Roxb.) ,*Afrocarpus gracilior* , *Sarcostigma kleinii* Wight & Arn., *Tabernaemontana alternifolia* L. , *Tectona grandis* L.f. and *Elletaria cardamomum* (L.)

Insects for testing

For rearing *Sitophilus granarius,* one liter transparent and cylindrical PET jar with about 9cm diameter and air tight screw cap was used. In the center of the lid a square hole with length of approximately 40% of lid diameter is made. To fill the square hole, a stainless steel square Mesh (mesh no.120) and having a length of about 1cm bigger than that of the hole length was used. It was tightly fixed to the outside surface of the lid using epoxy adhesive. Sterilized whole wheat was used for rearing S. granarius. It was sterilized by heating in a hot air oven to 60°C for 15 minutes.

Initial seed specimen of *Sitophilus granarius* was collected from market sourced infested wheat. Ten active adults were transferred to jar with 50 gram of wheat. After three weeks, the introduced adults are removed from the jar. When new adults emerge, Active and healthy insects were transferred to another jar containing 50g of wheat. These new adults were removed and discarded after three weeks. Similarly, another generation of adults were also removed and discarded. Then the remaining wheat in the jar containing eggs of the third generation were used for maintaining the culture.

For maintaining the culture, the top quarter of the infested wheat from the jar in which third or later generation insects or eggs were present was transferred to another jar and then 50 grams of sterilized wheat was added to the jar. There after culture is maintained by adding 75 grams of sterilized wheat every week up to one month. Then top quarter of the infested wheat and needed insects were transferred to a new jar. Another quarter from the top can also be used to inoculate another jar. Or if the insect population is much higher or more replicates were needed, 20 active adults alone can be transferred to another jar. Then 50g of sterilized wheat was added to each jar. Only excess adults were discarded. There after culture was maintained by adding 75 grams of new sterilized wheat every week up to one month. The above process is repeated to maintaining and/or multiplying the culture. The culture was maintained at 26±2 °C and 75±5% humidity.

Adults of Henosepilachna vigintioctopunctata (Hadda beetle) and Pyrrhocoridae family were collected from infested bitter gourd and seed-head of matured Sorghum plants respectively. Plants were grown in the agricultural land without insecticide application. In all the cases active adult insects of all sexes were selected for analysis.

Plant tissue extraction

Fresh plant tissues other than seeds were cut in to small pieces with knife or scissor, while seeds and small rhizomes pieces were crushed with mortar and pistil. 10 gram of this tissue were weighted and homogenized for 5 minute in an electric mixer with 100 ml Methanol - Water (4:1) solvent mixture. The homogenized material was filtered using grade1 filter paper. The filtrate was evaporated in room condition to about 70ml and if necessary the evaporated filtrate was made up to 70ml using methanol-water (4:1) solvent. And this filtrate was used as methanol-water extract. The residue obtained was kept in capped conical flask with 70ml ethyl acetate and gave mild intermittent shake for 24 hours and filtered. This filtrate was made up to 70ml using ethyl acetate and used as ethyl acetate extract .

Essential oil is extracted by hydro-distillation using clevenger apparatus. 300gm of cut or crushed fresh plant tissue or 100gm of crushed seed and 300ml of distilled water were added to 1000ml flat/round Bottom flask and distilled . For preliminary evaluation purpose 3% v/v essential oil in acetone was used, while for advanced analysis undiluted essential oil was used.

A-B sensitivity apparatus construction

A-B sensitivity apparatus is a modified T-maze, with a transparent tube having two sides or choices and an insect introduction tube inserted in the middle. Insect preparation: Only *Sitophilus granarius* was used for preliminary evaluation, but for advanced studies and photosensitivity tests *S. granarius, H.vigintioctopunctata* adult, *H.vigintioctopunctata* grub and insects of Pyrrhocoridae family were used. Ten adults of *S. granarius,* six adults of *H.vigintioctopunctata* and Pyrrhocoridae family and six grubs of *H.vigintioctopunctata* were used for their test. Just before the test, ten active adult insects of *Sitophilus granarius* were captured in to a ¼ inch transparent tube of 30 cm length, whose bottom end was tightly folded using a rubber band. After putting all the ten insects in to the tube, the top end was also tightly folded with a rubber band. The tube is called Insect Collection Tube. While for *Henosepilachna vigintioctopunctata* and Pyrrhocoridae family, active adults were gently captured and kept inside petridishes.

Contact Toxicity assay

For determining contact toxicity, a slightly modified Impregnated paper assay was used. The contact toxicity of essential oils was done in 9cm glass petridish. Active Insects for each test were collected in a collecting vessel, just before the test. *Sitophilus granarius*, *Sitophilus oryzae* and *Tribolium castaneum* were collected in the Insect Collection Tube . While others are collected in petridishes. Five *Cetonia aurata* adults were used for each test. For all others, 10 insects/larvae were used for each test.

Essential oil was impregnated to a Circular grade 1 filter paper with 9cm diameter placed inside the petridish. Direct application of pure essential oil avoids the chances of losing highly volatile compounds during the drying of solvent. Therefore, pure essential oil was applied to different optimally spaced spots in the filter paper for uniform spreading of the oil. Spots very near to the edges were avoided. After applying, waited for 10 seconds, so that the essential oil spreads more evenly. Thereafter insects were gently added from the collecting vessel to the petridish and the lid was closed tightly and then the edge was sealed with transparent adhesive tape to prevent disturbances from diffusion of gases from inside and outside.

