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Source: Florida Entomologist, 101(2) : 172-177
Published By: Florida Entomological Society
URL: https://doi.org/10.1653/024.101.0204
Parasitism of *Plutella xylostella* (Lepidoptera: Plutellidae) in southern Pakistan

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**Abstract**

Larvae of diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) cause severe economic loss to crucifer vegetables in Pakistan. Studies were conducted from 2007 to 2010 to identify and assess the abundance of parasitoids attacking *P. xylostella* in commercial, insecticide-treated cauliflower fields in southern Pakistan. In total, 6 parasitoid species were found attacking larvae and pupae of *P. xylostella*. These included the larval parasitoid *Cotesia plutellae* Kurdjumov (Hymenoptera: Braconidae); *Cotesia* sp. (Hymenoptera: Braconidae); *Diadegma* sp. (Hymenoptera: Ichneumonidae); a larval-pupal parasitoid *Oomyzus sokolowskii* Kurdjumov (Hymenoptera: Eulophidae); a pupal parasitoid *Diadromus collaris* Gravenhorst (Hymenoptera: Ichneumonidae); and a pupal parasitoid, *Brachymeria excarnata* Gahan (Hymenoptera: Chalcididae). *Cotesia plutellae* provided 2.3 to 52.2% parasitism, followed by *O. sokolowskii* with 2.1 to 6.7% parasitism. The average parasitism caused by *Cotesia* sp. was < 5%. The remaining parasitoids were scarce with < 0.5% parasitism. Additional studies on parasitism by *O. sokolowskii* showed that there was a significant positive correlation between *P. xylostella* pupal weight and the number of parasitoids emerging from a pupa. More than 50% of *P. xylostella* parasitized pupae supported 6 to 10 *O. sokolowskii* adults per pupa. Efforts should be undertaken to preserve these natural enemies through cultural practices and the use of soft insecticides to allow them to function more effectively in an integrated pest management program.

**Key Words:** biological control; *Oomyzus sokolowskii*, *Cotesia plutellae*

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The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), was a secondary pest of crucifer crops that has become a primary pest due to widespread application of insecticides that has reduced populations of natural enemies and caused widespread insecticide resistance in the host (Talekar & Shelton 1993). Now it is regarded as the most destructive insect of crucifers, occurring wherever crucifer crops are grown (Talekar & Shelton 1993; Shelton 2004). *Plutella xylostella* is one of the few insect species that have developed resistance to all major classes of insecticides, including recently introduced anthranilic diamides (Talekar & Shelton 1993; Shelton et al. 1993; Zhao et al. 2006; Wang & Wu 2012). *Plutella xylostella* ranks second for resistance to the highest number of insecticides with 95 active ingredients (APRD 2017). Two decades ago, the annual cost of controlling *P. xylostella* on a worldwide basis was estimated to be US$1 billion (Talekar & Shelton 1993). With monetary inflation and the increased production of crucifer vegetable and oil seed crops over the last 2 decades, the global management cost for *P. xylostella* is now estimated to be US$4 to 5 billion annually (Furlong et al. 2013).

Insecticide use to control *P. xylostella* by farmers in Asia and Africa generally is characterized by its overuse, with little regard to recommended integrated pest management (IPM) practices, and is seen as both increasingly expensive and unreliable (Grzywacz et al. 2010). A common problem with resource-poor farmers is their illiteracy and lack of understanding about how natural enemies work (Grzywacz et al. 2010). In Asia, another constraint to IPM programs is the availability of cheap, illegally imported insecticides (Sivapragasam 2004; Grzywacz et al. 2010). Failure of insecticides to provide adequate control has made *P. xylostella* the single most important constraint to commer-

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cial production of crucifer vegetables (Talekar & Shelton 1993). Outbreaks of *P. xylostella* in Southeast Asia can cause crop losses > 90% (Verkerk & Wright 1996). Production of crucifer vegetables in developing countries, including Pakistan, primarily is by smallholder farmers with intensive use of land, labor, and pesticides. The common method of insect management available to smallholder farmers in Pakistan is the frequent use of insecticides. Even with spraying every other day with cocktails of very toxic insecticides, farmers often are unable to save their crucifer crops, and resort to planting non-crucifer crops to avoid complete economic loss of the land (Abro et al. 1994).

