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HEYNEA TRIJUGA: POTENTIAL INSECTICIDAL & GROWTH AND DEVELOPMENT ACTIVITY AGAINST *SPILARCTIA OBLIQUA* (LEPIDOPTERA: ARCTIIDAE)

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Abstract

Plants are recognized as an important natural source of pesticides. They have a diverse range of chemicals to protect themselves against various pests/diseases. Extracts of the fruit pericarp of *Heynea trijuga* were evaluated for pesticidal and insect growth regulator (IGR) activity against *Spilarctia obliqua* under laboratory conditions. For three generations castor leaves treated with hexane and acetone extracts of *Heynea trijuga* (1-10%) were fed to 3rd instar larvae for five days and then untreated fresh leaves were given till pupation. An adverse morphogenic effect on various biological parameters (larval duration, pupal duration, adult emergence and fecundity) was observed in treated insects. The growth and development of insects were significantly affected in a dose-dependent manner. Larval duration, larval mortality, prepupal mortality, postpupal mortality and pupal duration increased with increase in concentration while, larval weight, adult emergence, fecundity and egg fertility decreased with increase in concentrations of extracts. The acetone extract showed better growth regulating activity than the hexane extract. The bioactive substances in *H. trijuga* extracts could be responsible for behavioral effects and post-ingestive toxicity and have good potential to be developed as insecticide/growth regulators.

KEY WORDS: *Heynea trijuga*, *Spilarctia obliqua*, Meliaceae, Fruit pericarp, IPM, Botanical pesticide

Introduction

With growing awareness and an interest in replacing synthetic chemicals with natural products with pesticidal properties, medicinal plants are proving to be an excellent source that could be exploited for several purposes (Joshi *et al*., 2011; Joshi & Tandon, 2021). Plants are biochemist par excellence and during their long evolution, they have synthesized a diverse array of chemicals to protect themselves from colonization by insects and other herbivores (Vasmatkar *et al*., 2014). These chemicals repel approaching insects, deter feeding and oviposition on plants, disrupt the behavior and physiology of insects in various ways and even prove to be toxic at different developmental stages of many insects. It is estimated that there are about 0.391 to 0.45 million different plant species in the world today and only 10 percent of these have been examined for their biological activities (Benner, 1993; Tandon *et al.,* 2005; Christenhusz & Byng, 2016; Corlett, 2016; Tandon & Mittal, 2018).

The plant world comprises a rich store of bio-chemicals that could be tapped for use as potential pesticides. Botanical pesticides have been utilized by man since ancient times (Koul & Dhaliwal 2001; Tandon *et al*., 2001; Tandon & Sand, 2016). Interest in plant products as insecticides has grown over the past decade, as several synthetic pesticides are being eliminated from use due to environmental and food safety concerns. Several plants have been identified for their pesticidal activities (Rao & Mani, 1995; Prakash & Rao, 1997; Roy & Saraf, 2006; Akhtar *et al*., 2008; Tamta *et al*., 2021a; Tamta *et al*., 2021b). The family Meliaceae has received much attention worldwide as a rich source of diverse phytochemicals that could be utilized for agrochemical activities (Tan & Luo, 2011). Neem (*Azadirachta indica* A. Juss.,) a member of the Meliaceae family, has been found to be active against more than 413 species of insect pests belonging to different orders (Isman, 2006).

Heynea trijuga Roxb. ex Sims. (syn. *Trichilia connaroides* (Wight & Arn.) Bentv.) (Family: Meliaceae) is a small tree found on the Indian subcontinent (The Plant List 2010e). It is widely distributed in sub-Himalayan tracts and Western Ghats of India, Burma, Tonkin, Cambodia and Sumatra. The extracts of this plant are used in Indian and Chinese folk medicine for treating various diseases and ailments. An excellent review on all aspects of the ethnobotany, phytochemistry and pharmacology of *Heynea trijuga* (*T. connaroides*) was provided by Garg (2011). Plants of the genus *Trichilia* are a potential source of many novel compounds that have been found to have insecticidal, antifeedant and growth inhibitory activities in numerous studies (Wheelar *et al*., 2000; Wheeler & Isman 2000; Wheeler & Isman 2001; Bogorni & Vendramim 2005; Singh & Singh 2008; Tandon *et al*., 2009; García-Gómez *et al*., 2018).

