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A Comparative Study on the Effect of Pheromone Baited Traps and *Acalypha indica* Extract on *Leucinodes orbonalis*

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ABSTRACT

Botanical insecticides are naturally occurring chemicals extracted from plants. This study aims to analyze the insecticidal activity of bioactive compounds present in the aqueous extract of *Acalypha indica* and to analyze the efficacy of pheromone baited traps in integrated pest management. In this study, various bioactive compounds present in the aqueous extract of *Acalypha indica* were analyzed by phytochemical screening and GC-MS analysis. The molecular properties of the compounds identified from GC-MS analysis were screened based on Tice rules using bioinformatics molecular property analysis tools. Out of 8, four compounds namely oleic acid, phytol, flavone and estra- 1, 3, 5[10]-trien-17a`-ol strictly follows Tice rules. The aqueous extracts were used for the screening of insecticidal activity against 3rd instar larvae of *Leucinodes orbonalis* by diet incorporation bioassay. On observation it was revealed that the aqueous extract of *Acalypha indica* induce antifeedant effect, which was very efficient at 80% concentration. Three pheromone baited traps namely yellow sticky trap, bucket trap and sleeve trap were used to evaluate its efficacy in pest management. From the evaluation, out of three pheromone baited traps, sleeve traps performed statistically better than others, trapping higher population of adult *Leucinodes orbonalis*. The sticky traps captured the highest number of non-target insects, mostly being ants, flies, and beetles. In bucket trap moderate non-target populations were present. Thus, on the comparative study the preliminary screening shows that *Acalypha indica* can be used to derive a novel insecticide and pheromone baited sleeve trap shows positive integrated pest management.

Keywords: *Acalypha indica*, *Leucinodes orbonalis*, GC-MS analysis, Tice rule, pheromone trapping

In Tamil Nadu, agriculture continues to be the most predominant sector of the state economy, as 70% of the population is engaged in agriculture and allied activities for their livelihood. Eggplant and tomato are the most commonly grown vegetable crops of the state. Tomatoes and eggplants are subjected to large number of pest attacks from the time of emergence to harvest. The most destructive pest is the shoot and fruit borer, *Leucinodes orbonalis*, commonly found in tropical and subtropical parts of Asia and Africa. *L.orbonalis* is a typical Lepidopteron belonging to the family Pyralidae. The adults are weak fliers and active at night. *L.orbonalis* undergoes four distinct life stages: egg, larvae, pupa and adult. They lay eggs on leaves and tender shoots of eggplants and tomatoes. Shortly after hatching, the neonate larvae migrate to the nearest shoot or fruit and bore inside. In fruit, the larvae typically enter just below the calyx. A *L.orbonalis* larva feeding inside a fruit completes its four larval instar stages in 15 to 20 days. A fully grown larvae bore back to the surface and emerges from the fruit or shoot leaving obvious exit holes. The larvae migrate to the soil surface to pupate in plant debris. The pupal period lasts for 7 to 10 days. The young *L.orbonalis* adults are generally found on the lower leaf following the emergence from the pupal cocoons. Farmers in Asia use large amounts of chemical pesticide to kill the pest in its larval stage. However without adequate pesticide on the plant, the newly hatched larva survives and crawls to the nearest shoot or fruit, and stays inside being safe from pesticide spray[1]. This leads farmers to spray chemicals frequently which cause biomagnifications in food chain.

The chemical pesticides have toxic effects on several non-target species and the growing environmental concerns raises the issues of safety and evaluation of toxicity. Increase in pesticide use increases pest resistance. Botanical pesticides are eco-friendly which is stored in plants as secondary metabolites. For the past 30 years plant extracts and their bioactive molecules have been intensively utilized as an alternative pest management component. Most of the noble bioactive components of plants are constituted by secondary metabolites like alkaloids, flavonoids, phenolic compounds, organic acids and lipids. Many of the defensive components of plant are biodegradable with non residual effects on the biological environment. In this study the insecticidal activity of *Acalypha indica* (Linn.) crude extract was studied.

