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Antifeedant Activity of Selected Ethno-botanicals used by Tribals of Vattal Hills on *Helicoverpa armigera* (Hübner)

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ABSTRACT

Phytochemicals, especially botanical insecticides are currently of interest because of their successful application in plant protection as potential biocontrol agents. Biological activity of leaf aqueous extract of twenty five medicinal plants were evaluated against the VI instar larvae of gram pod borer *Helicoverpa armigera* (Hübner), (Lepidoptera: Noctuidae). Antifeedant activity of leaf aqueous extracts of *Abutilon indicum, Acalypha fruticosa, Achyranthus aspera, Adhatoda zeylanica, Aegle marmelos, Aerva lanata, Albiziza amara, Andrographis lineaeta, Andrographis paniculata, Azadirachta indica, Cardiospermum halicacabum, Cassia tora, Catharanthus roseus, Datura metal, Gymnema sylvestre, Melia azedarach, Ocimum canum, Ocimum sanctum, Pergularia daemia, Plectranthus coleoides, Solanum nigrum, Solanum surattrense, Solanum trilobatum, Tribulus terrestris, Vitex negundo* were evaluated in this study. Preliminary screening after 24h of exposure to leaf aqueous extracts of the selected plants at a concentration of 1,000 ppm exhibited significant larval mortality rate. The percentage mortality rate varied considerably between 11.8 and 78.9. The mortality rate was observed in the decreasing order of *M. azedarach* > *A. indica* > *S. trilobatum* > *A. paniculata* > *A. marmelos* > *A. lineata* > *S. surattrense* > *C. roseus* > *A. zeylanica* > *A. fruticosa* > *D. metal* > *S. nigrum* > *O. canum* > *P. coleoides* > *O. sanctum* > *P. daemia* > *A. amara* > *G. sylvestre* > *C. halicacabum* > *V. negundo* > *A. indicum* > *C. tora* > *T. terrestris* > *A. aspera* > *A. lanata* against the larvae of *H. armigera*. The results imply that leaf aqueous extract of *M. azedarach, A. indica, S. trilobatum, A. paniculata, A. marmelos, A. lineata, S. surattrense, C. roseus,* A. *zeylanica, A. fruticosa* and *D. metal* can potentially be used as eco-friendly pest control agents against the larvae of *H. armigera*.

Keywords: Ethno-pharmacological activity; Antifeedant Activity; Medicinal Plants; *Helicoverpa armigera*; insect-pest management; Bio-pest control agents.

INTRODUCTION

India is basically an agro-based country more than 80% of Indian population depend on agriculture for their livelihood. Indian economy is largely determined by agricultural productivity. Insect-pests are known to cause significant damage to crops and affect agricultural returns. The monetary loss due to feeding by larvae and adult insects alone contributes to billion dollars per annum (Jacobson, 1982). *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) is a destructive polyphagous pest occurring on cotton, tomato, bhendi, chickpea, pigeonpea, chilli, maize, sorghum and many others, inflicting substantial loss every year (Reed and Pawar 1982; Manjunath et al.1985; Sharma 2001; Talekar *et al*., 2006). However, in polyphagous insects, colonization of a new host may induce the selection of adaptive characters and genetic differentiation in population (Rice 1987). The larvae feed on leaves initially and then bore into bolls/pods and seeds with its head thrust into the boll/pods, leaving the rest of the body outside.

H. armigera infests several plants like cereals, pulses, cotton, and vegetable besides it has been reported to attack wild hosts. In central and north India, it is the major pest affecting cotton. The larvae feed extensively on cotton plant parts including the newly emerging bolls causing severe damage to the crop. Bollworms are relatively safe from natural enemies because of the cryptic feeding habits of the larvae within cotton bolls. Therefore, large numbers of *H. armigera* in cotton and other vegetables survive to adults that may disperse widely, producing progeny that damage high-value crops (Cabanillas and Raulston 1995; Michael and Donald 1996). The ability of insect species to thrive on diverse host plants is an adaptive advantage for their better survival in the ecosystem. *H. armigera* is also characterized by its high mobility and fecundity and it has shown great capacity to develop resistance to synthetic insecticides used in its management (Armes et al. 1996; Kranthi 1997; Ramasubramaniam and Regupathy 2004).