The Bihar hairy caterpillar, *Spilarctia obliqua* Walker. (Syn: *Spilosoma obliqua*) (Lepidoptera: Arctiidae) is a polyphagous, voracious feeder and a destructive pest of soybean, cereals, fibers, pulses, castor, oilseed, vegetable, medicinal and ornamental plants in parts of India and the world (Bhadauria *et al*., 2001). During its life 2nd and 3rd instar larvae feed gregariously, leading to the death of the plant (Goel *et al*., 2004). This pest has developed resistance to the synthetic insecticides such as organophosphates, and pyrethroids and is therefore, difficult to control (Dhingra *et al*., 2007).

An alternate method for the management of this pest is needed since, plant products possess antifeedant, growth regulatory and insecticidal activities, that do not pose environmental pollution hazards, and are safe for beneficial organisms; hence, they could be utilized as potential pesticide. To the best of our knowledge, no growth regulatory activity of the *H. trijuga* fruit pericarp against *S. obliqua* has been reported. Keeping in mind the above and to our continued efforts to discover new eco-friendly pesticides from Indian medicinal plants, the present study was undertaken to evaluate the insecticidal and insect growth properties of *H. trijuga* fruit pericarp extracts against the polyphagous lepidopteran *S obliqua*, a devastating pest of many crops.

Materials and Methods

Collection and extraction of plant parts

Heynea trijuga (syn. *T. connaroides*) plants were identified by Prof. Y.P.S. Pangtey, Taxonomist, Department of Botany, Kumaun University, Nainital and reconfirmed at the Forest Research Institute (FRI) Dehradun vide herbarium no. M-29. The herbarium is maintained at the Department of Chemistry, G.B. Pant University of Agriculture and Technology, Pantnagar (India). Fully matured fruits (red-green in colour) were collected during the months of September-November in Nainital, Uttarakhand, India. The pericarp were removed from the fruits and shade dried. The material was finely powdered and subjected to extraction with solvents according to polarity.

Extraction was done in a Soxhlet apparatus using n-hexane and acetone separately until completion of extraction. The extracts (hexane and acetone) were concentrated to dryness on flash evaporator under reduced pressure at 35 ± 2^0C. The crude extracts obtained were diluted in hexane and acetone to prepare a 20% stock solution (w/v). Dilutions of extracts (v/v) were made from the stock solution.

Biological Assessments

Evaluation of biological activity of *H. trijuga* fruit pericarp hexane and acetone extracts against *S. obliqua* larvae was done in the laboratory in BOD incubator at 28 ± 2^0C, relative humidity 70 ± 5% and16:8 L:D for three generations. Fresh castor (*Ricinus communis*) leaves were washed thoroughly with water, air dried and dipped in extracts at different concentrations (1, 2.5, 5, 7.5 and 10%) for 10 min to give a thorough and uniform wetting of the leaves followed by shade drying. Twenty 3rd instar larvae (uniform size and age) were placed in pre-sterilized plastic jars (500 ml capacity) and introduced to treated castor leaves for five days (treated leaves were changed regularly every 24 h). After five days of treatment the larvae were removed from the jars and placed into new jars containing fresh untreated leaves. Fresh untreated leaves were given daily to the larvae till pupation. Leaves treated with hexane and acetone solvents, act as negative control. For the positive control a nimbicidin formulation (0.03% EC) was applied at a 0.25% concentration dose and neem oil (purchased from local market) was applied at a 0.5% concentration dose. All the treatments were replicated four times in completely randomized design (CRD) fashion. Records of the larval mortality (%), larval duration (days), larval weight (g/larva), pre-pupal mortality (%), post-pupal mortality (%), pupal duration (days), adult emergence (%), adult deformity (%), fecundity (per female) and egg fertility (%) were made for all replicates and treatments. The biological activities of the extracts were tested for three generations of insects.

Statistical Analysis

Data on the responses of the insects under different conditions were subjected to Analysis of Variance (ANOVA). The mean data obtained were subjected to Tukey's test to determine the level of significance between the control and the different concentrations and among different concentration treatments in a randomized completely block design (RCRD). The test of significance was carried out at 5% significance. The mean values followed by the same letter are not significantly different according to Tukey's test ($p<0.05$) and the degree of freedom (df) was 23 for the experiment. The data were analyzed using SPSS 16 (Statistical Package for Social Science). The data in percentage were also subjected to angular transformation because of binomial proportion (Snedeor & Cochran, 1968).

