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Biology and damage of Asian walnut moth, *Garella musculana* (Lepidoptera: Nolidae), a major insect pest of *Juglans regia* in Kashmir Himalaya

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Abstract. Over the past two decades, *Garella musculana* (Erschoff, 1874) (Lepidoptera: Nolidae) has surged as a major pest in Eurasia, infesting *Juglans regia*. Its caterpillar causes extensive damage to young fruits and shoots of natural and cultivated varieties of *J. regia*. The larval instars were seen feeding on nuts from May to August. In August, they started leaving nuts for pupation to continue the life cycle next year. The pupation took place inside the loose bark or crevices of walnut trees in white cocoons. In this study, the life cycle and extent of damage was studied. The larva goes through five growth stages (instars) and lasts for about 67.1 ± 2.26 days. It overwinters in the pupal stage in a dense white cocoon and then emerges in spring as adult when the environment becomes favorable. The mean generation time was 297.5 ± 4.45 days. It is univoltine in nature and its larva consumes young walnut pericarp resulting in deformed nuts and their early fall, leading to substantial yield losses. The deposition of excrement by *G. musculana* larva on the outer green thick layer (husk) degrades the quality of the walnut and thereby reduces its economic value. Knowledge of life-history is crucial for the design of management strategies. The management options of this pest are limited as the destructive feeding larvae are present within the shell or nut. Understanding the life cycle is important to spray timing and effective control. The emergence of the pest and egg laying are important attributes in controlling this pest. Besides, natural enemies as eco-friendly approaches acting as biocontrol agents are encouraged as the most viable alternative for management of this insect pest in this particular area. It is also important to promote the development of new walnut cultivars in newly planted walnut orchards with high potential output and higher shelling percentages.

Keywords: Asian walnut moth, damage, life cycle, rearing, walnut

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Introduction

Juglans regia L. is native to central Asia (Leslie & McGranahan, 1998) and consists of 7 to 45 species (Aradhya *et al.*, 2007). It is one of the 22 various walnut species that also grows in continents of Europe and America (Zhang, 2019). It is known for its wood, nutrient-rich kernels (Bayazit *et al.*, 2007) and, nutritionally a high-

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demand crop (Hassankhah *et al*., 2017) incorporated by FAO in the catalog of priority plants due to its nutritive values (Gandev, 2007; Raja *et al*., 2017). It flourishes well at an altitude of 900-3500 meters above sea level and the agro climatic conditions suitable for producing high quality walnut lies in the northwestern Himalayan region of India in which Jammu and Kashmir produces the main share of its export (about 98%). The walnut is grown as a cash crop around the world (Riedl *et al*., 1979; Leslie & McGranahan, 1998). The walnut industry has a significant role in J&K's economy as kernels and unshelled walnuts are being exported to about 23 foreign countries fetching crores of rupees annually. The walnut industry is an important sub sector in horticulture as it provides employment and income generation to poor farmers in rural areas.

The walnut crop in Kashmir Himalaya is grown organically; however, different insect pests damage their kernels, leaves, branches and trunk (Mir & Wani, 2005). Most pests are leaf defoliators (Abbas *et al*., 2015), resulting in decreased photosynthetic activity (Mohandas *et al*., 2004). Sometimes, inside the root and stem are long tunnels formed by larva and advance upwards (Khan *et al*., 2013). Some of the pests that cause damage to walnut trees include the codling moth- *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae), leopard moth- (*Zeuzera pyrina* Linnaeus) (Lepidoptera: Cossidae), gypsy moth- *Lymantria obfuscate* (Walker) (Lepidoptera: Lymantriidae), walnut blue butterfly *Chaetoprocta odata* (Hewitson) (Lepidoptera: Lycaenidae), *Aeolesthes sarta* (Solsky) (Coleoptera: Cerambycidae), walnut husk fly-*Rhagoletis completa* (Cresson) (Diptera: Tephritidae), walnut aphid *Chromaphis juglandicola* (Kaltenbach) (Hemiptera: Aphididae), and Asian walnut moth, *Garella musculana* (Erschoff) (Lepidoptera: Nolidae). In J&K, the Asian walnut moth was reported for the first time in 2011 (Khan *et al*., 2011) and is a severe pest as its larva attacks the fruits and young shoots of walnut trees. Usually, a single larva harbors the fruit, sometimes two, and rarely three or more. One caterpillar may destroy several fruits. The caterpillar of the insect pest also attacks shoots causing wilting of shoots which is more problematic for young trees. Usually, the green crust pericarp is eaten by the larva, which damages it and impedes normal fruit development. As a result, the yield in the walnut drops by about 70-80% (OEPP/EPPO, 2005). In Kyrgyzstan between, 1986 and 1988, it was figured out that the financial loss of the walnut growing companies was between 25500 and 52000 rubles per year. In plantation forests, approximately 60% of young shoots and 8% of fruits got spoiled. This damage is about 01 percent in young sprouts in natural forests and 42 percent in fruit trees (Orozumbekov & Moore, 2007). In studies conducted in Tajikistan, 66.7% of walnut fruits were damaged and it also has been found that yield loss varied between 20-50 kg due to the damage of *G. musculana* in walnut trees that are 20-30 years old (Sangov, 2015). *Garella musculana* also attacked the walnut trees of Europe (Ukraine in 2008, Bulgaria and Turkey in 2019, Romania in 2021 and Italy in 2022) (EPPO, 2022).

