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Field parasitism and biological characteristics of potent larval endoparasitiods of *Spilosoma obliqua* Walker in Jammu and Kashmir, India

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ABSTRACT: Three species of braconid parasitoids viz. *Glyptapanteles agamemnonis* Wilkinson, *Meteorus* sp. and *Cotesia ruficrus* (Haliday) were recovered from the field collected larvae of *Spilosoma obliqua* Walker feeding on cultivated *Vigna mungo* and wild weeds, *Xanthium strumarium* and *Parthenium hysterophorus* during *kharif* 2002 and 2003 in Jammu (J & K). Besides, the prevalence of one ichneumonid parasitoid *Agathis* sp. and viral disease (Nucleopolyhedrovirus) was also noticed. *Meteorus* sp. was considered as most promising parasitoid of this pest with a maximum parasitization of 77.0 per cent. Nevertheless, *G. agamemnonsis* is reported for the first time on *S. obliqua* in India. While, *G agamemnonsis* was recovered from the larvae feeding on all the three hosts *viz Vigna mungo*, *Parthenium hysterophorus* and *Xanthium strumarium*, *Meteorus* sp. was never recovered from *P. hysterophorus*. However, both these parasitoid co-existed in nature and found together attacking 3-4th instar larvae from September to October (37– 43 SW) with a peak activity within a minimum temperature range of 14.0 to 18.0°C. The natural parasitism by these parasitoids did not reveal significant correlation with aboitic factors, but the relationship between their respective K-values and larval density fit into density dependent model $K = aN^{b}$. However, *C. ruficrus* and *Agathis* sp. were noticed in later season when the mean minimum temperature reached below 10°C. The behaviour and developmental biology of all the three braconid parasitoids of *S. obliqua* is described.

KEY WORDS: *Cotesia ruficrus*, developmental biology, field parasitism, *Glyptapanteles agamemnonis*, *Meteorus* sp., *Spilosoma obliqua*

INTRODUCTION

Bihar hairy caterpillar, *Siplosoma obliqua* Walker is a sporadic pest and is widely distributed in the orient. It is highly polyphagous and feeds practically on all kinds of vegetation growing during *kharif* season (Kalra, 1984). For the past few years severe infestation of this pest was noticed on *kharif* pulses and mulberry plants at Jammu. While the young caterpillars preferred to eat the growing

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points of plants, the older one fed voraciously on all vegetation resulting in field devastation by moving full-grown caterpillars. The timely management of this pest is very important as delay may even lead to complete failure of crop if remain unchecked in field (Atwal, 1993). However, the use of chemicals against it is uneconomical as the pest feeds on many weeds (Sathpathi, 1999). Therefore, the role of natural enemies for management of this pest needs to be explored. The pest was reported to be parasitized by *Glyptapanteles obliqua*, *G. creatonoti*, *Meteorus* sp., *A vitripennis*, *A. obliqua* and *Carcelia* sp. in different regions of India (Singh and Gangrade, 1975; Kalra, 1984; Singh and Singh, 1995; Varatharajan *et al.*, 1998) with over all parasitism as high as 40.0 per cent. Therefore, conservation of these parasitoids is very important. Estimation of field parasitization is the foremost step to quantify the natural field mortality for any biocontrol programme. Although, few studies on natural enemies of this pest do exist, yet they are inadequate to assess the suitability of braconid parasitoids for its management. The present investigation on parasitization, developmental biology and biological attributes will provide vital information on mass rearing and actual field potential of the braconid parasitoids of this pest – a critical step in any field releases programme.

