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**Research article** 

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# Broccoli (*Brassica oleracea* L. var. *italica*) cultivars, *Palam Samridhi* and *Palam Vichitra* affect the growth of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae)



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# ABSTRACT

Effect of the ethyl acetate seed extracts of two cultivars of broccoli, *Brassica oleracea* Italica, *Palam Samridhi* (PS) and *Palam Vichitra* (PV) on growth, development and nutritional physiology of an economically important insect pest, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) was evaluated by conducting bioassays and nutritional assays. The insect larvae were fed on diets amended with the seed extracts of two cultivars at different concentrations viz. 5, 25, 125, 625 and 3125 ppm and taking water as control. The response of the insect varied with plant varieties. The extracts disrupted the developmental cycle of the pest. Larval mortality and total adult emergence were negatively affected. Larval period and total development period were also negatively influenced. Nutritional indices of *S. litura* also showed considerable decrease in the RGR, RCR, ECI and ECD as compared to control. The AD values were also enhanced with both the cultivars. The findings of the study revealed a considerable anti-insect potential of the two extracts and needs to be further explored for identification and isolation of bioactive constituents from broccoli for efficient management of the pest population.

# 1. Introduction

Currently, the control of insect pests is primarily dependent upon the use of numerous pesticides of synthetic origin such as organochlorines, carbamates, neonicotinoids, pyrethroids and organophosphates (Tudi et al., 2021). Their unregulated and indiscriminate use has affected the non-target organisms adversely, induced resistance to pesticides, led to outbreak of secondary insect pests, and in addition has jeopardized human as well as environmental health (Khater, 2012; Tudi et al., 2021). Consequently, throughout the world, the focus of research of the agricultural community is now on finding some alternate natural approaches based on inherent defence mechanisms of plants to control insect pests. Plants are a rich source of bioactive compounds (Loi et al., 2020) some of which have evolved to protect them from attack by insects, other herbivores and pathogens (War et al., 2012). Most of these compounds are eco-friendly and pose little threat to non-target organisms as well as human beings (Isman, 2006) and therefore, can serve as attractive alternatives to synthetic chemical pesticides. These bioactive compounds can act as repellents, toxicants, chemosterilants, feeding deterrents/

anti-feedants, growth retardants, as well as attractants (Khater, 2012; Mithöfer and Maffei, 2016). Some of these compounds have exhibited considerable insecticidal potential against insect pests (Sahayaraj and Kalidas, 2011).

Broccoli (*Brassica oleracea* L. var. *italica* Plenck) is a nutritionally important crop of family brassicaceae grown all over the world. It is a rich source of bioactive compounds such as glucosinolates, sulforaphane, polyphenols and minerals such as selenium (Moreno et al., 2006; Ferreira et al., 2018). Some of these compounds play an important role in plant defence against insect pests and other herbivores (Ahuja et al., 2011; Winde and Wittstock, 2011). Despite defensive functions and based on potential bioactivities, Broccoli is also suggested to be a valuable source of many functional foods as well as pharmaceutical products and carry health-promoting benefits (Le et al., 2019). Le et al. (2019) also recommended broccoli derived biocompounds to be applied in pre-clinical cancer studies as efficient cancer chemopreventive agents.

The common cutworm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) is an important pest of agricultural crops like sunflower, cauliflower, cabbage, groundnut, tomato, maize, pulses etc. in the Asian

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tropics (Javar et al., 2013; Yadav et al., 2014). The host range of *S. litura* includes 112 species of plants (Mallikarjuna et al., 2004; Yao, 2005). On most of the crops, the damage is caused by extensive feeding by larvae resulting in complete stripping of the plants. Currently, the management of this pest relies largely on synthetic chemical pesticides, leading to development of pesticide resistance properties in *S. litura* (Tong et al., 2013).

Therefore, the present study was envisaged to investigate the influence of extracts of two varieties of broccoli *viz*. *Palam Samridhi* (PS) and *Palam Vichitra* (PV) on growth and development of an economically important insect pest, *S. litura* so as to explore the possibility for using them as a source of efficient and eco-friendly botanical pesticides.

