Article

Insecticidal, food utilisation and biochemical effect of essential oils extracted from seeds of *Brassica juncea* (Czern.) against *Spodoptera litura* (Lepidoptera: Noctuidae) (Fabricius)

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Abstract

The glucosinolate rich hot and cold hexane extracts of *Brassica juncea* had a negative effect on the development of *Spodoptera litura* larvae when they were fed on different concentrations of the extracts. Both larval growth index and pupal growth index declined with treatment. However the hot extract with 3-butenyl isothiocyanate as the predominant compound had a more deleterious effect as at 3125ppm the larvae were unable to complete their development. The nutritional indices too were more adversely affected with hot extract as compared to cold extract. The RGR, RCR, ECI and ECD declined significantly with increase in concentration of the hot hexane extract. The activity of antioxidant enzymes, SOD and catalase decreased while that of phosphatases, GSTs, phenol oxidases increased during the initial treatment duration but decreased on prolonged treatment of the larvae with LC_{50} concentration of hot extract. A similar trend was observed for glutathione and lipid peroxides but a decrease in ascorbate content was observed as compared to control. The findings reveal a toxic effect of 3-butenylisothiocyanate rich hot hexane extract of *B. juncea* on *S. litura* larvae.

Keywords *Brassica juncea*; GC-MS analysis; hydrodistillation; essential oils; isothiocyanates; *Spodoptera litura*.

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1 Introduction

Glucosinolates are an important class of secondary metabolites found widely in plants of the family Brassicaceae. In the past decade, a lot of progress has been made in understanding the role of glucosinolates in insect-plant interactions. Like other secondary compounds, the defensive role of glucosinolates has been established in a number of studies (Kim and Jander, 2007). The defensive property of glucosinolates has been

attributed mainly to its degradation products viz. isothiocyanates (ITCs), nitriles and other biologically active products (Halkier and Gershenzon, 2006). The release of these compounds by a plant upon tissue damage by an insect has a negative impact on a generalist herbivore (Li et al., 2000; Hopkins et al., 2009; Muller et al., 2010). However, a number of specialist insects feed and complete their development on these plants and even use glucosinolates and their hydrolytic products such as isothiocyanates as oviposition and feeding stimulants (Hopkins et al., 2009; Muller et al., 2010). This has been attributed to evolution of various adaptive mechanisms in specialist insects such as enzymatic detoxification, excretion, sequestration or behavioural adaptations. In specialist insects, cabbage butterfly, *Pieris rapae* (Linnaeus) and diamondback moth, *Plutella xylostella* (Linnaeus) two distinct mechanisms have been observed which interfere with normal metabolism of glucosinolates. Cabbage white butterfly, *P. rapae* evades the problem of toxic glucosinolates by redirecting the normal course of hydrolysis reaction catalysed by myrosinases. They have a nitrile specifier protein in their gut which by a yet unknown mechanism can redirect the hydrolysis of glucosinolates into nitriles instead of isothiocyanates which can then be excreted out (Wittstock et al., 2004). In *P. xylostella*, a glucosinolate sulfatase enzyme in the gut of the larvae outcompetes myrosinase leading to the conversion of glucosinolates to desulfoglucosinolates instead of toxic nitriles and isothiocyanates. Similar studies on metabolic adaptations in generalist insects are very few and need to be studied so as to understand the ways in which their development and physiology is affected by glucosinolates and the ways by which they cope up with the ingested metabolites. Therefore, the present study was envisaged to investigate the influence of essential oils extracted from seeds of *Brassica juncea* (Czern.) on the development and biochemistry of a polyphagous pest, *Spodoptera litura* (Fabricius).

B. juncea (L.) (Czern.) (Indian mustard) is an important oilseed crop of India having high levels of erucic acid (oil) and glucosinolates (seed meal). The mustard oil has strong bactericidal and fungicidal properties and the seed meal is used as cattle feed and as a fertiliser (Duke, 1983; Negi et al., 2000).

S.litura (Fabricius) (Lepidoptera: Noctuidae) also known as taro caterpillar is an international polyphagous defoliator on many agro-economic crops. It is widely found in India and has a wide host range of about 120 plants such as cabbage, tomatoes, cotton, lettuce, etc. The caterpillars cause destruction to these crops.

2 Materials and Methods

2.1 Culturing of insects

The egg masses and larvae of *S. litura* were procured from the cauliflower fields located in the premises of Guru Nanak Dev University, Amritsar, India and 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 (15cm×10cm) on fresh castor leaves *Ricinus communis* L. collected from the university campus. The pupae were kept in pupation jars (15cm×15cm) having a moist layer of sterilized sand covered with filter paper. The adults emerged from pupae in pupation jars were transferred to oviposition jars in the ratio of 1:2 (male:female) and covered with fine muslin cloth. The oviposition sheets were lined in the jars 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. For our study we used 6days old second instar larvae.

