Impact of *Allium sativum* leaf lectin on the *Helicoverpa armigera* larval parasitoid *Campoletis chlorideae*

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Significant progress has been made over the past two decades in handling and introduction of novel genes into crop plants to increase yields, improve nutrition, and impart resistance to biotic and abiotic stresses (Sharma et al. 2004). The primary benefit to growers of adopting transgenics will be to control the insect species that have become resistant to commonly used insecticides. But, there are serious concerns about the potential influence of transgenic crops on non-target organisms (Sharma and Ortiz 2000). To ensure a sustainable deployment of transgenic insect resistant plants, it is important that they are compatible with other control methods, including biological control.

Plant lectins have been reported to affect survival and development of insect pests (Ferry et al. 2004). For example, the *Allium sativum* (garlic) leaf lectin (ASAL) has been reported to reduce pupal weight, pupal period, pupation and adult emergence of the pod borer *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) (Arora et al. 2005). Recently, the ASAL gene has been deployed to develop transgenic chickpea (*Cicer arietinum*) plants that show partial resistance to the aphid *Aphis craccivora* Koch (Hemiptera: Aphididae) (Romeis et al. 2004, Chakraborty et al. 2006). We have therefore studied the effects of ASAL on survival and development of the *H. armigera* larval parasitoid *Campoletis chlorideae* Uchida (Hymenoptera: Ichneumonidae) so as to help develop appropriate strategies for deployment of ASAL-transgenic crops for sustainable crop production.

The cocoons of *C. chlorideae* were collected from chickpea fields at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India, and the culture was maintained at 27±2ºC and 65–75% relative humidity under laboratory conditions on *H. armigera* reared on chickpea-based semi-synthetic artificial diet (Armes et al. 1992). The cocoons were placed individually in glass vials for adult emergence. The adult wasps were kept for three days in wooden cages for mating, and were fed on 10% honey solution. For oviposition, three randomly selected mated females were transferred to transparent plastic vials (15 ml capacity) kept in an inverted condition on a petri plate. A single *H. armigera* larva was offered for oviposition to the *C. chlorideae* females inside the vial. After oviposition, the *H. armigera* larvae were removed, and placed on chickpea-based artificial diet for further development. To observe the effects of ASAL on survival and development of *C. chlorideae*, the larvae of *H. armigera* were reared on ASAL (0.1%, w/v) intoxicated artificial diet. The ASAL was dissolved in distilled water and then mixed with the artificial diet using a magnetic stirrer. One cm^2 pieces of the intoxicated artificial diet were provided to the *H. armigera* larvae for 72 h before and/or after parasitization by *C. chlorideae* females. In the controls, the larvae were fed on artificial diet without ASAL. After parasitization, the *H. armigera* larvae were kept individually on the respective diets in 15 ml vials. The experiments were conducted in completely randomized design with a total of 45 larvae per treatment in three replications. The treatment combinations included: -- = *H. armigera* larvae fed on control diet before and after parasitization; +- = *H. armigera* larvae first fed on the ASAL intoxicated diet for 72 h before parasitization, and then fed on control diet till parasitoid cocoon formation; -+ = *H. armigera* larvae fed on control diet before parasitization, and then on ASAL intoxicated diet for 72 h; and ++ = *H. armigera* larvae fed on ASAL intoxicated diet for 72 h before and after parasitization. Observations were recorded on percentage of *H. armigera* larvae parasitized by the *C. chlorideae* females (% cocoon formation) and a number of parasitoid life-table parameters (egg and larval period, pupal period, adult emergence, adult weight and sex ratio).

