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Assessment of insecticidal action of 3-Isothiocyanato-1-propene on the growth and development of *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae)

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Abstract
The extensive use of chemical pesticides in agricultural practices have adversely affected environment as well a human health. Bioaccumulation of these pesticides at different trophic levels is one of the major concerns among agronomists. Consequently, there is need to consider natural plant products as an alternative source for pest management. Glucosinolates (GSLs) are secondary metabolites present in plants belonging to different families including brassicaceae. They are broken down into many isothiocyanates (ITCs) that play an important role in plant-insect-interaction. Among numerous known ITCs, 3-isothiocyanato-1-propene or allyl isothiocyanate (AITC) is known to possess biocidal activity against diverse range of pathogens. Therefore, present investigation was carried out to evaluate the insecticidal potential of AITC on growth and development of *Spodoptera litura* (Fab), a polyphagous pest. AITC resulted in 100% larval mortality at the highest concentration (3125ppm). It was also observed that AITC influenced larval period, pupal period, total development period and pupal weight. AITC decreased adult emergence along with larval growth indices. These alterations were further confirmed by the aberrations observed in pupae and adults of *S. litura*. Thus, our findings indicated that AITC possess insecticidal potential against *S. litura* and can be efficiently used for its management on large scale.

Keywords: Glucosinolate, allyl isothiocyanate, *Spodoptera litura,* biopesticide, pupal weight, larval mortality

1. Introduction
In recent decade, agronomic practices have intensified the use of synthetic pesticides in order to eliminate the problem associated with pests. Unfortunately, indiscriminate application of these chemicals has resulted in human and environmental hazards by directly affecting the sustainability of ecosystems [1]. Their property to accumulate in food chain along with other undesirable effects has generated a need to identify new active eco-friendly and non toxic compounds. In context to above facts, researchers have developed keen interest in plant derived allelochemicals to act as natural pest control agents.

Plants are associated with a unique phenomenon of synthesizing secondary metabolites that play an important role in plant defence system, thus protecting plants from herbivores. Interestingly, plant allelochemicals affect insects in several ways by inducing acute toxicity, growth disruption, hormonal imbalance, biochemical alterations and interference with consumption of diet, acting as toxic agents [2]. Literature survey has revealed that compounds/essential oils derived from plants such as *Azadirachta indica, Capsicum frutescence, Cerbera odollam, Cymbopogon nardus, Zingiber officinale, Ocimum sanctum, Chrysanthemum morifolium* have shown potential to act against various insect pests [3]. These plants have been known to act as biopesticide without posing threat to mammals and ecological system. Also, the tendency of such botanicals to biodegrade within a few hours of exposure supports their exploration as pesticides in place of synthetic chemicals [4].

Glucosinolates (GSLs) is a class of phytoconstituent present in diverse plant families including brassicaceae. These compounds play an active role in glucosinolate-myrosinase dependent plant-defence mechanism [5]. The breakdown products of GSLs are known as isothiocyanates (ITCs) that are well documented for their bioactivities *in-vitro* as well as *in-vivo* [6]. Among naturally occurring ITCs, Allyl isothiocyanate (AITC) is a major component of mustard oil, having high volatility, pungent smell and can be obtained by hydrodistillation [7].

It is known to possess protective activities such as anticancer, antitumor, antimutagenic, antimicrobial and antifungal activities [8]. AITC has also been reported to act against stored grain pests, pathogens, root-knot nematodes and weed seeds [9]. However, there are no reports regarding its insecticidal ability chiefly against *Spodoptera litura,* a destructive pest.

Spodoptera litura (Fab) (Lepidoptera: Noctuidae) is a widely distributed polyphagous pest throughout Asia, North Africa, Japan, Australia and New Zealand [10]. It has been reported to attack about 112 cultivated plant and vegetable species. With reference to India, it has known to affect almost 60 economically important cultivated plant species such as sunflower, tomato, tobacco, soybean, mungbean, cotton, cabbage and groundnut [11]. Keeping this in view, present investigation was designed to evaluate the insecticidal potential of AITC against *S. litura* and to calculate LD_{50} of AITC against *S. litura* using artificial diet.