MATERIALS AND METHODS

Field parasitism and seasonal incidence

Various stages of *S. obliqua* were collected from unsprayed cultivated *Vigna mungo* and wild weeds in and around FOA, Udheywalla during *kharif*, 2002 and 2003 and were kept separately in glass vials (50 x 5mm) for the emergence of parasitoids, if any. Larvae were collected at seven day interval and reared on mulberry leaves in pre-sterilized small screw cap plastic containers having a small circular hole on the lid covered with fine brass mesh, individually. The leaves were changed regularly as and when required until pupation of pest/ parasitoid was observed. Data recorded on per cent parasitism were correlated with the meteorological variables and subjected to multiple regression analysis. The larval density was also monitored during the crop growth by recording the total number of larvae from 10 randomly selected plants across *V. mungo* field. The density dependence of the parasitoid was examined by collecting all the larvae from 1m^2 area from 5 randomly selected spots in field. The larvae thus collected were reared separately as described above. The numbers of parasitised and surviving larvae were recorded on subsequent dates till completion of larval development. These estimates were used to determine the temporal trends in density dependence. The mortality due to larval parasitization was expressed as K-value (a measure of killing power) by the function $K = \log (N/S)$, where N = total number of larvae collected and S = number of unparasitized larvae. The relationship between K and larval density was fitted into density dependence model ($K = aN^b$) where a and b are constant (b determining the degree of density dependence). If the slopes were $0<b<1$, the parasitization was taken as the density dependent.

Rearing of host insect

A culture of host insect was maintained in the laboratory from field collected larvae which were grown in specimen jars measuring 20 x 10 cm provided with mulberry leaves daily. Pupating larvae were shifted to another jars provided with leaves and mixture of sand/soil. The pupae formed were thus collected, sexed, sterilized and kept for emergence. Emerging males and females were allowed to mate in separate jars @ 3: 3 and kept separately in small plastic containers for ovipositon. The laying females were rejected after first egg laying and freshly hatched larvae were fed on tender mulberry leaves.

Parasitoid multiplication

The nucleus culture of the parasitoid was maintained in the laboratory from field collected larvae of *S. obliqua* maintained separately in plastic vials. The respective parasitoids that emerged from the host body formed cocoons were then harvested, collected and placed in glass jars (20 x 15 cm) covered with muslin cloth till emergence. The adults were fed on honey (50 %) streaks supplemented with water soaked raisins and allowed to mate in glass chimneys. Laboratory reared optimum instar larvae were provided to the gravid female of each parasitoid for parasitization and oviposition. The parasitized larvae were fed on tender mulberry leaves in separate containers and maintained in laboratory at 26 ± 2°C and 70 ± 10 per cent relative humidity and 10: 14 (L: D) h photoperiod.

Mating and ovipositon behaviour

Ten sets of freshly emerged females were placed in clean glass tubes (5 x 2 cm) in the ratio of 1:1 and kept under observation every half and hour for period of 6 hours.

The biological attributes such as longevity, development biology, emergence rate and sex ratio were studied in as under:

Longevity

Ten of female of respective parasitoids were maintained individually in glass vials (2.5 x 7 cm) soon after emergence and provided with honey (50%) solution as adult food. The longevity was recorded daily until death of parasitoids.

Developmental period, emergence rate and sex ratio

The parasitized larvae were kept individually in plastic containers and observed daily for exit of final instar larvae spinning the cocoon for determining the egg-larval period of parasitoid. To record the pupal period, the newly formed cocoons were separated and observed after every 24 hours for emergence of parasitoid. The proportions of adults emerging from total no. of cocoons were multiplied by 100 to calculate the emergence rate and the number of males and females after emergence were counted and sex ratio was ascertained.

Interaction / competition among promising parasitoids

As the parasitoid, *G. agamemnonis* and *Meteorus* sp. appeared simultaneously attacking 3rd to 4th instar larvae and their activity period coincided with each other. Therefore, this stage larvae (N = 50) were exposed to female of both parasitic species simultaneously in order to know their natural co-existence or to find out any kind of interaction /direct competition among them. The parasitized larvae were reared on fresh mulberry leaves until the final exit of the parasitoid from host body.