More than 135 species of parasitoids attacking *P. xylostella* have been reported from various parts of the world (Delvare 2004), but only about 60 species appear to be important for biological control (Talekar & Shelton 1993). About a dozen species frequently are recovered in field studies (Furlong et al. 2013). Among larval parasitoids, the major ones, in order of decreasing importance, belong to the genera *Diadegma*, *Cotesia*, *Apanetes*, and *Microplitis*; among pupal parasitoids those in the genera *Diadromus* and *Oomyzus* appear to be most abundant (Lim 1986; Waterhouse 1992). *Cotesia plutellae* Kurdj (Hymenoptera: Braconidae) is native to Europe (Lloyd 1940) but has been reported from many countries (Sarfraz et al. 2005; Furlong et al. 2013). Similarly, *Oomyzus sokoloskii* Kurdj (Hymenoptera: Eulophidae), a gregarious larval-pupal primary parasitoid (Mushtaque 1990; Alam 1992; Fitton & Walker 1992), also has been recorded from many countries (Talekar & Shelton 1993; Sarfraz et al. 2005; Furlong et al. 2013).

No detailed research has been carried out on *P. xylostella* in this part of Pakistan, except for a few studies (Abro et al. 1994; Syed et al. 2012; Abro et al. 2013; Shah et al. 2013). Fundamental to all IPM programs should be the conservation of natural enemies (Furlong et al. 2013). As an alternative to reliance on broad-spectrum insecticides, natural enemies may provide an important component for an IPM program of this serious pest of crucifers in Pakistan. This study was undertaken to identify indigenous parasitoids attacking *P. xylostella* larvae and pupae in southern Pakistan.

### Materials and Methods

#### CROPS

Studies were performed in farmer fields in the Tando Allahyar District (25.4666°N, 68.7166°E) of Sindh located 200 km northeast of Karachi in southern Pakistan. The farmers are smallholders with 0.4 to 0.5 ha of field crops, and the leaves were changed daily. Development of larvae and pupae was monitored daily until a *P. xylostella* adult parasitoid emerged or the larva died. The percent parasitism was calculated by dividing the number of emerged parasitoids by the total number of parasitoids, and *P. xylostella* adults recovered after rearing. The larvae that were unaccounted for after rearing, due to disease or other causes, were not included in the denominator (Russell 1987; Rowell et al. 2005). There were 12 weekly collections beginning 15 d after transplanting and ending at crop harvest. The 12 collections were divided into 2 sets (6 early and 6 late season) and each set was averaged to obtain means for that period.

The parasitoids collected were identified by the International Commonwealth Institute of Biological Control, Rawalpindi, Pakistan. The weekly average temperature was obtained from the meteorological department of Sindh Agriculture University to study the effect of temperature on pupal parasitism by determining the correlation between these 2 variables. Correlation also was carried out between pupal weight and number of parasitoids emerging from a single pupa. The percent parasitism means of summer and winter 2007 crops were compared using a paired *t*-test. All data were analyzed using 2-way analyses of variance (ANOVA) for a randomized complete block design. Treatment means were separated by least significant differences (LSD) and were reported as means ± SE (Zar 2010) on a computer employing statistical software (STATISTIX® VERSION-8.1, Analytical Software, Inc., Tallahassee, Florida, USA).

#### PARASITISM CHARACTERISTICS OF *OMYZUS SOKOLOSSKII*

An experiment was conducted using weekly field collection of *P. xylostella* pupae parasitized by *O. sokoloskii* from Oct 2009 to Apr 2010. During each collection, 50 pupae were weighed individually on a Mettler AE 100 electronic balance (Mettler-Toledo, Columbus, Ohio, USA) and each pupa was placed into a labeled glass vial (3 × 2 cm) and observed daily until emergence of an adult *P. xylostella* or parasitoid, or death of the pupa. On emergence, parasitoids from each pupa were counted and recorded. The average weight of a *P. xylostella* pupa was calculated for those pupae that were parasitized at each collection date. Similarly, the number of parasitoids emerging from each pupa (= parasitoid load) was recorded. The number of parasitoids per pupa was grouped into parasitoid load classes (i.e., 1–5, 5–10) as shown in Figure 1. The average number of pupae parasitized per wk also was calculated.

