

Insecticidal effects of *Parthenium hysterophorus* and *Moringa oleifera* leaf extracts on digestibility indices and survival of *Spodoptera litura* (Lepidoptera: Noctuidae)

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Abstract

Insecticidal properties of *Parthenium hysterophorus* L. (family: Asteraceae), an annual herb, and *Moringa oleifera* (Lam.) (family: Moringaceae), a perennial tree, were assessed against different digestibility parameters and survival rate of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae). Effect of different extracts (based on three extraction solvents: ethanol, methanol, and acetone) of *P. hysterophorus* and *M. oleifera* on the digestibility indices and larval mortality of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) were studied. The extracts were tested at two different concentrations; 25 mg and 50 mg. The effect of methanol extract on *S. litura* larvae was greater than that of ethanol and acetone. The methanol extract of *P. hysterophorus* at 50 mg concentration significantly reduced the digestibility indices; 49.6% consumption rate, 80.4% growth rate, 63.9% efficiency of conversion of ingested food, 68.4% efficiency of conversion of digested food, 39.3% assimilation rate, and 12.3% approximate digestibility of *S. litura* larvae compared with the control (positive untreated) treatment. By using methanol extracts of *P. hysterophorus* at 50 mg concentration, larvae's survival rate was also found to be lower than those of other treatments. The performance of *M. oleifera* extracts was lower in terms of affecting the digestibility and survival of *S. litura* compared to *P. hysterophorus*. Among the two plant extracts, a higher concentration (50mg) of *P. hysterophorus* using methanol as a solvent represented a promising natural product for *S. litura* control.

Keywords: Botanicals, Biological control, Feeding indices, *Spodoptera litura*, Sustainable management

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Introduction

During the past few decades, the indiscriminate and excessive use of synthetic insecticides has led to resistance development in insect pests and the accumulation of toxic residues in the environment (Isman, 2006). Thus, environmentally safer alternate compounds are urgently needed, as endorsed by national and international legislation (Dougoud et al., 2019). The search for effective pesticides has resulted in a significant market opportunity for alternative products (Isman et al., 2011). Botanical pesticides are good alternatives to synthetic insecticides in integrated pest management (Nerio et al., 2010; Pavela and Benelli, 2016). Their multiple modes of action reduce both resistance development in insects and the risk to non-target organisms because of their rapid dissipation in the environment (Rajendran and Sriranjini, 2008).

Parthenium hysterophorus L. (family: Asteraceae) is a widely spread weed, commonly known as carrot weed (*chatak chandani* and *gajar ghas* in local vernacular) (Kumar et al., 2012). It has been reported to possess insect repellent, antioxidant, antibacterial, and antifungal properties (Gören et al., 1996; Bandonien et al., 2000). Because of its biological activities, *P. hysterophorus* was successfully used to control the Indian strain of mosquito, *Aedes aegypti* (Culicidae: Diptera) (Kumar et al., 2012). Lectins present in the *P. hysterophorus* affect the nutritional balance and retard the growth and development of *Aedes aegypti* larvae (Coelho et al., 2009). Parthenin, an active ingredient in *P. hysterophorus*, possesses a strong insecticidal property and has been reported to manage red cotton stainer (*Dysdercus koenigii* F.) effectively, red flour beetle (*Tribolium castaneum* Herbst.), potato tuber moth (*Phthorimaea operculella* Zell) and pulse beetle (*Callosobruchus chinensis* L.) (Sharma and Joshi, 1977). *Moringa oleifera* (Lam.) is a widely spread perennial tree, which belongs to the Moringaceae family (Anwar et al., 2007). Seeds of *M. oleifera* are a rich source of bioactive proteins, such as lectins, with various biological activities (de Lima Santos et al., 2014). A lectin isolated from *M. oleifera*, named cMoL (coagulant *M. oleifera* lectin), possesses insecticidal activity against the Mediterranean flour moth (*Anagasta kuehniella* Zeller (Lepidoptera: Pyralidae)) (Santos et al., 2009). Lectin interferes with the molting process in insects, such as *Zonocerus variegatus* (Lepidoptera: Pyralidae) and *Tribolium*

castaneum (Coleoptera: Tenebrionidae) (de Oliveira et al., 2011; Anita et al., 2012), causing growth disruption. Three lectins, SLL-1, SLL-2, and SLL-3, present in leaves of *M. oleifera*, are reported to have high antibacterial activity (Khatun et al., 2009).