Results

Effect of *H. trijuga* extracts on the larval stage

Feeding on *H. trijuga* hexane extract treated leaves by larvae caused 13.33-36.67% mortality whereas, acetone extract caused 13.33- 43.33% mortality and the trend observed was same as that of hexane extract (Table I and II). No concentrations were found to be statistically at par with control and all concentrations were significantly better to the control. In case of nimbicidin and neem oil, 100 percent larval mortality was recorded after 225 hours of treatment.

For larvae fed on the hexane extract, the maximum larval period (22.80 days) was observed at 10% concentration, whereas at concentrations 5, and 7.5 %, larval duration was significantly prolonged when compared to the control, concentrations of 1 and 2.5% were found to be statistically at par with the control (Table I). Similar results were obtained with the acetone extract. Prolonged larval duration up to 22.83 days was observed when larvae were fed with the 10% acetone extract concentration. All concentrations, except 1% were significantly better to the control. (Table II)

The results indicated there was a decrease in weight of *S. obliqua* larvae with the increase in concentrations in both *H. trijuga* fruit pericarp extracts (Table I-II). In the case of treatment with different concentrations of the hexane extract larval weight differed significantly as compared to the control. All concentrations showed better activity than the control (Table I). A similar trend was observed for the acetone extract. Reduction in weight of larvae was significantly higher compared to the control.

Effect of *H. trijuga* extracts on the pre-pupal and pupal stages

The data presented in Table I indicates that feeding larvae on the hexane extract of fruit pericarp *H. trijuga* at different concentrations caused maximum pre-pupal mortality at 10.0%. The mortality at other concentrations was also significant as compared with the control except at 1% which was at par with the control. The concentrations 5% and 7.5% were at par with each other (Table I). When feeding the larvae with the acetone extract, maximum mortality (13.33%) was observed at 7.5% concentration. The pre-pupal mortality results showed that 1% and 2.5 % concentrations were at par with the control, while significant activity was observed for all other treatments (Table II).

Both extracts resulted in post-pupal mortality. On feeding with the hexane extract treated leaves post pupal mortality of larvae was maximum (20.00%) at 7.5% and 10% concentrations and the extracts were significantly at par with each other while, at lower concentrations, post pupal mortality was also significantly different as compared to the control (Table I).In the case of acetone extract, a similar trend to that of the hexane extract was observed and all treatments were statistically having better activity than that of the control (Table II).

Pupal duration of *S. obliqua* was progressively prolonged with the increase in concentrations of *H. trijuga* fruit pericarp extracts (Table I and II). When the larvae were fed on the hexane extract, the maximum pupal duration (15.4 days) was observed at 10% concentration and all the other concentrations (except 1 %) showed significant prolongation of the pupal duration as compared to the control (Table I). Table II indicates a similar trend for the acetone extract of *H. trijuga* fruit pericarp, to that observed in the case of the hexane extract.

Effect of *H. trijuga* extracts on the adult stage

Data in Table I-II indicates that with the increase in concentration of fruit pericarp extracts of (hexane and acetone), there was a decrease in adult emergence, fecundity and egg hatchability/fertility in test insects, however, there was an increase in adult deformity with the increase in concentration. When *S. obliqua* larvae were fed on the hexane extract treated food for five days, the adult emergence recorded was 36.67% at 10% concentration compared to 83.33% in the control (Table I). All the treatments (1-10%) significantly reduced emergence compared to the control (Table I). With the acetone extract, the maximum significant reduction in adult emergence was observed at the highest concentration (10%) compared to the control (86.67%). All the lower concentrations treatments also showed significant reduction in adult emergence compared to the control (Table II).

Table I. Effect of the hexane extract of *H. trijuga* fruit pericarp on biological growth parameters of *Spilarctia obliqua*.