RESULTS AND DISCUSSION

Field parasitism

Laboratory rearing of field collected larvae of *S. obliqua* revealed parasitism by three braconid parasitoids *viz.*, *Meteorus* sp., *Glyptapanteles agamemnonis* (Wilkinson), and *Cotesia ruficrus* (Haliday) and one ichneumonid, *Agathis* sp. However, the nuclear polyhydrosis virus of this pest remained as the most important mortality factor during the period of study. Battu (1982) has reported natural incidence of this virus on *S. obliqua* up to 80.0 per cent in U. P. and Punjab. The prevalence of these parasitoids on *S. obliqua* is well established (Singh *et al.*, 1995; Varatharajan *et al.*, 1998) except *G. agamemnonis*, which is recorded for the first time. Mean percentage parasitization by *Meteorus* sp. ranged from 17.74% (2002) and 15.05 to 70.74% during 2003 which is in agreement with Pal *et al.* (1999) who reported its parasitism rate ranging from 37.5-52.2 per cent. Similarly, the mean parasitism by *G. agamemnonis* ranged from 6.45 to 12.20 and 4.3 to 13.72 per cent during the respective years (Fig. 1). According to Siddiqui (1981), the combined parasitism by this parasitoid and *Meteorus* sp. on *Amsacta moori* was about 30.8 per cent. *Cotesia ruficrus* parasitised a mean of 28.57 per cent larvae. Kalra (1984) found this parasitoid active from September to November with 8.0-10.0 per cent parasitism. The parasitic activity followed a definite succession wherein it was intresting to note that *G. agamemnonis* and *Meteorus* sp. were first to appear and cause parasitism on this pest (37-39 standard week - SW) in September with a maximum temperature range of 29.9 – 30.4° C and minimum temperature range of 18.9 to 20.9°C. Both these parasitoids remained active up to 43 SW when the minimum temperature reached below 14.0°C with a