Insecticidal activity of plant extracts may vary according to plant part, solvent, extraction methods and insect species. The antifeedant activity of these plant extracts could be different using different type of solvents. Baskar et al. (2009, 2010) showed the insecticidal properties of different plant extracts using acetone as a solvent against lepidopterous insect pest. The differences in insecticidal efficacy of plant extracts are more likely due to the extraction efficiency of the different solvents however hydrophobic compounds with bioactivity are reported to be more efficiently extracted by methanol than water (Mkindi et al., 2017; Dougoud et al., 2019). Similarly, Feng et al. (2012) reported strong antifeedant activity of thirty Chinese medicinal plants using ethanol as a solvent against *Spodoptera exigua* (Lepidoptera: Noctuidae).

Spodoptera litura Fabricius (Lepidoptera: Noctuidae), a polyphagous insect pest, attacks various crops in Pakistan (Ahmad et al., 2007). It is distributed worldwide, especially in tropical and temperate regions, including Asia, Australasia, Christmas Island, Cocos Island, Andaman, and Nicobar Islands (EPPO, 2014; Monobrullah and Shankar, 2008; Liu et al., 2018). It can cause 26 to 100% economic losses, depending upon the crop stage and infestation level (Khan et al., 2018). In Pakistan, *S. litura* is considered a devastating leaf-feeding insect pest (Khan et al., 2011), which causes heavy losses to various cultivated crops, ornamentals, vegetables, and fruits (Ahmad et al., 2013). So, there is a dire need for sustainable, environment-friendly control measures for this pest. The current study aimed at exploring the effectiveness of *M. oleifera* and *P. hysterophorus* against the gregarious plant pest, *S. litura* to determine their insecticidal effect. Leaf extracts of *M. oleifera* and *P. hysterophorus* prepared in different extraction solvents were used to determine their effect on the digestibility and survival of *S. litura*.

Material and Methods

The experiment was conducted in the Department of Entomology at the College of Agriculture, and



Department of Chemistry, University of Sargodha, Pakistan. Insect culture and treatments are described below;

Insect culture

Egg batches of *S. litura* were collected from soybean (*Glycine max* (L.) Merr.) field near the University. Eggs were kept in clean Petri plates under controlled conditions ($26\pm 2^{\circ}$ C temperature, and $64\pm 5\%$ RH). Newly hatched larvae were raised on an artificial diet (Sorour et al., 2011). Pupae were separated from Petri plates and kept in clean plastic jars ($120\text{ mm} \times 116\text{ mm} \times 95\text{ mm}$). On emergence, the adults were raised on 10% sugar solution. Cotton wool strips (1 cm wide, 5-10 cm long) were placed in plastic jars to facilitate oviposition. The F_3 generation of *S. litura* was used in further experiments.

Plant extract preparation

Fresh leaves of *P. hysterophorus* and *M. oleifera* were collected from the research area ($32^{\circ}07'49.3''\text{N}$ $72^{\circ}41'07.4''\text{E}$) near the University. The leaves were washed with distilled water to remove dust and contaminants. Leaves were air-dried for 48 hours, followed by oven drying at 50°C for 24 h. Dried leaves were ground to a fine powder in an electrical grinder (Moulinex, France). Three extraction solvents (acetone, ethanol, and methanol) were used individually to prepare plant extracts. A 10-gram powder sample of each plant material was mixed in 100 ml of each solvent. The solution was placed on an orbital shaker (OS-752 Pallscientific, Indonesia) for 12 h for thorough mixing. The solution was then filtered with a muslin cloth and Whatman No. 1 filter paper. After filtration, residues were mixed with 100 ml solvent, and the shaking procedure was repeated. The solution was kept in a rotary vacuum evaporator (BEV-1001V, Henan, China) to evaporate the solvent. After drying, extracts were stored in a refrigerator at 4°C temperature for further use.

