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Research Article

A NOVEL HERBAL PESTICIDE TO CONTROL ADULT SCALE INSECTS

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ABSTRACT
In recent years organic formulations involved in the biological control of insect-pests of economically important plants have been of great value in the field of agriculture to rule out the use of hazardous pesticides. Application of insecticides of botanical origin is becoming a simple and sustainable method of pest control. In this investigation *Wedelia chinensis* and *Cymbopogan citratus* were selected for mortality of scale insects. Bioactive principles in these herbs were analysed by GC-MS. Significant mortality of adult scale insects has been attributed to the presence of Butyl butyrate, Bis (3,3,5-Trimethyl hexyl) adipate in *Wedelia chinensis* and Benzofuran in *Cymbopogan citratus* in the herbal formulation.
Keywords: Herbal Pesticide; Biological Control; Scale insects; *Wedelia chinensis, Cymbopogan citratus,* GC-MS.

INTRODUCTION

Mealybugs (Hemiptera: Pseudococcidae) are small, soft-bodied plant sap-sucking insects that constitute the second largest family of scale insects (Hemiptera: Coccoidea), with more than 2,000 described species and 290 genera[1].Their common name is derived from the mealy wax secretion that usually covers their bodies. They are a specious and ubiquitous group of sap-sucking plant parasites, many of which are very serious agricultural pests. Mealybug is considered to be havoc in agricultural and horticultural crops. In the current decade, the trend of increased build up of various mealybug species in crop plants and in the wild is observed mainly due to certain abiotic changes in climate and environment. During the last few years mealybugs, which were considered to be minor pests in many crops have acquired the status of major pests especially in cotton, vegetables and fruits. In cotton crop, it is reported that the total damage in 2007 was estimated to range from US$400,000 to 500,000 in north India alone. Apart from the yield losses, the pest infestation increased the cost of insecticide application by US$250–375 per acre in both India and Pakistan[2]. Chemical control methods for mealy bug and fruit fly have been inefficient[3]. There has been consistent interest to evolve cultural and biological control methods. Phytochemicals are botanicals which are naturally occurring insecticides obtained from floral resources. Applications of phytochemicals in pest control were in use since the 1920s[4], but the discovery of synthetic insecticides such as DDT in 1939 side tracked the application of phytochemicals. Plants produce numerous chemicals, many of which have pesticidal properties. More than 2000 plant species have been known to produce chemical factors and metabolites of value in pest control programmes. Phytochemicals, especially botanical insecticides are currently of interest because of their successful application in plant protection as potential biocontrol agents[5]. Members of Solanaceae, Asteraceae, Labiatae and Rutaceae have insect repellent properties against different species of pests. Botanicals are basically secondary metabolites that serve as a means of defence mechanism of the plants to withstand the continuous selection pressure from herbivore predators and other environmental factors. Several groups of phytochemicals such as alkaloids, steroids, terpenoids, essential oils and phenolics from different plants have been reported previously for their insecticidal activities[6]. Since then, the search for new bioactive compounds from the plant kingdom and an effort to determine its structure and commercial production has been initiated. At present phytochemicals make up to 1% of world's pesticide market. The study aims at formulating, a novel pesticide by using locally available herbs. *Wedelia chinensis* and *Cymbopogan citratus* and *Apium graveolens* have many references for their insecticidal properties. *Wedelia chinensis* and *Cymbopogan citratus* are herbs of vast medicinal value too. After facing several problems due to injudicious and over application of synthetic insecticides in nature, re-focus on phytochemicals that are easily biodegradable and having no ill-effects on non-target organisms was formulated. Several studies have documented the efficacy of plant extracts as the bioactive toxic agents but the failure in the studies can be attributed to the lack of LC50 studies *in-vitro* conditions and application of the same during the right stage of dormancy of the mature adults.

MATERIALS AND METHODS

In the formulation of a green pesticide, the following steps were used

- Screening of the plant extracts
- Preparation of suitable form plant protection
- Admixture in the feeding deterrent
- Determination of the structure of active principle by gas chromatography and mass spectroscopy.

Collection of the plants and insects

Studies were carried out in the laboratory and garden at Natural Resource Management Centre (NRMC), Periyakulam, Theni, India using the population collected from unsprayed *Hibiscus* plants from the garden. Twigs of *Hibiscus* plant infested with reproducing females of *P. solenopsis* were brought to the laboratory. *Wedelia chinensis* and *Cymbopogan citratus* were collected from the wild, from the nearby area of Periyakulam, Theni district, India.