Stage of insect	Hexane extract concentration of *H. trijuga* fruit pericarp (±SD) #					Control (untreated)
	1%	2.5%	5%	7.5%	10%	
Larval mortality (%)	13.33^{a}±1.0 (21.14)	16.67^{b}±1.2 (23.85)	26.67^{c}±0.8 (30.99)	30.00^{d}±0.4 (33.00)	36.67^{e}±0.2 (37.14)	13.33^{a}±3.3 (21.14)
Larval duration (days)	20.92^{a}±1.3	21.32^{ab}±1.2	21.97^{bc}±1.6	22.17^{c}±1.0	22.80^{c}±1.2	20.90^{a}±1.0
Larval weight (g/larvae)	18.04^{d}±0.2	17.25^{c}±0.1	16.27^{b}±.5	15.91^{b}±0.4	15.27^{a}±0.2	19.35^{e}
Prepupal mortality (%)	0.00^{a}	3.33^{b}±0.2 (6.14)	6.67^{c}±0.2 (12.28)	6.67^{c}±0.5 (12.28)	10.00^{e}±0.5 (14.99)	0.00^{a}
Postpupal mortality (%)	6.67^{b}±0.6 (12.28)	16.67^{d}±0.4 (23.85)	13.33^{c}±0.4 17.21)	20.00^{e}±0.7 (26.07)	20.00^{e}±0.5 (26.07)	3.33^{a}±0.2 (6.14)
Pupal duration (days)	10.93^{a}±0.3	11.53^{b}±0.3	12.86^{c}±0.4	13.97^{d}±0.2	15.40^{e}±0.3	10.97^{a}±0.2
Adult emergence (%)	80.00^{e}±5.2 (63.93)	66.33^{d}±6.0 (52.77)	53.33^{c}±4.8 (46.92)	43.33^{b}±6.2 (41.07)	36.67^{a}±3.2 (36.93)	83.33^{f}±7.2 (66.14)
Adult deformity (%)	0.00^{a}	0.00^{a}	3.33^{b}±0.1 (6.14)	6.66^{c}±0.1 (12.28)	13.33^{d}±0.2 (21.14)	0.00^{a}
Fecundity/female	538.33^{e}±11.6	501.67^{d}±7.2	430.33^{c}±9.2	390.33^{b}±10.2	288.00^{a}±7.2	608.67^{f}±12.2
Egg fertility (%)	88.00^{e}±5.5 (69.74)	84.33^{d}±3.2 (66.71)	76.00^{c}±4.5 (60.09)	68.00^{b}±2.8 (56.79)	45.00^{a}±1.0 (36.82)	93.00^{f}±8.6 (74.75)

Values are average for three generations and mean of four replicates in each generation ± Standard Deviation; Values in parentheses are angular transformed values. Within a column, mean values followed by the same letter are not significantly different according to Tukey's test ($p<0.05$); df=23

Table II. Effect of the acetone extract of *H. trijuga* fruit pericarp on biological growth parameters of *Spilarctia obliqua*.

Stage of insect	Acetone extract concentration of *H. trijuga* fruit pericarp (±SD) #					Control (untreated)
	1%	2.5%	5%	7.5%	10%	
Larval mortality (%)	13.33^{a}±1.5 (21.14)	16.66^{b}±1.2 (23.85)	26.67^{c}±1.2 (30.99)	30.00^{d}±1.7 (33.00)	43.33^{e}±1.8 (41.15)	13.33^{a}±1.1 (21.14)
Larval duration (days)	20.93^{a}±0.8	21.20^{ab}±0.3	21.62^{bc}±0.2	21.90^{c}±0.4	22.83^{d}±0.2	20.75^{a}±0.5
Larval weight (g/larvae)	17.91^{e}±0.3	16.88^{d}±0.5	15.83^{c}±0.5	14.91^{b}±0.5	14.16^{a}±0.2	19.35^{f}±0.3
Prepupal mortality (%)	3.33^{b}±0.1 (6.14)	3.33^{b}±0.1 (6.14)	6.67^{c}±0.2 (12.28)	13.33^{e}±0.2 (17.21)	10.00^{d}±0.2 (14.99)	0.00^{a}
Postpupal mortality (%)	6.67^{b}±0.5 (12.28)	10.00^{c}±0.4 (18.43)	16.67^{d}±0.3 (19.92)	10.00^{c}±0.5 (14.99)	26.67^{e}±0.7 (30.99)	0.00^{a}
Pupal duration (days)	10.97^{a}±0.6	11.50^{b}±0.5	13.10^{c}±0.5	14.23^{d}±0.2	15.53^{e}±0.3	11.00^{a}±0.9
Adult emergence (%)	76.67^{e}±2.2 (61.21)	70.00^{d}±1.7 (56.78)	50.00^{c}±1.7 (45.29)	46.67^{b}±1.2 (43.07)	26.67^{a}±1.2 (30.99)	86.67^{f}±5.2 (68.85)
Adult deformity (%)	0.00^{a}	0.00^{a}	6.67^{b} (8.85)	13.33^{c} (21.14)	13.33^{c} (21.14)	0.00^{a}
Fecundity/female	518.33^{e}±9.8	458.00^{d}±9.2	408.67^{c}±6.5	306.00^{b}±4.2	137.00^{a}±3.7	586.67^{f}±11.2
Egg fertility (%)	90.00^{e}±7.6 (71.57)	87.00^{d}±8.0 (67.22)	77.67^{c}±7.1 (60.20)	65.00^{b}±6.6 (57.41)	23.00^{a}±3.4 (18.72)	91.67^{e}±7.4 (73.28)

