

## Insecticidal activity of *Peganum harmala* seed extract against tomato fruit borer [*Helicoverpa armigera* (Hubner)]

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### Abstract:

*The present work describes the insecticidal activity of aqueous, ethanol and benzene extract of Peganum harmala L. seeds along with one synthetic chemical, cypermethrin against tomato fruit borer [Helicoverpa armigera (Hubner)] (Lepidoptera: Noctuidae). Seeds extract have shown pronounced effect on larval mortality, the data collected after 24 hours. High rate mortality percentage of Helicoverpa armigera larva caused by cypermethrin 25% EC (100%), followed by Harmal seed benzene extract 30, 20% (80.00%), (73.33%), Harmal seed ethanol extract 30, 20% (66.67%), (60.00%), Harmal seed aqueous extract 30% (46.67) and Harmal seed aqueous extract 20% (40.00%), which was least effective among all the treatments.*

**Key words:** *Helicoverpa armigera*, tomato fruit borer, seed extract, *Peganum harmala*, Harmal, insecticidal effects.

### Introduction

*Helicoverpa armigera*, which is commonly known as gram pod borer, American bollworm and tomato fruit borer, is the most

destructive pest of tomato in India. African bollworm is a polyphagous insect attacking a wide range of crop including legumes, sorghum, cotton, tomato, pepper, sunflower, safflower, flax and niger seed. *Hibiscus spp*, *Baleria spp*, *Guziotia scabra*, *Amarantus spp* and *Gynandropsis gynandra* were recorded as alternative hosts (**Tebkew et al., 2002**). *H. armigera* has developed resistance against insecticides, and field failures resulting from pyrethroid resistance have been reported from Australia, Thailand, Turkey, India, Indonesia and Pakistan (**CAB 2003**). The development of resistance to insecticides has been documented most extensively for the synthetic pyrethroids, but (in some areas) *H. armigera* has also developed resistance to other insecticides i.e. endosulfan, the carbamates and organophosphates (**Martin et al., 2000, 2003 and Torres et al., 2002**). The practice of using plant derivatives, or botanical insecticides as we now know them, in agriculture dates back at least two millennia in ancient China, Egypt, Greece, and India (**Ware 1883 and Thacker 2002**).

Many naturally occurring plant extracts can be used for insect control as adult or larval repellents, oviposition deterrents, anti-feeding additives, growth regulators, and chemosterilants (**Isman 1993**). botanicals have been suggested as alternative sources for insect control because many products are selective to insect pests and have no or little harmful effects on non-target organisms and the environment. Furthermore, they are easily available and less expensive than synthetic insecticides (**Liang et al., 2003 , Charleston et al., 2005**).

The *peganum harmala* L. (Syrian rue) is a wild-growing flowering plant that belongs to the zygotryphaceae family commonly known as “Harmal” grows spontaneously in semi-arid rangeland, steppe areas and sandy soils (**Soliman and Fahmy 2001, Mahmoudian et al., 2002**). *Peganum harmala* is a perennial herb growing in Africa, the Middle East, India, Pakistan, South America, Mexico (**Kartal et al., 2003**). In Iraq it is growing wild in the middle and northern parts (**Muhi-**

