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Evaluation of Insecticidal Potential of 4-Methylthiobutyl Isothiocyanate on the Growth and Development of Polyphagous Pest, *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae)

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Abstract
4-Methylthiobutyl Isothiocyanate or Erucin obtained from *Eruca sativa* (Mill.) (Thell) seeds were investigated for its effect against the second instar larvae of common cutworm, *Spodoptera litura* (Fab.) by conducting artificial diet bioaasays and topical assays. The artificial diet bioassays conducted at different concentrations (40mg/l, 81mg/l, 121mg/l, 161mg/l and DMSO as control) showed inhibitory effects on percentage of pupation rate and percentage of adult emergence. Topical application of 161mg/l concentration of erucin to the larvae of *S. litura* delayed the larval and total development period and decreased the percentage adult emergence. These findings indicate a possible insecticidal role of erucin in insect pest management.

Keywords: 4-methylthiobutyl isothiocyanate, *spodoptera litura*, feeding bioassay, topical treatment

1. Introduction
The contribution made by synthetic insecticides in management of insect pests has been immense and has drastically improved crop yield. They are highly effective and provide rapid protection, repellency and killing of insects [1]. However, they are now being regarded as the greatest threat to the environment and human health as their indiscriminate use has elicited a wide spectra of problems such as poisoning of applicators and consumers, destruction of non-target animals, disruption of natural biological control agents and pollinators as well as contamination of groundwater [2]. Moreover, a number of pests have developed resistance to synthetic insecticides. Therefore, identification of novel effective compounds having insecticidal potential is essential to combat the problems created by overuse of synthetic pesticides.

Plants are stationary organisms and to defend themselves against herbivores, have developed a large array of secondary metabolites [3]. These compounds form an important defensive component in plants and impart resistance to crops against herbivory. Exploration of these compounds for the management of insect pests can reduce environmental damage to a large extent since compared to insecticides, plant compounds are relatively safe and biodegradable [4]. Some of these compounds have been found to be toxic and their deterrent effects on the growth of insect pests have been documented in literature [5].

Glucosinolates in plants are constitutive and induced defense chemicals that are activated in response to injury by herbivorous animals. The protective role of glucosinolates and their degradation products against phytophagous insect pests have been demonstrated in various experiments. Seo and Tang [6] had reported toxic effects of benzyl isothiocyanate against eggs and first instar larvae of Hawaiian fruit flies, *Dacus cucurbitae* (Coquillett), *Dacus dorsalis* (Hendel) and *Ceratitis capitata* (Wiedemann). In another study, toxicity of allyl isothiocyanate to diamondback moth, *Plutella xylostella* (Linneaus) and Southern armyworm, *Spodoptera eridania* (Stoll) was observed by Li *et* al [7]. Besides insects, toxic, growth inhibitory and feeding deterrent effects of glucosinolates have also been demonstrated against other plant enemies including mammals, birds, molluscs, aquatic invertebrates, nematodes, bacteria and fungi [8]. However, the very glucosinolates which are toxic and serve as feeding and growth deterrent for many herbivores can act as oviposition and feeding stimulants for insects specialised for feeding on their host plants [9]. Although, the specialists have evolved mechanisms to circumvent the toxic effect of the glucosinolate-myrosinase system, nevertheless higher concentrations of glucosinolates can adversely affect the growth of specialist insects and thus may result in plant resistance. Erucin is an isothiocyanate and an analog of sulforaphane found in cruciferous vegetables. Erucin has been reported to exhibit anti oxidative, anti-inflammatory, neuroprotective, anti-cancer and chemotherapeutic activities [10, 11, 12]. However its anti-insect activity has not been studied.

The tobacco caterpillar, *Spodoptera litura* (Fabricius) is one of the most important insect pests of agricultural crops in the Asian tropics. *S. litura* is widely distributed in India and its larvae are polyphagous defoliators [13]. The host plants recorded for *S. litura* are 120 species out of which 40 are crop plants [14]. Frequent use of insecticides to control *S. litura* has resulted in insecticide resistance [15] and has necessitated that alternative measures be explored for control of this pest. Therefore, the present study was envisaged to study the effect of erucin (4-Methylthiobutyl isothiocyanate) on the polyphagous pest, tobacco cutworm, *S. litura.*

2. Materials and Methods
Insect Culture
The egg masses and larvae of *S. litura* were collected from the cauliflower fields located in the premises of Guru Nanak Dev University, Amritsar, India and after identification, their subsequent generations were reared in the laboratory at 25 ±