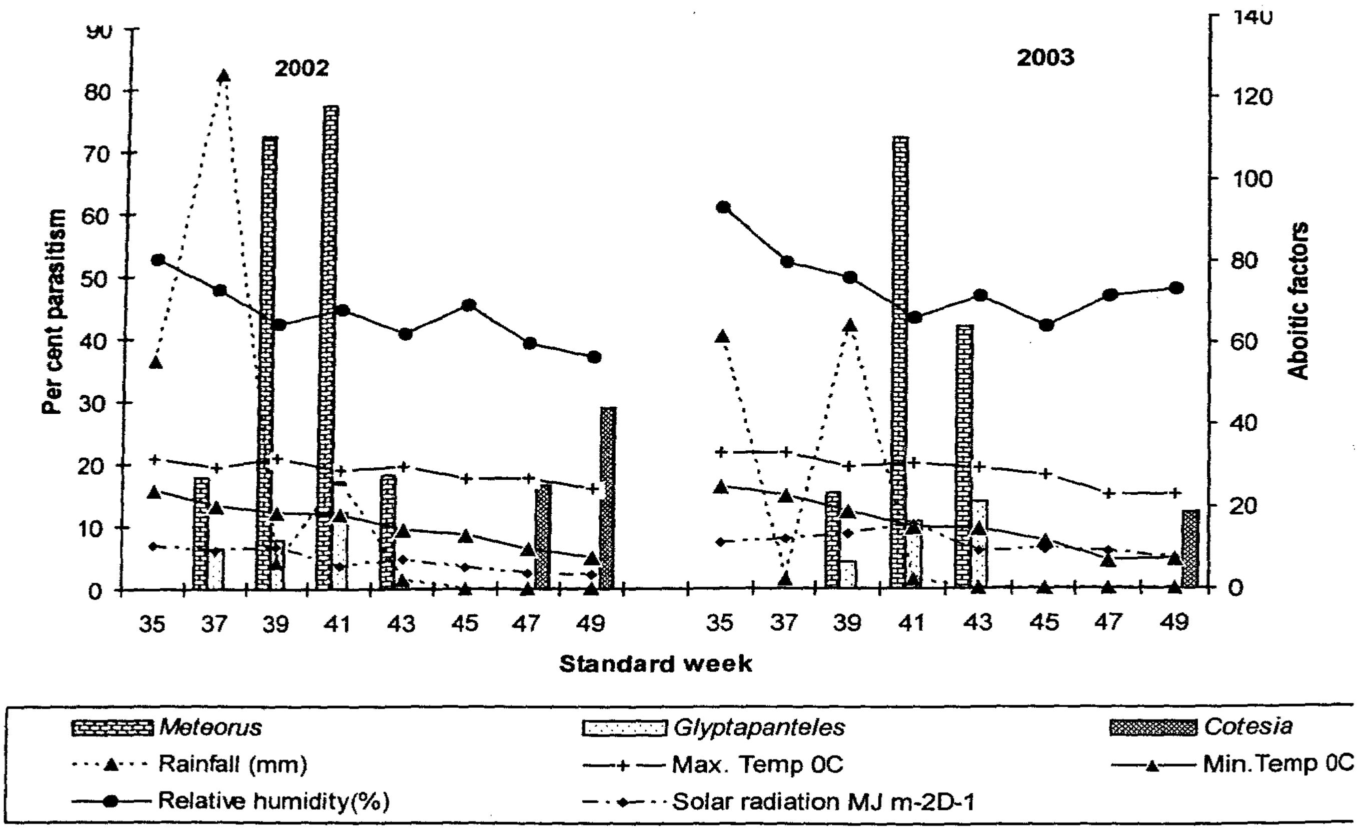

Fig1. Natural parasitism by braconid parasitoids of *S. obliqua*

peak parasitism coinciding with a temperature range of 29.2 to 30.6°C (Max. temp) and 14.7 to 18.5°C (Min. temp) and relative humidity ranging from 66.0 to 71.5 per cent. However, *C. ruficrus* and *Agathis* sp. were noticed later in the season (47-49 SW) when the mean minimum temperature reached below 10.0°C .The period of activity of these parasitoids is in agreement with (Kalra, 1984).

The simple correlation between aboitic factors and parasitism (pooled data) revealed that among the three braconid parasitoids recovered from this pest, the parasitic activity did not show any significant correlation with aboitic factors except *C. ruficrus*, which exhibited significant negative correlation with maximum (r = -0.55) and minimum (r= - 0.57) temperatures (Table 1). Regression studies on data pooled over two years indicated combined influence of aboitic factors on parasisitism by *Meteorus* sp. *G. agamemnonis* and *C. ruficrus* up to an extent of 42.5, 24.4 and 47.9 per cent, respectively. The regression equations for the respective parasitoids are presented in Table 2. The relationship between larval density of *S. obliqua* and K–value indicated negative slopes for *G. agamemnonis* (b = 0.25) and *Meteorus* sp. (b = 0.38) during the period of activity of these parasitoids. Gupta and Raj (2003) demonstrated similar kind of relationship in *Helicoverpa armigera* parasitized by *C. chlorideae*. The relationship fit into density dependent model $K = aN^b$ which implied that the parasitization was density dependent (Fig. 2) indicating the need for conservation of promising parasitoids through non-disruptive strategies. Larvae collected from different wild hosts revealed differential parasitism (Fig. 3). It was interesting to observed that *G. agamemnonsis* was recovered from the larvae feeding on all the three hosts *viz.*, *V. mungo*, *P. hysterophorus* and *X. strumarium*. However, *Meteorus* sp. was never recovered from *P. hysterophorus*.

