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Formulation of Herbal Pesticide towards effective Biological Control of Scale Insects in *Psidium guajava* L.

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Abstract:
Scale insects have attracted insect taxonomists and entomologists due to the invasive-destructive nature, morphological and biological variations and the need for developing an effective control strategy. As of now bio-insecticides have gained prominence as potential plant protecting agents. In the present study an attempt was made to develop a suitable formulation of a herbal pesticide towards effective biological control of scale insects using blends of two different herbal preparations. The prepared formulations were subjected to evaluation tests at varying concentrations on the insect pest. Insects treated with *Wedelia chinensis* ash + *Tridax procumbence* ash + coir pith at different concentration with 5 ml of citrulinic acid + 5 ml of soap solution exhibited highest rate of mortality. Results indicate that herbal preparation can effectively be used for the management of scale insects as an eco-friendly approach.

Keywords:
Herbal Pesticide; Biological Control; Scale Insects; Phenacoccus solenopsis; Psidium guajava; Wedelia chinensis; Tridax procumbence

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Introduction:
India is basically an agro-based country; more than 80% of Indian population depends on agriculture. Indian economy is largely determined by agricultural productivity. Insect-pests are known to cause significant damage to crops and affect agricultural productivity. Variety of chemical insecticides and pesticides are used to control insect-pests. However, harmful effects and persistent nature of the chemical pesticides demand for eco-friendly alternatives. The environmental hazards posed by synthetic pesticides provide an impetus for investigations into some eco-friendly and bio-rational alternatives [1]. Reproductive and dispersal modes of social insects mean that fundamentally different models may be required for predicting determinants of their invasions from those for sexually reproducing organisms. Many species of scale insects, social wasps, bees and ants are extraordinarily invasive in nature. The fact that drives invasiveness of social insects may be the flexibilities offered by both individual and colony behavior. Social insect invasion case studies have revealed strong evidence for biotic resistance to invasions in some instances [2]. However, the impact of invasions depends on the species, the host, environmental factors, and natural enemies.

Scale insects can be serious pests on trees, shrubs, and other perennials. Adult females and nymphs are immobile as they remain wingless. Immature scale insects and adult females have a characteristic round or oval to elongate and flattened or humped appearance. Immature males often have a different color and shape than females, especially in later nymphal stages (instars). Adult males are tiny,

delicate insects with a pair of wings. Since, the adult males are rare and have a short life span, the females reproduce without mating. At maturity, adult females produce eggs that are kept hidden under their body. Eggs hatch into tiny crawlers (mobile 1st instar nymphs, yellow to orange in color). Crawlers begin feeding within a day or two after emergence. Nymphs may spend their entire life in the same spot without moving as they mature into adults. However, are blown by wind to near by plants [3]. Scale insects are most present on the bark, fruit, or leaves. They insert a tiny straw like mouthpart into plants and suck fluids. Scale insects (Coccoidea) are diverse group of insects with 30 families and 8000 species.

Phenacoccus solenopsis Tinsley (Hemiptera: Pseudococcidae) is invasiveness pest with morphological and genetic variations that needs an effective management strategy. *P. solenopsis* has a wide spread geographical distribution [4-11]. In India, occurrence, severity, and epidemic forecast of mealybugs on several crops have been carried out by different groups [12-13]. Most mealybugs become permanent additions to ecological communities. Scale insects are often controlled by beneficial predators, parasites and application of broad-spectrum insecticides. However, chemical control is expensive. Alternatively, biological control of scale insects holds limited success rate. During the outbreak of scale insects, a well-timed and thorough spray of oil during the dormant season or soon after scale crawlers are active in late winter to early summer provide an effective control. Complete spray coverage of infested plant is required to obtain excellent control. Thorough spray coverage is especially critical when treating armored scales and oak pit scales, as these scales are generally less susceptible to pesticides than soft scales. Widespread infestation of *P. solenopsis* and economic damage to crops warrant the study to develop an effective management strategy.

The biological degradation and conversion of agroindustrial wastes by earthworms (vermicomposting), is becoming choice of recycling wastes [14]. Application of vermicompost not only benefits crop plants but also helps the plants to mobilize, acquire nutrients, promote plant growth and inhibit pathogenic microorganisms [15]. Biowash or vermiwash is the watery extract of vermicomposts rich in enzymes, plant growth promoting hormones, vitamins along with micro and macronutrients and beneficial microbes that increases the resistance of crops against various diseases and enhance the growth and productivity of crops [16].