Efficacy of plant extracts against *Spodoptera litura* larvae

The plant extracts of *P. hysterophorus* and *M. oleifera* were tested at two concentrations (25 mg and 50 mg) against *S. litura* larvae to determine their effect on digestibility and survival of the insect. Almost similar sizes of 3rd and 4th larval instars were tested for each concentration of plant extracts. Before starting the experiment, healthy larvae were selected and starved for 24 h. Leaves of soybean were collected from the field and washed with distilled

water to remove dust and contaminants. The leaves were dipped for 10 seconds in each solution and dried for 10 min at room temperature. Treated leaves were kept in Petri plates, and a single larva was released in each plate.

Treatment layout and data recording

Treatments, *P. hysterophorus* 25 mg, *P. hysterophorus* 50 mg, *M. oleifera* 25 mg, *M. oleifera* 50 mg, control (+ve) and (-ve), were arranged in a completely randomized design (CRD). Untreated larvae were considered control (+ve), and the respective solvent was applied in the control treatment (-ve). Each treatment was replicated five times, and 5 larvae were tested in each replication, totaling 25 larvae/treatment. Data on larval length and weight, the weight of leaves before and after 24 h of feeding, and feces weight were recorded. A daily account of the survival rate of larvae was also kept. The digestibility indices were calculated using the following formulae (Waldbauer, 1968)

Growth rate (GR)

The growth rate was expressed as the digested material available to the insect during the experimental period.

$$GR = \frac{\Delta B}{BI \times T}$$

Consumption rate (CR)

$$CR = \frac{I}{B \times T}$$

The efficiency of conversion of digested food (ECD)

$$ECD = \frac{G}{D} \times 100$$

The amount of food digested was calculated by subtracting the weight of feces from the weight of food ingested.

The efficiency of conversion of ingested food (ECI)

The conversion of ingested food measurement indicates the overall efficiency of the insect to utilize the food for growth.

$$ECI = \frac{G}{I} \times 100$$



The following formula calculated approximate digestibility (AD) and assimilation rate (AR):

$$AD = \frac{I - F}{I} \times 100$$

$$AR = CR \times AD$$

Where;

ΔB = Weight gained by an insect.

BI = Mean weight of insect during the feeding period

T = Duration of feeding period (days)

I = Weight of food ingested.

B = Average larval weight during the trial

G = Weight gained by the insect

D = Weight of food digested.

F = Weight of feces

Statistical analysis

Data normality was determined before the analysis. Digestibility and survival data were analysed using one-way analysis of variance (ANOVA) to check the effectiveness of botanicals using different solvents. Means were separated by using the Tukey HSD all-pairwise comparison test. All the analyses were performed using Minitab 17.0 software.

Results

There was a significant effect of plant extracts using methanol ($F = 238.0, P < 0.001$), ethanol ($F = 24.9, P < 0.001$) and acetone ($F = 140.0, P < 0.001$) as extraction solvents on consumption rate of *S. litura* larvae. The larvae's consumption rate was lower (0.19-0.21 mg/mg/day) when treated with *P. hysterothorus* at 50 mg concentration compared to other treatments. Among the extraction solvents, methanolic extracts considerably affected the consumption rate in comparison to ethanol and acetone (Figure 1). Larval growth rate was significantly reduced by consumption of plant extracts using methanol ($F = 177.0, P < 0.001$) and ethanol ($F = 125.0, P < 0.001$) as extraction solvents. However, acetone-based leaf extracts yielded insignificant ($F = 2.76, P > 0.05$) results for both plants. Among plants, *P. hysterothorus* significantly affected the growth rate at 50 mg concentration, wherein larval growth rate relative to control (+ve) treatment was reduced by 80.4%, 73.8%, and 65.4% using methanol, ethanol, and acetone as solvents, respectively (Figure 1).