Selection of plants was on the basis of absence of damage by the insect-pest. Healthy plant materials were wipe dried and stored in poly bags at the laboratory and their botanical identity was established. Flora of Presidency of Madras[7] and The Flora of Tamil Nadu Carnatic, India[8] were used for authentication of the plants. Further, *Wedelia chinensis* was identified and authenticated by Dr. John Bitto, Rapinath Herbarium, St Josephs College, Tiruchirappalli, Tamilnadu, India specimen plant has been deposited in the Herbaria with the voucher no. KAB001.

Insect rearing

Insect rearing is quite essential for detailed study of morphological, physiological and behavioural characteristics of insect. *P. solenopsis* insects were grown at 21.4-30.6ºC temperature and 52.5 to 90.6 % mean humidity range. Insects were reared by a modified method of [9]. An earthen pot with the volume of 4.8 cc was filled with 1 kg of coir pith. Potato with 4-6 sprouts were placed on the coir pith and water was sprinkled at 24 h intervals. 100 young crawlers were introduced on the coir pith and kept undisturbed for settlement. Adult females were collected in 6 months duration.

Herbal ash preparation

The dried plants of *Wedelia chinensis* were burnt in cake oven and after cooling; the ash was sieved in a 600 µm diameter and stored in sealed jar[10].

Preparation of Oil extract from *Cymbopogan Citratus*

One kg of *Cymbopogan* leaves were collected and soaked in water over night. The leaves were filled in soxhlet apparatus to extract the essential oil.

Herbal pesticide preparation

Wedelia chinensis ashes of different weights (1 g / 2 g / 3 g / 4 g / 5 g) were suspended in 100 ml of water. The ash solution (1 %, 2 %, 3 %, 4 %, 5 %) was filtered through Whatmann filter paper no.1.With 90 ml of ash filtrate 5 ml of *Cymbopogan citratus* extract and 5 ml of celery soap solution was added to prepare this herbal formulation. Control had 90 ml water with 5 ml *Cymbopogan* extract and 5 ml celery oil.

GC-MS analysis

GC-MS analysis of dried powdered ash of *Wedelia chinensis,* dried extract of *Cymbopogan citratus* was carried out with an electron impact mode. Injector and MS transfer line temperature were set at 220 and 290ºC. The components were identified based on the comparison of the relative retention time and mass spectra with the standards. Alkanes were used as reference points in the calculation of RRIC[11].

Spray toxicity assay

In spray toxicity assay, leaves containing slowly moving crawlers and immobile crawlers of late instar nymphs and adult mealy bugs were placed into plastic Petri dishes (10 cm dia × 2 cm ht). The leaves were sprayed with a sprayer with 5 pores of 0.01 mm diameter. Each spray release 0.5 ml pesticidal solution and wet 2 cm diameter of leaf surface and encounter 2.8 ± 0.3 numbers of crawlers of moving instars and 5.8 ± 0. 3 numbers of immobile instar nymphs including adult individuals. Adult individuals need 2 number of spray to wet the waxy body surface. Treatment was replicated for 6 times along with distilled water as a control group. Following the spray on each leaf sample, the insects on the leaf were observed at every 10 min interval for its mortality or survival[12]. Insects were treated with *Wedelia chinensis* ash in 1 %, 2 %, 3 %, 4 %, 5 % solution. 90 ml ash filtrate was added with 5 ml *Cymbopogan citratus* extract + 5 ml *Celery* oil.

Table 1: Effect of *Wedelia chinensis* Ash and *Cymbopogan citratus* Extracts with Celery Oil on Mortality Rate of Scale Insects

Treatment groups	Mortality rate (%)
Group I	Control
Group II	90.8 ± 2.13
GroupIII	91.1 ± 1.68
Group IV	94.1 ± 0.86
Group V	97.7 ± 0.95
Group VI	98.8 ± 1.56
Level of significance	F>0.05

Group I 100 ml of plain water; **Group II** 1 % ash 90 ml +5 ml Cymbo oil + 5 ml celery oil;
Group III 2 % ash 90 ml + 5 ml Cymbo oil + 5 ml celery oil; **Group 1V** 3 % ash 90 ml + 5 ml Cymbo oil + 5 ml celery oil;
Group V 4 % ash 90 ml + 5 ml Cymbo oil + 5 ml celery oil; **Group V1** 5 % ash 90 ml + 5 ml Cymbo oil + 5 ml celery oil