**eldeen et al., 2008**). In India it is usually found in drier parts of Jammu and Kashmir, Punjab, Haryana, Rajasthan, Uttar Pradesh and Delhi (**Hajra et al., 1997**). Since ancient times, it has been claimed that this plant has medical compounds harmalol and Harman (**Bellakhdar 1997**), its seed extracts also contain anthroquinones and a small quantity of flavonoid glycosides (**Prashanth and John 1999, Sharef et al., 1997**) which are found especially in the seeds and the roots (**Soliman and Fahmy 2001**). **Bellakhdar (1997)** had reported that *P. harmala* and *A.iva* are used for the treatment of diabetes. The biological activities of these alkaloids have been studied in phytopharmacology, phytotherapy, and toxicology for treating various human and animal diseases (**Fan et al., 1997, Shahverdi et al., 2005, Arshad et al., 2008**). Recently, *P. harmala* extracts have shown Pesticidal effects on plant fungal pathogens as well as various mite, nematode, and insect species. For example, *P.harmala* extracts were used to control citrus fungal diseases in Morocco (**Ameziane et al., 2007**), varroa mite in honey bee colonies (**Shaddel et al., 2008**), and root-knot nematodes, *Meloidogyne* spp. (**Allagui et al., 2007**). When adult desert locusts, *Schistocerca gregaria* Forskal, were fed the alkaloids extracted from *P.harmala* leaves, there was significant mortality, reduced food intake, weight loss, delayed sexual maturity, and reduced female fecundity, Moreover, *P. harmala* plants were avoided by the locusts under natural conditions in Morocco (**Abbassi et al., 2003**). **ABBASIPOUR et al. (2010)** reported that the larvicidal activity of different concentrations of seed extract of Harmal against the third instar larvae of *P. xylostella* differed significantly among the tested concentrations after 48 where a mortality of 66 and 100% was obtained from the ethanol extract of Harmal at concentrations of 30 and 40 mg/ml, respectively. Concentrations of 10 and 20 mg/ml showed little larvicidal activity that was not significantly different from the control.

However, there have been no studies on the effect of *Peganum harmala* against the tomato fruit borer.

## **Materials and Methods**

### **Experimental site:**

The present investigation was conducted at the Department of Entomology-Sam Higginbottom Institute of Agriculture, Technology and Sciences" Allahabad, Uttar Pradesh.

### **Insect rearing**

Large numbers of tomato fruit borer larvae were collected from unsprayed field of gram crop at Sam Higginbottom Institute of Agriculture, Technology and Sciences (SHIATS) - Allahabad. Each larva was reared separately in a plastic cups (10 cm X 7 cm) under laboratory conditions ( $26 \pm 1^\circ\text{C}$  temperature;  $65 \pm 5\%$  RH, and 14hours light and 10hours dark photoperiod) , placed in each cup 2 cm of light soil for pupation purpose. The colony was maintained on tomato fruit and leaves. Fresh diet was provided after a regular interval of 24 hours. Rearing of insect on natural diet is season bound labor intensive and limited by the development of microbial diseases which often eradicate the laboratory culture. (Gopali *et al.*, 2001).Larvae of F2 generation was used in laboratory experiment.

### **Plant material extraction.**

Harmal seeds were collected from local market (Chowk market) in Allahabad. The collected seeds were washed under running water in the laboratory and left to dry in the shade. Dried seeds were ground and powdered with an electric stainless steel blender for 5 min, then sieving by sieve measuring 60 to remove the hard parts that have been crushed again, and then used for preparation of aqueous extract, ethanol and benzene extracts of 20 and 30% concentrations were prepared. The experimental insect sets were treated with these extracts and mortality was

noted along with these sets normal and control sets were also maintained. The results were statistically analyzed using one-way ANOVA.

### **Bioassay**

A leaf dipping bioassay method described by Tabashnik and Cushing (1987) (27) was adapted to evaluate the insecticidal activity of Harmal seed extract against *Helicoverpa armigera* larvae. Tomato leaves were washed with distilled water and air dried. Two concentrations 20 and 30 g/lit. of the seed aqueous, ethanol and benzene extract were prepared with hot water, ethanol and benzene. Normal water was used for the control. Three replicate were done and in each replicate 40 third instar larvae were used that is number of total larvae were used 120 for each treatment. The leaves were dipped for 30 seconds in the test solutions and control. The treated leaves were placed into the petri dishes (8.5 cm diameter) on moistened filter paper (one leave per petri dish) with the adaxial surface upper most. *Helicoverpa* larvae were then placed onto the leaf disc and then a cover was put onto the dish. The dishes mention under laboratory conditions ( $26 \pm 1^{\circ}\text{C}$  temperature;  $65 \pm 5\%$  RH, and 14hours light and 10hours dark photoperiod).