2.2 Plant material and extraction

The seeds of *B. juncea* were purchased from Pusa Bold IARI, Karnal, Haryana. The seed oil was prepared using cold and hot extraction methods. In the cold extraction, 500 grams of seeds were crushed using grinder. The crushed seeds were placed in a flask having 1.2 litres of hexane as a solvent. The flask was sealed with aluminium foil and allowed to stand for 24 hours. After 24 hours, the solvent was filtered using Whatmann

filter paper No. 1. The solvent was collected in another storage bottle and was dried using rotary evaporator at 30˚C. Fresh 1.2 litres of solvent was added in the remaining seed meal, which was sealed and kept as such for another 24 hours. After 24 hours, the hexane fraction was collected and dried again. Both the extracts were pooled together and the total weight of the extracts was noted to calculate the final yield.

The hot extraction of glucosinolates hydrolytic products was performed using a slightly modified hydrodistillation method using Clevenger apparatus (Arora et al., 2016). The seeds of *B. juncea* were washed and air dried to remove any kind of dust particles and stored at -20˚C until extraction. The seeds (100 grams) were grinded and dipped in round bottom flask containing 1000ml distilled water. It was then boiled using hot magnetic stirrer and the condensed vapours were collected as oil from the inner tube of Clevenger apparatus. The total mixture collected was extracted twice with 50 ml of hexane and salted with 2.5 g of anhydrous sodium sulfate. The fraction was then dried at 30˚C by using rotary evaporator to obtain 1 ml of the extract. The oil extract thus obtained was further dissolved in 1 ml of Dichloromethane and then passed through 0.22µm membrane filter and analysed for the presence of ITCs by Gas Chromatography-Mass spectrometry.

2.3 Calculation of yield collected after extraction from both methods

The yield of the seed extract was calculated by the formula given below:

Yield (%) = amount of extract collected / total amount of seeds used × 100

2.4 Gas Chromatography – Mass Spectrometry

The GC-MS analysis was carried out with slight modifications in the protocol (Arora et al., 2016) using a Shimadzu (QP2010 series) gas chromatograph-mass spectrometer (Tokyo, Japan) equipped with an AOC-20i autosampler coupled to a DB-5 MS capillary column (30 m × 0.25 mm i.d., 0.25 μm). The temperature gradient started at 40 °C, held for 4 min, and programmed to 230 °C at 4 °C/min, then held for 15 min at 230 °C; the sample injection temperature was 40 °C and volume was 2 μL in GC grade DCM. Inlet pressure was 97.1 kPa and helium was used as a carrier gas at a flow rate of 1.1 mL/min in split mode (1: 50). MS interface temperature: 250 °C; MS mode: EI; detector voltage: 0.9 kV; mass range: 40 to 800 u; scan speed: 1666 u/s; interval: 0.50 s (2 Hz).

2.5 Larvicidal bioassay

Second instar larvae (six days old) of *S. litura* that were reared on amended diet (containing various concentrations (5, 25, 125, 625 and 3125 ppm) of hexane oil extract of *B. juncea* obtained from both extraction procedures and unamended diet (control) were used. The methodology of Koul et al. (1997) was followed for making artificial diet. The ingredients used for this diet comprised of: bran, kidney bean flour, yeast, agar, vegetable oil, formaldehyde, and rest containing appropriate mixture of streptomycin, vitamin-B complex, sorbic acid, ascorbic acid, methyl para- benzoic acid and distilled water. Six replications were taken with 5 larvae in each replication for each concentration. Observations were made daily and larval growth index and pupal growth index were determined from calculations made for various parameters such as larval period, total development period, percentage pupation and percentage adult emergence.

2.6 Nutritional performances

The nutritional indices of *S. litura* were assessed based on the method given by Koul et al. (2005). The second instar larvae were starved for 3–4 hrs so that their digestive tracts were clear. These larvae were weighed and then released into the sterilised plastic experimental vials containing known weights of treated and control diets. Each concentration was replicated six times with five larvae in each replication. Experiment was carried out for 3 days interval in which weight of each larva, uneaten diet and frass (faecal matter) were observed and weighed at the end of the experiment. Dry weight of each larva, uneaten diet left and frass were observed after incubation of 72 hours at 60˚C at the end of experiment. Nutritional indices such as Relative growth rate (RGR), Relative consumption rate (RCR), Efficiency of conversion of ingested food (ECI), Efficiency of

conversion of digested food (ECD), Approximate digestibility (AD) were then calculated according to the expressions given by Waldbauer (1968) using their dry weights.

2.7 Biochemical studies

The LC_{50} concentration was used for biochemical studies which entailed investigating its influence on the activity of some antioxidant and detoxifying enzymes in the six days old larvae. In addition, the levels of lipid peroxide content (LPC), glutathione content (GC) and ascorbate content (ASC) were ascertained in the larvae of *S. litura.*

Superoxide dismutase (SOD)

The activity of superoxide dismutase (SOD) was estimated and extracted by following the protocol of Kono (1978). The second instar larvae of *S. litura* (10% w/v) were homogenised in sodium carbonate buffer (50mM, pH 10.00). The whole assay comprised of 1.3ml of sodium carbonate buffer (50mM, pH 10.00), 0.5 ml of nitroblue tetrazolium dye (NBT), 0.1 ml of 0.6% Triton-X 100, 0.1 ml of 20mM hydroxylamine hydrochloride (pH 6.0) and the absorbance was measured at 540nm.