# 2. Materials and methods

# 2.1. Insect culture

The culture of *S. litura* was maintained on castor leaves in the B.O.D. (Biological oxygen demand) incubator at  $25 \pm 2$  °C temperature,  $65 \pm 2\%$  relative humidity and 12:12 hrs photoperiod (Light: Dark) (Thakur et al., 2013).

#### 2.2. Plant material and plant extract

The seeds of *Brassica oleracea* L. var. *italica* viz. *Palam Samridhi* (PS) and *Palam Vichitra* (PV) were procured from Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya (CSKHPV), Palampur. Seeds were surface sterilized by rinsing them in 70% ethanol for 1-2 min followed by a 15-minute exposure to 1.3% sodium hypochlorite. The seeds were then placed in seed germinator for about three days. All sprouts were grown with a 16:8 hrs light/dark photoperiod at 22 °C temperature. Sprouts were then gently collected from the trays and homogenized with the grinder.

Thereafter, extraction of plant material was done using the method prescribed by Liang et al. (2007) with slight modifications. The seeds were homogenized along with water for 5 min and autolyzed at room temperature for 30 min. After autolyzing, the meals were extracted two times with ethyl acetate which was further combined and salted with 2.5g anhydrous sodium sulfate and then the fraction was dried at 30 °C under vacuum on a rotary evaporator.

#### 2.3. Gas chromatography-mass spectrometry

The GC-MS analysis of the extracts obtained was carried out on a Shimadzu (QP, 2010) series Gas Chromatogram-Mass Spectrometer (Tokyo, Japan), AOC-20i auto-sampler coupled with DB-5MS capillary column, (30 m  $\times$  0.25 mm i.d., 0.25 µm) according to the protocol given by Arora et al. (2014). The temperature of the column was maintained between 70°C-230 °C at the rate of 4 °C/min, and then held for 15 min at 230 °C. The sample injection volume was 2 µl at 40°c in GC grade DCM. Helium was used as carrier gas at a flow rate of 1.1 ml/min in split mode (1:50).

#### 2.4. Bioassays

In order to study the insecticidal and feeding deterrent effect of these two naturally occurring varieties of broccoli, the bioassays were conducted on the second instar larvae of *S.litura* by feeding them with artificial diets incorporated with different concentrations of the extracts viz. 5, 25, 125, 625, 3125 ppm and water as control.

The artificial diet of *S. litura* was prepared using the methodology proposed by Koul et al. (1997). All the experiments were carried out using six replications for each concentration with 5 larvae of *S. litura* per replication. Similarly, for control, there were a total of six replications with 5 larvae of *S. litura* in each control replication. A total of 30 individuals (5 larvae x 6 replications) were used for control and for each concentration. Each larva was placed in a separate plastic rearing cup and

the diet was regularly changed. Observations regarding various development parameters like larval period, total development period, percent adult emergence and percent larval mortality were taken on a daily basis.

#### 2.5. Nutritional assays

In order to determine the efficacy of food utilisation, the insect nutritional assays were conducted by feeding second instar *S. litura* larvae (6 days old) on freshly prepared artificial diet (treated and untreated) placed in plastic cups after recording their initial weights. The larvae, faecal matter, and remaining uneaten diet were separated, dried and weighed after 72 hrs. The experiment was designed for three days (72 hrs) interval with 5 larvae per replication for each concentration. There were six replications for each experiment.