2ºC temperature, 65 ± 5% relative humidity (RH) and 12:12 (D: L) photoperiod. The rearing was carried out in glass jars (15 cm (height) × 10 cm (diameter) on fresh castor leaves (*Ricinus communis* L.) collected from the university campus. The pupae were separated and kept in pupation jars (15 cm × 15 cm) having 2–3 cm layer of moist sterilized sand covered with filter paper. The adults emerged from these pupation jars were transferred to oviposition jars in the ratio of 1 male: 2 females (identified on the basis of difference in size with males being smaller than females and the presence of ovipositor in females) and covered with muslin cloth. The jars were lined with filter paper to facilitate egg laying and adults were provided with 10% sugar solution as food source. The eggs were kept in petri plates having a moist cotton swab. Six days old second instar larvae have been used in the experiment.

Procurement of Erucin

Erucin was isolated from *Eruca sativa* (L.) in the Botanical and Environmental Sciences Department, Guru Nanak Dev University, Amritsar. The extraction of erucin (4-methylthiobutyl isothiocyanate) was done using seeds of *Eruca sativa* (Mill.) Thell. Var. RTM-314 using the method given by Arora *et al.* [16]. The seeds (50g) were crushed and dissolved in 1000 ml distilled water in a flat bottom flask using a magnetic stirrer at a speed of 500rpm The flask was kept on a hot plate at a temperature of 500 °C. Once the contents of the flask started boiling, the temperature was reduced to 400 °C and rotation was set at 350 rpm. The setup was kept for 3 h and the oil along with the water was collected in the outer tube. The glucosinolate hydrolytic products were then extracted using equal volume of dichloromethane (DCM). The solvent was then evaporated under vacuum using rotary evaporator at 30 °C and the extract was obtained. The extract was later analyzed using gas chromatography-mass spectrometry (GC-MS) and the major hydrolytic product observed was erucin at a concentration ≥90%.Continuous extraction was done so as to obtain 3gm of the extract containing glucosinolate hydrolytic products. The extract was then added to silica (60-120 mesh size) and slurry was obtained. A glass column was taken and packed with silica (60-120 mesh size). Hexane was allowed to pass through the column and saturate the silica gel. Once the gel was set, the slurry along with hexane was passed through the column and fractions (50 ml each) were obtained. The polarity of the solvent was changed to 1: 99: ethyl acetate: hexane, and was allowed to pass through the column and fractions were obtained. The fractions were partially evaporated and analyzed for the presence of pure compound using TLC (dip in annisaldehyde reagent). The fractions containing pure compound with same color were pooled and later the solvent was evaporated under vacuum using rotary evaporator. The obtained compound was dissolved in 1 ml GC grade DCM and analyzed using GC-MS and gas chromatography-flame ionization detector (GC-FID). The presence of erucin and its structure was later confirmed using ultra high pressure liquid chromatography with photo diode array detector (UHPLC-PDA) and nuclear magnetic resonance (NMR) spectroscopy (^{1}H and ^{13}C). The compound was observed to be in a purity of ≥99% [17, 18].

Experimental Method

The experiments were conducted in a Biological Oxygen Demand (B.O.D.) incubator (Caltan, Narang Scientific Works Pvt. Limited, New Delhi) maintained under controlled conditions of temperature, relative humidity (RH) and photoperiod as mentioned in the section of insect culture. Bioassays were carried out using 6-day old second-instar larvae of *S. litura* by two different methods.

Artificial Diet Bioassay

The six-day old second-instar larvae of *S. litura* were reared on amended diet (containing various concentrations of the compound erucin i.e. 40mg/l, 81mg/l, 121mg/l and 161mg/land unamended diet (control). The compound, erucin was oily in nature and hence was not readily soluble in water. The compound was dissolved in 0.5% dimethyl sulfoxide (DMSO). For control, 0.5% dimethyl sulfoxide was used. The methodology for making artificial diet was adapted from Koul *et al*. [19]. The ingredients of artificial diet were bran (6g), kidney bean flour (30g), vegetable oil (375µl), formaldehyde (600µl), yeast (3g) and rest (2.4g). All the ingredients were mixed properly with water. Boiled agar (3g) was added to the ingredients mixture above mentioned. The hot diet mixture prepared was poured in a petridish and placed in open for its solidification. This diet was further cut into cubes and used for the experient. The compound erucin was incorporated into the diet by mixing it with the ingredients of the diet chart formulated by Koul *et al*. [19] and the amount of diet (approximately 300-400 mg/larva) given to these larvae put in solocup or plastic container (40ml) was changed regularly. Observations were made daily for recording various parameters such as larval period, pupal period,total development period, percent pupation and percentage of adult emergence. Also the larvae which did not show any movement were considered as dead and abnormalities were also observed. There were 6 replications (30 larvae) with 5 larvae in each replication for each concentration.