Common guava (*Psidium guajava* L.) is a low evergreen tree 6 to 25 feet high, with wide-spreading branches and square, downy twigs, is a native of tropics. Branches crooked, bringing opposite leaves , flowers white, incurved petals, 2 or 3 in the leaf axils, fragrant, with four to six petals and yellow anthers, fruits small, 3-6 cm long, pear-shaped, reddish-yellow in color when ripe [17].

The yield in *P. guajava* is drastically reduced due to the infestation of scale insects as a result there is a huge economic loss to the farmers annually. Though several methods have been used to control the scale insects on *P. guajava* none has been effective practically. With this information, the present study has been carried out on *P. guajava* using the extracts obtained from selected botanicals and formulations.

Materials and Methods:

Collection of the plants and insects

Studies on were carried out in the laboratory at NRMC, Periyakulam, Theni using the population collected from unsprayed Guava trees in the orchards. Twigs of *Psidium guajava* L. plants infested with reproducing females of *P. solenopsis* were brought to the laboratory. Plants - *Wedelia chinensis* and *Tridax procumbence* collected from the wild, from the nearby area Periyakulam, Theni district. Selection of plants was made on the basis of absence of damage by the insect-pest. Healthy plant materials were collected in poly bags and brought to lab and their botanical identity was established. Flora of Presidency of Madras [18] and The Flora of Tamil Nadu Carnatic [19] were used for authentication of the plants.

Insect rearing

Individual females were separated, and fed on Guava leaves in Petri plates. Leaves without insecticidal spray and free from infestation, were washed with tap water and kept in shade and used as food source. Base of the petiole of individual leaves was left water soaked cotton swab to prevent desiccation of the leaf, since parthenogenetic, emerging individual neonate crawlers were used in the study. Young individuals maturing on the same day were transferred to separate glass Petri plates containing Guava leaf. The study was conducted between June and July 2011 in the laboratory when temperature and mean relative humidity ranged between 21.4 to 30.6°C and 52.5 to 90.6 % respectively.

Preparation of herbal pesticide

Container for vermicomposting was constructed by cutting a 200 L plastic barrel (collected from scrap) into two halves. A metal grill of about 10cm was placed at the bottom of the barrel and air dried foliages of *Wedelia chinensis* and *Tridax procumbence*, coir pith collected from Dolly Fibers,

Periyakulam, Theni district were composted on top with earthworms (*Eisenia foetida*). The plastic barrel was fixed with an outlet at the base to collect biowash (vermiwash). The whole setup was left for two months until all the foliages of herbals were digested.

Extraction of biowash:

At the end of two months, when the foliages of *Wedelia chinensis*, *Tridax procumbence* and coir pith were completely composted, biowash was extracted. To the completely prepared compost, water was sprinkled slowly and uniformly. The quantity of water sprinkled was determined by the volume of compost in the tank. The watery extract of vermicompost drained out of the container was collected at the bottom of the drum and called biowash. The biowash was stored until further processed for evaluation.

The crude biowash collected from the vermicompost contained many solid particles as well as microorganisms. In order to remove these, the biowash were centrifuged at 9000 g for 30 min and the supernatants collected. After adjusting the pH to 3.0 the supernatant was partitioned three times against ethyl acetate (EtOAc).

Observations on survival and molt of the crawlers were recorded daily under stereoscopic microscope until they became adults. Unless the crawlers were in a pre-molt stage, Petri plates along with fresh leaves were changed on alternate days or else transferred after the molt. Transferring leaf disc along with crawler obviated their direct handling using a camel hair brush (No.1). Petri plates with missing crawlers were discarded and excluded from the final data. Developmental time of each instar was recorded based on an observation. Daily monitoring of crawlers, those that had stopped further molting and reached adult stage was done to determine the pre-reproductive and reproductive periods, fecundity, and longevity. As the eggs or neonate crawlers were counted and discarded, the individual adults that had produced them were transferred to new Petri plates for further observations. When eggs were observed they were separated along with the leaf disc and observed until they hatched. The data obtained were subjected to statistical analysis.