The various nutritional parameters of consumption, digestion, and utilization of food were calculated by the methods documented by Waldbauer (1968) and Koul et al. (2005). The nutritional parameters such as the relative growth rate (RGR), relative consumption rate (RCR), efficiency of conversion of ingested food (ECI), efficiency of conversion of digested food (ECD), and approximate digestibility (AD) were calculated as:

$$RGR = \frac{Change in larval dry weight per day}{Initial dry weight of larvae}$$

$$RCR = \frac{Change in dry weight of diet per day}{Initial dry weight of larvae}$$

$$ECI = \frac{Dry \text{ weight (Gain of insect)}}{Dry \text{ weight (Food ingested)}} \times 100$$

$$ECD = \frac{Dry \text{ weight (Gain of insect)}}{Dry \text{ weight (Food ingested)} - Dry \text{ weight (Fecal matter)}} \times 100$$

$$AD = \frac{Dry \text{ weight (Gain of insect)} - Dry \text{ weight (Fecal matter)}}{Dry \text{ weight (Food ingested)}} \times 100$$

#### 2.6. Statistical analysis

The data obtained was subjected to one-way ANOVA and wherever F-values were found to be significant, the means were compared by the Tukey's honestly difference test ( $P \le 0.05$ ) using Assistat (7.7).

# 3. Results

#### 3.1. GC-MS analysis

The GC-MS data showed the presence of various compounds in *Palam Samridhi* (PS) and *Palam Vichitra* (PV) extracts (Figures 1 and 2). The PS extract contained 3-Butenyl isothiocyanate, erucin, sulforaphane and PV extract contained erucin, 2-Phenylethyl isothiocyanate, sulforaphane and their mass spectra (MS) matched with NIST database (Tables 1 and 2; Figures 1 and 2). The components of extracts were also identified by matching their mass spectra in the computer library such as Wiley, New York mass spectral (MS) library and their retention indices (RI) were compared either with authentic compounds or with published data in the literature.

# 3.2. Bioassays

Bioassays conducted with ethyl acetate extracts of both PS and PV showed growth inhibitory effects on the larvae of *S. litura*. Significant inhibitory effects of PS and PV on larval mortality and percent adult emergence of *S. litura* were observed (Table 3). In the larvae fed on PS



Figure 1. GC-MS chromatogram of Palam Samridhi.



Figure 2. GC-MS chromatogram of Palam Vicihitra.

incorporated diet, the larval mortality was observed to be greater than control at higher concentrations with maximum increase observed at 3125 ppm. However, in the larvae fed on PV amended diet, the larval mortality initially decreased at lower concentrations as compared to control but then increased significantly with maximum mortality of 46.7% at 3125 ppm.

The adult emergence of *S. litura* decreased significantly with both PS and PV, but the decrease was not concentration dependent (Table 3). In larvae fed on PS incorporated diet, the maximum decrease in percentage adult emergence was observed at 3125 ppm where it was reduced to 49% of the control. In larvae fed on PV incorporated diet, the percentage adult emergence was lowest at 5 ppm followed by 3125 ppm.

PS significantly delayed the larval period at all concentrations except at highest concentration of 3125 ppm where it was shortened as compared to control (Table 4). On the other hand, PV incorporated diet decreased the larval period at lower concentrations as compared to control while at highest concentration of 3125 ppm, it was delayed by 0.35 days as compared to control, however the results obtained were found to be non-significant.

For PS incorporated diet, the total development period prolonged by 3.07 days at 625 ppm concentration as compared to control while in the

highest concentration of 3125 ppm, it again decreased (Table 4). On the other hand, the PV incorporated diet decreased the total development period as compared to control but the decrease was concentration in-dependent (Table 4).

# 3.3. Nutritional assays

The RGR, ECI, and ECD were significantly reduced in the larvae fed on PS and PV incorporated diet when compared to control (Figures 3, 4, and 5). As compared to control, RGR decreased by 31.21% at 3125 ppm for PS and by 12.67% at 3125 ppm in case of PV. The RCR too was significantly reduced in larvae treated with PS but in larvae fed on diet having PV, the RCR initially decreased by 14.46% up to 25 ppm but then again increased up to 77.17% at 3125 ppm as compared to control (Figure 6). In response to PV amended diet, AD of the larvae showed a maximum of 16.66% increase at 125 ppm and about 15.46% increase at 3125 ppm as compared to control (Figure 7). Although, AD was found to be increased with PS diets but the results were statistically non-significant (Figure 7).