Acalypha indica is an annual erect herb commonly called as "Kuppai meni". It belongs to the family Euphorbiaceae. It is a common shrub in Indian gardens, backyards of houses and waste places through the plains of India. The root, stem and leaf of *A.indica* possess herbal activity. The leaves and roots of the plant have abundant bioactive components, namely acalyphine, cyanogenic glucosides, alkaloids and triacetoneamine. The root of *A.indica* has the lowest water activity and moisture content and it contributes to better storage and longer shelf life[2]. The plant offer a wide array of bioactive compounds which are sufficiently toxic and alters the intestinal electrolyte, energy source (glucose and total protein) and eluate detoxification enzyme level of pest[3].

Due to intensive use of insecticides in arthropod control, many pest populations have developed resistance to these compounds and residual effects, respectively, resulting in worldwide efforts of finding alternative pest management. Insects use complex communication system to cue them in variety of complex social behaviors. The odour which is responsible for this complex behavior is known as pheromones. Pheromones are a class of semiochemical that insects and other animals release to communicate with other individuals of the same species. The key to all of these behavioral chemicals is that they leave the body of the first organism, pass through the air or water and reach the second organism, where they are detected by the receiver. In insects, these pheromones are detected by the antennae on the head. The signals can be effective in attracting faraway mates, and in some cases, can be very persistent, remaining in place and active for days. Long-lasting pheromones allow marking of territorial boundaries or food sources. Other signals are very short-lived, and are intended to provide an immediate message, such as a short-term warning of danger or a brief period of reproductive readiness. Pheromones can be of many different chemical types, to serve different functions. As such, pheromones can range from small hydrophobic molecules to water-soluble peptides. Pheromones have major application in integrated pest management. The most important application is in monitoring a population of insects to determine if they are present or absent in an area or to determine if enough insects are present to warrant a costly treatment. This monitoring function is the keystone of integrated pest management. A second major use of pheromones is to mass trap insects to remove large numbers of insects from the breeding and feeding population. Massive reductions in the population density of pest insects ultimately help to protect resources such as food or fiber for human use. The major application of pheromones is the disruption of mating in populations of insects. This has been most effectively used with agriculturally important moth pests[4]. The use of pheromones reduces the amount of insecticide applied and also increases the yield of crop. The current study is to evaluate the effect of pheromone baits and *Acalypha indica* extract on *Leucinodes Orbonalis* over chemical such as Chlorpyrifos.

MATERIALS AND METHODS

Collection of plant

Acalypha indica was collected from sandrorkuppam, Ambur, Vellore district, Tamil Nadu, India. The collected plants were washed thoroughly under tap water and shade dried. The shade dried plants were pulverized using electric blender and stored for further studies.

Preliminary phytochemical analysis of *A. indica*

From the pulverized plant material 100g powder was soaked in 250 mL distilled water and boiled for overnight with occasional shaking. Then the content was filtered using Whatmann filter paper to obtain filtrate. The filtrate was centrifuged to obtain aqueous extract. The aqueous extract was divided into different test tubes and various phytoconstituents were analyzed according to methods described by Allen[5] and Harborne[6]. The different phytoconstituents tested for Tannins, Polyphenols, Saponin, Glycosides, Alkaloids, Triterpenes and Flavonoids.

GC-MS Analysis

GC-MS analysis was carried out on an Agilent 6980 gas chromatogram equipped with a straight deactivated 2mm direct injector liner and a 15 m Alltech EC-5 column (250µ I.D.,0.25 µ film thickness). A split injection was used for sample introduction and the split ratio was set to 10:1. The oven temperature program was programmed to start at 350C, hold on for 2 minutes, and then ramp at 200C per minute to 3000C and hold for 5 minutes. The helium carrier gas was set to 2ml/minute flow rate (constant flow mode). A JEOL GC-mate II bench top double - focusing magnetic sector mass spectrometer operating in electron ionization (EI) mode with TSS-2000 software was used for all analyses. Low-resolution mass spectra were acquired at a resolving power of 1000 (20% height definition) and scanning from m/z 25 to m/z 700 at 0.3 seconds per scan with a 0.2 second inter-scan delay. High resolution mass spectra were acquired at a resolving power of 5000 (20% height definition) and scanning magnet from m/z 65 to m/z 750 at 1 second per scan. Identification of components Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were determined.