The garlic leaf lectin (0.1%) treatment had a significant influence on larval and pupal periods (Table 1), and emergence (Fig. 1) of *C. chlorideae* reared on *H. armigera* larvae fed for 72 h on ASAL intoxicated diet. ASAL fed *H. armigera* larvae increased the larval period of the parasitoid by 0.8 day as compared to the control diet. The *H. armigera* larvae fed on ASAL impregnated

diet after parasitization decreased the pupal period by one day as compared to that on control diet (Table 1). The *H. armigera* larvae fed on *Bt* proteins Cry1Ab and Cry1Ac in artificial diet have been reported to prolong the larval period of the parasitoid by 2 days (Arora et al. 2005, Sharma et al. 2006). ASAL intoxicated diet fed to *H. armigera* larvae before and after parasitization decreased pupation of *C. chlorideae* by 22.2% over control, but the differences were not significant (Fig. 1). However, adult emergence from ASAL-treated larvae was significantly decreased (28.9%) as compared to that from untreated control larvae (44.4 to 51.1%) (Fig. 1). For comparison, the *Bt* proteins also result in a significant reduction in cocoon formation and adult emergence of *C. chlorideae*, when reared on intoxicated *H. armigera* larvae (Arora et al. 2005, Sharma et al. 2006). The feeding of *H. armigera* larvae on any of the ASAL intoxicated diet protocols did not affect the weight of *C. chlorideae* adults, and the trend in sex ratio was inconsistent (Table 1).

Table 1. Effect of *Allium sativum* leaf lectin (ASAL) on life-table parameters of the parasitoid *Campoletis chlorideae* through intoxicated *Helicoverpa armigera* larvae.

Treatment[1]	Larval period (days)	Pupal period (days)	Adult weight (mg)	Sex ratio (male:female)
Control --	8.0	6.5	2.4	1:0.44
ASAL +-	8.3	6.3	2.4	–
ASAL -+	8.0	5.5	2.4	1:1
ASAL ++	8.8	6.5	2.4	1:0.44
F-probability	<0.001	0.008	0.954	–
LSD (P = 0.05)	0.23	0.49	NS[2]	–

1. *Helicoverpa armigera* larvae fed on: -- = control diet only; +- = ASAL diet before parasitization only; -+ = ASAL diet after parasitization only; ++ = ASAL diet before and after parasitization.
2. NS = Not significant.

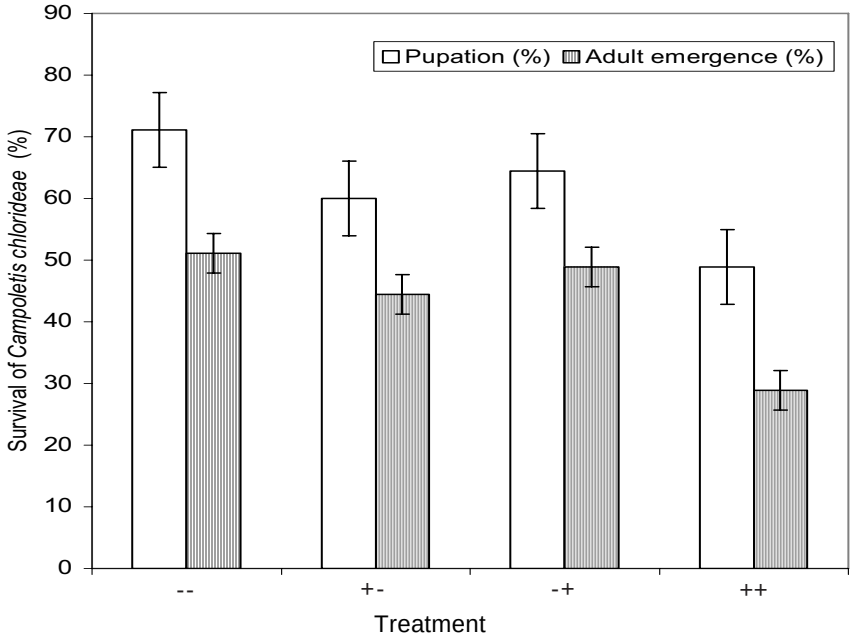

Figure 1. Survival of *Campoletis chlorideae* reared on ASAL intoxicated *Helicoverpa armigera* larvae. (*Helicoverpa armigera* larvae fed on: -- = Control diet only; +- = ASAL diet before parasitization only; -+ = ASAL diet after parasitization only; ++ = ASAL diet before and after parasitization.)