Catalase (CAT)

The extraction and estimation of catalase (CAT) activity was done according to the protocol of Bergmeyer (1974) by homogenising the larvae (5% w/v) in 0.05 M potassium phosphate buffer (pH 7.0). The enzyme extract (0.1 ml) was added to 0.05% H_2O_2 (2.9 ml) and the decrease in absorbance was recorded at 240 nm.

Phenol oxidase (PO)

To assay the activity of phenol oxidase (PO), the methodology given by Zimmer (2005) was used. The larvae (1% w/v) were homogenised in 0.05M sodium phosphate buffer (pH 6.2). Around 300µl of enzyme extract was mixed thoroughly with 700µl of catechol prepared in the extraction buffer. The rise in the level of absorbance was recorded at 340nm at 1 minute interval for 10 minutes.

Alkaline phosphatase (AkP)

The alkaline phosphatase (AkP) was extracted by homogenising the larvae (1% w/v) in 0.05M Tris buffer (pH 8.6) as per the methodology proposed by Mac Intyre (1971). The assay mixture comprised of 2ml of 0.005M sodium naphthyl phosphate prepared in 0.05M Tris buffer, 2ml of post coupling solution and 0.2ml of enzyme extract. The absorbance was recorded at 540nm.

Acid Phosphatase (AcP)

The procedure used for the extraction and estimation of AcP was similar to that of AkP except that 0.05M acetate buffer (pH 5.0) was used instead of 0.05M Tris buffer (pH 8.6). The larvae (1% w/v) were homogenised in the buffer for estimating the AcP activity.

Glutathione-S-Transferase (GST)

The activity of glutathione-S-transferase (GST) was assessed according to the methodology given by Chien and Dauterman (1991). The larvae (2% w/v) were homogenised in 0.1M sodium phosphate buffer (pH 7.6) having 0.1mM phenyl thiourea (PTU). The assay mixture consisted of 60µl of ethanolic CDNB solution, 200µl GSH (reduced glutathione), 100µl of enzyme extract and 40µl of 0.1M sodium phosphate buffer (pH 7.6). The increase in absorbance was spectrophotometrically recorded at 340nm at intervals of 1 minute for 5 minutes at 25˚C.

Esterases (EST)

The activity of esterases (EST) was determined by the protocol of Katezenellenbogen and Kafatos (1971). The larvae (1% w/v) were homogenised in chilled 0.1M sodium phosphate buffer (pH 6.5). The assay mixture comprised of 2ml of 1mM α-naphthyl acetate prepared in buffer, 0.05ml of enzyme extract and 1ml of post-coupling solution. The absorbance was measured at 540nm.

Lipid peroxide content (LPC)

The lipid peroxide content was extracted and estimated according to the methodology given by Hermes-Lima et al. (1995) and Vontas et al. (2001). The larvae (20% w/v) were homogenised in ice-cold methanol. The assay mixture consisted of 30µl enzyme extract, 250µl ferrous sulphate, 100µl xylenol orange and 520µl distilled water. The absorbance was measured at 520nm.

Glutathione content (GC)

The content of glutathione was measured according to the protocol given by Allen et al. (1983) by homogenising the larvae (20% w/v) in 0.01M HCL. The homogenate obtained was further deproteinised with equal amount of HCLO4 (10% w/v). The whole assay mixture comprised of 60µl of enzyme extract, 90µl of Ellmans's reagent, 60µl of glutathione reductase and 300µl of and NADPH disodium salt. The optical density was read at 412nm.

Ascorbate content (ASC)

The ascorbate content was extracted and estimated by homogenising the larvae (10% w/v) in 67mM potassium phosphate buffer (pH 6.5) (Omaye et al., 1979). The whole assay mixture comprised of 270µl of enzyme extract, 80µl of orthophosphoric acid, 1.37ml of α,α'-dipirydyl and 280µl of ferric chloride. The optical density was recorded at 525nm.

3 Statistical Analysis

The data obtained in larvicidal and nutritional assays were subjected to one-way ANOVA. The means were compared by means of the Tukey's honestly difference test ($p \leq 0.05$) using Assistat (7.7 beta). Data obtained from biochemical studies was analysed by means of student 't' test using SPSS-16.

4 Results and Discussion

More than 20 different aliphatic and aromatic isothiocyanates together with other potential allelochemicals have been identified among the degradation products of glucosinolates originating from *Brassica napus*, *Brassica hirta*, *Brassica compestris*, *B. juncea* and *Brassica nigra* (Spencer and Daxenbichler, 1980; Brown et al., 1991; Brown and Morra, 1995). The GC-MS data collected for the chemical characterisation of hexane extract of *B. juncea* showed the presence of various volatile compounds in the hot and cold extracts (Figs 1 and 2). The total yield obtained after extraction by both the methods was 28.23% in case of cold hexane extract and 9.856% of hot extract. The studies revealed allyl isothiocyanate and 3- Butenyl isothiocyanate as the major compounds in the cold hexane extract of *B. juncea* while 3-Butenyl isothiocyanate was the predominant compound in the hot hexane extract of *B. juncea* and their mass spectra (MS) was matched with NIST database (Table 1). The compounds were also identified and analysed by matching their mass spectra in the computer library [Wiley, New Yorkmass spectral (MS) library] and their retention indices (RI) were compared either with published data in the literature or authentic compounds available. Li et al. (2000) too had reported that the dominanat glucosinolate, allyl or 3-butenyl comprised of at least 90% of the total glucosinolate in high glucosinolate concentration *B. juncea* lines.

