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PERFORMANCE OF FIVE PLANT EXTRACT ON-LEAF-CUTTINGBEETLE *Hypomeces squamosus* on *Harumanis* VARIETY OF MANGO IN PERLIS, MALAYSIA

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ABSTRACT

Application of plant extracts as alternative to commercial insecticides provide an eco friendly non hazardous control method. The gold dust beetle (*Hypomeces squamosus* L.) is among the insects that produce cuts, holes, or leaf falls, as they feed on mango leaves. A field trial (RBD, 4 replicates) was conducted to evaluate the effect of ethanol extracts of five plants [*Azadirachta indica* L. (neem), *Cymbopogon citrates* L. (lemon grass), *Capsicum frutescent* L. (hot chilly), *Zingiber officinale* L. (ginger), and *Syzygium aromaticum* L.(clove)] on the leaf damage caused by this beetle. Four, 4-5 year old, mango plants per treatment, were sprayed with 0.5% of each extract at weekly intervals for five weeks, with water treatment as control. The plant extracts from neem and chilly showed remarkable reduction ($p \leq 0.05$) in the number of fallen and damaged leaves while other treatments recorded high number of fallen and damaged leaves. The data on the number of insects visiting the plants, one week after each spray for each treatment, showed chilly extract to be a good treatment in recording low number of insects when compared with the control. Lemon grass and ginger also recorded low insect count.

KEYWORDS: Plant Extracts, Pest Control, Leaf-Cutting Beetle, *Hypomeces Squamosus,* Mango

INTRODUCTION

The heavy use of insecticides has resulted in increased costs, environmental pollution, disputes among neighbors, and the reduction in natural enemies of insect pests and pollinators (Spalding 1982). There is a strong need to re-evaluate existing practices to chemicals for pest control (Rola and Pingali 1993) amid the fear of the danger to public health and environment pollution. Bioactive compounds from plant extracts, usually the limuloids group, can serve as effective and useful alternative to the conventional methods in pest control. These compounds work on the insect hormonal system (Robert et al. 1996; Fernandez and Jens 1999) and may prevent resistance to these compounds since they do not react on the digestive or nervous system of the insects. Neem oil has been effectively used to control Bruchid - beetle larvae in stored beans and other legumes (Ellis et al.1992). recorded a 100% killing of *Callosobruchus maculates* (beetle) when the stored crop was treated with 0.5% v/w of neem oil 2 days after of treatment (Ram and Gopal 2000).

Plant extracts and oils can delay the egg-laying by the female of *Callosobruchus* adult and prevent its damage in stored legumes (Golob and Webley, 1980). Other plant extracts, such as, *Anacadium occidentale, Zingiber officinale* and *Azadirachta indica* and peels of citrus significantly decreased infestation and emergence of *C. maculatus* (Onu et al. 1997). Findings by other researchers (Ofuya et al. 1992; Banner 1993, Aku et al. 1998, Adedire and Akinneye 2004, Rahman and Talukder 2006) also supported the use of plant extracts in reducing the beetle infestation in stored crops like legumes or maize. In study by (Rahman and Talukder 2006) bioefficacy of plant extract, powder, ash and oil from nishinda (*Vitex negundo L.*), eucalyptus (*Eucalyptus globules L*), bankalmi (*Ipomoea sepiaria K.*), neem (*Azadirachti indica L.*), safflower (*Carthamus tinctorius L.*), sesame (*Sesamum indicum L.*) and bablah *(Acacia Arabica L.*) and found that the plant oils were effective against insect infestation of the pulse beetle *Callosobruchus maculates* Leaf extracts from *Phytolacca*

dodecandra showed a high adult mortality of the maize weevil *Sitophilus zeamais Motsch* (Firdissa and Abraham, 1999) reported that. Plant extracts from groundnut, coconut, and oil from fresh garlic showed significant inhibitory effectiveness against insect's beetles (Lale1995, Ho et al. 1997).