Table 1. Correlation co-efficients of *S. obliqua* larval parasitism by braconids and aboitic factors (Pooled data)

Parasitiod	Temperature °C		R.H. (%)	Total rainfall (mm) $MJ/m^{-2}/d^{-1}$	Solar radiations
	Maximum	Minimum			
Meteorus sp.	0.32	0.19	-0.23	-0.05	0.25
G. agamemnonis	0.25	0.19	-0.10	0.10	0.26
C. ruficrus	-0.55*	-0.57*	-0.49	-0.27	-0.06

*Significant at P = 0.05

Table 2. Multiple Regression analysis of larval parasitism by braconids and abiotic factors (Pooled data)

Parasitoids	Regression equation	Co-efficient of multiple determination (R^2)
Meteorus sp.	$Y = 306.65 - 0.23\ x_1 - 6.88\ x_2 + 7.74\ x_3 - 3.36\ x_4 + 3.38 x_5$	0.42
G. agamemnonis	$Y = 38.71 - 2.57 \times 10^{-3}\ x_1 - 0.83\ x_2 + 0.87\ x_3 - 0.42\ x_4 + 0.60\ x_5$	0.24
C. ruficrus	$Y = 16.65 + 3.07 \times 10^{-2}\ x_1 + 0.34\ x_2 - 0.73\ x_3 - 3.78 \times 10^{2}\ x_4 - 1.08\ x_5$	0.48

x_1 = Total Rainfall (mm); x_2 = Maximum Temperature(°C); x_3 = Minimum Temperature(°C);
x_4 = Relative Humidity(%); x_5 = Solar Radiations ($MJ/m^{-2}/d^{-1}$)

Interaction among promising parasitoids

Studies on interaction/ competition among promising parasitoids revealed that in general no progeny was obtained owing to the early death of host except few cases (3 larvae) wherein progeny of *Meteorus* survived. However, in nature simultaneous occurrence of these parasitoids were never obtained. Similar kind of interaction has been observed between *A. ruficrus* and *Meteorus rubens* on *Agrotis ipsilon* (Awadallah *et al.*, 1995).

Parasitic behaviour, oviposition and developmental biology

G. agamemnonis

The field studies revealed that the adult parasitoids were noticed in quite large numbers on *Xanthium strumarium* plants supporting gregariously feeding larvae of susceptible stage, especially during morning hours. It was found that the female parasitoids (3-12 nos.) displayed continuous efforts for parasitizing a single group of 105 to 213 larvae found on a single plant. Although, the parasitoid was very active and made several attempts to oviposit in the host larvae, yet the success rate was low due to typical defensive behaviour/ mechanism exhibited by the host larvae. Firstly, the silken threads around larvae made the parasitiod inaccessible to host and secondly the host larvae displayed typical behaviour wherein all the larvae feeding gregariously on a single plant reacted sharply to parasitizing adults by lifting their anterior body upwards in the air in a synchronized manner resembling snake like posture with a typical vibrating sound. Due to this kind of group behaviour, the gravid female preferred to parasitize the few larvae feeding in isolated manner on certain leaves. The defensive behaviour in various hosts

