Research Article



Effect of Emamectin Benzoate with Leaf Extracts of *Parthenium hysterophorus* and *Moringa oleifera* on the Digestibility and Survival Indices of *Spodoptera litura* (Lepidoptera: Noctuidae)

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Abstract | Botanical insecticides are an environment-friendly approach and have been used in integrated pest management for various insect pests. Here we tested the effect of emamectin benzoate 1.9% EC alone and with botanicals; *Parthenium hysterophorus* L. and *Moringa oleifera* L. on the digestibility indices and survival of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae). The *P. hysterophorus* extract at 50 mg concentration was comparable to the alone application of emamectin benzoate in terms of reduced digestibility and survival of *S. litura*. Overall, 42.7% relative consumption rate (RCR), 74.3% efficiency of conversion of ingested food (ECI), 72.3% efficiency of conversion of digested food (ECD), 87.5% relative growth rate (RGR), 11.04% approximate digestibility (AD), and 47.6% assimilation rate (AR) were reduced after application of a mixture of emamectin benzoate and *P. hysterophorus* in comparison to control +ve treatment (untreated). The survival rate was also decreased by using this combination compared to the alone application of insecticide. These findings reflect *P. hysterophorus* extract's interference with the regulation of feeding and metabolism of *S. litura*. Further, the results promote *P. hysterophorus* extract in combination with emamectin benzoate for integrated management of *S. litura*.

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Introduction

Spodoptera litura Fabricius (Lepidoptera: Noctuidae) is a serious polyphagous insect pest (Ahmad *et al.*, 2007; Liu *et al.*, 2018). It is commonly known as tobacco caterpillar, armyworm and cutworm (Seth and Sharma, 2002). It is distributed worldwide, especially in tropical and temperate regions (Lin *et al.*, 2019). It feeds on more than 120 host plants, including 112 cultivated food plants from 44 families (Ahmad *et* *al.*, 2013; Abdullah *et al.*, 2019). Major host plants of this pest are chilies, tobacco, cotton, okra, sunflower, soybeans, potato, lucerne, and castor (Sharma and Bisht, 2008). It may cause 26 to 100% economic losses in Pakistan's agro-ecological condition relying upon the specific to stage of crop and level of infestation (Khan *et al.*, 2018). During 2003, the outbreak of *S. litura* occurred in Pakistan throughout the cotton belt and has resulted in heavy damage to crops (Ahmad *et al.*, 2007).



To manage the *S. litura* population in field use of synthetic insecticides is still being consider a good option (Ismail *et al.*, 2017). Emamectin benzoate is a novel semi-synthetic bioinsecticide derived from the naturally occurring compound avermectin (Zaka *et al.*, 2014). It is highly toxic against different insect pests, including; lepidopterans, thrips, fruit borer, cockroaches, and leafminer with both contact and stomach action (Muthukrishnan *et al.*, 2012). It acts as a chloride channel activator that affects the nervous system of insects.

The use of these chemicals is wide-ranging that has provided an ideal environment of resistance development for the insects (Ahmad et al., 2008). Resistance development in S. litura against newer insecticides has been reported previously from different countries including Pakistan (Shad et al., 2012). Further, the harmful effect of chemicals on our environment and human is another threat (Kumar et al., 2013). Increasing risks with the insecticidal application, there is a need to use some other alternate and effective methods for the management of insect pests that should be safer for our environment and human health. Plants are the rich source of bioactive chemicals that can provide an alternative to synthetic chemicals (Qin et al., 2010). Many plants have insecticidal properties and are being used in integrated pest management programs (Mathesius, 2018; Trivedi et al., 2018). Moringa oleifera (Lam.), or drumstick tree' (Anwar and Bhanger, 2003), is a perennial tree and belongs to the family Moringaceae (Anwar et al., 2007; Ramachandran et al., 1980). Many bioactive proteins are present in this plant that have different biological activities such as lectins (de Lima Santos et al., 2009, 2014) named cMoL, which showed insecticidal activity against Anagasta kuehniella Zeller (Lepidoptera; Pyralidae). Parthenium hysterophorus L. (Family: Asteraceae), commonly known as chatak chandani, gajar ghas (Kumar et al., 2012), is a widely spread weed and has been used as an insect repellent (Wiesner et al., 2007). All plant's parts contain toxins known as sesquiterpene lactones (Datta and Saxena, 2001), parthenin and other phenolics (Oudhia, 2001) have insecticidal properties (Wu et al., 2016).