![Fig. 1. Frequency distribution of number of Oomyzus sokoloskii adults emerging from a single Plutella xylostella pupa.](image-url)
Results

LARVAL PARASITISM

Six species (3 larval and 3 pupal) of parasitoids were collected and 4 of these were identified to species level, the remaining 2 were identified to genus (Table 1). During the summer and winter seasons, *Cotesia plutellae* was the most dominant larval parasitoid (Table 2). The parasitism rate of *Cotesia plutellae* fluctuated between 2.3 and 52.2%. Early season larval parasitism of *P. xylostella* by *C. plutellae* from the 2007 summer crop averaged 8.2 (SE ± 1.4), and individual collections ranged from 2.7 to 12.3% (data not shown in Table 2). The late season parasitism averaged 11.0 (SE ± 0.8), and individual collections ranged from 8.2 and 13.7% (data not shown in Table 2). Although more parasitism by *C. plutellae* occurred in winter than in the summer crop, the difference was not significant. *Plutella xylostella* larval parasitism during the early winter crop of 2008 was lower than the late season but the difference was not significant. The percent parasitism recorded on winter-sown crops for 3 yr indicated a significant difference for collection dates within a season and for different seasons (*F* = 3.0; df = 11, 22; *P* < 0.05 for dates; *F* = 22.46; df = 2, 22, *P* < 0.01 for seasons). The parasitism rate of the larval parasitoid *Cotesia* sp. was low and remained in the range of 0.3 to 3.2%. The larval parasitoid *Diadegma* sp. was not present in the summer crop and its rate of parasitism remained ≤ 0.5% (Table 2).

PUPAL PARASITISM

Pupal parasitism was lower than larval parasitism. The larval-pupal parasitoid *Oomyzus sokolowskii* was one of the other dominant parasitoids of *P. xylostella* occurring during the summer and winter seasons. Its parasitism range was 2.1 to 6.7%. The percent parasitism recorded on winter-sown crops for 3 yr was not significantly different for seasons (*F* = 0.00; df = 2, 105; *P* = 1.000). *Diadromus collaris* Gravenhorst, a pupal parasitoid of *P. xylostella*, was rare and found only in the winter crop during Jan when temperatures were low. Its parasitism range was 0.3 to 0.5%. *Brachymeria excarinata* Gahan, a pupal parasitoid of *P. xylostella* and a hyperparasitoid on *C. plutellae*, was also rare and not found in the summer season crop. In the winter crop, its parasitism was only 0.2% (Table 2).

PARASITISM CHARACTERISTICS OF *OOMYZUS SOKOLOWSKII*

A total of 1,150 *P. xylostella* pupae were collected from Oct 2009 to Apr 2010. From these, 182 were parasitized by *O. sokolowskii* and data were recorded for 167 parasitized pupae. The overall average parasitoid load per pupa was 8.4 ± 0.3 of *O. sokolowskii* adults. However, the parasitoid load varied enormously (Fig. 1). The majority (51.5%) of *P. xylostella* pupae had a parasitoid load in the 6–10 class intervals (average 7.9 ± 1.3). The maximum number of *O. sokolowskii* recorded per *P. xylostella* pupa was 21.0. There was a significant negative correlation between temperature and number of *O. sokolowskii* adults emerged (*r* = −0.538; df = 18; *P* = 0.02) (Fig. 2) and a significant positive correlation (*r* = 0.626; df = 18; *P* = 0.01) between *P. xylostella* pupal weight and the number of *O. sokolowskii* adults emerged per pupa (Fig. 3).