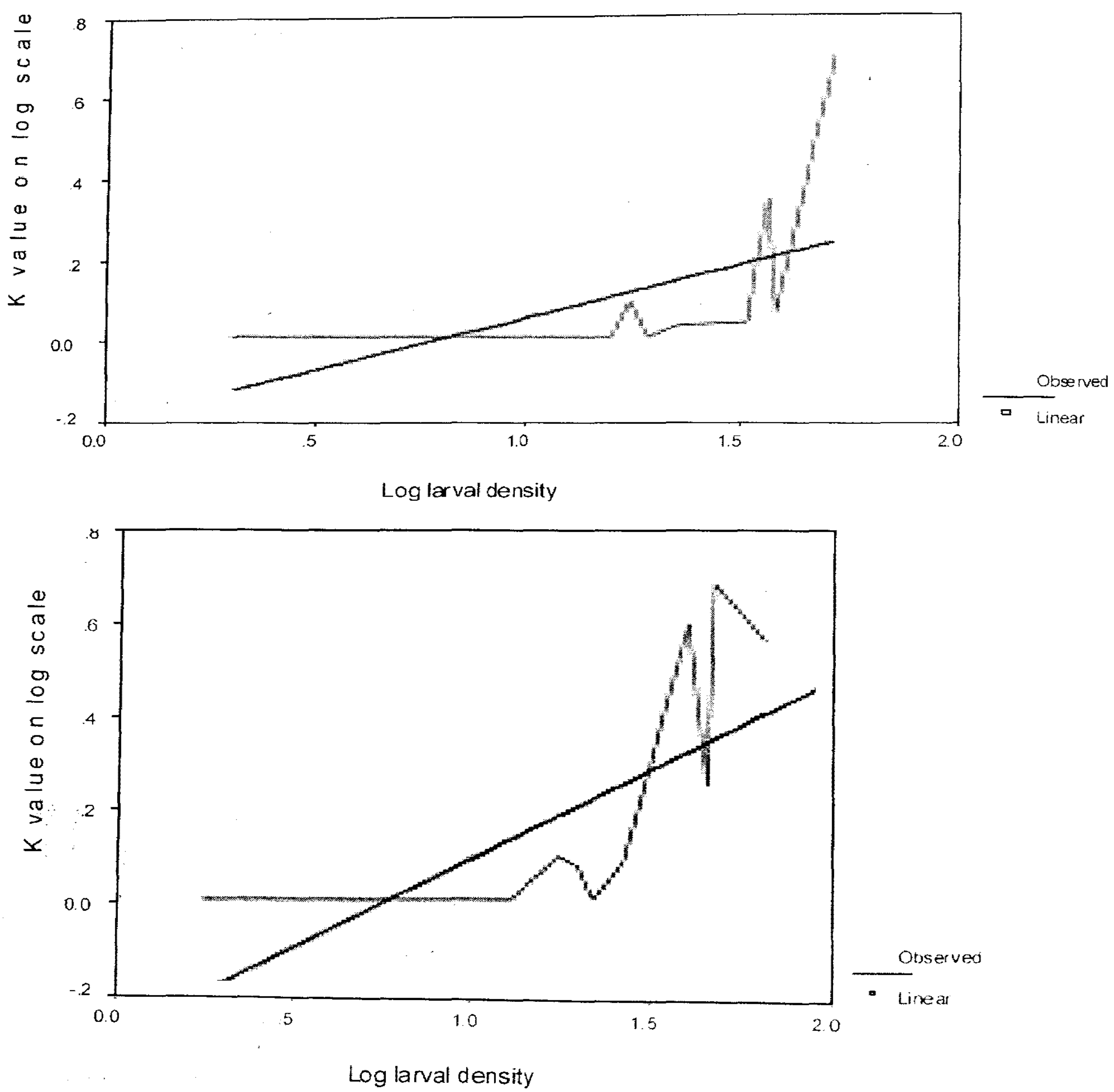

Fig 2. Relationship between log larval density of *S. obliqua* and K-value of the parasitoids

against braconid parasitoids is well documented (Narendaran, 2001), but the present findings add to our understanding on the newly reported parasitic behaviour exhibited in field.

In laboratory, it was found that the adult after emergence, were ready for mating immediately. Females were receptive soon after emergence and the males were strongly attracted towards the female, displayed excitement vibrating its antennae and fluttering its wings internally upon location of female and mounted over them within 10-20 seconds. The receptive female stopped further necessary movement and co-operated for successful insemination, which took about 30-50 seconds. The gravid female on becoming aware of