However, the increasing trend of plants use for high volumes of extract stock solution, inconsistent efficacy, and lower effectiveness against some target pests are challenging compared to synthetic insecticides (Isman, 2006; Pavela, 2015). About deficiencies in

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pesticides and plant extracts, new strategies for both methods are constantly sought. The joint effect of two substances showing synergistic effect has already been documented (Pavela, 2015). Previously plant extracts have been used in combination with synthetic insecticides (Harve and Kamath, 2014; Shaalan *et al.*, 2005; Mohan *et al.*, 2010). Silva *et al.* (2017) reported the synergistic and combined effect of deltamethrin-*Ocimum basilicum* against the *S. frugiperda* by lowering 80% LD₅₀ of the deltamethrin.

So far, no report is available on the combinatorial effect of insecticide with *P. hysterophorus* and *M. oleifera* against *S. litura*. Here, we determined the impact of emamectin benzoate alone and incombination with *M. oleifera* and *P. hysterophorus*, leaf extracts on digestibility indices of *S. litura*. We are expecting that the combinations of botanicals with insecticides will provide an alternative strategy in minimizing the insecticidal application, than alone application of insecticides and plant extracts.

Materials and Methods

Insect culture

The S. litura eggs were obtained from a field of soybean Glycine max (L.) in September, 2018 nearby University. The collected eggs were kept in glass Petri plates at 26 ± 2 °C temperature and $64\pm5\%$ RH. The neonate larvae were provided with artificial diet prepared by following the method of Sorour (Sorour *et al.*, 2011). Pupal culture was shifted to plastic jars (120 mm × 116 mm × 95 mm) from Petri plates. On emergence, adult's culture was provided with a sugar solution (10%). For oviposition, Muslin cloth strips (1 cm wide, 5-10 cm long) were hanged in oviposition plastic jars. The F₃ generation was used for further experiments.

Plant extracts preparation

Fresh and healthy leaves of *P. hysterophorus* and *M. oleifera* were obtained from a nearby field. Leaves were washed with distilled water and air-dried for 48 hours and then kept in oven at 50°C for 24 hours. The dried leaves were crushed in the electrical grinder (Moulinex, France) to make a fine powder. Ten-gram powder was added in 100 mL of methanol for making solution for 12 hours this solution was kept on an orbital shaker (OS-752 Pallscientific, Indonesia) to mix thoroughly. Then muslin cloth was used for filtering the solution and using Whatman No. 1 filter

paper. After filtration, the remaining material was mixed with 100 mL methanol solvent, and again kept on electrical shaker and repeated the shaking procedure. For solvent evaporation, the solution was kept in a rotary vacuum evaporator (BEV-1001V, Henan, China). After drying, the prepared extracts were stored in refrigerator at 4°C temperature.

Leaf dip bioassay

The recommended dose (0.5ml/liter of water) of emamectin benzoate 1.9% EC (GREEN ZONE), and 50mg of each plant extracts were used in this study. A preliminary experiment was performed to check the mortality of S. litura using two different concentrations (25mg, 50mg) of each plant extract. The higher concentration of each extract showed more significant toxicity against S. litura. So, we useda higher concentration of plant extracts in this study. An almost similar size of 3rd and 4th larval instars was tested and before the experiment, samesized and healthy larvae were selected and kept at starvation for 24 hours. The leaves (approximately 400mg) of soybean G. max were obtained from the field. To remove the contaminants and dust particles from the collected leaves were washed with distilled water. The washed leaves were dipped in each solution for 10 seconds and dried at room temperature for 10 minutes. In each Petri plate placed the treated leaves and then single larvae was kept in each plate. The completely randomized design (CRD) was used to arrange the treatments. In control (+ve) treatment the untreated larvae were used, and in control (-ve) treatment only methanol was applied. Each treatment was replicated five times having five larvae in each replication.