2. Material and methods
2.1 Chemicals
The extracts of seeds and sprouts of *Brassica juncea* were prepared using a modified hydro distillation method using dichloromethane [12]. Since the extracts contained AITC (Fig 1) in minor amounts, it was procured from Sigma Aldrich, St. Louis Missouri, United States for further use.

2.2 Rearing of *S. litura* in laboratory
To determine the above mentioned objectives, present investigation was carried out for period of about 6-7 weeks. The culture of *S. litura* was maintained using B.O.D incubator via regulating the temperature at 27°C± 2°C and relative humidity of 60 % using natural diet or artificial diet [13].

2.3 Bioassays
The bioassays were carried out using methodology given by Koul *et al* [13]. The test compound AITC, was initially dissolved in 1ml of 0.5% dimethyl sulfoxide (DMSO) and further dissolved in water to prepare final concentrations. Since, water was used for dissolving AITC, it was considered as control. The effect of AITC on growth and development of second instar larvae (6 days old) of *S. litura* was further determined by feeding the larvae on artificial diet incorporated with different concentrations of AITC (5 ppm, 25 ppm, 125 ppm, 625 ppm, 3125 ppm). For this purpose, the second instar larvae (6 days old) were allowed to feed on treated diets as well as untreated diets. The experimental design comprised of 6 replicates with 5 larvae in each replicate. Each experiment was repeated twice. For determining the insecticidal activity observations were made daily on biological parameters i.e., larval, pupal and total development period, larval mortality (%), adult emergence (%), pupal weight, larval growth index and total growth index. In addition to this, LC_{50} was also calculated using larval mortality (Fig. 2).

2.4 Statistical analysis
The data was subjected to one way ANOVA. The means obtained were compared by the Tukey's Honestly difference test (HSD) at 0.05 level of significance using Asistat (7.7).

3. Results and discussion
During the past few years, glucosinolates have attracted the interest of researchers to act as biopesticide, owing to the presence of various biological activities [14]. The property of allyl isothiocyanate to act against pathogens has made it important target for research in field of pest control and management, especially against economically notorious insects such as *S. litura*. In present investigation, the LC_{50} of AITC against *S litura* was obtained to be 1212.5 ppm as indicated by regression equation and larval mortality (Fig. 3). In addition, the treatment with highest concentration of AITC (3125 ppm) also resulted in 100% mortality. This was in accordance with Sukovata *et al*. evaluating the toxicity of *B. juncea* seed meal in the presence of semi-natural di*et al*ong with field conditions showing efficacy and lethal concentrations of GSLs against Melolontha grubs due to presence ITCs [15].

Our study demonstrated that, ingestion of AITC by second instar larvae (6 days old) resulted in an increased larval period, pupal period and total development period (Fig. 4A). These observations were in agreement with the toxicity of AITC against neonates of diamondback moth, *Plutella xylostella* in combination with cypermethrin and chlorpyrifos, being used as sprays that was almost equivalent to commercial available fumigant "phosphine" [16]. Aggarwal and Kurashige also studied the insecticidal activity of AITC incorporated in artificial diet that resulted in delayed development period and growth of small white butterfly, *Pieris rapae* (L) [17]. Interestingly, such anti insect activity of AITC can be attributed to the presence of pungent odour of ITCs, which is released on hydrolysis of GSLs due to myrosinase coming into action [18]. Larvicidal action of AITC also significantly influenced overall percentage emergence of adults. The percentage of adult emergence calculated was 36.67% at the concentration of 625 ppm as compared to the control (untreated) larvae where the emergence was 90.00% (Fig. 4 B). In addition, male and female emergence was also significant when compared to the control, thus depicting anti insect tendency of AITC. These observations regarding AITC are similar to reports regarding regulation of emergence by *B. juncea* seed meal against *Bradysia impatiens* [19]. Paes *et al*., found mortality of all the developmental stages of *S. zeamais* that were assessed using synthetic mustard essential oil (SMEO), ultimately affecting the adult emergence [20].Thus providing strong evidence in favour of AITC to play an important role in regulation of emergence in treated *S. litura* population.