Variety of chemical insecticides and pesticides are used to control *H. armigera*. *H. armigera* has a long history of resistance to conventional insecticides. However, harmful effects and persistent nature of the chemical pesticides demand for eco-friendly alternatives. Economic loss due to this pest in India accounts for 5,000 cores (Manjunath *et al*., 1985). During the last 50 years, worldwide use of synthetic insecticides to control insect pests has led to both insecticide resistance and environmental persistence (Roush, 1990). The environmental hazards posed by synthetic pesticides provide an impetus for investigations into some eco-friendly and bio-rational alternatives (Subashini et al., 2004).

Plant derived phytochemicals have been widely used in the management of agricultural pest (Choudhary et al., 2001). Plant derived pesticides are eco-friendly, non-toxic to non target organisms, non persistent in nature, besides they do not promote drug resistance (Liu *et al*., 2000). Therefore, researchers world over are engaged

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in a mission to hunt for novel phytochemicals that could potentially be used in the management of insect-pests.

Plants are endowed with a potential to produce a range of secondary metabolites like alkaloids, terpenoids, flavonoids, phenols, glycosides, sitosterols and tannins. These phytochemicals are known to protect the plants from the attack of insect-pests (Ahmad, 2007). However, production of phytochemicals varies from plant to plant. Further, parameters like age of plant, part of plant (root, stem, leaf, fruit, flower, seed and bark) have been reported to affect the production of phytochemicals. The phytochemicals produced in response to insect-pest attack, affect feeding and ovi-position of insects on the plants. Application of bio-pesticides has been reported to have positive impacts on bollworm population management (Ge and Ding 1996).

India has a vibrant repository of medicinal plants and perhaps the world's most sophisticated indigenous medical heritages. The wealth is not only in terms of the number of unique species documented so far for their medicinal use but also the depth of the traditional knowledge base about the uses for human, veterinary health care and crop protection (Ved and Goraya, 2008).

Malayali's are predominant hill tribes of Vattal Hills, Dharmapuri, Tamilnadu. Tribals of this community use local herbs for various aliments, veterinary health care and for protecting the plants. Besides hold a vibrant knowledge base with regard to the use of the local plants. Their reliance on herbs for medicine has prompted document and further investigate the usage of the plants as described by them (Table 1, 2).

Use of plants in present day agricultural practice in India has changed dramatically over the years, due to its non affordability, non-availability, non-accessibility of plants from the wild. Further, the use of plant products in modern agriculture practices is limited owing to the limitations over the chemical fertilizers and pesticides. Studies on the use of and hunt for plant based products as potential plant protecting agents have accelerated in recent times as they are safe and are non persistent in nature. Botanists, agriculturists, and chemists are to comb in the hunt for novel bioactive "leads" which could be developed as an effective plant protecting agents against the attack of various pests and pathogens Jermy (1990).

A number of plants have been shown to have pesticidal and antifeedant activity against *H. armigera* (Ramya *et al.,* 2008) of which Neem has been subjected to extensive investigation by Chopra *et al.,* (1994). Studies have shown that *Acorus calamus, Annona squamosa, Vitex negundo* are effective in the management of *H. armigera* (Murugan *et al.,* 1998). Sundararajan and Kumuthakalavalli, (2001) evaluated antifeedant activity of aqueous extract of *Gnidia glauca* and *Toddalia asiatica* against *H. armigera.* In this view, effect of leaf aqueous extracts of selected medicinal plants have been evaluated in the present study for their efficacy in the management of the insect pest *H. armigera*.

Materials and Methods

Preparation of plant extract

Following plants *A. indicum, A. fruticosa, A. aspera, A. zeylanica, A. marmelos, A. lanata, A. amara, A. lineaeta, A. paniculata, A. indica, C. halicacabum, C. tora, C. roseus, D. metal, G. sylvestre, M. azedarach, O. canum, O. sanctum, P. daemia, P. coleoides, S. nigrum, S. surattrense, S. trilobatum, T. terrestris, V. negundo* were selected from the wild in Vattal Hills, Dharmapuri District, TN, India. Selection of plants used in the present study was made on the basis of their availability and absence of damage by the insect-pest. Healthy plant materials were collected from the wild in poly bags, brought to lab and their botanical identity was established using the Flora (Gamble, 1993; Mathew, 1985).