Bioassays conducted with both the extracts showed significant adverse effects on the development of *S. litura* larvae. The LGI and TGI of the larvae fed on diet containing the cold hexane extract decreased with increase in concentration (Table 2). At 3125ppm, the LGI reduced to 36.58% of the control and TGI decreased to 30.34% of the control. The anti-insect activity of the cold extracts might be due to the toxic effect of isothiocyanates (allyl and 3-butenyl) present in them. Previous laboratory studies conducted with the purified compound allyl isothiocyanate on *S. litura* larvae had revealed 100% mortality at 3125 ppm concentration (Bhushan et al., 2016). They observed that the growth indices of the larvae were negatively affected with increasing concentration of allyl isothiocyanate. The growth indices also declined when the larvae were given

artificial diet having different concentrations of hot hexane extract of *B. juncea* (Table 3). At the highest concentration (3125ppm) no larvae survived to form pupae which indicated the toxic effect of 3- Butenyl isothiocyanate rich hot hexane extract. Similar findings were reported by Ulmer and Dosdall (2006) who had observed slow larval development of cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) on *B. juncea* cv-H-butenyl, which contained substantial amounts of 3-butenyl glucosinolate in both its pericarp (4.76µM/g) and seed (101.20µM/g). On the contrary, 3-butenyl and 4-pentyl glucosinolates have been reported in various studies to play an important role in the acceptance of the host plant (Evans and Allen-Williams, 1992; Bartlet et al., 1993; McCaffrey et al., 1995; Blight et al., 1995). It has been observed that specialists feed equally well on both high and low glucosinolate lines of *B. juncea* but a generalist insect like bertha armyworm, *Mamestra configurata* (Walker) prefers low glucosinolate *B. juncea* to the high glucosinolate line (Bodnaryk, 1992). *S. litura* which is also a polyphagous pest too seemed to the susceptible to the toxic effects of glucosinolates at higher concentrations.

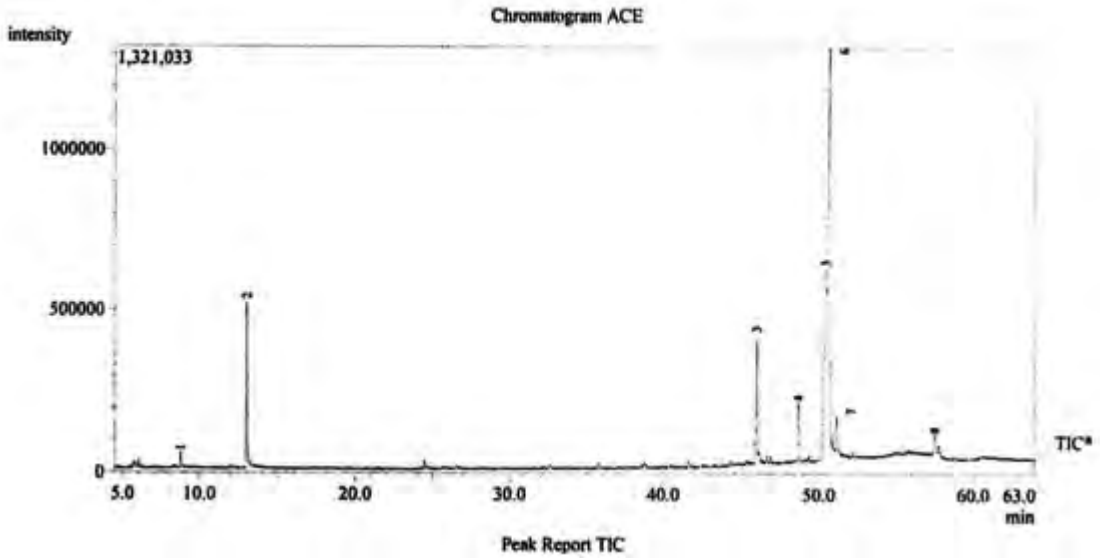

Fig. 1 GC-MS chromatogram of cold hexane extract of *B. juncea*.