In bioassays to evaluate the potential of 13 plants extracts for the control of the red palm weevil, (*Rhynchophorus ferrugineus).* Their results indicated that the ethanol extracts of sea ambrosia (*Ambrosia maritima*), French cotton (*Calotropis procera*) and curcuma (*Curcuma longa*) have superior toxicity as compared to the extracts of other tested plants. (Salama and Ismail 2007)

The present research intended to apply neem, ginger, clove, hot chili, and lemon grass, extracts in the pest control of a leaf-feeding beetle on mango foliage. Mango var. *Harumanis* (family Anacardiaceae) having 60 genera of which 15 genera were reported in Malaysia (Abidin and Malik 1996), was selected for the study. This variety is noted for its pleasant taste, texture and aroma among consumers, in the northern state of Perlis, Malaysia, and is popular both at the local and international markets. But it is prone to several insect pests mainly beetles, (Bagshaw et al.1989), at all stages of its life cycle and it became the primary objective of the research to address the damage caused by *Hypomeces squamosus* L. (Gold dust weevil), one of the prominent leaf cutters in Harumanis.

METHOD AND MATERIALS

Selection of Insect Pest

Hypomeces squamosus L. [Coleoptera: Cuculionidae, Plate 1] is among the insects that feed on mango leaves and cause leaf fall or damage. They are covered with shiny yellow or green scales that give them a dusted appearance (CABI 2004). This weevil was the most prevalent insect found on the mango plants during the onset of the field experiment.

Selection of Field Site

Field studies were conducted between February 2011 and March 2011 at the Agriculture Research Station of the Agro technology unit of the University Malaysia Perlis on *Mangiferae indica* var *Harumanis*. The trees were of an average age between 4 and 5 years and approximately of an average height of 2.5 m.

Trees Classification and Selection

Trees under study were divided into six major groups depending on the number of treatments used (five plant extracts + water control) using the RBD design with 4 replicate. For the spraying schedule, **four** plants were selected at random and numbered 1 to 4 for each treatment to distinguish the trees and to facilitate data collection by using different colors (Neem [Blue], Chilly [Red], Lemon grass [Orange] Ginger[Green] Clove[Yellow] and Control [white]; Plate 2.) The total number of plants used in the 4 replicates was: 96 [4 plants/treatment/replicate x 6 treatments x 4 replicates]. Hence the total number of plants used for each replicate for the 6 treatments was 4 plants/ treatment x (6) trees.

Preparation of Plants for Extraction

Leaves from Neem were collected from trees growing at the river side park near the Kangar town. The other plants, leaves and stem of lemon grass, hot chilly fruits, ginger tubers and clove fruits were purchased from the wet market at Kangar.

These samples were brought to laboratory and washed under running water, to get rid of dirt, insects and plankton. They were dried overnight in the laboratory- electric oven at 40^0C. One hundred grams of the material were pulverized by an electric mixer, and preserved in labelled glass bottles that were sealed until use.

Preparation of Plant Extracts

The extraction technique used was a modification of Ruch's (2001) method .Up to 50g each of the oven dried and pulverized powered material from plants were treated with 500 ml of 95% alcohol with constant stirring for 30 min. After stirring, the solutions were filtered through 2 layers of cheese-cloth gauze and Whitman's (No.2) filter paper before the filtrates were subjected to evaporation using a rotary evaporator at 60 ^{o}C degree for 60 min to removed the ethanol. The dark spongy materials were dried in an oven at 37 ^{o}C for 2 days. The dried powder was stored in small and sterilized 5ml screw-capped glass bottles that were refrigerated (4^{o}C) until further use. The extraction yield was calculated as Yield (%) = (WE/WP) × 100

Where WE and WP are the weights of the dried extract and the dried powder before extraction respectively, [Table 1]

Preparation of Plant Extract-Dilutions

The ethanol-plant extracts that were prepared earlier and kept in the refrigerator were used to prepare the dilutions for spraying the plants. Aliquots of 5g of each powder from *Azadirachta indica* (Neem), *Capsicum frutescens* (Chilly), *Cymbopogon citratus* (Lemon grass), *Zingiber officinale* (ginger), and *Syzygium aromaticum* (clove) were brought into concentrated solution by adding 2ml of organic solvent DMSO (solvent dim ethyl sulfoxide) and further diluted by mixing with a liter of water to get a concentration of 0.5% or 5000ppm. The purpose of adding DMSO to dissolve non-polar materials that do not dissolve in water (Raja et al.2011).

The 1 liter plant extracts [conc.0.5%] from each treatment were then poured into 2L spray pumps, stirred by hand thoroughly using circular movement and taken to the field to be sprayed directly on the entire whole plan in addition to above or under the leaves of the labeled plants from the different treatments. The experiment was conducted using the RBD statistical design with four replicates. Fresh dilutions of conc. 0.5% for each treatment were prepared weekly from the extracts that were stored in the refrigerator temperature of 4^{o}C (Ruch, 2001) for the consecutive spraying schedule of 5 weeks.