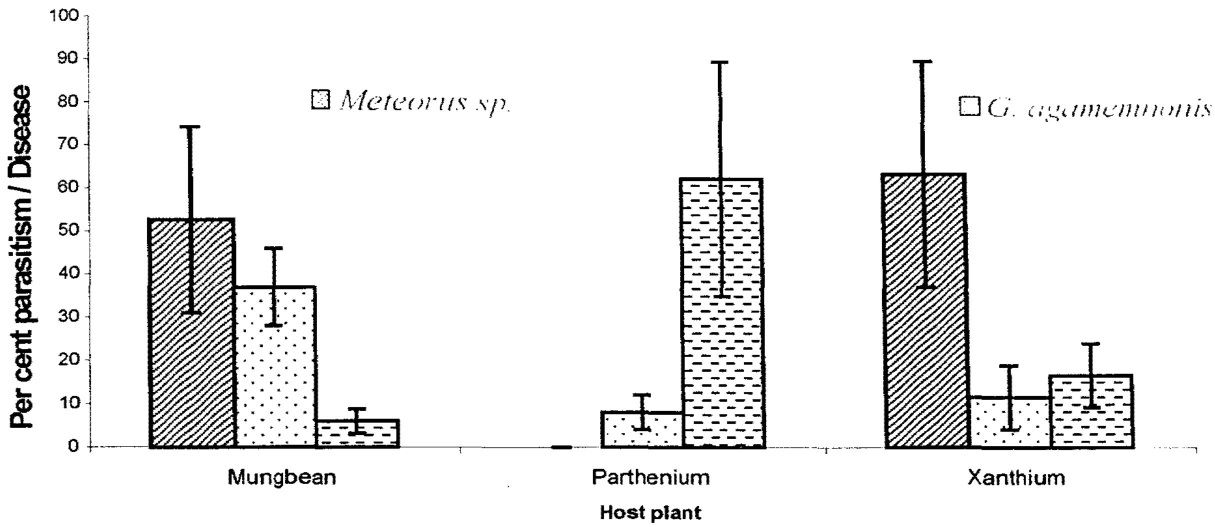

Fig 3. Prevalence of natural enemies *S. obliqua* on cultivated and wild hosts

the presence of suitable host, began to rub its hind leg against the tip of its abdomen and palpated the host by tapping the antennae on it and took 2-11 seconds for successful oviposition with *an* average of 6.12 ± 1.89 seconds. The ovipositon behaviour described above is common in parasitic hymenoptera especially genus *Apanteles* sp. (Gosh, 1999).

The ovipositon commenced on the same day of emergence and female parasitoid were found to oviposit anywhere in the body cavity of host larvae except head region. The maximum oviposition was found in the anterior dorsal region followed by posterior dorsal region, but on ventral side it was rarely observed. The egg larval period ranged from 18-21 days with a mean value of 19.1 ± 1.20 days (Table 3). After the larval period, the pre-pupa cut its way out of host from either side of latero-ventral region of larvae and started spinning of cocoon. The external case of cocoon was loosely spun while the inner one reinforced and tough. Cocoons were cylindrical and pale whitish or white. The total period from cocoon formation to adult emergence ranged from 5-8 days with a mean value of 6.8 ± 1.03 days. The adult emerged by cutting a circular opening at the cephalic end of the capsule. The emergence rate was 85.0-97.0 (mean 92.6 ± 6.2) per cent with a sex ratio of 1.6:1 (F:M). Female survived for longer period than males. If not provided food the adult survived for 3-5 days with a mean value of 4.5 ± 0.53 and the longevity increased up to 8 days, if honey was provided to the adults.

Host larvae were parasitized during pre-moult to the 2nd instar, 3rd instar and even up to 4th instar larvae but the percentage of success decreased markedly with the age of host at the time of parasitization. Superparasitism was common in pre-moult and 1st instar of larvae offered and the super parasitized larvae died without further development. Gosh (1999) made similar observations on biology and parasitism of this parasitoid on *Perricallia ricini*.