#### 4. Discussion

Larval mortality was increased while adult emergence was reduced with both the cultivars however the results varied with respect to cultivars. Broccoli like other plants of the Brassicaceae family is rich in glucosinolates which carry diverse functions such as growth inhibitors, toxins or feeding deterrents against a large number of herbivores ranging from phloem-feeding aphids to leaf chewing lepidopterans' larvae (Poelman et al., 2008; Hopkins et al., 2009). S. litura is a generalist insect pest which feeds on a wide range of crops. Generalist insect pests fed on glucosinolates and isothiocyanate (ITC) incoporated diets commonly suffer inhibition of growth and development (Jeschke et al., 2015, 2016, 2017). In larvae of many generalist lepidopteran insects, including the cotton leafworm, S. littoralis Boisduval, the predominant glucosinolate hydrolysis derived products, ITCs, at low concentration are detoxified to some level by conjugation to GSH in the midgut and the subsequent excretion with the faeces (Wadleigh and Simon, 1988; Schramm et al., 2012; Zalucki et al., 2021). As a result of such metabolic adaptations to the toxic effects of glucosinolates present in the plant extracts, S. litura larvae might be able to prevent mortality to some extent but could not prevent glucosinolates and their hydrolysis products from disturbing and delaying their growth and development (Jeschke et al., 2017). Glucosinolates may still serve as potential defenses as the increase in development time increases the risk of predatory attacks (Jeschke et al., 2017). After feeding on PS incorporated diet, both the larval period and total development period of S. litura larvae were prolonged. It has already been reported that glucosinolates significantly influence the duration of the developmental stages (Smallegange et al., 2007). A negative effect of glucosinolates on eggs was detected for brassica pod midge, Dasineura

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|---------------------|---------------------------------|-------------------------|--|
| Table 1. GC-MS anal | vsis of Palam Samridhi (PS) for | isothiocyanates and glu | cosinolates hydrolytic products.   |

|      | •              | \$ 8  | 5 5 1                    |  |
|------|----------------|-------|--------------------------|--|
| Peak | Retention Time | Area% | Isothiocyanates          | Mass spectra                             |
| 1    | 6.178          | 0.18% | 3-Butenyl isothiocyanate | 113 [M],85,72,60,55,53                   |
| 3    | 13.287         | 1.53% | Erucin                   | 161 [M], 146, 115, 100, 85,72,61, 55, 53 |
| 4    | 16.871         | 0.66% | Sulforaphane             | 177 [M],160,114,85,72,64,55              |

| Table 2. | GC-MS analy | ysis of Palam | Vicihitra (PV) | for isothiocy | vanates and g | glucosinolates | hydrolytic products. |
|----------|-------------|---------------|----------------|---------------|---------------|----------------|----------------------|
|----------|-------------|---------------|----------------|---------------|---------------|----------------|----------------------|

| Peak | Retention Time | Area% | Isothiocyanates              | Mass spectra                             |
|------|----------------|-------|------------------------------|--|
| 3    | 13.299         | 1.10% | Erucin                       | 161 [M], 146, 115, 100, 85,72,61, 55, 53 |
| 4    | 13.716         | 0.07% | 2-Phenylethyl isothiocyanate | 163 [M], 105, 91, 77, 72,65, 50          |
| 6    | 16.887         | 0.23% | Sulforaphane                 | 177 [M],160,114,85,72,64,55              |

Table 3. Larval mortality (%) and adult emergence (%) of *S.litura* after ad-libitum feeding given to second instar larvae on *Palam Samridhi* (PS) and *Palam Vichitra* (PV) incorporated artificial diet.