### **Results**

#### **Larvicidal activity**

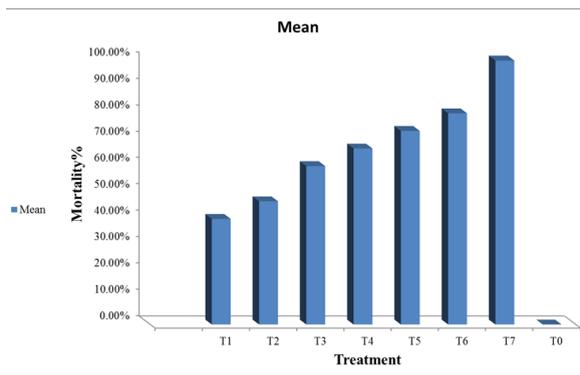
The larvicidal activity of different concentrations and extracts of Harmal seed against the third instar larvae of *Helicoverpa armigera* differed significantly. Among the tested concentrations after 24 hours (table 1) All the treatments were found significantly superior over control. Cypermethrin 25 EC (100.00%) was found the most effective and gave large mortality percentage of *Helicoverpa armigera* larvae followed by Harmal seed benzene extract 30% (80.00%), Harmal seed benzene extract 20% (73.33%), Harmal seed ethanol extract 30% (6.67%),

Harmal seed ethanol extract 20%(60.00%), Harmal seed aqueous extract 30%(46.67) and Harmal seed aqueous extract 20%(40.00%), was least effective among all the treatments. Treatments T<sub>1</sub>and T<sub>2</sub> are at par with each other and non-significant statistically. Treatments T<sub>3</sub> and T<sub>4</sub> are at par with each other and non-significant statistically.

**Table 1: Insecticidal activity of Peganum Harmal a seed extract against third instar of tomato fruit borer.**

Treatment			Corrected Mortality%			Mean
			R1	R2	R3	
T1	Harmal Aqueous extract 20%	40.00%	40.00%	40.00%	40.00%	
T2	Harmal Aqueous extract 30%	40.00%	60.00%	40.00%	46.67%	
T3	Harmal Ethanol extract20%	60.00%	60.00%	60.00%	60.00%	
T4	Harmal Ethanol extract30%	60.00%	60.00%	80.00%	66.67%	
T5	Harmal Benzene extract20%	80.00%	60.00%	80.00%	73.33%	
T6	Harmal Benzene extract30%	80.00%	80.00%	80.00%	80.00%	
T7	cypermethrin	100.00%	100.00%	100.00%	100.00%	
T0	Control	0.00%	0.00%	0.00%	0.00%	

**Fig 1: Insecticidal activity of *Peganum Harmal a* seed extract against tomato fruit borer, in laboratory condition.**



## Discussion and conclusion

All the treatments were found significantly superior over control. Cypermethrin 25 EC (100.00%) was found the most effective and gave large mortality percent of *Helicoverpa armigera* larvae followed by Harmal Benzene extract 30, 20% (80.00%), (73.33%); %, Harmal seed ethanol extract 30, 20% (66.67%), (60.00%); Harmal seed aqueous extract 30, 20 % (46.67%), (40.00%) respectively. Harmal seed aqueous extract was least effective among all the treatments during 24 hours.

Results of this study demonstrated that the Harmal seed extract was effective on *Helicoverpa armigera* larval mortality. Naturally, with increasing the concentrations, the extract effect was increased. In previous studies, larvicidal activity deterrence of many plant extracts on *Helicoverpa armigera* were investigated (Lulie *et al.*, 2012, Basavaraj *et al.*, 2014). In the other hand, in some studies effects of alkaloids of Harmal on lepidopteran insects were investigated. El-Gengaihi *et al.* (1997) studied effect of each alkaloid of Harmal extract separately on cotton leaf worm, *Spodoptera littoralis* Boisduval (Lepidoptera Noctuidae) and found that harmine alkaloid caused an increase in larval period and larval mortality. Larval mortality of their study accepted our results using different insect and indicated that this plant through ingestion can be effective on larvae of Lepidoptera. Also in other research, similar to our results, Abbassi *et al.* (2003) reported that the alkaloids extracted from Harmal with ethanol caused significant mortality, reduction in fecundity of female and egg hatching in desert locust, *S. gregaria*. In this study, the percentage of egg hatching significantly decreased after treating the leaves by harmal. The results reported on desert locust by Abbassi *et al.* (2003), similar to ours obtained on diamondback moth, demonstrated the harmal ovicidal effect on different insect species. Abbasipour *et al.* (2010) found a mortality of 66 and 100% in the third instar larvae that had fed

for two days on the cabbage leaves treated with the ethanol extract at concentrations of 30 and 40 mg/ml, respectively. Significant dose response was observed on larval and pupal weight; pupal and adult emergence rate.