Fresh leaves from the selected plants were collected and washed separately. About 1gm of fresh leaf material was ground with distilled water using mortar and pestle. The extract was filtered and the filtrate was made up to 100ml and was maintained as stock. The leaf aqueous extract was diluted with distilled water to obtain 1% test solution used in the bioassay studies.

Test organism

The larvae used for the study were collected from the host plants in the cotton fields and brought to lab. They were reared on artificial diet under laboratory conditions. **S**tudies were carried out using VI-instar larvae of *H. armigera* against the aqueous extract of all the selected plant species. The percentage mortality was calculated after a period of 24h by using bioassay studies.

Bioassay studies

Studies were conducted for a period of 24h in the laboratory in transparent plastic containers of 4x2.5 cm size capped with perforated plastic lids. Fresh leaves of cotton plant were collected from the field and washed in water. Excess moisture was removed from the leaves and the leaves were dipped in 1% test solution, shade dried and served to the VI-instar larvae of *H. armigera.* Extract free leaves served as control. For each treatment 10 larvae were singly introduced in separate containers after six hour starvation. Three replicates each of ten larvae were maintained for each treatment. All the experiments were conducted at $27^0C \pm 2$, 75% humidity and 14h dark period. Twenty four hour larval mortality was observed and the percentage mortalities were corrected using Abbott's formula (Abbott, 1925).

Results

The aqueous extracts of selected plant species collected from Dharmapuri District of Tamil Nadu were subjected to bioassay studies against the VI instar larva of *H. armigera* (24h) and the percentage mortality rate was observed. The results are given in Table 3. It was observed that all the selected plant species exhibited antifeedant activity against the larvae of *H. armigera.* However, the percentage mortality rate varied significantly among the plants tested. The percentage mortality rate of the insects ranged from 11.8 to 78.9.

The mortality rate was observed in the decreasing order of *M. azedarach* > *A. indica* > *S. trilobatum* > *A. paniculata* > *A. marmelos* > *A. lineata* > *S. surattrense* > *C. roseus* > *A. zeylanica* > *A. fruticosa* > *D. metal* > *S. nigrum* > *O. canum* > *P. coleoides* > *O. sanctum* > *P. daemia* > *A. amara* > *G. sylvestre* > *C. halicacabum* > *V. negundo* > *A. indicum* > *C. tora* > *T. terrestris* > *A. aspera* > *A. lanata.* The results imply that leaf aqueous extract of *M. azedarach, A. indica, S. trilobatum, A. paniculata, A. marmelos, A. lineata, S. surattrense, C. roseus, A. zeylanica, A. fruticosa* and *D. metal* can potentially be used as eco-friendly pest control agents against the larvae of *H. armigera*.

Table 1. Ethnomedicinal perspectives of botanicals used by Malayali Tribes of Vattal Hills

Botanical name	Family	Vernacular	Part	Habit
Abutilon indicum L.	Malvaceae	Thuththi	WP	Herb
Acalypha fruticosa Forsskal.	Euphorbiaceae	Chinni chedi	Leaf	Herb
Achyranthus aspera L.	Amaranthaceae	Naayuruvi	Leaf	Herb
Adhatoda zeylanica Medicus.	Acanthaceae	Adathodai	Leaf	Herb
Aegle marmelos (L.) Correa	Rutaceae	Vilvam	Leaf	Tree
Aerva lanata L.	Amaranthaceae	Kooraipoo	WP	Herb
Albiziz amara (Roxb).	Mimosaceae	Usilai	Leaf	Tree
Andrographis lineata Wallich ex Nees.	Acanthaceae	Siriyanangai	Leaf	Herb
Andrographis paniculata (Burm.f.) Wall. ex Nees	Acanthaceae	Periyanangai	Leaf	Herb
Azadirachta indica A. Juss	Meliaceae	Veppamaram	Leaf	Tree
Cardiospermum halicacabum L.	Sapindaceae	Mudakkaththan	Leaf	Climber
Cassia tora L.	Caesalpiniaceae	Aavarai	Leaf	Shrub
Catharanthus roseus L (G) Don.	Apocanaceae	Nithyakalyani	WP	Herb
Datura metal L.	Solanaceae	Oomathai	Leaf	Herb
Gymnema sylvestre (Retz.)R.Br.ex Roem.&Schult.	Asclepiadaceae	Sirukurinjan	Leaf	Herb
Melia azedarach L.	Meliaceae	Malaivembu	Leaf	Tree
Ocimum canum Sims	Lamiaceae	Naithulasi	Leaf	Herb
Ocimum sanctum Linn.	Lamiaceae	Thulasi	WP	Herb
Pergularia daemia (Fors.) Chiov.	Asclepiadaceae	Veli parutthi	Leaf	Climber
Plectranthus coleoides Benth.	Lamiaceae	Omavalli chedi	Leaf	Herb
Solanum nigrum L.	Solanaceae	Manathakkali	Leaf	Herb
Solanum surattrense Burm. f.	Solanaceae	Kandankathiri	WP	Climber
Solanum trilobatum L.	Solanaceae	Thoodhuvalai	Leaf	Climber
Tribulus terrestris L.	Zygophylaceae	Nerunji	WP	Herb
Vitex negundo L.	Verbenaceae	Karunochchi	Leaf	Tree