Topical Treatment

For topical treatment, 1mM concentration of erucin (2 µl) prepared in 0.5% DMSO and 0.5% DMSO as a control were applied on the dorsal surface of second instar larvae with the help of a syringe. After topical application, the treated larvae were fed on unamended artificial diet without erucin compound. There were 5 replications for each experiment and each replication had 5 larvae. Observations were recorded daily for the parameters as given above. The experiments were repeated twice.

Statistical analysis

The data recorded for larval period, pupal period, total development period, percentage of pupation rate and percentage of adult emergence was subjected to one-way ANOVA and wherever F values were found to be significant the means were separated by the Tukey's honestly difference test ($P \leq 0.05$) according to Assistat (7.7).

3. Results and Discussion

Glucosinolates are gaining widespread recognition as a class of natural pesticides [20]. Glucosinolates are converted to toxic

hydrolytic products when acted upon by myrosinases,spatially separated from glucosinolates in plant tissue [21]. The most toxic glucosinolate derivatives are isothiocyanates. Allyl and benzyl isothiocyanates have been found to be more toxic against eggs of black wine weevil, *Otiorhynchus sulcatus* (Coleoptera: Curculionidae) (Fabricius) [22] than their intact counterparts. Artificial diet bioassay in present study demonstrated that when erucin at higher concentrations of 121mg/l and 161mg/lwas incorporated in the artificial diet of second instar larvae of *S. litura*, it significantly decreased the percentage pupation rate (Table 1). At these concentrations, percentage pupation rate was inhibited by 35.71% when compared to control. The percentage of adults which emerged from treated larvae also decreased with increasing erucin concentration in the artificial diet. Wadleigh and Yu [23] had also reported increased larval mortality of armyworm, *Spodoptera frugiperda* (J. E. S.), cabbage looper, *Trichopulsia ni* Hb. and velvet bean caterpillar, *Anticarsia gemmatalis* (Hub.) after treatment with allyl (0.005%), benzyl (0.01%) and 2-phenylethyl isothiocyanate (0.05%). Agrawal and Kurashige [24] also had observed increased larval mortality, development period and growth of small white butterfly, *Pieris rapae* (L.) after the larvae were fed artificial diet supplemented with various concentrations of allyl isothiocyanate(0, 0.282, 0.565, 1.129, and 1.693 µmol/g). However, erucin had no significant effect on the larval and total development period of *S. litura* larvae indicating absence of antifeedant activity. The pupal period was significantly affected with erucin treatment but no consistent trend was observed. It decreased at lower concentrations of 40mg/l and 81mg/l but increased at 121mg/l as compared to control. Generalist lepidopteran larvae have been reported to detoxify ingested glucosinolates and their hydrolytic products via conjugation to glutathione (GSH) and can survive on low glucosinolate diets [25, 26]. However the metabolic cost incurred in detoxification of glucosinolates can have a negative impact on the development of an insect. Also GSH-dependent detoxification might become less efficient with increase in dietary isothiocyanate concentrations and so the latter can disrupt cell structure and function [27]. This could be an explanation for the detrimental effect of erucin on pupation and emergence of *S. litura* larvae at higher concentrations.

Topical application of the highest concentration of erucin (161mg/l) to the larvae of *S. litura* produced significant effects on its larval period, total development period and percentage adult emergence (Table 2). The larval period and total development period were delayed by 2.17 and 1.96 days respectively as compared to control. Moreover, significantly lower percentage of adult emergence (40%) from topically erucin-treated larvae was observed than that in control treatment (96%). Abnormalities too were observed in the treated larvae which were in the form of malformed larvae, pupae and adults (Fig.1).These findings may suggest that erucin when applied topically to the larvae of *S. litura* was readily absorbed by the insect., The results of the present study indicate that erucin at higher concentration adversely affects the growth and development of *S. litura* larvae. Various studies have documented that while specialist lepidopterans such as *P. rapae* can biochemically adapt to glucosinolates through a gut nitrile-specific protein [28], the generalist lepidopteran has a poor physiological adaptation to glucosinolates and show a wide range of responses to glucosinolates, including absence of effect [29], existence of effects similar to these experienced by specialist herbivores [30], and the presence of negative effects on larval survival, especially when fed higher concentrations of aliphatic glucosinolates [31]. In studies carried out on a specialist insect *Plutella xylostella* (L.) and generalist *Spodoptera eridania* (Cramer), Li *et al* [32] had compared the effects of variable glucosinolate and myrosinase activity in plant tissues of fourteen *Brassica juncea* lines on their feeding and growth. It was found that although the relative growth rate of both species was lower on lines of *B. juncea* with higher glucosinolate concentrations, the effect was stronger for generalist *S. eridania* than specialist *P. xylostella*. The present findings clearly imply that the compound could be useful for its exploitation for development of resistance in plants against the insect pest.