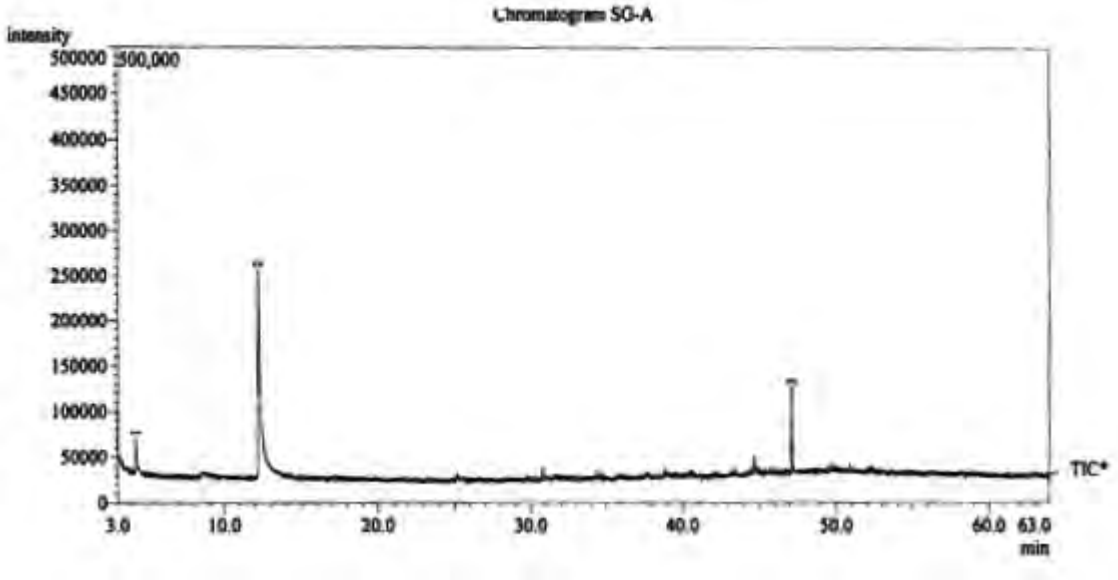

Fig. 2 GC-MS chromatogram of hot hexane extract of *B. juncea*.

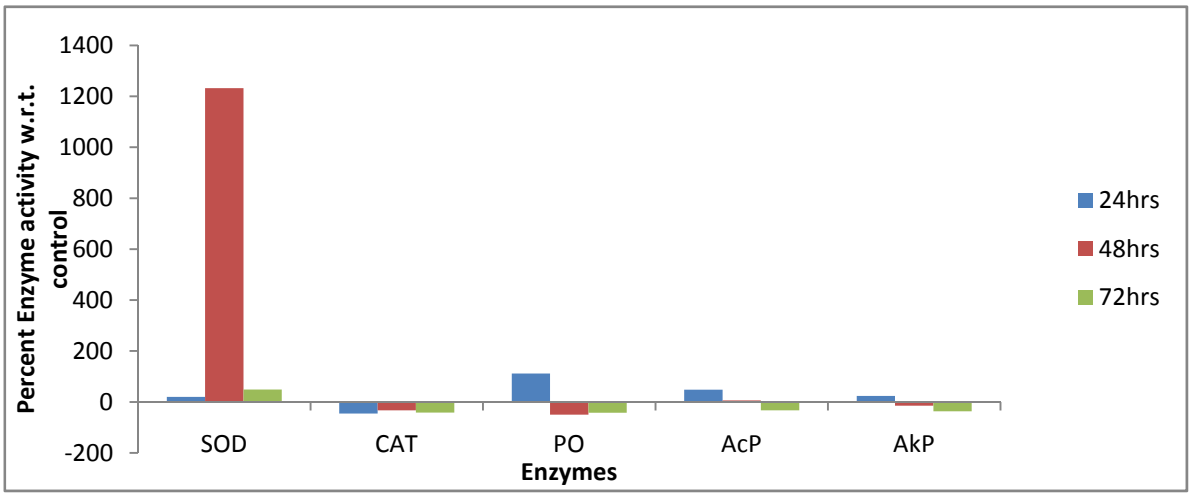

Fig. 3 Enzyme activity after the second instar larvae (6 days old) of *S. litura* were fed on hot hexane extract of the *B. juncea* incorporated in artificial diet.