Table 2. Ethno-pharmacological uses of the botanicals from Vattal Hills

Botanical Name	Ethno-pharmacological Use(s)
Abutilon indicum (L.) Sweet	WP - febrifuge, anti-helmintic and diuretic, anti inflammatory - Leaf – cure bleeding piles, relieve pain. Flowers - antibacterial, anti-inflammatory.
Acalypha fruticosa Forssk.	Leaves treat stomach ache, alleviate dysentery and digestive disorders. Leaf and root - skin infections.
Achyranthes aspera Linn.	Roots - relieve gum pain and clean teeth, prevent tooth ache. Leaf extract with janggery – treat menorrhagia. Inflorescence scratched against breasts to increase lactation.
Adhatoda zeylanica Medicus.	Extracts of root, bark, leaves, and flowers used for bronchial, asthmatic and pulmonary affections, bronchodilatory action. Leaves - treat scabies, ringworm and skin infections, common cold, fever.
Aegle marmelos (L.) Correa ex Roxb.	Leaf - hypoglycemic activity. Leaves paste is applied externally to get relief itches, Stem Bark/ Seed - anti-diarrhoeal, stomachic, arrest diarrhoea, colitis dysentery and other enteric infections.
Aerva lanata (L.) Juss. ex Schult.	WP - Decoction taken internally to treat urinary problems. Leaf paste applied externally on insect bitten site to alleviate pain; leaves chewed to relieve toothache. Leaves - hepatoprotective, anti-helmintic, anti-diarrhoeal, anti-calculus. Flowers and roots - alleviate headache.
Albizia amara (Roxb.) Boivin	Dried leaf powder – hair wash, antidandruff, reduces heat.
Andrographis lineata Wallich ex Nees.	Leaf paste antidote against scorpion and snake poison. Leaf powder mixed with goat's milk taken orally to treat diabetes.
Andrographis paniculata (Burm.f.) Wallich ex Nees	Leaf paste antidote against scorpion and snake poison. Fresh leaf paste applied externally to treat leprosy, scabies, eczema and ringworm infection. Leaf juice taken orally during menstruation to prevent excessive bleeding.
Azadirachta indica A. Juss	Leaf paste with turmeric powder is applied externally to treat skin infections, small pox and chicken pox. Tender leaf consumed to eliminate stomach worms.
Cardiospermum halicacabum L.	Leaf paste applied on keen relives joint and rheumatic pains.
Cassia tora L	Leaf juice taken internally to reduce body heat, Flower paste with lime promotes wound healing, Dried flowers – hair wash
Catharanthus roseus L (G) Don.	Leaf paste is applied externally for tumor – anticancer activity
Datura metal L.	Leaf paste cures pimples and other skin infections.
Gymnema sylvestre (Retz.)R.Br.ex Roem.&Schult.	Leaf infusion - cure diabetes, reduce blood sugar level. Paste of leaves applied externally to alleviate nerve disorders.
Melia azedarach L.	Bark/ Leaf taken to relieve stomach pain - anti-helmintic.
Ocimum canum Sims	Leaf - relieve headache and fever
Ocimum sanctum Linn.	Leaf infusion - relieve cold and cough
Pergularia daemia (Fors.) Chiov.	Leaf paste - relieve stomach pain. Fresh leaves boiled in water vapor inhaled to alleviate headache.
Plectranthus coleoides Benth.	Leaf - common cold, improves digestion, alleviate stomach ache and digestion problem, lessen delivery pain, promote wound healing.
Solanum nigrum L.	Leaf - cure stomachache, stomach ulcer; leaf paste - cure rabies, wound healing.
Solanum surattrense Burm. f.	WP - cure cough, cold and wheezing. Tender fruits - treat cold, cough and fever. Leaf paste - treat intestinal worms.
Solanum trilobatum L.	Leaf juice – relive cough and cold in children. Leaf juice - cure asthma.
Tribulus terrestris L.	WP extract - remove kidney stones.
Vitex negundo L.	Vapor of leaf inhaled - relieve nausea, cold, cough, fever and headache.