#Values are average of three generations with mean of four replicates in each generation ± Standard Deviation; Values in parentheses are angular transformed values. Within a column, mean values followed by the same letter are not significantly different according to Tukey's test ($p<0.05$); df=23

Adult deformity data indicates that there was not significant difference in adult deformity at the different concentrations of hexane and acetone extracts (Table I and II). However, in treatments with higher concentrations i.e., at 5-10% concentration, significant adult deformity was observed as compared to the control (Table I and II).

Data on fecundity and egg hatchability/fertility showed there was a decrease in the egg laying capacity and egg hatchability/fertility in all treatments for both hexane and acetone extracts. There was a significant reduction in fecundity per female and egg fertility/adult emergence of the test insect with the increase in concentration of both fruit pericarp extracts of *H. trijuga* compared to control (Table I and II).

Discussion

The results indicate that *H. trijuga* hexane and acetone extracts have significant growth regulatory properties suggesting the presence of some bioactive compounds in both extracts that possess behavioral effects and post-ingestive toxicity against *S obliqua*. The data presented in Table I and II, showed that all the biological parameters of *S. obliqua* were significantly influenced by the *H. trijuga* extracts to varying degree. The larval duration, larval mortality, prepupal mortality, post pupal mortality and pupal duration increased with the increase in concentration of the extracts. The larvae weight, adult emergence, fecundity and egg fertility decreased with the increase in the concentrations of extracts. The prolonged larval period and mortality of the insect at various stages may be attributed to antifeedant and insecticidal properties of the extracts that could be due to the presence of triterpenoids or limonoids in plant. *H. trijuga* (*T. connaroides*) belongs to the family Meliaceae, the members of which are characterized by the presence of structurally diverse terpenoids such as triterpenoids or limonoids which have significant biological activities. It is reported in the literature that the triterpenes and limonoids present in genus the *Trichilia* spp. are responsible for different biological activities against insects (Kubo & Klocke, 1982; Purushothaman *et al*., 1987; Mikolajezak *et al*., 1989; Mary *et al*., 2004; Cunha *et al*., 2005; Cunha *et al*., 2008; Geng *et al*., 2009; Matos *et al*., 2009; Vieira *et al*., 2014; Tandon & Mittal, 2017; García-Gómez *et al*., 2018). The insecticidal activities of benzene, methanol, ethyl acetate and hexane extracts of *T. connaroides* (*H. trijuga)* against 3rd instar larvae of *Henosepilachna vigintioctopunctata, Plutella xylostella, Amsacta albistriga* and *Spodoptera litura* have been reported by Singh *et al*., (2011). Insecticidal activity of *T. connaroides* (*H. trijuga)* and *Melia azedarach* extracts have also been reported against *Plutella xylostella* and *Spodoptera litura* (Singh & Singh, 2008). Xie *et al.,* (1994) demonstrated growth inhibitory activity of ethanolic extracts of wood, bark, seed and exocarp of *T. connaroides* (*H. trijuga)* in *Peridroma saucia* and *Spodoptera litura*. A seed extract of *T. havanensis* showed activity against lepidopteran larvae i.e., *Spodoptera littoralis* and *Helicoverpa armigera* (Lopez *et al*., 1997, Lopez *et al*., 1998), and acetone and aqueous fraction extracts of *T. havanensis* seed kernels showed toxicity in larvae and adults and a decreased fecundity rate of adults in *Chrysoperla carnea* (Huerta *et al*., 2003a, 2003b). Tandon *et al*., (2009) found *T connaroides* leaf extracts to possess significant IGR activity against *Spilosoma obliqua,* and the acetone extract was found to be better to the hexane extract. A methanolic leaf extract of *T. connaroides* was found to be more effective against red flour beetle and lesser grain borer than the commercial product teflubenzuron (an insect growth regulator) (Guruprasad & Akmal 2014). The methanolic extracts of leaf, bark and flower of *T. silvatica* were found to decrease viability in larvae, the period of oviposition, number of eggs, pupal biomass and egg viability of *S. frugiperda* (Freitas *et al*., 2014).