***Meteorus* sp.**

The parasite was found attacking 4th to 5th instar larvae. The behaviour of parasitization was typically of braconid type, comprised the tapping of host larvae with the help of antennae followed by ovipositional attempts. In some cases, many attempts were made and the abdomen was even inserted at 2-5 places in the abdomen of single larvae. Unlike, *G. agamemnonis* defensive behaviour was exhibited by quick movement of body in zig zag manner by the larvae, which though remain sluggish otherwise. The incubation period lasted for 4 -5 days and the larvae came out of the

Table 3. Developmental biology, emergence and sex ratio of parasitods of *S. obliqua*

Attribute	Parasitoid species		
	Meteorus sp.	*G. agamemnonis*	*C. ruficrus*
Egg – larval period (days)*	10.50± 1.08 (9-12)	19.1 ± 1.20 (18-21)	10.0± 1.05 (9-12)
Pupal period (days)*	7.90± 1.10 (6-9)	6.80 ± 1.03 (5-8)	7.90 ± 0.87 (6-9)
Longevity with out food (days)*	4 .20± 1.03 (3-6)	4.50 ± 0.53 (3-5)	4.10 ± 0.99 (3-6)
Longevity with food (days)*	10.2 ± 1.05 (8-13)	6.50 ± 0.80 (5-8)	11.8 ± 1.61 (10-13)
Emergence (%) +	91.8 ± 6.1 (83-95)	92.6 ± 6.2 (85-97)	89.0 ± 5.30 (79-94)
Sex ratio (F: M) +	1.3 : 1	1.6: 1	1.5 :1

*Values are mean of 20 observations; + N = 50 values in parentheses indicate range.

body of the host within 9-12 days (mean 10.5 ± 1.08) and pupated immediately within 1 to 3 hours of emergence. The total duration of egg to adult stage in 2nd to 3rd instar larvae was found to be 22.6 and 25.5 days, respectively (Pal *et al.*, 1999). It was interesting to observe that although parasitoid grub emerged from the host larvae provided with the leaves placed on moist agar in individual container, yet none could spin its cocoon. The parasitized caterpillar remained alive from 4-6 days of emergence of parasitoid from its body, though remained sluggish/ sedentary and didn't feed. Ghosh (1999) reported that the *Perricilia ricini* larvae parasitized by *G. agamemnonis* lived for 4-5 days after exit of parasitoid grub.

The mature grub was cream colored and measure about 4.01-5.10 mm long and 1.50 to 1.75 mm wide and started spinning of cocoon by typical backward and forward movement of head. In all the containers only one larva came out of the body of caterpillar. The parasite suspended the cocoon by a slender swollen thread attached to the muslin cloth tied on top of container. The cocoons were usually brown, and elliptical and measure about 4.0-5.0 mm long and 1.68 to 2.20 mm wide. In most of the case the solitary cocoons were found suspended by a long silken thread from top cover, but few field-collected cocoons were found attached in clusters to the host larvae. The Pupal period ranged from 6-9 days and adult parasitoid survived only for 3-5 days only. The observations regarding developmental biology of this parasitoid corroborate the findings of (Singh and Singh, 1995). The mean emergence rate of the parasitoid varied from 83.0 -95.0 per cent with a sex ratio of 1:1.3 and the adults survived for 8–13 days with food.

C. ruficrus

It emerged from 3rd to 4th instar larvae as endo-parasitoid with a parasitism rate varying from 4.0 to 8.5 per cent during November to December. Oviposition to larval egression in 3-5 day old larvae took 10.0 ± 1.05 days (9-12) with a pupal period lasting for 6-9 days (7.9 ± 0.87). Adult survived for 4.1 ± 0.99 days without food and 11.8 ± 1.61 days with food. The cocoon emergence rate was 89 per cent. Naganagoud and Kulkarni (1988) found that the parasitoid took a period of 16.1 days for adult emergence with a egg-larval period of 11.9 days and pupal period of 4.1 days on *Mythimna separata*.

It was concluded that the braconid parasitoids are essential biotic component that contribute to the natural regulation of *S.obliqua* population in existing agro ecosystem of Jammu plains. Due to polyphagous nature of the pest, the use of chemical pesticides will not only bring insignificant suppression of this pest on a particular crop but may also prove disruptive to the parasitoids. Henceforth, the present investigations warrant more attention towards the conservation of these parasitoids as well as to explore the bio control potential of promising parasitoids and nucleopolyhedrovirus (NPV) of this pest

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