Damage Assessment on the Trees Sprayed

The actual assessment of leaf damage in the field is often quite difficult to determine as it is dependent on the feeding preference and flight pattern of the insect and the environment factors, such as cloudy, rainy, or hot dry weather, during the time of spray.

Keeping in view of the above factors and the natural leaf fall caused by matured leaves or strong winds, we considered the leaf fall on the ground at a radius of 2m from the trunk of the tree and insects visiting the 4 trees from each treatment as the criteria to record insect damage one week after each spray.

It was laborious to determine the intensity of damage and the actual leaf area consumed by the insects on the plant or in fallen leaves for insect damage in the present experimentation. Hence, we used the leaf area consumed by the insects, as indicated by the fallen leaves showing holes or cut in the leaves, to record leaf damage data. The fallen leaves were collected and counted for leaf damage based on the above symptoms.

Later, after the count they were collected in plastic bags and removed from the 2m radius periphery of the tree trunk so as to minimize error in data collection for the following week. The number of insect- damaged fallen leaves per week after spray was considered as the data for insect damage.

Determining the Repellent, Attractant, or Neutral Properties of the Extracts

To observe the effectiveness of the extracts to see whether they repelled, attracted or were neutral to the application of the plant extracts, a visual insect count of the insects on the sprayed trees, one week of the spray, was recoded, for each treatment in the 4 replicates. The repellent property was considered by the presence of insects on the leaves or any other part of the plant but did not cause much damage on the leaves due to the possible anti-feeding property of the extract.

If the number of insects was comparable with the population of the insects causing normal damage as seen in the water control spray, then that treatment was considered as of 'no effect' or neutral. High population of insects on the plants after spray indicated possible attractant property of the plant extract. Sunlight and high temperature can often degrade the active compounds in the plant extracts (Lo et al.2005) and keeping in view this consideration and the nocturnal behavior of the insects, the spraying of the plants was done at weekly intervals for 5 weeks, in the evening around 5.30 pm.

Statistical Analysis

Experimental data was subjected to ANOVA using SPSS (SPSS Inc., Chicago, USA). (Sbeghen-Loss et al. 2011). Significant difference between mean values were determined using Duncan's Multiple Range test (P= 0.05) following ANOVA.

RESULTS

Effect of Plant Extracts to Reduce Fallen Leaves

The bar chart data obtained from the accumulative mean of the 5-week spray period showed that the concentration of *Azadirachta indica* L. (neem), *Capsicum frutescens* L. (hot chilly), and *Cymbopogon citrates* (Lemon grass) extracts (5000 ppm) gave at significant impact ($p \leq 0.01$) in reducing the number of fallen leaves on the ground recorded at a radius of 2 meters from the trunk of the labeled tree. (Figure1). Other extracts did not show much effect when compared with the control treatment.

The 5-week data on the accumulative mean of damaged leaves (Fig.2) showed that neem extract, and hot chilly extracts showed a significant reduction in the number of damaged leaves ($p \leq 0.01$). Lemon grass also recorded reduction in the number of damaged leaves ($p \leq 0.05$) when compared with the control water- spray treatment. The effects of ginger and clove were not significant.

The data from Figure.1 and Figure.2 seems to complement one another and can be regarded as reliable to access the efficacy of the plant extracts in minimizing the damage caused by the pest.

Effect of Plant Extracts to Reduce Insect Count

The data on the average number of insects visiting the sprayed plant after 5 weeks of weekly sprays has however showed a different trend (Fig.3) 2 plant extracts apparently increase insect abundance, while the other treatments did not deter the insects from visiting the leaves on the mango plants.

Effect of Plant Extracts to Reduce Damaged Leaves

The data on the average number of insects visiting the sprayed plant after 5 weeks of weekly sprays has however showed a different trend (Fig.3) 2 plant extracts apparently increase insect abundance, while the other treatments did not deter the insects from visiting the leaves on the mango plants.

DISCUSSIONS

The exploitary, *in vivo,* experiment performed at the Agro technology Unit of the university was subjected to factors, such as temperature, light, and humidity of the 5- week duration of the experiment, flight pattern and eating habit of the leaf-eating beetle. These factors may have a direct or indirect effect on the result of the experiment. Table 1 summarizes the results of the entire experiment.