Discussion

The results indicated that larval parasitism could be an important mortality factor of *P. xylostella* in southern Pakistan, even in commercial fields that are treated routinely with broad-spectrum insecticides. With the use of softer insecticides, one would expect parasitism rates to increase. The rate of larval parasitism of *P. xylostella* was higher in the winter-spring crop compared with the summer crop. The highest larval parasitism of 65.2% was recorded 85 d after transplanting the winter crop, in the last wk of Feb. In a previous report from southern Pakistan, Mushtaque and Mohyuddin (1987) recorded 57.2% parasitism of *P. xylostella* larvae by *C. plutellae* during the month of Jan. In Australia, Goodwin (1979) also reported high parasitoid activity during spring and summer, although extremely high temperatures during summer reduced the number of both the host and its parasitoids. In China, Liu et al. (2000a) demonstrated that *C. plutellae* was active throughout the yr but was less abundant and developed slowly during winter months. In the present studies, parasitism by *C. plutellae* ranged between 1.9 to 48.8% during different periods, which might be due to host density, because it has been reported that a density-dependent relationship exists between *C. plutellae* and its host (Nagarketti & Jayanth 1982) and *C. plutellae* are responsive numerically to increasing populations of *P. xylostella* (Ooi 1992; Rowell et al. 1992). *Cotesia plutellae* thrive in environments that have not been sprayed with insecticides (Alam 1992), but also has been reported to be a dominant parasitoid of *P. xylostella* in China, India, Thailand, and other countries, where it has been collected from fields sprayed with insecticides (Liu et al. 1997; Liu et al. 2000a; Krishnamoorthy 2004; Rowell et al. 2005; Sarfraz et al. 2005).

The other *Cotesia* sp. was found parasitizing larvae of *P. xylostella* during the season when temperatures were comparatively low. Additional studies are needed to confirm its identity and evaluate its effectiveness. *Diadegma* sp. also was observed during Dec and Jan only, when temperatures were low and its rate of parasitism was < 2%. *Diadegma seminalis* Hellen is an important European species parasitizing *P. xylostella*, and has been introduced in many tropical and subtropical Asian countries, but it has become established only in cooler areas where temperatures rarely exceed 28 °C (Talekar & Hu 1996; Rowell et al. 2005).

The parasitism of *P. xylostella* by *O. sokolowskii* was greater during the winter-spring crop than the summer crop. This could be due to high temperatures during the summer season compared with the winter-spring crop. Mushtaque (1990) reported low parasitism of *P. xylostella* by *O. sokolowskii* during Jul and Aug on cauliflower at Sahiwal, Pakistan. *Oomyzus sokolowskii* is an effective parasitoid found in many countries of the world (Talekar & Hu 1996; Wang et al. 1999; Silva-Torres et al. 2009). Because *C. plutellae* and *O. sokolowskii* share similar

| Species       | Family       | Type                          |
|---------------|--------------|-------------------------------|
| *Cotesia*     | Braconidae   | Solitary larval endoparasitoid |
| plutellae     |              |                               |
| *Cotesia*     | Braconidae   | Solitary larval endoparasitoid |
| sp.           |              |                               |
| *Diadegma*    | Ichneumonidae| Solitary larval endoparasitoid |
| sp.           |              |                               |
| *Oomyzus*     | Eulophidae   | Gregarious larval-pupal endoparasitoid |
| sokolowskii   |              |                               |
| *Diadromus*   | Ichneumonidae| Solitary pupal endoparasitoid  |
| collaris      |              |                               |
| *Gravenhorst* |              |                               |
| *Brachymeria* | Chalcididae  | Solitary pupal parasitoid      |
| excarinata    |              |                               |
| Gahan         |              |                               |

Table 1. Hymenopteran parasitoids of *Plutella xylostella* collected at Tando Allahyar, Southern Sindh, Pakistan, 2007–2010.
ecological niches and co-exist in many countries (Talekar & Hu 1996), and both attack *P. xylostella* larvae with a slight overlap in their host stage preference (Talekar & Hu 1996), it is possible that both parasitoids may compete for host larvae in the field (Wang et al. 1999). Talekar & Hu (1996) reported that when *P. xylostella* larvae parasitized by *C. plutellae* were offered to *O. sokolowskii*, it readily parasitized the larvae and developed to the adult stage only if the *C. plutellae* parasitism was < 24 h old. Liu et al. (2000b) observed negative correlation in rates of parasitism of *P. xylostella* larvae by *C. plutellae* and *O. sokolowskii*, suggesting potential competition. Similar interactions also have been recorded by Shi et al. (2004) for *D. semicalusum* and *O. sokolowskii*.