Table 1: Developmental parameters of *Spodoptera litura* after its second instar larvae were fed on artificial diet amended with Erucin.

Concentration (mg/l)	Larval period (Days) Mean ± S.E	Pupal period (Days) Mean ± S.E	Total Development Period (Days) Mean ± S.E	Percentage of pupation rate Mean ± S.E	Percentage of Adult Emergence Mean ± S.E
Control	14.47±0.16^{a}	11.53±0.20^{a}	27.13±0.32^{a}	93.33±6.67^{a}	93.33±6.67^{a}
40	15.02±0.13^{a}	10.37±0.22^{b}	25.12±0.07^{a}	90.00±5.77^{a}	50.00±17.3^{a}
81	14.25±0.72^{a}	10.37±0.22^{b}	24.62±0.94^{a}	80.00±0.00^{ab}	50.00±5.77^{a}
121	14.33±0.19^{a}	12.33±0.78^{ab}	26.33±0.77^{a}	60.00±0.00^{b}	40.00±11.50^{a}
161	15.00±0.29^{a}	11.00±0.00^{a}	23.16±0.09^{a}	60.00±11.5^{b}	40.00±11.50^{a}
F value	2.32$^{N.S.}$	4.76*	3.15$^{N.S.}$	6.05**	3.82*

* Indicates significant difference at P≤0.05;**Indicates significant difference at P≤0.01; $^{N.S.}$ non-significant
Means followed by the same letter within columns are not significantly different according to the Tukey test at P=0.05

Table 2: Developmental parameters of *Spodoptera litura* after its second instar larvae were topically treated with erucin.

Concentration (mg/l)	Larval period (Days) Mean ± S.E	Pupal period (Days) Mean ± S.E	Total Development Period (Days) Mean ± S.E	Percentage of Pupation Rate Mean ± S.E	Percentage of Adult Emergence Mean ±S.E
Control	15.18±0.15^{a}	10.94±0.28^{a}	26.12±0.30^{a}	96.00±4.00^{a}	96.00±4.00^{a}
161	17.20±0.19^{b}	10.83±0.34^{a}	28.08±0.35^{b}	80.00±6.32^{a}	40.00±8.94^{b}
F value	68.63**	0.06 $^{N.S.}$	17.46**	4.57*	32.67**

*Indicates significant difference at P≤0.05;** Indicates significant difference at P≤0.01; $^{N.S.}$ non-significant.
Means followed by the same letter within columns are not significantly different according to the Tukey test at P=0.05

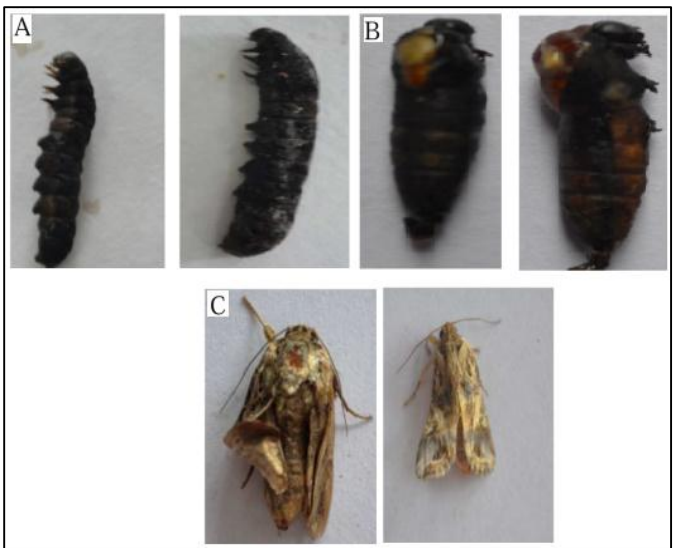

Fig 1: Abnormalities in (A) larvae, (B) pupae and (C) adults formed after topical treatment with Erucin.

4. Conclusion

The research on glucosinolates has gained widespread attention in the past decade and can provide a natural solution to overcome pest populations. Our study has highlighted the potential of erucin in managing insect pests and can form the basis for manipulating glucosinolates and theit hydrolytic products for imparting resistance in crops against generalist insect pests.

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