It is often difficult to evaluate the damage factor on the leaves caused by the beetle considering the eating habit, the flight pattern of the insect pest, the weather fluctuations, and the aging leaf fall. Hence data of both fallen leaves and damaged leaves showing holes or cuts in the leaves was taken into account and the pattern of the results complemented each other with slight variations. The effect of the treatments for fallen and damaged leaves in the *ascending order* was: Neem, Chilly, Lemon grass, Ginger, Clove, and Control [Fig.1 and Fig.2]. Neem, chilly and lemon grass recorded low number of damaged leaves.

Volatile plant secondary metabolites are detected by the highly sensitive olfactory system of the insect. The olfactory receptor neurons [ORNs] present in the sensillae of the antennae, are used by the insects to locate suitable or unsuitable plant hosts. Perception of blends of plant volatiles plays a pivotal role in host recognition, non-host avoidance and ensuing behavioral responses as different responses to these volatile compounds (Bruce and Pickett 2011). The insect count on the leaves after the 5-week spray period exhibited a set of interesting pattern.

Neem showed reduction in leaf damage indicating its repellent property (Chio and Yang 2008) but an increase in insect count when compared to control. It is difficult to explain this trend. The most likely reason could be the availability of more leaf foliage for the insect to feed but deterred from feeding due to its bitter taste and repellent property. Capsaicin as reported in the *Capsaicin Technical Fact Sheet*, NPIC, from Oregon State University, is technically a phenylpropanoid, which makes hot chili peppers hot. It is increasingly being used as an insect repellent for organic growers who wish to avoid treating their plants with artificial chemicals. (CTA 2008). The chilly extract from hot pepper in the present study performed well in recording low number of damaged leaves besides low insect count after the spraying schedule.

Repellent property of Lemon grass proven using GC-MS analysis and found that the components, geraniol, neral and geranial, isolated from the essential oils exhibited repellent property against Tribolium castaneum Herbst (Coleoptera: Tenebrionidae) after 4hr exposure to the dose of 0.021 ml/L when compared with the commercial product IR3535 dose of 0.668 ml/L . The present study indicated some repellent or anti feeding property of lemon grass crude extract against mango leaf cutting beetle (Hypomeces squamosus) of mango at 0.5% concentration [Table 2] (Olvero-Verbal et al .2010).

Ginger extract analyzed with GC-MS [Gas chromatography, mass spectrometry] had bioactive compounds heptanol, heptyl acetate and linalool that repelled stored maize weevil (Ukeh et al. 2009). But the trend to repel *Hypomeces* beetle was not observed in the experiment [Table 2] possibly due to less number of leaves available for insects to feed on. The low number of leaves on the trees could have been caused by the high number of fallen and damaged leaves recorded in this treatment that was comparable with the control.

Clove which showed a potential as a grain protectant against stored rice and maize (Ho et al.1994) was not effective against mango leaf cutter in the present study. It in fact attracted more insect than the control [$p \leq 0.01$] indicating that it has a potential to be used as an attractant in integrated pest management. Methyl Eugenol has been used in trapping fruit-flies (Chuah and Goh 1997). It is present in cloves (Myint et al. 1996) and this could be the cause for the high insect count from this treatment.

CONCLUSIONS

Chilly and clove showed a consistent results in both the damaged and visiting insects data. From this, it could be concluded that hot chilly extract possess a great potential to be an effective bio pesticide to minimize the damage caused by the Gold dust beetle and the clove extract could be employed as an insect attractant in Integrated Pest Management.

FUTURE RESERCH

Development of new agents for insect control based on bioactive chemical compounds from indigenous plant sources, eco-friendly and may serve as suitable alternative to synthetic insecticides as the are relatively safe, inexpensive and are readily available in many of the world.