The pupal parasitoid *D. collaris* was observed only during the month of Jan when temperatures were cooler in Southern Sindh, Pakistan, and its parasitism was < 1.0%. This pupal parasitoid has been introduced in many countries where it has shown some effectiveness in controlling *P. xylostella* in cooler highland crucifer vegetable production (Talekar & Hu 1996; Rowell et al. 2005).

*Oomyzus sokolowskii* is a larval-pupal gregarious parasitoid naturally found parasitizing *P. xylostella* worldwide (Silva-Torres et al. 2009). This study indicated that on average, 8.4 parasitoids emerged from a single host pupa. Uematsu and Yamashita (2000) collected *P. xylostella* pupae from cabbage fields, and reported the average number of parasitoids emerging from a single host changed seasonally from 8.6 to 10.9 during autumn-winter and spring-summer cabbage crops, respectively. Under laboratory studies, Ooi (1988) recorded an average of 8.6 *O. sokolowskii* adults emerging from a single host. In the laboratory, Wang et al. (1999) parasitized different larval instars of *P. xylostella* and found the average number of *O. sokolowskii* emerging per host varied from 7.6 to 9.5. Liu et al. (2000b) found 7.8 ± 3.3 *O. sokolowskii* per *P. xylostella* pupa. In the laboratory, Silva-Torres et al. (2009) observed that progeny per host depended on the number of ovipositions per host by female *O. sokolowskii*. The number of progeny per host ranged from < 10 per 1 oviposition to > 20 per host per 3 ovipositions.

Resource availability is assumed higher in larger hosts based on the amount of food available inside the host for a developing parasitoid (Hardy et al. 1992; Zaviezo & Mills 2000). Greater resource availability is expected to promote parasitoid survival and produce offspring with

**Table 2.** Percent larval and pupal parasitism of *Plutella xylostella* collected at Tando Allahyra, Southern Sindh, Pakistan, 2007–2010.

| Season/parasitoids | Summer 2007 | Winter 2007 | Winter 2008 | Winter 2009 |
|--------------------|-------------|-------------|-------------|-------------|
|                    | Larval      | Pupal       | Larval      | Pupal       | Larval      | Pupal       | Larval      | Pupal       |
| Early              |             |             |             |             |             |             |             |             |
| *C. plutellae*     | 8.2 ± 1.4   | 18.2 ± 3.5  | 4.7 ± 0.5   | 2.3 ± 0.2   |
| *Cotesia sp.*      | 0           | 3.2 ± 1.0   | 2.1 ± 0.4   | 0.3 ± 0.1   |
| *Diadegma sp.*     | 0           | 0.4 ± 0.2   | 0.3 ± 0.0   | 0           |
| *O. sokolowskii*   | 2.1 ± 0.4   | 2.2 ± 0.2   | 2.1 ± 0.2   | 3.1 ± 0.6   |
| *D. collaris*      | 0           | 0.3 ± 0.0   | 0.4 ± 0.1   | 0.4 ± 0.2   |
| *B. excarinata*    | 0           | 0           | 0           | 0.2 ± 0.0   |
| Late               |             |             |             |             |             |             |             |             |
| *C. plutellae*     | 11.0 ± 0.8  | 52.2 ± 5.4  | 31.5 ± 6.4  | 3.1 ± 0.4   |
| *Cotesia sp.*      | 0           | 2.4 ± 0.5   | 2.6 ± 0.5   | 0.7 ± 0.2   |
| *Diadegma sp.*     | 0           | 0.4 ± 0.6   | 0.4 ± 0.1   | 0           |
| *O. sokolowskii*   | 3.2 ± 0.4   | 6.2 ± 0.5   | 5.6 ± 0.4   | 6.7 ± 0.6   |
| *D. collaris*      | 0           | 0.5 ± 0.1   | 0.3 ± 0.2   | 0.4 ± 0.2   |
| *B. excarinata*    | 0           | 0.2 ± 0.2   | 0           | 0.2 ± 0.1   |
| Overall mean       | 9.6 ± 0.8   | 2.7 ± 0.3   | 12.8 ± 8.3a | 1.6 ± 0.6   |
|                    | 6.9 ± 4.9ab | 1.4 ± 0.9   | 1.1 ± 0.5b  | 1.8 ± 1.1   |