Molecular property analysis

The molecular properties of the compounds identified from the GC-MS were analyzed based on the Tice rules using Mol inspiration server and NCBI PUBCHEM database. Mol inspiration is a cheminformatics software tools supporting molecule manipulation and processing. Molecular properties through these databases also give information on bioactivities of compounds. All compounds identified by the GC-MS screening were assessed for their insecticidal potential using physico-chemical property calculations according to Tice rules[7].

Phytotoxicity assay

In this assay a comparative study on the phytotoxicity of *Acalypha indica* and commercially used synthetic insecticides namely Chlorpyrifos was observed on the plant seeds of *Solanum melongena*. The seeds of *Solanum melongena* were collected from Thiruvallur, TamilNadu, India. The seeds with uniform size were chosen for the experimental purpose. The seeds were washed thoroughly using distilled water. The petridishes were sterilized in autoclave at 15 Psi for 20 minutes at 120°C. Five different concentration of the aqueous plant extract (10, 20, 40, 60 and 80 in percentage) was prepared and 10 seeds per concentration were soaked for 24 hours. Whatmann filter paper was placed in the sterilized petridishes and wetted with 1 mL of distilled water. After 24 hrs of incubation the seeds were transferred to the petridishes (20/plate) containing Whatmann filter paper. Same procedure was repeated for commercial chemical insecticide Chlorpyrifos which were compared with *A.indica*. The seeds incubated with distilled water were used as control. The germination rate of the seeds was observed every five days for 15 days. The germination percent (GP) and speed of emergence (SE) was calculated by the following equation

$$GP\ (\%) = \frac{\text{No. of germinated seeds at final count}}{\text{Total no. of seeds set for bioassay}}$$

$$SE\ (\%) = \frac{\text{No. of germinated seeds at the starting day of germination}}{\text{No. of germinated seeds at the final days of measurement}}$$

Insecticidal Activity

The larvae of *Leucinodes orbonalis* were collected from the egg plant field at Vepampattu, Ayathur, Tamil Nadu, India. The collected insects were reared in plastic cups closed with transparent polythene sheet. The insects were reared under laboratory conditions (26°C +2°C, 57-67% RH) separately and they were provided with fresh egg plant fruit placed in plastic cups closed with polythene sheets with holes for aeration. The feed was changed every two days till it reached the pupal stage. The adults emerged from the pupae were reared in a plastic container with netted lid and 10% of honey solution mixed with Multivitamin drops in a cotton swab was placed as feed for adults. Folded filter paper sheets and fresh cleaned eggplant fruits were kept inside the plastic jar for egg laying. The egg masses were incubated in a separate container for hatching. The newly hatched first instar larvae were carefully transferred using a paint brush to rearing plastic cups containing fresh eggplant fruits. First instar larvae were reared in groups until the third instar stage and they were used for testing**.**

Insecticidal activity of *A.indica* aqueous extract on *Leucinodes orbonalis*

Toxicity bioassay[8] was carried out with *Leucinodes orbonalis* population using different concentration of the plant extract. Third instar larvae of uniform size were taken from the mass culture maintained in the laboratory. From the stock solution 1 mL was taken and diluted with 9mL distilled water to prepare 10% concentration of test extract and similarly 40% and 80% concentrated test extracts were prepared. By diet incorporation method the insecticidal bioassay was carried out. For each treatment 5 Larvae starved for 4 hours were introduced in separate containers containing the feed. Two replicates were maintained for each treatment. Feed with chemical insecticide Chlorpyrifos was used to comparatively analyze the insecticidal activity of *A. indica* with commercially used synthetic insecticides. The entire experiment was conducted at 26°C +2°C, 57-67% RH. After 24 hours of feeding, the weight of the leftover feed, feces and larvae were recorded. Data percentage of larval mortality using Abbott's formula[9], feeding deterrence index using Ben Jannet *et al.* formula[10], and metabolic rate using Waldbauer *et al.*[11-12] were estimated using the following formula. All the indices were calculated on the dry weight basis. To find the dry weights, the feed, feces, and larvae (freeze killed larvae, 5 specimens for each stage) were weighed, oven-dried (48 hours at 60° C), and then re-weighed to establish a percentage of their dry weight.