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Table 1: Plant Species, Parts and Concentration used in Experiment

Common Name	Plan Species	Family Name	Nature of the Extract	Yield(%)	Conc used in Spray(ppm)
Neem	Azadirachta indica L.	Meliaceae	Leaves powder	8.4	5000
Chilly	Capsicum frutescence L.	Solanaceae	Fruit powder	6.4	5000
Lemon grass	Cymbopogon nardus L.	Poaceae	Leaves powder	6.8	5000
Clove	Syzygyum aromaticum L.	Myrtaceae	Fruit powder	4.9	5000
Ginger	Zingiber officinale L	Zingiberaceae	Tuber powder	11	5000

Table2. Effect of Plant Extracts (5000ppm) on the Hypomeces squamosus (Fallen Leaves, Damage Leaf and Visiting Insect) After Weekly Spraying on Leaves for a Period of five Weeks

Plant Extract	Fallen Leaves	Damaged Leaves	Insect Count	Remarks
Control	High	High	Low	Neutral
Neem	Low	Low	Medium	Anti-feeding
Lemon grass	Medium	Medium	Low	Anti -feeding

Tablec 2:Contd.,

Chilly	Low	Low	Low	anti -feeding
Ginger	High	High	Low	Neutral
Clove	High	High	High	Attractant

Plate1: Photographs of the Gold Dust Beetle Feeding on the Leaf and Inflorescence of Mango

Plate 2: Photographs of some Labeled Randomly-Selected Mango Plants used in the Study

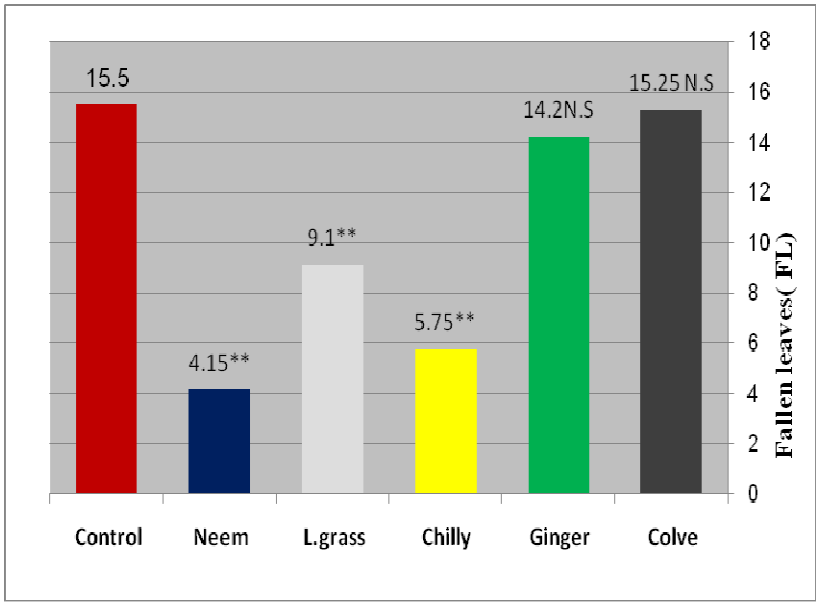

Figure1: Effect of Plant Extracts 5000ppm (Fallen Leaves) after Spraying on Leaves Directly for a Period of Five Week