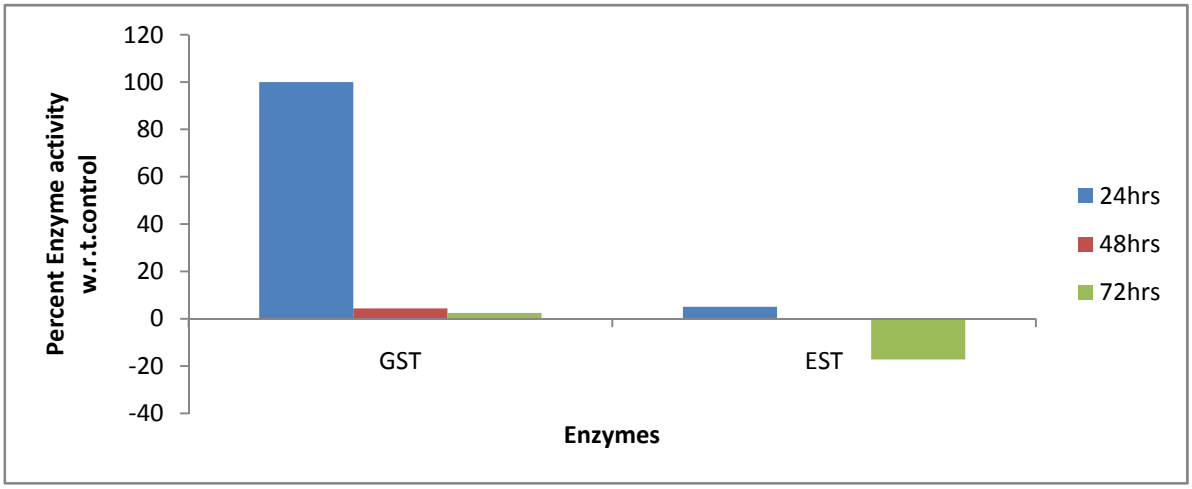

Fig. 4 Enzyme activity after the second instar larvae (6 days old) of *S. litura* were fed on hot hexane extract of the *B. juncea* incorporated in artificial diet.

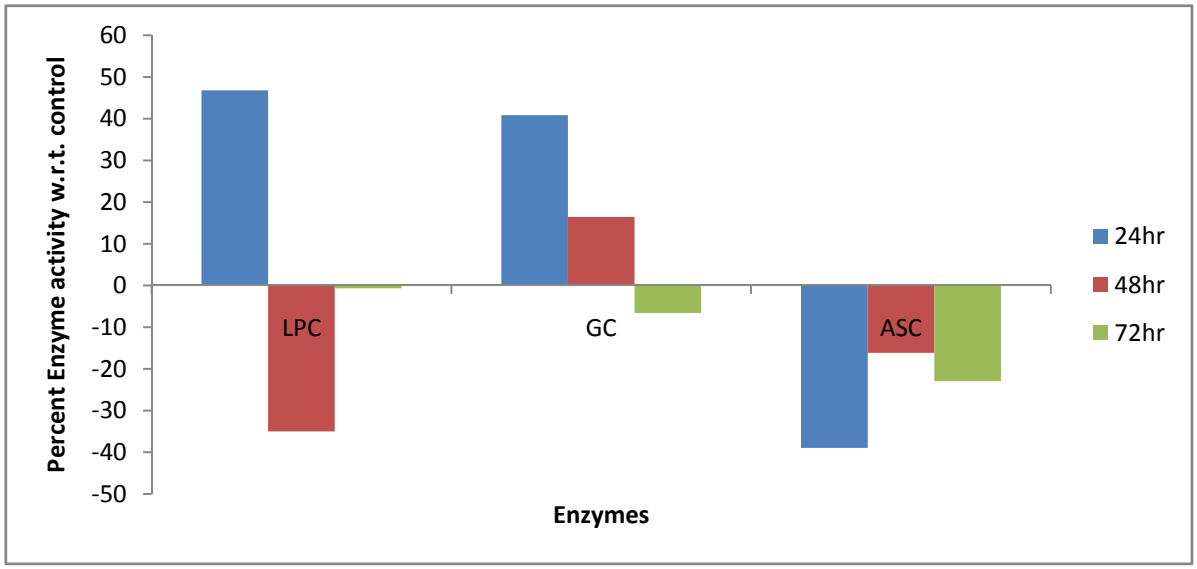

Fig. 5 Enzyme activity after the second instar larvae (6 days old) of *S. litura* were fed on hot hexane extract of the *B. juncea* incorporated in artificial diet.

Table 1 GC-MS analysis of glucosinolates in the cold and hot hexane extract of *B. juncea.*

Peak (Cold)	**Retention Time**	**Area %**	**Isothiocyanates**	**Mass Spectra**
1	8.775	8.90%	Allyl isothiocyanate	99[M], 98,72,71,45,41
2	13.079	10.09%	3-Butenyl isothiocyanate	113[M],85,72,60,55,53
Peak (Hot)	Retention Time	Area %	Isothiocyanates	Mass Spectra
2	12.203	77.15%	3-Butenyl isothiocyanate	113[M],85,72,60,55,53

Table 2 Larval Growth Index (LGI) and Total Growth Index (TGI) of *S.litura* after ad-libitum feeding of second instar larvae (6 days old) on cold hexane extract of *B. juncea* incorporated in artificial diet.

Concentrations (ppm)	**LGI Mean±S.E.**	**TGI Mean±S.E.**
Control	4.51±0.38^{a}	2.90±0.21^{a}
5	4.18±0.24^{a}	1.96±0.19^{ab}
25	3.63±0.30^{a}	1.77±0.28^{ab}
125	3.83±0.64^{a}	2.16±0.45^{a}
625	3.00±0.27^{ab}	1.93±0.33^{ab}
3125	1.65±0.06^{b}	0.88±0.16
F value	8.31**	5.03**

**=Significant at 1% level of significance. Means within a column followed by the same letter are not significantly different, p<0.05; based on Tukey's test.

Table 3 Larval Growth Index (LGI) and Total Growth Index (TGI) of *S.litura* after ad-libitum feeding of second instar larvae (6 days old) on hot hexane extract of *B. juncea* incorporated in artificial diet.