Data recording

Data for larval weight and length, faeces weight and leaves' weight before and after 24 hours of feeding were recorded. Data for the survival rate of larvae were also recorded daily.

The digestibility indices were calculated by following formulas (Waldbauer, 1969);

Relative growth rate (RGR) = $\frac{B-A}{B} \times days$ Relative consumption rate (RCR) = $\frac{D}{B} \times days$ Efficiency of conversion of ingested food (ECI) = $\frac{B}{D} \times 100$ Effeciency of conersion of digested food (ECD) = $\frac{A}{E} \times 100$ Approximate digestibility (AD) = $\frac{D - F}{D} \times 100$ Assimilation rate (AR) = $RCR \times AD$

Where: A= mean weight (g) of the insects on last day; B= original mean weight of insects (g); D= food biomass ingested (g) per insect); E = weight of food digested (g) calculated by subtracting the weight of feces from the weight of food ingested, F= feces biomass produced (g) per insect.

Statistical analysis

Data normality was assessed before the analysis. Digestibility and survival data were analyzed using one-way analysis of variance (ANOVA) by keeping the treatment/chemicals as the main factors. Means were separated by using Fisher's least significant difference (LSD) all-pairwise comparison test. The data was analysedby using Minitab 17.0 software.

Results and Discussion

Spodoptera litura larvae gained maximum weight in control +ve (344.9mg) and controlled-ve (330.1mg) treatment over time. Emamectin benzoate plus *P. hysterophorus* 50mg reduced the larval weight more than control treatments, and the larval weight was found 266.8mg on the last day of feeding on treated leaves. However, larvae gained 277.7mg weight feeding on treated leaves with emamectin benzoate alone (Figure 1).

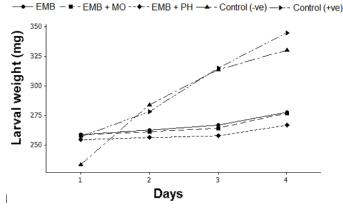


Figure 1: Effect of emamectin benzoate (EMB) insecticide alone and in combination with Parthenium hysterophorus (PH), and Moringa oleifera (MO), on larval weight of Spodoptera litura, control (+ve) is untreated larvae, control (-ve) is methanol application.



The mixture of emamectin benzoate and P. hysterophorus reduced the maximum larval growth rate to 87.5% compared to the control +ve treatment. Similarly, the combined application of insecticide with M. oliefera reduced the larval growth rate to 82.5%. While about 67.5% reduction in larval growth rate was occurwiththe application of insecticide alone. The consumption rate of S. litura larvae was reduced maximum up to 42.7% after applying a mixture of emamectin benzoate and *P. hysterophorus* in comparison to control +ve. However, larvae consumed 33.9% less when they fed on leaves treated insecticide alone. A similar trend in reducing ECI and ECD was found when larvae fed on treated leaves with a mixture of emamectin benzoate and P. hysterophorus. About 74.3% ECI and 72.3% ECD were reduced by applying this mixture application in comparison to control +ve. Combined application of emamectin benzoate and P. hysterophorus reduced maximum assimilation rate up to 47.6% compared to control +ve. Maximum reduction (14.9%) of AD rate was found by applying emamectin benzoate in comparison to control +ve. About 11.04% AD rate was reduced using the mixture of emamectin benzoate with M. oliefera and 8.61% with P. hysterophorus (Table 1).