Sub-lethal effects of *Bt* proteins on the host larvae (sick host) reduce their nutritional quality for the parasitoid, and poor nutritional quality of the host results in detrimental effects on the development and survival of natural enemies (Murugan et al. 2000). Such effects have generally been reported for parasitoids that developed in sub-lethal affected host larvae (Romeis et al. 2006). Although, *H. armigera* fed on ASAL proteins showed some adverse effects on the *C. chlorideae* fitness and survival, these effects are far lower than those of broad-spectrum pesticides. There is need to establish whether the reduced cocoon formation and adult emergence of *C. chlorideae* were due to poor nutritional quality of the host larvae and their early mortality or due to direct toxicity of ASAL to the parasitoid.

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References

Armes NJ, Bond GS and **Cooters RJ.** 1992. The laboratory culture and development of *Helicoverpa armigera*. Natural Resources Institute Bulletin No. 57. Chatham, UK: Natural Resources Institute.

Arora R, Sharma HC, Dhillon MK and **Romeis J.** 2005. Influence of Cry1Ab and Cry1Ac intoxicated *Helicoverpa armigera* larvae on the survival and development of the parasitoid, *Campoletis chlorideae*. Page 341 *in* Abstracts, IVth International Food Legumes Research Conference: Food Legumes for Nutritional Security and Sustainable Agriculture, 18–22 Oct 2005, New Delhi, India. New Delhi, India: Indian Agricultural Research Institute.

Arora R, Sharma HC, Van Dreissche E and **Sharma KK.** 2005. Biological activity of lectins from grain legumes and garlic against the legume pod borer, *Helicoverpa armigera*. International Chickpea and Pigeonpea Newsletter 12:50–52.

Chakraborty D, Sarkar A and **Das S.** 2006. The problem and prospect of insect resistance development in chickpea through *Bt* and *ASAL* gene expression. Page 12 *in* Annual Pulse Network Meeting, 2–4 February 2006, Indo-Swiss Collaboration in Biotechnology, ICRISAT, Patancheru, India. Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Ferry N, Edwards MG, Mulligan EA, Emami K, Petrova AS, Frantescu M, Davison GM and **Gatehouse AMR.** 2004. Engineering resistance to insect pests. Pages 373–394 *in* Handbook of plant biotechnology (Christou P and Klee H, eds.). Vol. 1. Chichester, UK: John Wiley & Sons Ltd.

Murugan K, Senthil KN, Jeyabalan D, Senthil NS, Sivaramakrishnan S and **Swamiappan M.** 2000. Influence of *Helicoverpa armigera* (Hübner) diet on its parasitoid *Campoletis chlorideae* Uchida. Insect Science and its Application 20:23–31.

Romeis J, **Meissle M** and **Bigler F.** 2006. Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. Nature Biotechnology 24:63–71.

Romeis J, **Sharma HC**, **Sharma KK**, **Sampa Das** and **Sarmah BK.** 2004. The potential of transgenic chickpeas for pest control and possible effects on non-target arthropods. Crop Protection 23:923–938.

Sharma HC, Dhillon MK and **Romeis J.** 2006. Influence of Cry1Ab and Cry1Ac intoxicated *Helicoverpa armigera* larvae on the survival and development of the parasitoid, *Campoletis chlorideae.* Page 25 *in* Annual Pulse Network Meeting, 2–4 February 2006, Indo-Swiss Collaboration in Biotechnology, ICRISAT, Patancheru, India. Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Sharma HC and **Ortiz R.** 2000. Transgenics, pest management, and the environment. Current Science 79:421–437.

Sharma HC, Sharma KK and **Crouch JH.** 2004. Genetic transformation of crops for insect resistance: Potential and limitations. Critical Reviews in Plant Sciences 23:47–72.