| Concentrations (ppm) | Larval mortality (%)<br>Mean $\pm$ S.E. (PS) | Larval mortality (%)<br>Mean $\pm$ S.E. (PV) | Adult emergence (%)<br>Mean $\pm$ S.E. (PS) | Adult emergence (%)<br>Mean $\pm$ S.E. (PV) |
|----------------------|--|--|---|---|
| Control              | $20.00\pm0.00^{\rm a}$                       | $33.33\pm2.98^a$                             | $73.33\pm8.43^{\rm a}$                      | $76.67\pm8.03^a$                            |
| 5                    | $26.67\pm4.22^a$                             | $33.33\pm2.98^{\rm a}$                       | $50.00\pm6.83^{abc}$                        | $30.00 \pm 6.83^{\mathrm{b}}$               |
| 25                   | $20.00\pm0.00^a$                             | $20.00\pm0.00^{\rm b}$                       | $76.67\pm8.03^{ab}$                         | $50.00\pm3.65^{ab}$                         |
| 125                  | $35.00\pm6.06^{ab}$                          | $20.00\pm0.00^{\rm b}$                       | $56.70\pm10.9^{abc}$                        | $43.33\pm6.15^{b}$                          |
| 625                  | $32.00\pm6.53^{ab}$                          | $20.00\pm0.00^{\rm b}$                       | $40.00\pm8.94^{bc}$                         | $40.00\pm10.3^{b}$                          |
| 3125                 | $46.66\pm2.98^{\rm b}$                       | $46.7\pm11.2^{ab}$                           | $36.00 \pm 3.27^{c}$                        | $32.00\pm4.00^{b}$                          |
| F- value             | 5.86**                                       | 4.99**                                       | 4.35**                                      | 6.10**                                      |

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

Table 4. Larval period (days) and total development period (days) of *S. litura* after ad-libitum feeding given to second instar larvae on *Palam Samridhi* (PS) and *Palam Vichitra* (PV) incorporated artificial diet.

| Concentrations (ppm) | Larval period<br>Mean±S.E. (PS) | Larval period Mean $\pm$ S.E. (PV) | Total development period Mean $\pm$ S.E. (PS) | Total development period Mean $\pm$ S.E. (PV) |
|----------------------|---------------------------------|------------------------------------|---|---|
| Control              | $13.58\pm0.25^{\rm a}$          | $15.91\pm0.21^a$                   | $20.68\pm0.28^a$                              | $26.54\pm0.82^a$                              |
| 5                    | $13.03\pm0.13^{c}$              | $15.12\pm0.48^a$                   | $22.40 \pm 0.49^{ab}$                         | $25.00 \pm 0.52^{ab}$                         |
| 25                   | $13.79\pm0.10^a$                | $15.24\pm0.25^a$                   | $22.55 \pm 0.27^{ab}$                         | $24.95\pm0.31^{ab}$                           |
| 125                  | $14.41\pm0.33^{abc}$            | $14.99\pm0.13^a$                   | $23.58\pm0.28^{\rm b}$                        | $26.19\pm0.41^a$                              |
| 625                  | $14.00\pm0.21^{ab}$             | $15.54\pm0.17^a$                   | $23.75\pm0.80^b$                              | $25.28 \pm 0.29^{ab}$                         |
| 3125                 | $13.33\pm0.11^{bc}$             | $16.26\pm0.85^a$                   | $21.60\pm0.23^a$                              | $24.00\pm0.43^b$                              |
| F- value             | 5.49**                          | N.S.                               | 6.86**  | 3.43*   |

\*\* = Significant at 1%, \* = Significant at 5% and N.S. = Non-significant. Means within a column followed by the same letter are not significantly different, p  $\leq$  0.05; based on Tukey's test.



Figure 3. RGR (mg/mg/day) of *S. litura* after the larvae were fed on *Palam Samridhi (PS)* and *Palam Vichitra (PV)* incorporated artificial diet.



**Figure 4.** ECI (%) of *S. litura* after the larvae fed on *Palam Samridhi* (PS) and *Palam Vicihitra* (PV) incorporated artificial diet.



Figure 5. ECD (%) of *S. litura* after the larvae fed on *Palam Samridhi* (PS) and *Palam Vicihitra* (PV) incorporated artificial diet.



Figure 6. RCR (mg/mg/day) of *S. litura* after the larvae were fed on *Palam Samridhi (PS)* and *Palam Vichitra (PV)* incorporated artificial diet.