Results:

Change in the nutrient content due to infestation of scale insects is given in Table 1. The mortality rate of crawlers of *P. solenopsis* at various concentrations in different treatment groups varied considerably in a time dependent manner across different treatment groups as shown in Table 2. The mortality rate increased significantly with time, reaching a maximum of 25 % in all the treatment groups with a maximum of 30.2 ± 11.77 in $1T_1$. Since no significant effect due to the addition of olive oil was observed in Phase I, a combination of citrulinic acid and soap solution was used along with herbal vermiwash in Phase II. Mortality rate of scale insects varied significantly at various concentrations of the plant material in different treatment groups (Table 3). Maximum mortality rate of 82.4 ± 32.7 was observed in $2T_1$ with supplementation of 2ml herbal extract followed by 66.2 ±22.61 in $2T_2$ with supplementation of 2ml herbal extract respectively. However, maximum mortality rate of 55.2 ± 5.81 was observed in $2T_3$ with supplementation of 4ml herbal extract. The overall percentage mortality rate observed was 95.9, 94.8 and 82.7 in 2T1, 2T2 and 2T3 respectively. Since the mortality rate in Phase II was significantly high, a combinatorial effect using the vermiextract of the selected botanicals was elucidated in Phase III. Mortality rate of scale insects at various concentrations of ash in different treatment groups (*Wedelia chinensis* ash + *Tridax procumbence* ash + coir pith at different concentration with 5 ml of citrulinic acid + 5 ml of soap solution in 90 ml water (3T1 - 1 gm ash; 3T2 - 2 gm ash; 3T3 - 3 gm ash; 3T4 - 4 gm ash; 3T5 - 5 gm ash) varied significantly. Maximum mortality rate (90±23.33) and percentage mortality (98.90) was observed in 3T5 (Table 4; Fig. 1).

Further, it was observed that females had a wider range of developmental periods than males. Females after the final moult took about 2-8 days for reproduction. Reproduction by *P. solenopsis* was parthenogenetic with 96.5 and 3.5% of offspring produced as crawlers and eggs through ovoviviparity and oviparity, respectively.

Discussion:

Natural products with pesticidal activity have been and are being explored in order to make pesticides which are easily biodegradable, selective and can be locally produced, especially for farmers who can not afford expensive synthetic pesticides. At present, serious attention is drawn to extracts from vermicomposts of higher plants that contain antifungal substances in the form of alkaloids or prohibitins, which help in resisting the pathogens. Earthworms also promote microbial activity and diversity in organic wastes to levels even greater than those in thermophilic composts (Edwards, 1998). Hence, there seems to be an even greater potential for suppression of plant disease by vermicomposts than by composts, probably due to stimulatory effects of soil microbial activity [15].

Although the reports of occurrence and epidemics of *P. solenopsis* have been documented on cotton from several countries [10, 13], details of the biological parameters were not explored on other plants due to the need for extensive standardization of culture materials and methods. It has been reported that *P. solenopsis* on *H. rosa-sinensis* progressively develop in a time dependent manner [9]. Since, study pertaining to the life history and pattern of biological activities are difficult under field conditions, laboratory studies have become indispensable [20]. However, under laboratory conditions developmental patterns vary significantly, indicating the influence of ecological conditions and the role of host plant on the development of the insects. Furthermore, the development duration is species dependent [21]. A wide range of host plants and short developmental duration as observed suggests that *P. solenopsis* is more acclimatized to tropical conditions across the sub-continent [22].

Nevertheless, less number of male populations is an indication to the fact that that male has no role in reproduction, although, under field conditions sexual reproduction may occur. Viewed in juxtaposition with the biology of *P. solenopsis* it is evident that longevity of adults, and their larger size with increased waxy coating, and higher food requirement, result in visibility of the pest and symptoms on the host plant. Therefore, all aspects related to the developmental biology of *P. solenopsis* and the mode of infestation on host plants is essential to make management decisions for formulation of appropriate eco-friendly herbal pesticide. Higher mortality of the crawlers, the longer effective reproductive period and increased longevity of adult females along with the expected natural mortality factors such as predation, parasitization and action of abiotic factors on crawlers and adults under natural field conditions, suggest that management interventions should be focused on adult females rather than males and crawlers to prevent the multiplication and spread of this insect pest [23].

Conclusion:

Results indicate that management of *P. solenopsis* is variable in terms of behavioral and developmental patterns and therefore, the choice of plant material, combination in the formulation of the spray and the prevailing ecological conditions besides that host plant must be taken into consideration for effective management of the insect pest. In conclusion, an attempt has been made to evaluate the role of selected botanicals and their formulations can be used as bio-pesticidal agent in insect-pest management programs. The results of the present study hold a promising possibility of further investigations of efficacy natural plant products of their bio-pesticidal properties.