Pheromone baited traps to capture *Leucinodes orbonalis* (Lepidoptera: Pyralidae)

A field site in Vepampattu near Thiruvallur district, Tamil Nadu, India was used in the study. The field comprise 0.5 acre and has continuous production of eggplant from November through May. Traps were placed February through April 2017. Three trap blocks (Fig. 1) were placed in quadrant position among the crops. Each trap block included one of each pheromone baited trap: a yellow sticky, a Bucket trap, a sleeve trap. The yellow sticky trap measured 150 × 200 mm. Through the holes provided in the sticky sheet the pheromone lures were fixed to it via bungee cord. The sleeve traps used in our study measured 50 cm tall with a bottom diameter of 30 cm that narrowed to an opening of 30 cm. The top portion was attached with Lures that were pinned to a cork. Therefore, moths attracted to the pheromone flew upwards through the base cone and

A: Bucket Trap **B:** Sleeve Trap **C:** Yellow Sticky Trap

Fig. 1: Pheromone baited traps

fall downwards into the apex cone, where they were captured. The sticky, sleeve cone, and bucket traps were hung on wooden stick poles so that the lure height was approximately 1.5 m above the ground. Because they had to be tied to the poles rather than placed on top.

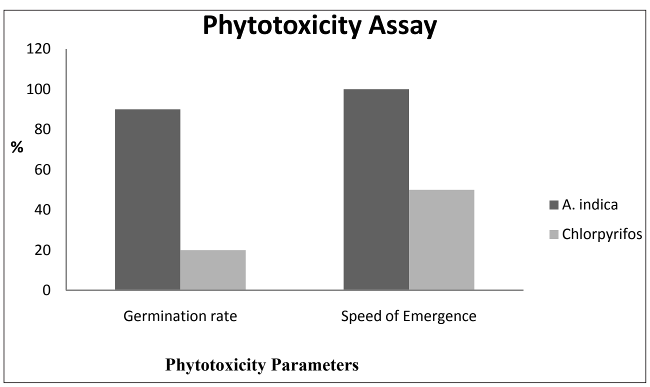

Fig. 2: Effect of *A. indica* extract and Chlorpyrifos on Germination rate and Speed of emergence of *Solanum melongena* seeds at 80% concentration on 15th Day

Traps within each trap block were rotated on a weekly basis. Traps within blocks were at least 30 m apart; the separation among blocks was at least 100 m. Three calculations were made to measure trap sensitivity. First, the overall trap efficiency of the three different Pheromone baited traps based on the number of moths captured after installation. The second calculation was the number of times that a particular trap was the only trap of the 3 with target moths. The final calculation was the number of times that a trap contained non target insects.

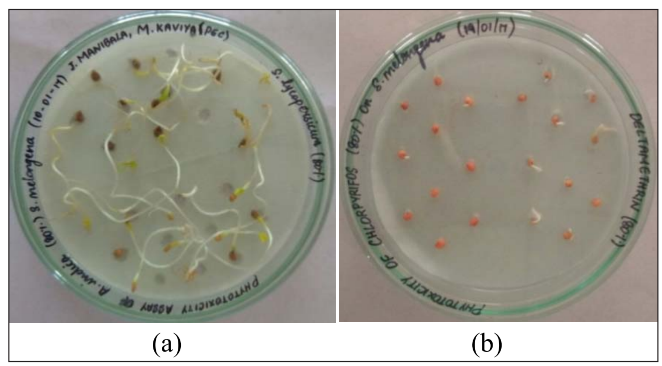

Fig. 3: Germination of *Solanum melongena* seeds treated with (a) *Acalypha indica* extract (b) Chlorpyrifo sin 80% concentration on Day 15

RESULTS AND DISCUSSION

Phytochemical analysis of *A. indica* and GC-MS analysis:

The qualitative phytochemical screening was carried out by observing different color reaction that reflects the presence of compounds. The analysis showed the presence of various secondary metabolites, namely alkaloids, flavonoids, tannins, polyphenols, saponins, glycosides and terpenes in the aqueous extract of *A.indica*. From the analysis two of the major groups namely the terpenes and phenols are present in the plant extract of *Acalypha indica*. Eight compounds were identified by GC-MS analysis such as Flavone, Tridecanoic acid methyl ester, Estra-1,3,5 (10)-trien-17 a-ol, Phytol, Oleic acid, Non adecanoic acid 18-oxo methyl ester, E-13-Docosenoic acid, Docosanoic acid methyl ester.