The assay was engineered to find the Absolute lethal concentration (LC 100) . To avoid the confusion with fumigant toxicity it was assumed that the toxicity is due to the direct contact of the insect with the essential oil impregnated in the filter paper only and all the applied oil spreads evenly in the filter paper. And therefore, surface concentration was used for mathematical analysis. Hence, Absolute lethal surface concentration was used instead of Absolute lethal concentration. The identification of exact absolute lethal surface concentration was done by screening a range. The maximum value of essential oil applied was limited to 150µl(2.359µl/cm2) and the test duration was limited to be around 1hr in all possible tests. The insects were considered dead if they were immobile in strong white light and when tickled with a soft feather. The insect were visibly tested under strong white light after 30min, 45 min, 1 hr, 2hr, 3hr, 4hr, 6hr, 8hr, 10hr, 12hr, 16hr, 20hr and 24hr. If that test showed 100% mortality, petridish was opened and the insect were tickled with soft feather. If that also fails to give 100% mortality, that experiment was repeated with the next time interval(duration) or next higher dose of essential oil. All experiments with 100% mortality were repeated for three times to confirm the results.

IMPORTANT RESULTS

The study was to evaluate the insect repellent activity of certain plants and to compare the sensitivity of certain insects towards selected essential oils, composite light and a few colors. While on the experimental arena, chemical and photo sensitivity tests were carried out using a ‘modified T-maze’ called as ‘A-B sensitivity apparatus’. Plant parts were extracted using methanol-water and ethyl acetate. Essential oil was extracted with hydro distillation method. Preliminary evaluation procedure was focused on repellent activity against *Sitophilus granarius*, but further studies used more insect species. Using new indices, the repellent and insect potential were compared. In Preliminary evaluation, 18 out of 19 plants showed repellent activity. Further studies on the essential oil of available plants showed that, *Merremia vitifolia* has the highest repellent activity than the *Peperomia Pellucida* and the positive control, *Elettaria cardamomum*. *Merremia vitifolia* essential oil has the lowest absolute effective surface concentration and highest repellent index. Considering the Insect properties, *Sitophilus granarius* is the strongest and hence had the highest Anti-Repellent Index of 5.8421 µl/gcm2 against *Elettaria cardamomum* essential oil. In photo sensitivity test all the insects showed more affinity towards the middle of the visible spectrum and are repelled by darkness.

Considering certain methodologies with diffusion related errors, the percentage repellent activity wasn’t directly proportional to the quantity of the repellent and also there were reduction in repellent activity with time. The A-B sensitivity apparatus and its methodologies, preserves the ideal and preliminary condition of pure diffusion but eliminate the errors due to diffusion of volatile compounds. The experiments showed that even one micro-liter of essential oil gave 100% repellent activity. The certain extracts in Preliminary evaluation test also gave respectable results of around 90%.

Elletaria cardamom, Merremia vitifolia and *Peperomia pellucida* Essential oils were tested against insects of 6 orders viz. Coleoptera, Diptera, Hemiptera, Hymenoptera, Isoptera and Lepidoptera. And were found to be very effective contact toxins. For the test, a slightly modified Impregnated paper assay was used in which Absolute lethal surface concentration and duration of the test was recorded. Based on this and other parameters the toxicity potential and insect strength were compared using new indices. It was found that *Merremia vitifolia* essential oil was the most powerful contact toxin. Among tested Insects, Coleoptera pests of stored products was the strongest followed by larvae of Lepidoptera. While Pyrrhocoridae family was the weakest one. The contact toxicity of *Peperomia pellucida* essential oil was slightly lower than that of the positive control, *Elletaria cardamom* essential oil. Comparing contact toxicity with the potential of chemical sensitivity using certain indices, it was found that there was a huge increase in the insecticidal potential of the essential oils, when used as a repellent.

The result of this study can pave way for many further researches in the realm of instrumentation, indices, more insect species, potential/positive plants, phyto-chemicals, photo sensitivity with diverse wavelengths & luminosity, and pre-infestation effect of the repellent(s) &/attractant (s) application. Most plants selected for preliminary evaluation, doesn't had noticeable pest problem in its habitat. Therefore, the survival mechanism of plants with even negative test results also deserves further research. The A-B sensitivity apparatus with a modified IiT and/or IcT and/or Choice sides can effectively be used for analyzing preliminary repellent activity and anti-feedent activity of mosquito repellents. Pest's sensitivity to chemical and photo stimulus can be utilized to improve integrated pest management and is in the realm of further study. The method allows even enterprising farmer to test and then utilize the repellent activity of aqueous extract of weeds around their field. The result of the study emphasizes that if the purity and diversity of nature exists, effective nature friendly pest control strategies are possible

The results here show that the botanical insecticides are toxic to all the tested insect orders. In that MV essential oil was the most effective contact toxin and repellent. While EC essential oil was a slightly better contact toxin than PP essential oil. But the repellent activity potential of PP essential oil was much better than EC essential oil. It seems that a repellent, especially a spacial repellent is the most eco-friendly alternative to safe guard the natural predators, non-target insects and especially the weak beneficial insects. Even crude extracts of potential plant parts can become helpful insect repellent and/or toxin especially in pre infestation period. Botanicals together with the natural responses to check pest population like natural predator population and host plant adaptability has to be further explored.

The study shows the comparative potential of essential oils, comparative strength of insects of different orders and comparison of contact toxicity and chemical sensitivity. The study and its methodologies paves the way for further research in these fields and also for comparing photo sensitivity having a measurable luminescence per square cm with contact toxicity and chemical sensitivity.

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