AITC also showed effect on weight acquired by pupae as compared to untreated control (Table 1**).** Similar findings were reported by Carbone *et al*. by investigating the larval development, body weight, mortaliy and feeding rate of *Mamestra brassicae* and *Pieris rapae* against 6 genotypes of *Brassica oleracea* [21]. It was found that pupal weight and mortality rate increased on mature plants, irrespective of the genotype. Ingestion of AITC resulted in a significant increase of the larval mortality in a dose dependent manner (Table 1**).** The larval mortality was observed to be least in untreated larvae i.e., 10.00 % that was augmented at the highest concentrations. The toxicity of AITC against larvae of *S. litura* in present study was in accordance with study including Indian mustard and AITC against Masked Chafer Beetle Larvae (*Cyclocephala* spp.) [22]. Da costa *et al*., also observed susceptibility of larvae and pupae of *Sitophilus zeamais* to vapours of *B juncea* that can be attributed to inhibitory effect of AITC against oxidative phosphorylation, leading to interruption of ATP synthesis [23]. Incorporation of AITC in the artificial diet resulted in the significant inhibition in the larval and total growth index (Table 2). Both the indices showed overall a dose-dependent decrease as compared to control larvae. Such observations are also seen in the reports showing insecticidal activity of gallic acid [24].

Assimilation of AITC into the artificial diet also led to the aberrations in the pupae and adults (Fig. 5), in the form of blackened and compressed head pupae, partially emerged pupae, pupae with wrinkled and deformed wings supporting the adverse effect of AITC against *S. litura.* Similar observations were also observed by Santos *et al*., who reported that exposure of immature stages (larvae and pupae) to AITC resulted in morphological deformity in adults of *Tribolium castaneum* (Coleoptera: Tenebrionidae) [25]. Thus, the overall findings from present investigation indicated the insecticidal action of AITC against the larvae as well as adults of *S. litura.* Consequently, it can be used as biopesticide along with other natural compounds for elimination of such insect pests.

Table 1: Pupal weight and larval mortality (Mean±SE) of *S. litura* when second instar larvae were fed on different concentrations of AITC. Means varying in alphabets show significant difference at $p \leq 0.05$.

Concentrations (ppm)	Pupal weight (Mean ± SE)	Larval mortality (%) (Mean ± SE)
Control	232.37±7.57^{a}	10.00±4.47^{a}
5	218.88±3.03^{a}	12.77±6.15^{a}
25	219.79±8.28^{a}	12.77±3.35^{a}
125	225.06±5.59^{a}	20.00±7.30^{a}
625	232.22±2.53^{a}	33.33±3.35^{b}
3125	-	100.00%±0.00
F value	1.22 N.S.	6.16**

Table 2: Larval growth index (LGI) and total growth index (TGI) (Mean±SE.) of *S. litura* when second instar larvae were fed on different concentrations of AITC. Means varying in alphabets show significant difference at $p \leq 0.05$.

Concentrations (ppm)	Larval Growth Index (LGI) (Mean ± SE)	Total Growth Index (TGI) (Mean ± SE)
Control	5.49±0.19^{a}	3.16±0.14^{a}
5	4.85±0.38^{a}	2.46±0.12^{ab}
25	5.16±0.28^{a}	2.24±0.27^{b}
125	4.78±0.50^{ab}	1.68±0.24^{bc}
625	3.45±0.25^{b}	1.23±0.23^{c}
3125	-	-
F value	5.24**	12.36**