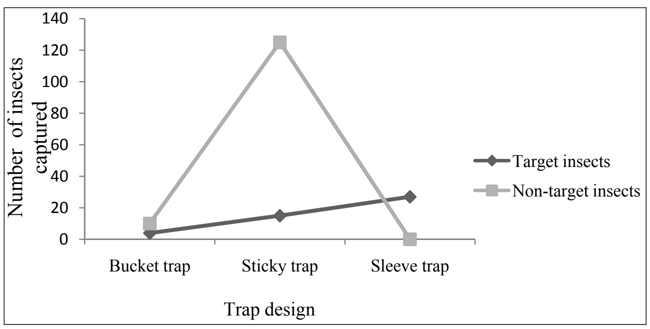

Fig. 4: Effect of trap design on number of target and Non-target insects captured

Molecular property analysis

The compounds identified through GC-MS analysis were assessed for insecticidal activity based on Tice rule. The compounds namely Flavone, Oleic acid, Phytol, Estra- 1,3,5[10] trien 17a`ol respectively has molecular mass >150 and <500 g/mol, log P value relatively equal to 5, hydrogen bond donor less than 2, hydrogen bond acceptor within 1-8 and number of rotatable bond relatively equal to 12 (Table 1). Thus, the shortlisted compounds satisfies the Tice rule and implies that the aqueous extract of *Acalypha indica* possess insecticidal activity. The compounds listed above acts as feeding deterrents to a large number of plant feeding insects. Many bioinformatics approaches available on the study of insecticidal activity of plant extract. The insecticidal property analysis of phytochemicals derived from Chloroform, Ethyl acetate and Methanol extracts of root-bark and fruit of *Morinda tinctoria* against the cotton bollworm *Helicoverpa armigera* using bioinformatics approaches such as molecular structure property analysis, homology modeling and docking studies was investigated[13].

Phytotoxicity assessment of *Acalypha indica*

In the Phytotoxicity assay two different parameters were determined, namely radicle length and seed germination percent. The etiolated seedlings having long radicle of different lengths were measured after five days period of dark treatment, showing differences in time of germination of seedlings with their cotyledons attached. The overall test period was 15 days. A comparative Phytotoxicity assay was carried out between aqueous extract of *A.indica* and synthetic pesticides Chlorpyrifos. The different parameters like germination rate, speed of emergence and shoot length were significantly different from each other. The seeds treated with *A.indica* aqueous extract showed maximum germination rate 90% and speed of emergence of 100% at 80% concentration compared to the seeds treated with Chlorpyrifos (Fig 2, 3). In both maximum and minimum concentrations of Chlorpyrifos the germination rate and speed of emergence was lower, exhibiting high phytotoxic

Table 1: Molecular properties of bioactive compounds present in plant extract

Sl. No.	Name of the compound	Molecular weight g/mol	Log p	No. of H bond donors	No. of H bond acceptors	No. of Rotatable bonds
1	Flavone	222.243	3.6	0	2	1
2	Oleic acid	282.468	6.5	1	2	15
3	Phytol	296.539	8.2	1	1	13
4	Estra- 1,3,5[10] trien 17a`ol	254.373	5.1	1	1	0

effect and growth inhibition activity, whereas *A.indica* promoted the growth of the seedlings and acts as a growth promoter. The assessment of the phytotoxicity of insecticides, herbicides or any other plant protection product is an essential element in its efficacy evaluation. The phytotoxicity level of insecticides over plants can be screened accurately through seed treatments[14].