Table 2: Results of GC-MS Analysis for *Wedelia chinensis* and *Cymbopogan citratus*

Plant	Compound	IUPSC Name	Functions	Reference
Wedelia chinensis	Butonic acid	Butyl butyrate	Arthropod control compound	Moshav Sitrva, 1998
	Butane 1,1-dibutoxy	DL-3-amino butonic acid	composition in fungicide	Yigalcohen, 1993
	Adipic acid, bis (3,3,5-trimethylhexyl) ester	Bis (3,3,5-trimethylhexyl) Adipate	Arthropod control composition	Moshav Sitrva, 1998
Cymbopogon citrus	Benzofuran-2-one,4-amino-2,3-dihydro	4amino benzofuran-2(3H)-one	Feeding deterrent and Toxic	Rice and Coats, 1994

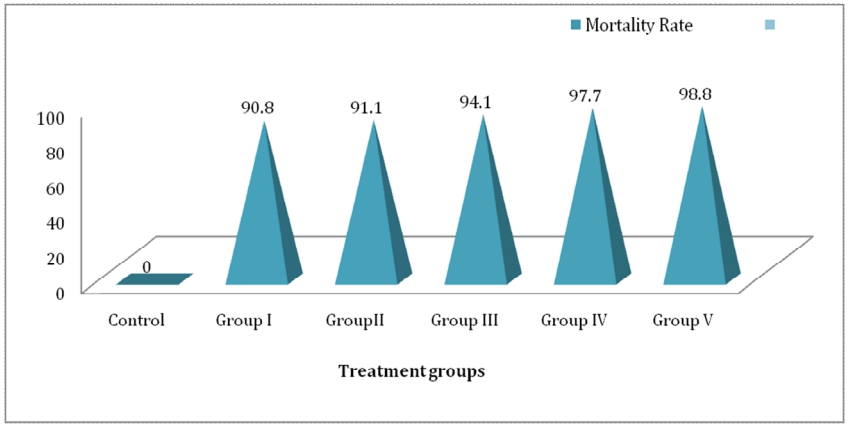

Figure 1: Effect of *Wedelia chinensis* ash and *Cymbopogan citratus* extracts with celery oil on mortality rate of Scale insects