Plant extracts using methanol ($F = 66.1, P < 0.001$) and ethanol ($F = 57.6, P < 0.001$) had a significant effect on the efficiency of conversion of ingested food of *S. litura* larvae. However, using acetone ($F = 0.76, P > 0.05$) as extraction solvent, no significant difference was found among treatments. The efficiency of conversion of ingested food was reduced by 63.9% and 40.2%, respectively, using methanol and ethanol extracts of *P. hysterothorus* at 50 mg concentration (Figure 2). Similarly, a significant effect of methanolic ($F = 81.9, P < 0.001$) and ethanolic ($F = 84.0, P < 0.001$) plant extracts was found on the efficiency of conversion of digested food of *S. litura* larvae. An application of *P. hysterothorus* at 50 mg concentration reduced the efficiency of conversion of digested food by about 68.4% with methanolic and by 47.6% with ethanol extract (Figure 2).

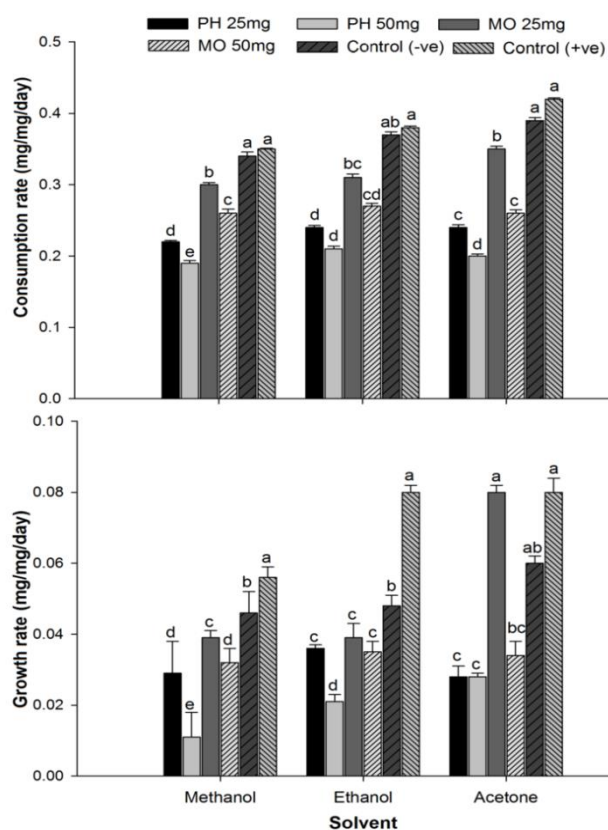


Figure-1: Effect (Mean ± SE) of plant extracts with different extraction solvents on the consumption and growth rate of *Spodoptera litura* larvae, PH = *Parthenium hysterothorus*, MO = *Moringa oleifera*, Control (-ve) is respective solvent only, Control (+ve) is untreated, means sharing similar letters are not significantly different at P = 0.05