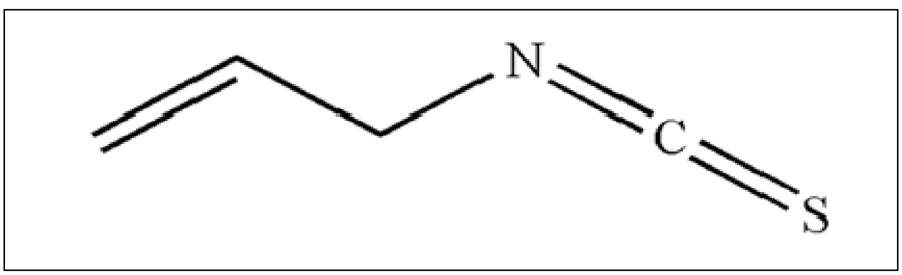

Fig 1: Chemical structure of Allyl isothiocyanate

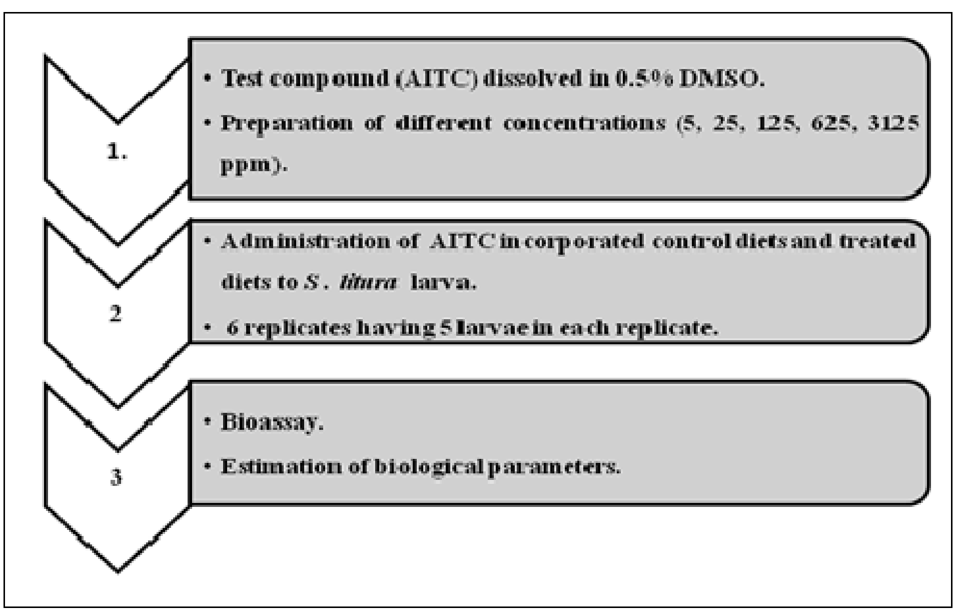

Fig 2: Schematic representation of protocol followed to determine the insecticidal activity of Allyl isothiocyanate.

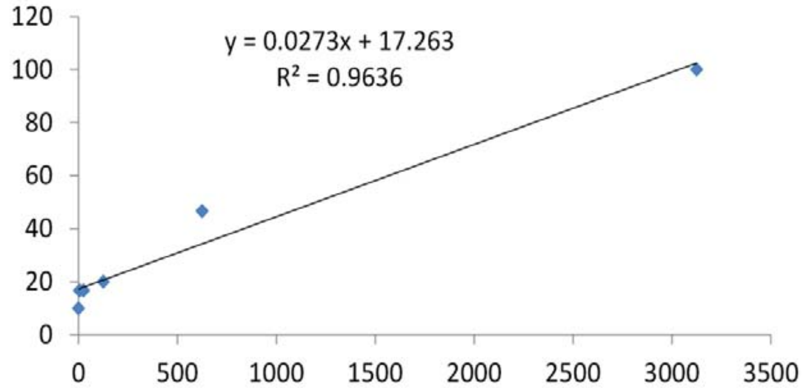

Fig 3: Regression equation used for calculating LD_{50} value of AITC.