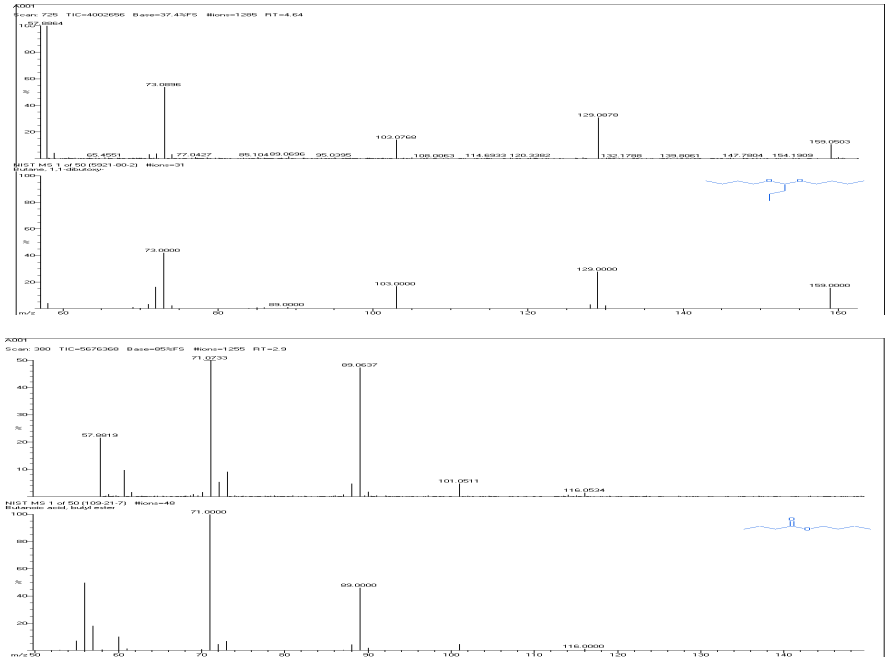

Figure 2: GC-MS profile of aqueous ash extract of *Wedelia Chinensis*

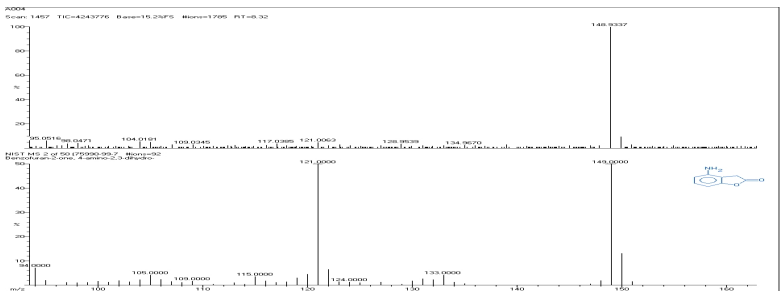

Figure 3: GC-MS profile of aqueous extract of *Cymbopogan citratus*

RESULT AND DISCUSSION

The susceptibility of mealy bugs to this formulation was dose dependent. Increasing the dosage of ash concentrations from 1 to 5 g increased the nymphal and adult mortality range from 91-99 % (Figure 1). High mortality values were observed at 5 g /100 ml. No mortality was observed in control or untreated groups (Table 1). Formulated herbal pesticide with *Wedelia chinensis* and *Cymbopogan citratus* is highly effective on adult mealybugs. The compounds Butyl butyrate, Bis (3,3,5-Trimethyl hexyl) adipate (Figure 2) and 4aminobenzofuran-2(3H)one (Table 2) which are present in *Wedelia chinensis* and *Cymbopogan citratus* (Figure 3) were responsible for the insecticidal activity of the herbal formulation. It is reported that the alcohol and methanol extracts of *Wedelia chinensis* had given good protectent effect against *Plutella xylostella* and on *Aphis medicaginis* Koch[13]. *Wedelia* herb or root has shown 40.9 % insecticidal activity [14]. The literature says that the compound butyl butyrate present in *Wedelia chinensis*

have been used in the pesticidal formulations in arthropod control[15]. Results are in confirmity with the earlier reports. Essential oils derived from lemongrass have been found effective as animal repellents, antifeedants, insecticides, miticides and antimicrobial products; thus finding use as disinfectants, sanitizers, bacteriostats, microbiocides, fungicides[16]. Celery oil is used as a synergist and sometimes a plant-safe detergent and dispersing the insecticides evenly and as a mild caustic against the insects. Insecticidal soaps disrupt the waxy cuticle or "skin" of the insect, which eventually causes the insect to dry out (desiccate) and die. Nirma powder with neem oil in the control of long tailed mealy bug was reported by[17] . The above reports support the present work. The essential oil constituents are primarily lipophilic compounds that act as toxins, feeding deterrents and oviposition deterrents to a wide variety of insect pests. Insecticidal properties of several monoterpenoids to the housefly, red flour beetle and southern corn root-worm have been reported[18]. Although many monoterpenoids have insecticidal properties, the degree of toxicity of different compounds to one species differs considerably. Cornelius *et al.,* (1997) evaluated the toxicity of monoterpenoids against *Coptotermes formosanus* (a subterranean termite). The benzofuron form *Cymbopogan citratus* is the basic compound in monoterpenes and these compounds naturally show toxic effect on insects[19]. Similar effect is seen in the scale insect study.

CONCLUSION

The major tool in biological control operation is the application of synthetic insecticides, such as organophosphate compounds. This has not been very successful due to operational, economic and ecological factors. Due to lack of cost effective pesticide with environmental sustainability and harmful effect to plants, a novel pesticide to control scale insect is formulated. The present work deals with selection of suitable herbs with a maximum mortality rate of scale insects. GC-MS confirmed the presence of Butyl butyrate, Bis (3,3,5-Trimethyl hexyl) adipate and Benzofuran which are arthropod control chemicals. Adult mortality ranges from 91-99 % at 5 g /100 ml. This could go a long way in controlling adult females to prevent further production of eggs in consecutive generations. Since phytochemicals are safe, easily available round the year and cost effective, these stimulate local efforts to enhance the magnitude of pest control. Such an effective formulation can be used for large scale field study by agriculturists to decimate scale insect menace during flowering and fruition.

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