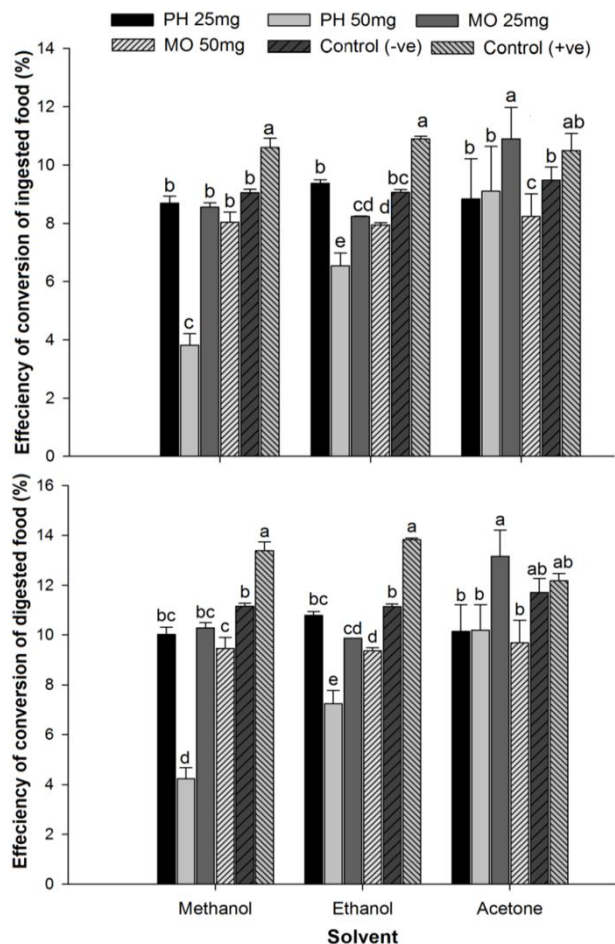


Figure-2: Effect (Mean ± SE) of plant extracts with different extraction solvents on the efficiency of conversion of ingested and digested food of *Spodoptera litura* larvae, PH = *Parthenium hysterophorus*, MO = *Moringa oleifera*, Control (-ve) is respective solvent only, Control (+ve) is untreated, means sharing similar letters are not significantly different at P = 0.05

Plant extracts had significant (methanol, $F = 118.0, P < 0.001$; ethanol, $F = 36.7, P < 0.00$ and acetone, $F = 78.2, P < 0.001$) effect on approximate digestibility of *S. litura* larvae. *P. hysterophorus* at 50 mg concentration reduced digestibility by 9.0-13.0% approximately, relative to control (+ve) treatment (Figure 3). Furthermore, assimilation rate was also significantly affected after application of plant extracts with different solvents (methanol, $F = 134.0, P < 0.001$; ethanol, $F = 21.8, P < 0.001$ and acetone, $F = 141.0, P < 0.001$). Assimilation rate was higher when treated with *P. hysterophorus* at 50 mg

concentration compared with other plant extracts (Figure 3).

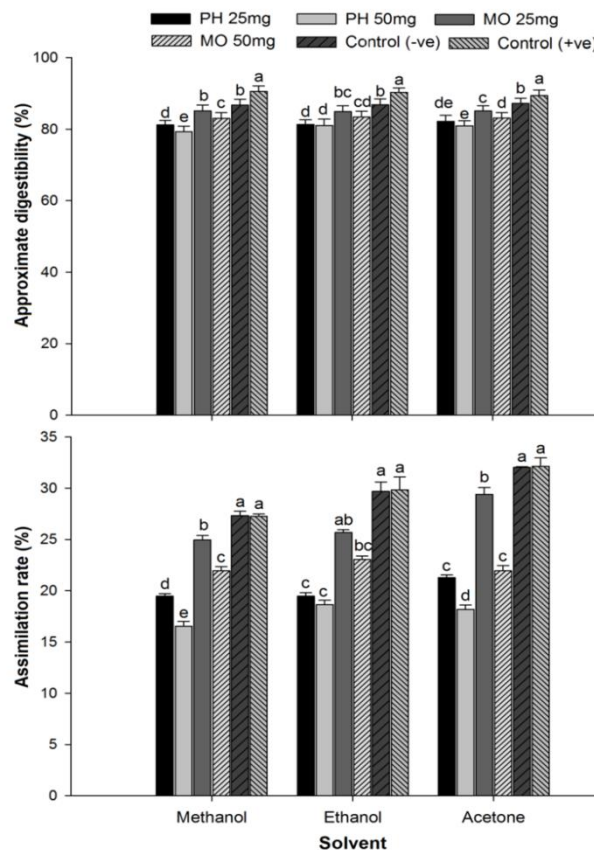


Figure-3: Effect (Mean ± SE) of plant extracts with different extraction solvents on the approximate digestibility and assimilation rate of *Spodoptera litura* larvae, PH = *Parthenium hysterophorus*, MO = *Moringa oleifera*, Control (-ve) is respective solvent only, Control (+ve) is untreated, means sharing similar letters are not significantly different at P = 0.05

The survival rate of larvae was higher in control treatments compared to those treated with botanicals. With time, the survival rate decreased to 6.0 – 14.0% in *P. hysterophorus* using three different solvents after 4th day of exposure. In *M. oleifera*, the larval survival rate was 20.0-26.0% at 50 mg concentration and 26.0-46.0% at 25 mg concentration after the 4th day of exposure (Figure 4).