S. litura larvae's survival rate was higher (86.0-94.0%) in both control treatments. However, with time, larvae's survival rate was decreased maximum in the treatment where emamectin insecticide plus *P. hysterophorus* extract was applied. Using emamectin alone and mixture of emamectin with *M. oliefera* extract, 6.7% larvae were survived on the last day of exposure (Figure 2).

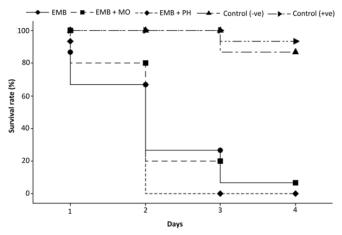


Figure 2: Survival rate of Spodoptera litura larvae after application of emamectin benzoate (EMB) alone and in combination with plant extracts, PH = Parthenium hysterophorus, MO = Moringa oleifera, untreated larvae were considered control (+ve) and methanol solvent was applied in control (-ve) treatment.

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The comparison of plant extracts with insecticides has been immensely studied to minimize the use of synthetic insecticides, better toxicity, overcome insecticide resistance, and safer for the environment (López et al., 2005; Khater, 2012). We studied the toxicity of emamectin benzoate insecticides combined with P. hysterophorus and M. oleifera plant extracts against S. litura. Our findings showed that the combined effect of insecticide with P. hysterophorus negatively affected the S. litura in terms of growth disruption and survival rate. The S. litura larvae's growth is disrupted not only because of feeding inhibition (by the reduction in uptake of food and relative consumption rate) but also by digestibility (by reduction of S. litura efficiency in converting ingested food to growth) (War et al., 2011; Mahmoodi et al., 2020). Larval feeding inhibits and couldn't indigest food due to the presence of active principles in the plants (Jeyasankar and Premalatha, 2012). There is a disturbance in insect's physiology and its capability to digest food. However, detailed nutritional analyses are needed to understand the antifeedant effect of P. hysterophorus better. Our findings are similar to previous studies that reported feeding reduction of different insect pests using various plant extracts alone and in combination with insecticides. However, the reduction level was dependent upon species and dose (Silva et al., 2013; Olaitan and Abiodun, 2011; Nathan et al., 2005). After feeding on treated leaves, larvae showed variation in the conversion of ingested food into biomass. Results showed that the larvae fed on untreated leaves highly utilized the food compared to treated with insecticide plus plant extracts. Reduction in larvae's consumption rate due to application of botanicals was also reported by previous studies (Adel and Zaki, 2010; Ahmed et al., 2013).

Botanical insecticides have adverse effects on the insects' metabolic process; however, detailed study on these precise effect mechanisms is still needed. It could be either due to insects' inability to digest the food treated with botanical insecticides or decreased the conversion ability of ingested food into nutrients (Martinez and van Emden, 2001). In this study, the digestion (food nutrients absorption), rate of ingested food, assimilation rate was decreased due to feeding of larvae on leaves treated with emamectin plus *P*. *hysterophorus*. The combination of *P. hysterophorus* and synthetic insecticide suppressed the larvae growth and development of *S. litura* and reduced the body weight in terms of RGR. The plant extracts application proved

Table 1: Effect (means±SE) of emamectin benzoate insecticide alone and in combination with plant extracts on relative consumption rate (RCR), relative growth rate (RGR), efficiency of conversion of ingested food (ECI), efficiency of conversion of digested food (ECD), approximate digestibility (AD) and assimilation rate (AR) of Spodoptera litura, untreated larvae were considered control (+ve) and methanol solvent was applied in control (-ve) treatment.