Insecticidal Activity

The percentage of larval mortality was calculated in 24 hrs and 48 hrs time interval (Table 2).

Table 2: Larval mortality in percentage

Concentration (v/v) %	Larval mortality %			
	A.indica		Chlorpyrifos	
	24hrs	48hrs	24hrs	48hrs
10	20	52	40	72
40	30	70	60	100
80	68	85	72	100
Control	0	0	0	0

The maximum larval mortality 85% was observed in 80% concentration of *A.indica* extract in 48 hrs. The feeding deterrence index (FDI) values of *A. indica* and the chemical pesticide are tabulated (Table 3).

Table 3: Feeding deterrence index and consumption rate of *A. indica* and Chlorpyrifos

Concentration (v/v) %	FDI*		CR*	
	A. indica	Chlorpyrifos	*A. indica*	Chlorpyrifos
10	10.5 ± 1.41	13.09 ± 0.17	0.15 ± 0.07	0.26 ± 0.34
40	14.89 ± 0.49	18.16 ± 0.367	0.225 ± 0.106	0.225±0.106
80	17.83 ± 0.84	20.45 ± 1.34	0.135 ± 0.063	0.3±0.70
Control	100	100	0.6 ± 0.28	0.6±0.28

*Values obtained from Mean + standard deviation on triplet assay.

The comparative study shows the FDI value is higher in 80% concentration in both *A.indica* extract and Chlorpyrifos. This shows that the *A.indica* extract is almost equivalent to chemical pesticide Chlorpyrifos. The consumption rate shows that lower the consumption rate higher the efficiency of the pesticide. The consumption rate was lower in both Chlorpyrifos and *A. indica* but maximum lower consumption rate was seen in 80% concentration of *A.indica* aqueous extract. Similar results were found by Wubie *et.al.*[15] who worked on *Brevicoryne brassicae* that maximum insecticidal activity of *Mentha piperita (L.)* plant extract was found in the highest concentration and it was increased from 24 hours to 72 hours exposure period.

Pheromone trap efficacy

The pheromone traps did attract a few male moths when set up in a field. Over the study period each trap captured average amount of moths as mentioned but among them sleeve trap captured the target adult *L.orbonalis*, whereas the sticky and bucket trap were less efficient in capturing target pest (Fig. 4). Similar study was conducted by Geetha Lakshmi, *et al.*[16] but the result showed that use of pheromone lures could not check the incidence of *L. orbonalis* satisfactorily in the peri-urban areas of Chennai during summer months. But in our study the trap efficiency was comparatively higher even though the experiment was carried out for a short period of six weeks. Of all the traps, sleeve trap shows higher trap efficiency than the other traps and the yellow sticky trap gives an average result. Thus the trap efficiency shows the positive pest integrated management.

CONCLUSION

The Phytochemicals present in the aquesous extract of *A.indica* were analyzed using qualitative color reactions as well as GC-MS. The Phytotoxicity assay was carried out to check the toxic effect of *A.indica* where they proved to be growth promoter unlike chemical pesticide which acted as inhibitor. The insecticidal activity of the extracts was assessed by the bioassays using diet incorporation method against the 3rd instar larvae for *Leucinodes orbonalis*. The molecular properties of the obtained Phytochemicals from the aqueous extracts were analyzed based on Tice rules. The lecture review showed that the compounds follow Tice rules strictly more likely to be an insecticide. The larvicidal activity may due the presence of compounds like oleic acid, flavones, Estra- 1,3,5[10] trien17a`ol, Phytol of aqueous extract of *Acalypha indica*. Scale up of the production shall also be considered for vast application of bio pesticides using field trials. The trap efficiency is observed using three pheromone baits such as sleeve trap, bucket trap and the yellow sticky trap. Of all the traps, sleeve trap shows higher trap efficiency than the other traps and the yellow sticky trap gives an average result. Thus, the trap efficiency shows the positive pest integrated management. Botanical insecticides generally act quickly, degrade rapidly and have low mammalian toxicity. Hence, the extract of *Acalypha indica* with the promising insecticidal activity could be studied further for the development of novel insecticide along with Sleeve pheromone trap which has a positive effect on integrated pest management.

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