NS= Insignificant, = significant at 0.01, **= significant at 0.05*

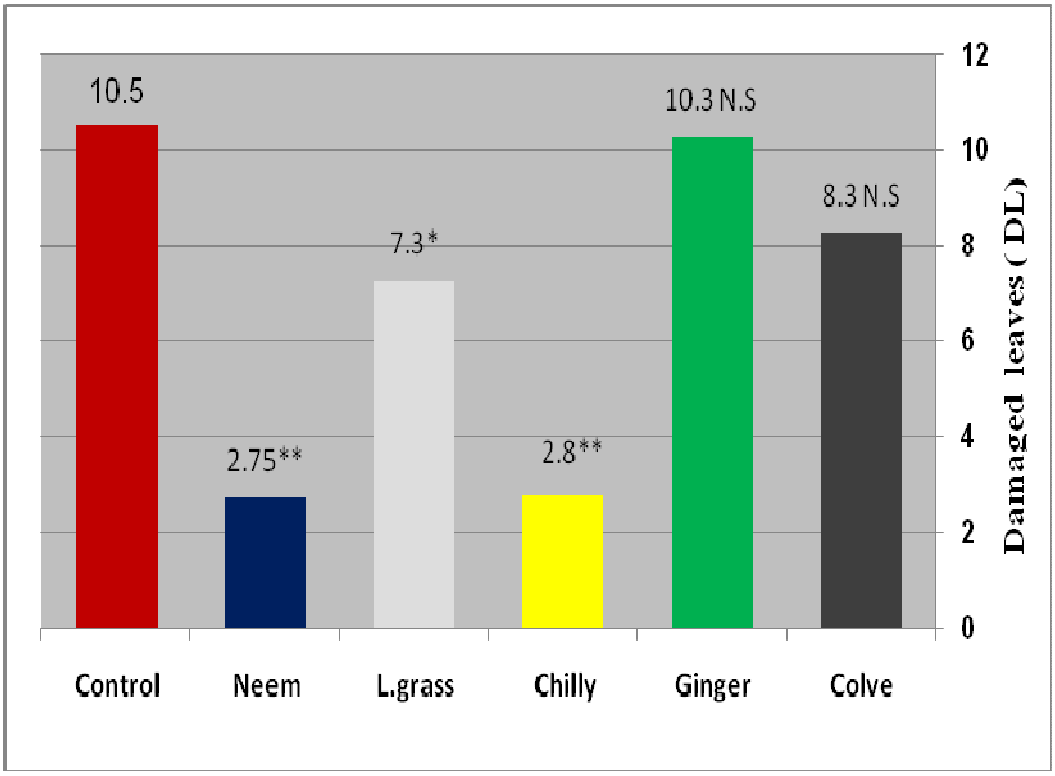

Figure 2: Effect of Plant Extracts 5000ppm (Damaged Leaves) after Spraying on Leaves Directly for a Period of Five Weeks

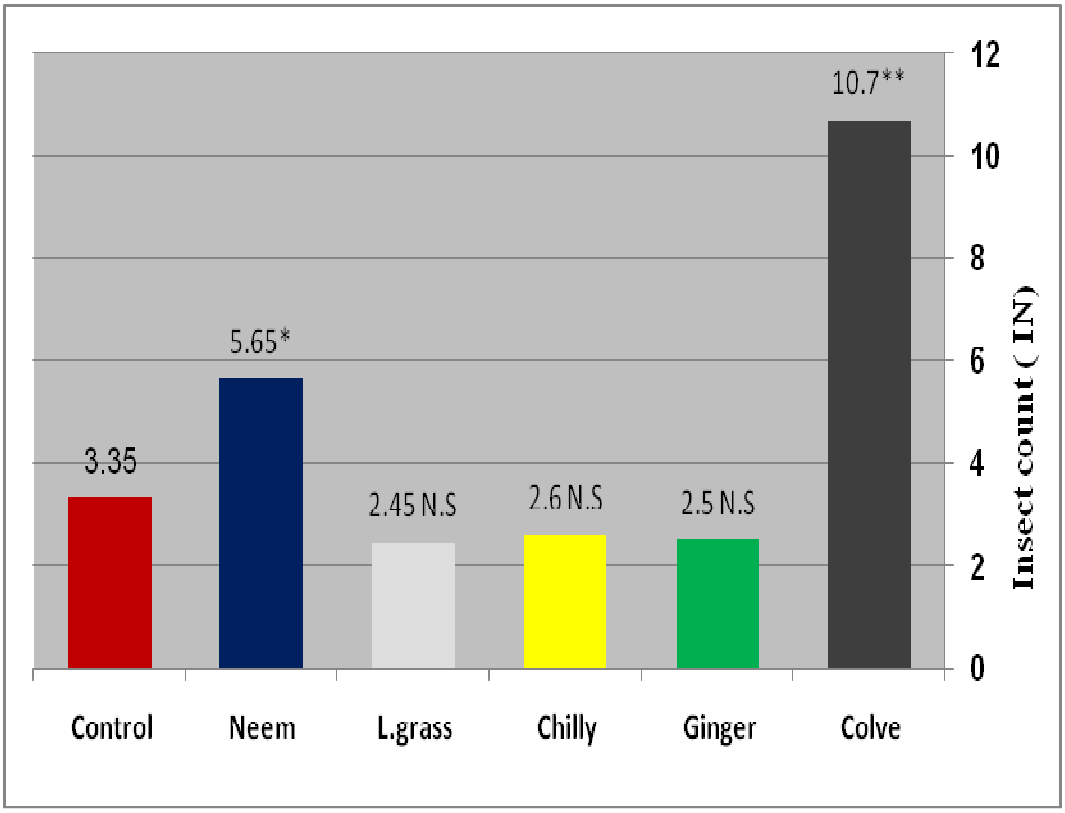

Figure 3: Effect of Plant Extracts 5000ppm (Visiting Insect Count) after Spraying on Leaves Directly for a Period of Five Weeks