Concentrations (ppm)	**LGI Mean±S.E.**	**TGI Mean±S.E.**
Control	4.86±0.34^{a}	2.99±0.14^{a}
5	3.93±0.31^{ab}	2.04±0.21^{b}
25	3.96±0.38^{ab}	1.97±0.21^{b}
125	3.60±0.16^{bc}	1.84±0.01^{b}
625	2.49±0.23^{c}	1.56±0.15^{b}
3125	0.00±0.00^{d}	0.00±0.00^{c}
F value	40.44**	43.74**

**=Significant at 1% level of significance. Means within a column followed by the same letter are not significantly different, p<0.05; based on Tukey's test.

Table 4 Nutritional parameters of *S. litura* when the second instar larvae (6 days old) were given artificial diet incorporated with different concentrations of *B. juncea* cold hexane extract.

Concentration (ppm)	RGR (mg/mg/day) Mean ± S.E.	RCR (mg/mg/day) Mean ± S.E.	ECI (%) Mean ±S.E.	ECD (%) Mean± S.E.	AD (%) Mean ±S.E.
ntrol	0.54±0.04^{a}	6.84±0.41^{a}	8.17±0.96^{a}	11.18±2.15^{a}	90.06±0.92^{a}
5	0.26±0.02^{b}	5.73±0.27^{b}	3.98±0.27^{b}	4.67±0.26^{b}	82.37±2.68^{c}
25	0.26±0.03^{b}	5.93±0.11^{a}	4.48±0.52^{b}	5.22±0.66^{b}	88.65±0.57^{b}
125	0.30±0.05^{b}	6.43±0.18^{a}	4.64±0.65^{b}	5.36±0.81^{b}	87.49±1.14^{bc}
625	0.37±0.04^{ab}	7.49±0.64^{ab}	5.35±0.60^{b}	5.88±0.76^{b}	93.14±0.74^{a}
3125	0.38±0.04^{ab}	7.58±0.56^{ab}	5.91±0.36^{ab}	7.05±0.73^{ab}	95.31±0.58^{ab}
F-value	6.30**	3.63*	6.24**	5.00**	11.75**

The averages followed by the same superscripts (a, b and c) do not differ statistically between themselves, Tukey's test ($p<0.05$).*= Significant at 5% level and ** = Significant at 1% level.

Table 5 Nutritional parameters of *S. litura* when the second instar larvae (6 six days old) were given artificial diet incorporated with different concentrations of *B. juncea* hexane hot extract.

Concentration (ppm)	RGR (mg/mg/day) Mean ± S.E.	RCR (mg/mg/day) Mean ± S.E.	ECI (%) Mean ±S.E.	ECD (%) Mean± S.E.	AD (%) Mean ±S.E.
Control	0.54±0.04^{a}	6.84±0.41^{a}	8.17±0.96^{a}	11.18±2.15^{a}	90.06±0.92^{a}
5	0.37±0.05^{b}	5.88±0.70^{b}	6.58±1.982^{b}	6.08±0.53^{b}	113.77±1.18^{c}
25	0.33±0.06^{bc}	4.57±0.64^{c}	4.91±2.14^{c}	3.76±1.19^{c}	119.63±0.81^{bc}
125	0.31±0.01^{c}	4.04±0.20^{c}	4.76±1.08^{c}	3.72±1.36^{c}	122.84±1.18^{b}
625	0.08±0.02^{d}	2.71±0.96^{d}	2.70±3.55^{d}	2.01±1.14^{d}	123.01±1.75^{b}
3125	0.03±0.01^{e}	1.76±0.58^{e}	0.92±1.98^{e}	0.62±1.25^{e}	137.09±0.62^{d}
F value	207.28**	215.49**	321.66**	747.60**	111.47**

The averages followed by the same superscripts (a, b, c, d and e) do not differ statistically between themselves, Tukey's test ($p<0.05$). ** = Significant at 1% level.

A contrasting trend was observed in the nutritional indices of the larvae fed on diets containing cold and hot hexane extracts of *B. juncea* (Tables 4 and 5). In the larvae fed on cold hexane extract of *B. juncea*, all the nutritional indices which were significantly less than control at the highest concentration increased as the concentration of the extract was increased to 3125 ppm, but remained less than control. However, the RGR, RCR, ECI and ECD of larvae fed on diet containing hot hexane extract of *B. juncea* decreased significantly with increase in concentration. These findings indicate that 3-Butenyl isothiocyanate, a dominant glucosinolate found in the hot hexane extract was exercising a considerably more deleterious effect on the larvae at higher concentrations. Corroboratory results were obtained by Li et al. (2000) who had reported lower RGR of *S. eridania* on *B. juncea* lines with 3-butenyl as the dominant glucosinolate. Wolfson (1980) and McCloskey and Isman (1993) had also reported similar findings in *S. eridania* and *M. configurata*, respectively. The RGR of *S. litura* decreased by 94% at 3125 ppm when fed on hot hexane extract of *B. juncea* compared to control. The decline in RGR was accompanied by a concominant decrease in RCR. ECD and ECI too reduced significantly

while AD increased in the larvae fed on hot hexane extract treated diet. AD denotes the proportion of ingested food that is actually digested whereas the proportion of digested food that is actually transformed into net insect biomass is denoted by ECD. ECI indicates the efficiency of conversion of ingested food. A decrease in ECD and ECI indicates that more food is being diverted for detoxification of the extract and less is being converted to body mass. Low ECD and ECI are compensated by increase in AD (Lindroth et al., 1993). Low consumption of food decreases its passage through the gut so as to enhance digestibility to overcome the lacunae of the food. Similar studies on the effects of extracts from plants on food consumption have been reported earlier (Wheeler et al., 2001; Nathan et al., 2005; Senthil-Nathan et al., 2006).