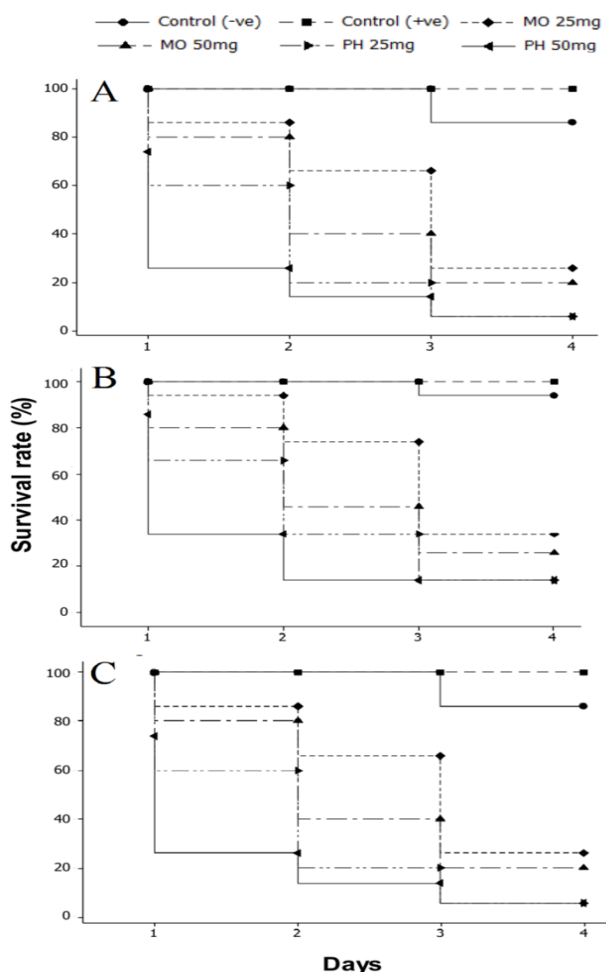


Figure-4: Effect (Mean \pm SE) of plant extracts with different extraction solvent; methanol (A), ethanol (B) and acetone (C) on the survival rate of *Spodoptera litura* larvae, PH = *Parthenium hysterophorus*, MO = *Moringa oleifera*, Control (-ve) is respective solvent only, Control (+ve) is untreated.

Discussion

Botanical insecticides are considered a potential method to manage diverse insect pests (Pavela, 2014). Insects feeding upon plants' toxic materials suffer a reduction in their growth and development, resulting in insect mortality (Shaheen and Khaliq, 2005). In this study, *P. hysterophorus* showed adverse effects on larval development and the growth rate of *S. litura*. This was most significant at 50 mg concentration, wherein larval growth rate relative to control (+ve) treatment was reduced by 65-85% using different extraction solvents. Different leaf extracts of

M. oleifera were also tested for their insecticidal properties, but their effect on *S. litura* larvae was much less than *P. hysterophorus*. Previously, *M. oleifera* has been reported as an effective substance in controlling the field and stored grain insect pests (Prabhu et al., 2011). However, the current study yielded moderate results on its efficacy in comparison to *P. hysterophorus*. *P. hysterophorus* is reported to be a very effective substance to control the mosquito population on account of its biological activities (Kumar et al., 2012). All parts of this weed, including trichomes and pollen, contain toxins known as sesquiterpene lactones that show promising insecticidal activity (Datta and Saxena, 2001). Sesquiterpene lactones consist of cytotoxic, antifeedant, phytotoxic, antimicrobial, and insecticidal properties (Rodriguez et al., 1976), but the major compound of these toxins is parthenin and other phenolics (Oudhia, 2001).