The findings of *H. trijuga* fruit pericarp extracts having an adverse morphogenic effect on the test insect pest are in conformity with the work of other researchers who have shown the adverse effects of *H. trijuga* extracts on other insects. Studies on *Trichilia* have shown that higher concentrations tri/tetra terpenoids or limonoids are found in seeds/fruit compared to other parts of plant (Vieira *et al*., 2014). Thus, from results it could be revealed that insect growth regulatory activity shown by *H. trijuga* pericarp could be due to presence of triterpenoids or limonoids compounds which are characteristics of this genus and family.

Conclusion

It could be concluded from our study that fruit pericarp extracts had adverse morphogenic effects on the biological parameters (larval duration, pupal duration adult emergence and fecundity) of *S. obliqua*. The acetone extract of the fruit pericarp was found to be more effective than the hexane extract. The differences in the efficacy of hexane and acetone extracts could be attributed to the phenomenon of polarity. The hexane extracts were less effective than the acetone extract, as the latter has extracted medium polar compounds like triterpenoids and/or limonoids as active principles, while the former has mostly nonpolar and waxes and fatty substances, which are less active. From these results, it can be inferred that both extracts of *H. trijuga* fruit pericarp, would adversely affect the biological parameters of *S obliqua* to varying levels. The *H. trijuga* extracts could be useful as a natural crop protectant against insect pests to replace synthetic insecticides. Isolation of pure bioactive substances from the extract would be an added benefit for developing/synthesizing new potential growth regulators/pesticides. *H. trijuga* is a strong candidate to replace the synthetic chemicals used in the industry and could serve as a novel template for the development of a new synthetic pesticide/IGR.

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HEYNEA TRIJUGA: ПОТЕНЦИЈАЛНА ИНСЕКТИЦИДНА ВРСТА И ЊЕНО ДЕЛОВАЊЕ НА РАСТ И РАЗВОЈ *SPILARCTIA OBLIQUA* (LEPIDOPTERA: ARCTIIDAE)

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Извод

Биљке представљају важан природни извор пестицида. Имају широк спектар хемикалија за заштиту од разних штеточина и болести. Урађена је процена екстракта перикарпа плода *Heynea trijuga* на пестицидну активност и активност регулатора раста инсеката (IGR) против *Spilarctia obliqua* у лабораторијским условима. Листови рицинуса третирани хексаном и ацетонским екстрактима (1-10%) су коришћени за исхрану ларви трећег ступња *Heynea trijuga* у периоду од пет дана, а затим су необрађени, свежи листови давани до улуткавања. Код третираних инсеката уочен је негативан морфогени ефекат на различите биолошке параметре (дужина стадијума ларве и лутке, појаву одраслих јединки и плодност). На раст и развој инсеката је значајно утицала доза третирања. Трајање ларвеног стадијума, морталитет ларви, морталитет пре улуткавања, смртност након улуткавања и дужина стадијума лутке су се повећавали са повећањем концентрације, док су се тежина ларве, излазак адулта, плодност и вијабилност јаја смањивали са повећањем концентрације екстраката. Екстракт ацетона је показао бољу активност регулације раста од екстракта хексана. Биоактивне супстанце у екстрактима *Heynea trijuga* могу бити одговорне за ефекте понашања и токсичност након ингестије и имају добар потенцијал да се развију као инсектициди/регулатори раста.

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