Treatment	RGR	RCR	ECD (%)	ECI (%)	AR	AD (%)
EMB	0.013±0.009b	0.413±0.012b	5.29±0.442b	3.17±0.274b	24.88±0.651b	60.05±0.496c
EMB+MO	0.007±0.003bc	0.377±0.007bc	4.21±0.302bc	2.66±0.175b	23.49±0.450b	62.76±0.340b
EMB+PH	0.005±0.007c	0.358±0.008c	3.17±0.552 c	2.04±0.357b	23.17±0.490b	64.47±0.055b
Control (-ve)	0.037±0.018a	0.602±0.004a	11.02±0.545 a	7.72±0.314a	42.02±0.536a	69.45±0.578a
Control (+ve)	0.040±0.019a	0.625±0.011a	11.46±0.143a	7.95±0.149a	44.20±0.713a	70.55±0.392a
Significance	168.0***	211.0***	84.5***	117.0***	338.0***	116***

*** shows the significance at P = 0.05, means sharing similar letters are not significantly different at P > 0.05; EMB: emamectin benzoate, PH: Parthenium hysterophorus, MO: Moringa oleifera.

poisonous for the growth of insects having insect growth regulator hormones disruption ability and insect stop feeding (Nasr *et al.*, 2010), due to which insect ingestion becomes minimum and reduction in digestion occur (Shannag *et al.*, 2015). Plant extracts application combined with other control methods has been considered a useful technique for the management of lepidopterous insect pests (Adel and Zaki, 2010; Arivoli and Tennyson, 2013; Ahmed *et al.*, 2013).

Plant extracts act as a synergism when combined with insecticides (Mansour *et al.*, 2012). The synergistic action could be due to the organism's least resistance ability and the toxic nature of the chemical composition (Chansang *et al.*, 2018). Combining synthetic insecticides with plant material interrupts the different target sites (Madhu and Vijayan, 2010). Synthetic insecticides like emamectin benzoate cause the hormonal regulation of molting and developmental processes (Ishaaya *et al.*, 2007), whereas plant extracts causeprevention or inhibition oviposition, growth hindering, repelling, and mimic juvenile hormones (Suwannayod *et al.*, 2019).

According to Ali *et al.* (2017), botanicals, as alone or in combination with synthetic insecticides cause negative effects on the food consumption, growth, and development of the cabbage white butterfly, *Pieris brassicae* (L., 1758) (Lepidoptera: Pieridae). Combiningdifferent plant extracts with some selected synthetic chemicals showed highly toxic results against both larvae and adult *Anopheles pharoensis* (Mansour *et al.*, 2010). Synergism is the process that helps in disrupting the integrity of an insect's midgut epithelial cells with specific enzymes deltaendotoxins fixation, due to which insects stop feeding and dead (Mhalla *et al.*, 2018). The synergism effect of botanicals with insecticides could be cost-effective and long-lasting bio-pesticides (Shaalan *et al.*, 2005). The combined application of synthetic and botanicals pesticides could be proved an ideal tool for managing resistance due to the repetition of conventional pesticides (Chandler *et al.*, 2011).

Conclusions and Recommendations

In summary, it can be observed that *P. hysterophorus* extract can be used with emamectin benzoate insecticide in the integrated management of *S. litura* due to their negative effect on the digestibility and survival of the larvae. Mixing *P. hysterophorus* extract with insecticide may be a perfect solution to delay or reduce the insect resistance to synthetic insecticides. For the control of bollworms, the combination of these chemicals may be used as insecticidal formulation.

However, further study is needed to understand better action and cost-effectiveness of these combinations in the fields.

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Novelty Statement

The findings showed that a mixture of emamectin benzoate and plant extracts, especially *Parthenium hysterophorus*at 50mg concentration, significantly reduce the digestibility and survival of *Spodoptera*

litura. Thus, this combination of insecticides can be used in controlling this insect pest, which will be cost-effective.

Author's Contribution

Muhammad Irfan Ullah and Sana Majeed: experimented.

Muhammad Arshad, Nimra Altaf and Muhammad Luqman: Wrote the first draft of the manuscripts.

Asad Abdullah: Designed the experiments. Muhammad Afzal and Sana Majeed: Analyzed the data.

All authors read and approved the final manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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