In the present case, the methanol extract of *P. hysterophorus* prepared from the aerial parts of the plant proved to be most effective against the growth rate of *S. litura*. The consumption rate of larvae was reduced when they fed on *P. hysterophorus*-treated leaves. The larvae's food uptake capacity was affected more while feeding on *P. hysterophorus*-treated leaves, which led to the growth retardation/suspension of *S. litura* larvae. Similarly, the reduced efficiency of converting ingested food indicated the disruption of digestibility, showing long-term effects on larval growth and development. We believe that there was a disturbance in the physiology of *S. litura*, which rendered them unable to digest the available food. The adverse effects of *P. hysterophorus* extracts on the conversion efficiency of ingested food of *S. litura* larvae could be attributed to either having the ability to inhibit the conversion of nutrients for growth or reduction of larval ability to absorb and digest the nutrients from the food treated with pesticidal plant extracts. Reduction in the ability of insects to convert food into biomass or reduction in food intake ultimately extends the larval developmental period, which occurs most probably due to the effect of botanicals on the hormones like ecdysteroids and juvenile (Morgan, 2009). Previously, the insecticidal and acaricidal activities of *P. hysterophorus* extracts have been shown to affect survival rate, inhibit growth, and act as antifeedant against *S. littoralis* larvae (Pavela et al., 2010). Furthermore, the *P. hysterophorus* extracts have also shown an acaricidal effect on *Tetranychus urticae*

(Trombidiformes; Tetranychidae) by causing greater mortality mites (Erdogan et al., 2012). In the current study, plant extracts showed considerable effects on the digestibility parameters of *S. litura* that caused a significant reduction of food intake (CR) and growth rate (GR) of larvae compared to control treatment. Many studies have reported the antifeedant effect of different plant extracts (Mogg et al., 2008; Pavela, 2009, 2010) and different secondary metabolites, such as alkaloids (Pavela, 2010), tannins (Lingathurai et al., 2011), terpenes (Kumar et al., 2013) and phenolics (Pavela, 2009).

Approximate digestibility of *S. litura* was indicated by the reduced rate of ingested food, which was affected more when larvae fed on *P. hysterophorus*-treated leaves. Because of the detrimental effects of *P. hysterophorus* extracts with methanol as a solvent on the feeding behavior of *S. litura* larvae, the survival rate was markedly reduced in comparison to other treatments. Overall, the growth rate of larvae, calculated as growth rate and total weight gained, was inhibited by *P. hysterophorus*, but the effect depended on the concentration and the type of extraction solvent. The higher concentration (50mg) of *P. hysterophorus* had a greater effect on the digestibility indices of *S. litura* compared to other treatments. The diluted extracts might have enhanced the metabolism, whereas the more concentrated extract might have slowed down the metabolic rate (Bashir et al., 2013).

The *P. hysterophorus* extract using methanol as an extraction solvent was more toxic to *S. litura* larvae than another extraction solvent (ethanol and acetone). The solvent's polarity is also an essential factor in toxicology studies (Khan et al., 2015). However, variations in the larval growth and development, and survival could be attributable to *P. hysterophorus* extract's dissolving nature in different solvents (Rizvi et al., 2012; Koubala et al., 2013). The active ingredients in plant extracts in different extraction solvents may have different dissolving nature and may show the synergistic effect with a respective solvent against a particular pest (Oyedokun et al., 2011). These explanations can be efficient in terms of the compound's polarity being extracted in each solvent. However, methanol is better in dissolving organic compounds than other solvents and shows higher bioactivity against pests as it has more toxic compounds (Abdulhay et al., 2012). In fact, as observed in the current study, ethanol and acetone extracts showed lower toxicity towards different

indices of *S. litura*. Variation in the toxicity of plant extracts obtained using different polarity solvents was also observed in many previous studies (Boussaada et al., 2008; Germinara et al., 2011). However, chemical analysis of methanol extract is needed to support this hypothesis. Furthermore, the knowledge of the composition of extraction solvent and its dissolution abilities and binding with phytochemicals should be focused on closer insight into plant-based insecticides' development.

Conclusion

Botanicals had wide application in the past, but synthetic pesticides gradually replaced these. However, synthetic pesticides' detrimental effects on the environment and human health have called for reemployment of plant-derived products to control pest infestation of important food commodities. The effectiveness of plant-based toxins becomes more prominent when insects develop resistance against synthetic pesticides. The current study reported the efficacy of 50mg methanolic extract of *P. hysterophorus* as an insect growth regulator and antifeedant against the common pest, *S. litura*. Eco-friendly botanical preparations like those used in this study, prepared from local flora, can be easily available to the farmers advocating use as eco-friendly alternatives to synthetic pesticides.

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Ullah MI, Majeed S & Arshad M: Conceived idea, designed research methodology, data analysis, literature review and manuscript write up
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