Table 1 Change in Nutrient Content (mg/g) of Guava fruit

	Nutrient Content (mg/g)		Level of significance
	Uninfected	Infected	
Carbohydrate	275.4 ± 99.420	162.00 ± 27.370	F > 0.05
Protein	25.8 ± 2.008	14.4 ± 2.244	F > 0.05
Calcium	1.275 ± 0.326	1.8 ± 0.424	F < 0.05
Phosphate	0.2325 ± 0.044	0.375 ± 0.077	F < 0.05

Table 2 Phase I - Mortality rate of scale insects at different concentrations in different treatment grp

Time interval (min)	$1T_1$	$1T_2$	$1T_3$	Level of significance (F)
5	5.8 ± 3.25	7.6 ± 2.57	8.6 ± 3.77	<0.05
10	14.0 ± 5.69	14.4 ± 4.07	14.4 ± 5.89	<0.05
15	22.0 ± 5.76	20.4 ± 5.92	25.0 ± 9.32	<0.05
20	30.2 ± 11.77	25.0 ± 8.67	25.0 ± 9.32	<0.05

Insects were treated with a mixer of 20 ml *Wedelia chinensis* vermi wash + 20 ml *Tridax procumbence* vermi wash + 10 ml of coir pith vermi wash along with olive oil at different concentration ($1T_1$ – 1ml of olive oil; $1T_2$ – 2ml of olive oil; $1T_3$ – 3 ml of olive oil).

Table 3 Phase II - Mortality rate of insects at various concentrations of the plant material in different treatment groups

Concentration of herbal extract (ml)	$2T_1$	$2T_2$	$2T_3$	Level of significance (F)
1	7.91 ± 12.4	29.0 ± 11.1	26.8 ± 3.65	F > 0.05
2	82.4 ± 32.7	66.2 ±22.61	39.0 ± 6.54	F > 0.05
3	50.6 ± 12.3	63.0 ±28.78	43.6 ±18.09	F < 0.05
4	49.6 ± 18.6	46.0 ±11.52	55.2 ± 5.81	F < 0.05
5	31.6 ± 5.3	26.8 ± 7.27	33.4 ± 7.22	F < 0.05
% mortality	**95.9**	**94.8**	**82.7**	

Insects were treated with a mixer of 5ml of citrulinic acid + 5 ml of soap solution with ashes at different weights in 90 ml of water (2T1 – *Tridax procumbence*; 2T2 – *Wedelia chinensis*; 2T3 – Coir pith).

Table 4 Phase III - Mortality rate of scale insects at various concentrations of ash in different treatment groups

Treatment Groups	Mortality rate
$3T_1$	63.4 ± 9.93
$3T_2$	52.4 ± 19.65
$3T_3$	58.2 ± 36.57
$3T_4$	56.6 ± 18.07
$3T_5$	90 ± 23.33
Level of significance	F > 0.05

Insects were treated with *Wedelia chinensis* ash + *Tridax procumbence* ash + coir pith at different concentration with 5 ml of citrulinic acid + 5 ml of soap solution in 90 ml water ($3T_1$ - 1 gm ash; $3T_2$ - 2 gm ash; $3T_3$ - 3 gm ash; $3T_4$ - 4 gm ash; $3T_5$ - 5 gm ash).

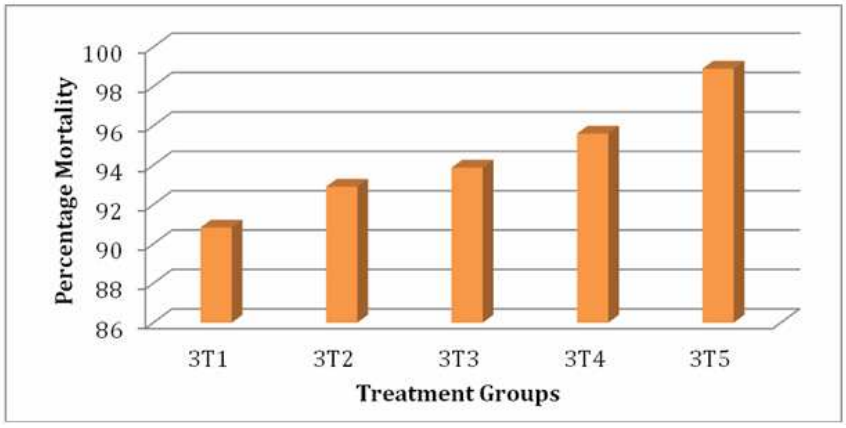

Fig. 1 Percentage mortality rate of the insects with various treatments.

Insects were treated with *Wedelia chinensis* ash + *Tridax procumbence* ash + coir pith at different concentration with 5 ml of citrulinic acid + 5 ml of soap solution in 90 ml water ($3T_1$ - 1g ash; $3T_2$ - 2g ash; $3T_3$ - 3g ash; $3T_4$ - 4g ash; $3T_5$ - 5g ash)

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