In conclusion, seed extracts of Harmal have been demonstrated to have a strong insecticidal activity against *Helicoverpa armigera* larvae. Thus, this plant has excellent potential to be utilized as a naturally occurring agent for *Helicoverpa armigera* control.

## REFERENCES

1. Abbasipour, Habib; Mohammad Mahmoudvand; Fahimeh Rastegar and Moslem Basij (2010) Insecticidal activity of *Peganum harmala* seed extract against the diamondback moth, *Plutella xylostella*. *Bulletin of Insectology* 63 (2): 259-263, 2010-ISSN 1721-8861
2. Abbassi, K., Z. Atay-Kadiri, and S. Ghaout. 2003. Biological effects of alkaloids extracted from three plants of Moroccan arid areas on the desert locust. *Physiol. Entomol.* 28: 232-236.
3. Allagui, N., S. Tahrouch, M. Bourijate, and A. Hatimi. 2007. Action of plant extracts on root-knot nematode (*Meloidogyne* ssp.) mortality. *Acta Bot. Gallica* 154: 503-509.
4. Ameziane, N., H. Boubaker, H. Boudyach, F. Msanda, A. Jilal, and A. A. Benaoumar. 2007. Antifungal activity of Moroccan plants against citrus fruit pathogens. *Agron. Sustain.Dev.* 27: 273-277.
5. Arshad, N., C. Neubauer, S. Hasnain, and M. Hess. 2008. *Peganum harmala* can minimize *Escherichia coli* infection in poultry, but long-term feeding may induce side effects. *Poult. Sci.* 87: 240-249.

6. Basavaraj K.; Naik, Mohan I.; Jagadish K.S.; Geetha S. and Shadakshari Y.G.(2014). Efficacy of biorationals and botanical formulations against *Helicoverpa armigera* Hub. in sunflower. *JBiopest* 7 (Supp.):94-98(2014).
7. Bellakhdar J (1997). La pharmacopée traditionnelle. Médecine arabe ancienne et savoirs populaires. Paris, (eds) Ibis presse.
8. CAB (2003). *Crop protection compendium: global module*. Commonwealth Agricultural.
9. Charleston D. S., Kfir R., Dicke M., Vet L. E. M., 2005.- Impact of botanical pesticide derived from *Melia azedarach* and *Azadirachta indica* on the biology of two parasitoid species of the diamondback moth.- *Biological Control*, 33: 131-142.
10. El-gengaihi S. E., D# N. Z., Mohamed S. M., 1997-. Chemical and biological investigation of harmal plant. 2. Alkaloidal investigation.- *Journal of Applied Entomology*,121: 165-167.
11. Fan, B. T., J. L. Liang, J. C. Men, F. Gao, G. L. Li, S. X. Zhao, T. J. Hu, P. Dang, and L. Zhang. 1997. Effect of total alkaloid of *Peganum harmala* L. in the treatment of experimental haemosporidian infections in cattle. *Trop. Anim. Health Prod.* 27: 77-83.
12. Gopali J.B. and Lingappa S.(2001) Refinement of Mass Rearing Techniques for *Helicoverpa armigera* (Hubner). *Karnataka J. Agri/ Sci*, 14(2):(336-341).
13. Hajra, P. K., Nair, V. J. and Daniel, P. (1997). *Flora of India*. Vol. 4, Botanical Survey of India, Calcutta.
14. Isman, M. B. (1993). Growth inhibitory and antifeedant effects of azadirachtin on six noctuids of regional economic importance. *Pesticide Sci.*, 38: 57-63.
15. Kartal, M., M. L. Altun, and S. Kurucu. (2003). HPLC methods for the analysis of harmol, harmalol, harmine