Table 3. Effect of leaf aqueous solutions on the larvae of *H. armigera.*

Botanical name	% Mortality
Melia azedarach L.	78.9 ± 0.837
Azadirachta indica A. Juss	76.8 ± 0.867
Solanum trilobatum L.	74.8 ± 2.702
Andrographis paniculata (Burm.f.) Wall. ex Nees	72.8 ± 3.115
Aegle marmelos (L.) Correa	72.2 ± 1.974
Andrographis lineata Wallich ex Nees.	71.5 ± 1.483
Solanum surattrense Burm. f.	70.3 ± 1.581
Catharanthus roseus L (G) Don.	67.8 ± 2.588
Adhatoda zeylanica Medicus.	65.2 ± 1.924
Acalypha fruticosa Forsskal.	63.3 ± 2.387
Datura metal L.	62.9 ± 2.881
Solanum nigrum L.	61.8 ± 2.280
Ocimum canum Sims	58.2 ± 2.074
Ocimum sanctum Linn.	57.9 ± 1.304
Plectranthus coleoides Benth.	56.8 ± 1.923
Pergularia daemia (Fors.) Chiov.	55.9 ± 2.916
Gymnema sylvestre (Retz.)R.Br.ex Roem.&Schult.	51.8 ± 1.483
Albiziz amara (Roxb).	51.5 ± 2.702
Cardiospermum halicacabum L.	51.1 ± 3.198
Vitex negundo L.	43.5 ± 3.873
Abutilon indicum L.	37.7 ± 3.564
Cassia tora L.	32.8 ± 4.301
Tribulus terrestris L.	24.2 ± 1.925
Achyranthus aspera L.	15.8 ± 1.871
Aerva lanata L.	11.8 ± 1.924

Discussion

Prohibitive expense to meet the challenges of increasing resistance in insects, resurgence of pests and escalating environmental pollution caused by synthetic pesticides call for the discovery of less-expensive, non-hazardous alternatives in the management of insect-pests. Plants are endowed with a potential to produce a wide spectrum of allelo-chemicals (Norduland and Sauls, 1981). Insects have been influential in the evolution of allelo-chemicals in plants which in turn affects the insects. Some of compounds affect the feeding behavior of the insects and inhibit feeding, while few others disrupt hormonal balance there by inhibits growth, metamorphosis and reproduction. Due to aforesaid reasons there is resurgence of interest in plant derived compounds for developing novel eco-friendly insecticides (Jacobson and Crosby, 1971).

Despite hundreds of plants have insecticidal property, only few compounds like Azadirachtin (known to disrupt the action of moulting hormone) and pyrethroids (brings about paralysis of the insects) have been commercialized. However, an understanding of structure-activity relationship and knowledge on the mode of action is required for large-scale production. For successful exploitation of a bio-insecticide, screening of phytochemicals for wide spectrum of behavioral and physiological activities in poly-phagous insects is essential.

Koul *et al* (2000) administered phytochemical extracts orally through food to determine the toxicity or efficacy of plant materials for antifeedancy, inhibition of growth or emergence as adults. Murugan *et al* (1998), followed no-choice test method in which the test insect was provided with treated leaf disc. Dual choice method mimics the situation in the field, and is valuable in assessing the antifeedancy of phytochemicals. However, this method does not facilitate administration of apt quantity of plant extract to the insects. On the other hand, no-choice bioassay method provides on opportunity to precisely administer the desired quantity of extract to the insect. Hence in the present study, no-choice bioassay method was followed for assessing the insecticidal activity of the different plant extracts.