Figure 7. AD (%) of *S. litura* after the larvae fed on *Palam Samridhi* (PS) and *Palam Vicihitra* (PV) incorporated artificial diet.

*brassicae* (Wim) (Åhman, 1985; Björkman et al., 2011) and on the feeding of rape beetle, *Meligethes aeneus* (Fabricius) (Cook et al., 2007).

Also, it was noticed that the toxic effect of PS on the insect pest was more than PV as per the results obtained for larval mortality, larval period and total development period. This could be due to variations in the glucosinolates content in the two species of broccoli or due to the ability of the insects to detoxify, sequester or excrete different plant defensive compounds. The glucosinolates content varies between individual plant species (Moyes et al., 2000; Chaplin-Kramer et al., 2011), between organs of the same plant species and between the developmental stages of individual plant species (De Villena et al., 2007; Cartea and Velasco, 2008). This suggests that the glucosinolate concentration may differentially influence the insect pests. Moreover, Jeschke et al. (2017) reported that glucosinolates affect insects by deterrence, growth inhibition and increasing susceptibility to predation as a result of delayed development time.

Incorporation of the extracts of two cultivars of broccoli was found to significantly affect the various nutritional parameters of *S. litura* larvae. The analysis of nutritional indices of *S. litura* larvae revealed that the RCR of *S. litura* larvae fed on PV incorporated diet was more as compared to control. But the RCR of larvae fed on PS diet was lesser than control. This could account for the prolonged development time of the larvae of *S. litura* fed on PS incorporated diet.

The ECI and ECD decreased in the larvae of *S. litura* when fed on both species of broccoli incorporated diet. ECI is a measure of the insect's capability to utilize the food it ingests for growth. Decreased ECI is an indication of more food being metabolized for energy to perform defensive functions or to detoxify the toxic effect of the diets and less energy being converted into body mass. The low ECI values as compared to control in the larvae of S. litura could be due to the energetic cost for detoxification or due to impaired metabolism (Yazdani et al., 2013; Aljabr et al., 2017). The latter can have an adverse effect on insect's conversion efficiency (Scriber and Slansky, 1981). Decrease in ECD values usually results from either the presence of toxins in the diet or due to the lack or unsuitability of food constituents required by the insects for proper growth (Koul et al., 2004). Low ECI and ECD probably account for low RGR of S. litura larvae. AD increased in the S. litura larvae when fed on diet amended with the two species of broccoli. This increased AD possibly serves to fulfil the increased demand for nutrients (Koul et al., 2003) and to compensate for the reduction in food stuff conversion resulting from lowered ECI and ECD, probably by switching biomass production towards detoxification process (Wheeler and Isman, 2001). Our results are in accordance with the findings of Datta et al. (2019) who also reported a significant decline in nutritional parameters viz. RGR, RCR, ECI and ECD in S. litura larvae in response to crude plant extracts. Punia et al. (2020) also reported similar anti-nutritional effect of plant secondary metabolite against S. litura. A disturbed feeding pattern in the cotton bollworm, Helicoverpa armigera Hübner larvae while feeding on leaves of A. Thaliana, has already been reported (Shroff et al., 2008). The larvae avoided the glucosinolate-enriched parts especially, midvein and the edge of the leaf demonstrating deterrent activity of glucosinolates (Shroff et al., 2008).

#### 5. Conclusion

The present findings revealed toxic or deterrent effects of the two broccoli varieties on the insect pest, *S. litura*. These studies can provide the baseline data for further exploration of bioactive compounds from broccoli which can be used by plant breeders for enhancing their expression through transgenic plants so as to confer resistance against the insect pests.

#### Declarations

#### Author contribution statement

Shallina Gupta: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Ashun Chaudhary, Saroj Arora: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Sumit Singh: Analyzed and interpreted the data; Wrote the paper. Satwinder Kaur Sohal: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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#### Data availability statement

Data included in article/supplementary material/referenced in article.

# Declaration of interests statement

The authors declare no conflict of interest.

#### Additional information

No additional information is available for this paper.

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