- and harmaline in the seeds of *Peganum harmala* L. J. Pharm. Biomed. Anal. 31: 263-269.
16. Liang G. M., Chen W., Liu T. X., (2003). Effect of three neem-based insecticides on diamondback moth (Lepidoptera: Plutellidae).- *Crop Protection*, 22: 333-340.
  17. Lulie, Nigussie and Raja, Nagappan(2012) Evaluation of Certain Botanical Preparations against African Bollworm, *Helicoverpa armigera* Hubner (Lepidoptera: noctuidae) and Non Target Organisms in Chickpea, *Cicer arietinum* L. *J Biofertil Biopestici* 2012 Volume 3 • Issue 5, 3:5.
  18. Mahmoudian, M.; Jalilpour, H. and Salehian, P. Toxicity of peganum harmala: review and a case report. *Iran. J. Pharm. Ther.*, 2002; 1: 1- 4.
  19. Martin T Ochou O G Hala N'klo F Vassal J M & Vaissayre M (2000). Pyrethroid resistance in the cotton bollworm, *Helicoverpa armigera* (Hubner), in West Africa. *Pest Management Science* 56, 549-554.
  20. Martin, T., Ochou, O. G., Vaissayre, M. and Fournier, D., (2003). Organophosphorus insecticides synergize pyrethroids in the resistant strain of cotton bollworm, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) from West Africa. *Journal of Economic Entomology* 96 (2): 468-474.
  21. Muhi- eldeen, Z.; Al- Shamma, K. J.; Al- Hussainy, T. M.; Al- Kaissi, E. N.; Al- Daraji, A. M. and Ibrahim, H. Acute toxicological studies on the extract of Iraqi *peganum harmala* in rats. *Eur. J. Sci. Res.*, 2008; 4: 494-500.
  22. Prashanth, D. and john, S. Antibacterial activity of peganum harmala. *Fitoter.* 1999; 70: 438- 439.
  23. Shaddel-Telli, A. A., N. Maheri-Sis, A. Aghajanzadeh-Golshani, A. Asadi-Dizaji, H. Cheragi, and M. Mousavi. 2008. Using medical plants for controlling varroa mite in honey bee colonies. *J. Anim. Vet. Adv.* 7: 328-330.

24. Shahverdi, A. R., H. R. Monsef-Esfahani, B. Nickavar, L. Bitarafan, S. Khodae, and N. Khoshakhlagh. 2005. Antimicrobial activity and main chemical composition of two smoke condensates from *Peganum harmala* seeds. *J. Biosci.* 60: 707-710.
25. Sharef, M.; el- Ansari, M. A. and Saleh, N. A. Four flavonoid glycosids from *peganum harmala*. *Phyto. Chem.*, 1997; 44: 533- 536.
26. Soliman, A. M. and Fahmy, S. R. Protective and curative effects of the 15 KD isolated protein from the *peganum harmala* L. seeds against carbon tetrachloride induced oxidative stress in brain, tests and erythrocytes of rats. *Eur. Rec. Med. Pharm. Sci.*, 2001; 15: 888- 899.
27. Tabashnik B. E., Cushing N. L., 1987.- Leaf residue vs. topical bioassays for assessing insecticide resistance in the diamondback moth, *Plutella xylostella* L.- *FAO Plant Protection Bulletin*, 35: 11-14.
28. Tebkew Damte, Adane Tesyaye and Asmare Dejen. (2002). Potentials for botanicals in controlling the African bollworm. In: *Proceedings of the National Workshop on African bollworm management in Ethiopia: Status and Need*, April 17-19, 2002, pp. 106- 114.
29. Thacker JMR. 2002. An Introduction to Arthropod Pest Control. Cambridge, UK: Cambridge Univ. Press. 343 pp.
30. Torres Vila, L. M., Rodriguez Molina, M. C., Lacasa Plasencia, A., Bielza Lino, P., Rodriguez del Rincon, A., (2002b). Pyrethroid resistance of *Helicoverpa armigera* in Spain: current status and agro ecological perspective. *Agriculture Ecosystems and Environment* 93: 55-66 (abstract).
31. Ware GW. 1883. Pesticides. Theory and Application. San Francisco: Freeman. 308.