Usually larger doses of plant extracts inflict mortality either by inhibiting feeding or reducing digestibility or inhibiting growth. Smaller doses of extracts may not be adequate for killing the insects however; it may sometimes induce malformation (Ahmad, 2007). Induction of morphogenetic deformities during larval development or metamorphosis has greater impact on population build up. Malformed adults are unable to participate in reproductive activities and hence do not help building up the population. High antifeedancy (low ED_{50}) has been reported for pure compounds isolated from different plants by (Simmonds *et al.*,1990). Aqueous extracts of *Calotropis procera* and *Datura stromonium* display about 90% feeding protection against *H. armigera* (Dodia *et al.,* 1998).

Likewise, it has been reported that the effectiveness of the phytochemicals depend on the extraction method used. Janardhan *et al*(1999) reported that petroleum ether extracts of *Parthenium histerophorus* at a concentration of 0.2 and 0.5% cause 100% mortality in the larvae of *H. armigera*. Antifeedant property of plant extracts brings about retardation of growth and ultimately results in death of the insect. However, compounds which do not display antifeedant property are reported to have growth regulatory activity (Kraus *et al.*, 1987). On the other hand, a few plant extracts display bimodal activity. At high concentrations they are as feeding deterrents and at low concentrations as growth inhibitors (Nawrot *et al.*, 1991). Jermy (1990) reported that extracts with antifeedant and toxic effect are more successful in practical application as they evoke behavioral effect of antifeedancy. Jaglan *et al* (1997) evaluated the effect of *Azadirachta indica* extracts against *H. armigera* and reported that Chloroform : methanol (9:1) extracts of Neem seed kernels and leaves showed better insecticidal properties than methanol extracts.

On the other hand Koul *et al* (2000) reported that *H. armigera* larva fed on azadirachtin treated leaf suffered dose dependant effect on growth. Young larvae fed on 4 ppm azadirachtin treated leaf initially gained weight however, older larvae fed on 4 ppm azadirachtin leaf suffered 75% decrease in growth compared with the control larva (Koul, 1985). Similar observations were made by Murugan and Babu (1998) on growth and feeding physiology of *H. armigera* larva on extracts of *Ricinus communis*, *Glycosmis pentaphylla*, *Vitex negundo* and *Nerium oleander*. Murugan *et al*(1998) reported that larvae fed on 0.3% ethylaceteate fraction of *Glycosmis pentaphylla, Vitex negundo* and *Nerium oleander* showed significant effect on initial weight (259, 181 and 177%) in 48h compared to weight gained by the control larva (898, 972 and 890%). Earlier, it has been reported that the aqueous leaf extracts of *Gnidia glauca* showed more than 50% larval mortality at 0.8-1.0% and 86.1% mortality observed at 1.0% on *Toddalia asiatica* extract against the sixth-instar larvae of *H. armigera* (Sundararajan and Kumuthakalavalli, 2001; Ramya *et al.*, 2008).

In the present study all the tested plant extracts exhibited antifeedant activity on *H. armigera.* Of the 25 species of the plants screened for insecticidal property, ten plant species showed high rate of mortality against the VI-instar larva of *H. armigera.* In conclusion, the study reveals that leaf aqueous extracts of *M. azedarach, A. indica, S. trilobatum, A. paniculata, A. lineata, S. surattrense, C. roseus,* A. *zeylanica, A. fruticosa* and *D. metal* can potentially be used as eco friendly bio-pesticide to control the devastating damage caused by final instar larvae of *H. armigera.*

Conclusion

The use of natural products may be considered as an important alternative antifeedant for the control of *H. armigera*. Results from this study demonstrated that the leaf aqueous extracts of *M. azedarach*, *A. indica*, *S. trilobatum*, *A. paniculata*, *A. lineata*, *S. surattrense*, *C. roseus*, A. *zeylanica*, *A. fruticosa* and *D. metal* have excellent antifeedant activity and that secondary metabolites present in these medicinal plants is responsible for such activity. However, further investigations for the antifeedant activity and its effects on non-target organisms and field evaluation are needed. Accordingly, the results of present investigation could form the basis for selecting novel, more specific, biodegradable natural compounds.

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