Means ± SE followed by different letters are significantly (P < 0.05) different from each other by LSD.

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**Fig. 2.** Relationship between average temperature and average number of *Oomyzus sokolowskii* adults emerged per *Plutella xylostella* pupa.

**Fig. 3.** Relationship between average *Plutella xylostella* pupal weight and average number of *Oomyzus sokolowskii* adults emerged per pupa.
larger body size, increased longevity, and higher fecundity rates than parasitoids emerging from low quality hosts (King 2000; Silva-Torres et al. 2009). There was a significant positive relationship between host pupal weight and number of *O. sokolowskii* parasitoids emerging per host pupa (Fig. 2). Nakamura and Noda (2002) also reported that number of progeny of *O. sokolowskii* per host increased significantly with host size. The study by Li et al. (2017) also suggested that *O. sokolowskii* females might optimize their clutch size in response to *P. xylostella* larval body size, and their sex allocation in response to clutch size. On the other hand, Silva-Torres et al. (2009) found no effect of host size on number of progeny per host in *O. sokolowskii*.

The present study found a negative relationship between the temperature and number of parasitoids emerging per host pupa. Working on parasitism of *P. xylostella* by *O. sokolowskii*, Talekar and Hu (1996) found that increasing temperatures from 10 to 35 °C significantly increased the parasitism of *P. xylostella* by *O. sokolowskii*. Wang et al. (1999) observed that the favorable temperature range for development, survival and reproduction of *O. sokolowskii* was 20 to 30 °C and observed that adult longevity of *O. sokolowskii* was significantly reduced when temperature increased from 20 to 30 °C. Furthermore, emergence was sharply reduced above 32.5 °C and parasitoids failed to emerge at 15 °C. Under laboratory experiments, Liu et al. (2000b) showed that the most favorable temperature for development, survival and reproduction of *O. sokolowskii* ranged from 20 to 30 °C, and temperatures below 20 °C or above 30 °C were unfavorable for its survival. The studies of Talekar & Hu (1996), Wang et al. (1999), and Liu et al. (2000b) were conducted under laboratory conditions, whereas our studies were conducted under field conditions where weather conditions vary enormously, and this could explain the differences.

This study indicated that *C. plutellae* and *O. sokolowskii* are important larval and pupal parasitoids and could play an important role in IPM of *P. xylostella* in Pakistan if they are conserved and, if possible, increased. This could be accomplished by habitat manipulation, diversifying the landscape, and providing plants that can offer nectar and other resources to natural enemies (Juric et al. 2017). Studies conducted by Johanowicz and Mitchell (2000) showed that sweet alyssum, *Lobularia maritima* (L.) Desv. (Brassicaceae: Brassicaceae), planted in cabbage fields increased adult parasitoid longevity and improved biological control of lepidopteran pests in Florida. Under field conditions, Lavandero et al. (2005) observed that the percentage of *D. semicalus* that parasitized *P. xylostella* larvae in a treatment that included flowering plants was more than twice that in a non-flower treatment. Winkler et al. (2010) found significantly more *D. semicalus* adults within a field of Brussels sprout fields bordered by dill, *Anethum graveolens* L. (Apiaceae: Apioideae), as compared to the control. Application of softer, less toxic insecticides to natural enemies should be encouraged, instead of the older insecticides including organophosphates and carbamates that are widely used in Pakistan (Abro et al. 2013). These measures also should help reduce the risk to farmers, the environment, and the pesticide residue problem on crucifer vegetables in developing countries (Grzywacz et al. 2010), including Pakistan.

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