In order to gain some knowledge regarding the physiological changes induced by ingestion of glucosinolate rich hot hexane extracts by *S. litura* which were comparatively more toxic than cold extracts, enzymatic assays were carried out to ascertain the activity of some enzymes involved in general antioxidant and detoxification mechanisms. SOD and catalase are two important antioxidant enzymes which scavenge the reactive oxygen species (ROS) formed during the interaction of insect with the toxic components of their host plants, leading to insect adaptation. However, the present study revealed no significant induction of SOD and decrease in catalase activity after treatment of the *S. litura* larvae with hot hexane extract of *B. juncea* (Fig. 3). Rather SOD activity was significantly suppressed after 48h of treatment indicating that these enzymes had no defensive role against glucosinolates rich extracts of *B. juncea*.

Phenol oxidase is an oxidative enzyme which plays a crucial role in insect defense via melanisation (Soderhall and Cerenius, 1998). In the larvae of *S. litura*, the phenol oxidase activity after showing a significant induction at 24h feeding interval got suppressed with increase in treatment time to 72h (Fig. 3). These findings indicated that the glucosinolate rich hexane extract might have affected the immune system in insects. Zibaee and Bandani (2010) had demonstrated a negative effect of *Atemisia annua* extract on phenoloxidase activity of sunn pest or corn bug, *Eurygaster integriceps* (Puton). A decline in immunity can make an insect more susceptible to secondary infection.

The enzymes, phosphatases, esterases and GSTs have been reported to possess detoxification ability against botanical insecticides (Zibaee, 2011). Acid and alkaline phosphatases are hydrolases which hydrolyse phosphomonoesters under acid and alkaline conditions, respectively (Janda and Benesova, 1991; Bai et al., 1988; Janda and Benesova, 1991). Alkaline phosphatase is mainly found in the intestinal epithelium of animals and its role is to provide phosphate ions from mononucleotides and ribonucleoproteins for different metabolic processes. Compared to other tissues, midgut has the highest acid and alkaline phosphatase activity (Sakharov et al., 1989). Our findings showed significant differences in acid and alkaline phosphatase during the initial and later treatment intervals when compared to control (Fig. 3). Both the enzymes were induced in *S. litura* larvae within 24h of treatment relative to control. These findings indicate a possible role of phosphatases in detoxification of the toxicants present in the hot hexane extract. However, prolonged exposure of the larvae to extracts resulted in a decrease in enzyme activity which indicated a toxic effect of the extract on the insect. Senthil Nathan et al. (2005) had suggested that reduced AcP activity reduced phosphorous liberation for energy metabolism, decreased the rate of metabolism as well as decreased the rate of enzyme regulation.

Among the two detoxification enzymes, esterases and GSTs whose activity was ascertained at all the three treatment durations, the induction observed in GST was significantly greater than esterase after 24h of treatment (Fig.4). Thereafter, the activity of both the enzymes declined with prolonged treatment. But while the level of GST remained higher than control, that of esterases decreased more than the control. The levels of glutathione too were found to increase significantly after 24h and 48h of treatment when compared to control. A common detoxification of ITCs is conjugation to the nucleophilic thiol (-SH) group of GSH mediated by GSTs (Boyland and Chasseau, 1969; Habig et al., 1974; Yu, 1984; Traka and Mithen, 2009). Several

lepidopteran species utilise GSH conjugation for detoxification of glucosinolate-derived ITCs, including the generalist herbivore, *Spodoptera littoralis* (Boisduval) (Yu, 1984; Wadleigh and Yu, 1988; Schramm et al., 2012). The present findings indicate that GSH-dependent detoxification might have become proportionally less efficient with increase in feeding interval.

Ascorbic acid is essential for both nutritive and antioxidative function in phytophagous insects. The ascorbate content declined in the larvae of *S. litura* at all the treatment durations (Fig. 5). The decrease in ascorbate prevents it from functioning as an important antioxidant against dietary plant compounds.

The level of lipid peroxide increased in *S.litura* larvae at 24h treatment interval (Fig. 5). The increased lipid peroxidation level is a result of increased oxidative stress which is beyond the ability of insect antioxidant defense to combat and neutralise the reactive oxygen species.

The findings revealed a comparatively more toxic effect of hot hexane extract on the growth and development of larvae of *S. litura*. Impaired larval growth with increasing concentration of the extract reflects metabolic imbalances and an ineffective defense system of the *S. litura* larvae. These studies can form the basis for breeding plants expressing higher concentrations of glucosinolates so as to enhance the control of insect pests.

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