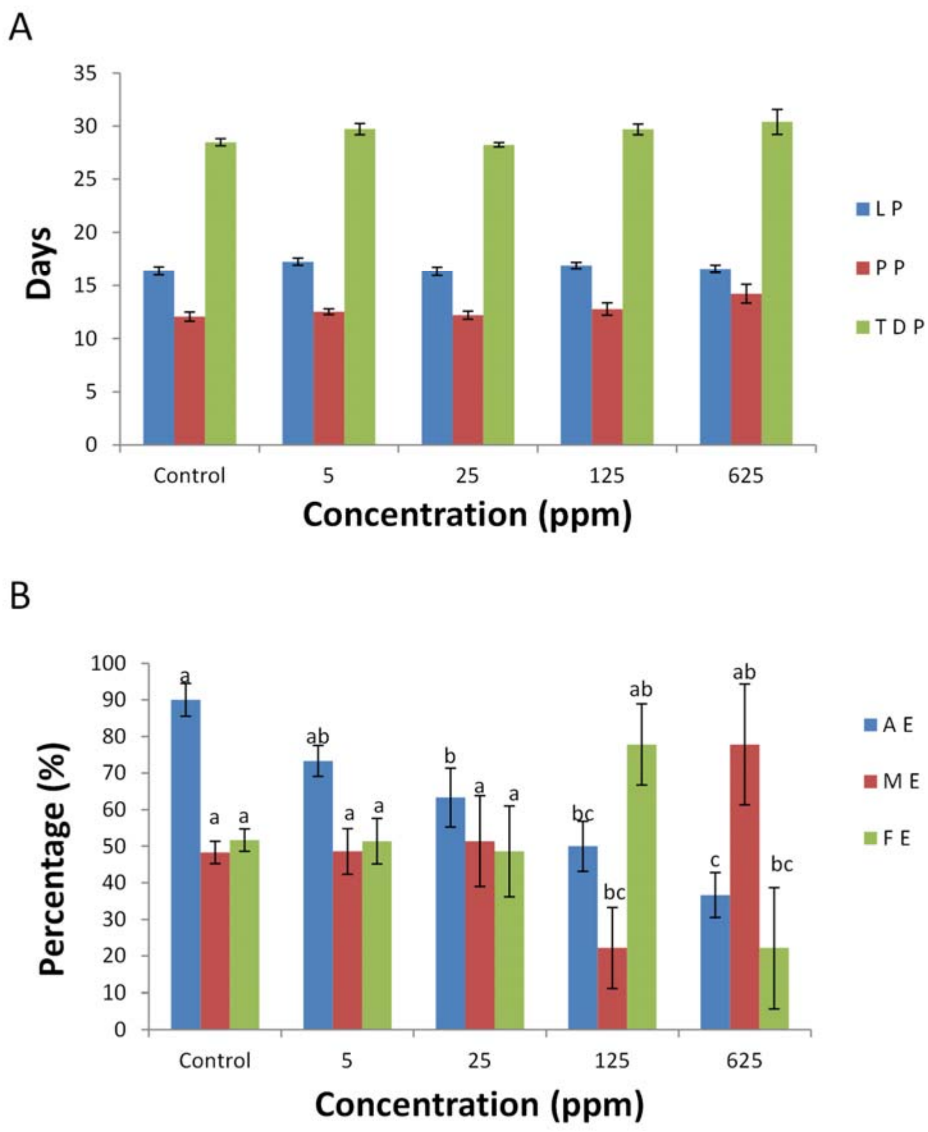

Fig 4: Representation of A) Larval period, pupal period and total development period of *S. litura* when second instar larvae were fed on different concentrations of AITC. The values are expressed as Mean±SE. B) Adult emergence, Male emergence and female emergence of *S. litura* when second instar larvae were fed on different concentrations of AITC. The values are expressed as Mean±SE.

Fig 5: Morphological changes normal vs abnormal pupae/adult as a result of treatment with different concentrations of AITC supporting its insecticidal activity A) Normal pupae B) Normal adult C) Deformed pupa D) Dead larva E) Compressed and wrinkled pupa F) Adult with abnormal wings.

4. Conclusion

Our study has highlighted the efficient insecticidal potential of AITC in managing the population of *S. litura* by showing 100% mortality at highest concentration. It also influenced all the biological parameters required by *S. litura* for growth and development. Thus, it can be concluded that Allyl isothiocyanate (AITC) can provide important arsenal to overcome problem associated with Lepidoptera species.

5. Acknowledgement

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6. Conflict of Interest

Authors declare no conflict of interest.

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