Our study aim was to observe the complete life cycle of *G. musculana* and the extent of damage it causes to *J. regia*. The results may provide a guideline for integrated pest management and could be used in predicting the occurrence of larvae and overwintering pupae so that actions could be taken to reduce the loss caused by this pest. It will also help to establish standard procedures for producing *G. masculana* laboratory colonies that provide the baseline for insect management for production agriculture.

Materials and methods

Study area

The field surveys were conducted in various districts of Kashmir to record the infestation of the *G. musculana.* At all places, walnut trees suffering from *G. musculana* attack were selected randomly and shoots/fruits were examined for any egg, larva, or pupa. Sampling of adult moths was done in the field by using the insect nets.

Laboratory Rearing

The biological studies of *G. musculana* were conducted in a makeshift laboratory established at district Budgam of the study area. The adult moths collected from the field were endorsed to copulate separately in wire meshed cages with dimensions of 2.0 ft, 1.5 ft and 1.0 ft in length, breadth and height, respectively, in the laboratory. Each pair was observed for pre and post mating behavior. Some fresh leaves, paired nutlets, and tender branches were collected from the canopy of several nearby trees and kept in a container for egg laying. All these were

changed till egg laying was observed. Artificial food was provided to the moths by hanging cotton balls soaked in honey solution in the center of the meshed cages. The eggs laid were transferred carefully to sterile petri dishes of dimensions (09×01) cm, lined with wet filter paper and provided with fresh walnut leaves and small twigs. The food and filter paper lining were changed with fresh stock on subsequent days. Besides, egg laden fruits and shoot masses from the fields were plucked carefully and kept in petri dishes of dimensions (12.0×1.2) cm lined by moist filter paper. Paired nutlets and tender branches with some large nuts were kept for feeding. The neonate larvae were observed daily and examined for their biological information from molting to emergence. A total of ten larvae were used to determine the number of instars each representing one replication. The larvae lengths were measured carefully by digital vernier caliper and or scale and the mean was calculated. Furthermore, the size and color of pupal cocoons were also measured/observed and the mean were calculated. Identification and labeling were made using standard taxonomic literature of Vassiliev, 1912; Degtyareva, 1964; Dzhaparov, 1990. Hind wing shows Sc+R1 arrangement which is the characteristic feature of the family Nolidae. Collected insects were identified with the help of running keys of Erschoff, 1874; Fibiger *et al.*, 2009.

Data analysis

Biological parameters such as duration and size of each developmental stage were analyzed using descriptive statistics with the calculation of means and SD.

Results

Adult morphology

The adult size of the body ranges between 8-9 mm (8.5±0.52). The adult wing span is 18-23 mm (20.7±1.88) in females, whereas it is 15-18 mm (16.8±1.22) in males. The measurement is based on ten adults each. The thorax is grey or brownish grey with dark transverse stripes. The wing fringe is grey with black points. The fore wings are in general laden with grey transverse brown, white, and black bands and lines while the hind wings are monochrome grey (Fig. 1A &B).

Copulation

The male exhibits pre-copulatory behavior such as twitching of the antennae and shaking of the body to persuade the females for courtship. Female moths respond by protracting and retracting the last abdominal segment of their bodies at the same time. Mating was observed in the afternoon as well as in the evening and lasted for about 30 minutes to 60 minutes under lab conditions.

Oviposition

The females lay their eggs on young nuts at the point where a pair of nuts are touching or on the buds of one-year-old shoots of walnut. It usually lays 2 or 3 eggs at a time. The egg is yellowish-grey, spherical, 0.5mm in diameter and, dorso-ventrally flattened.

Incubation period and hatching

The incubation period was recorded between 5-8 days (6.5±1.26 days). The minimum and maximum temperature was recorded as 3-27 °C (15 °C) and minimum and maximum humidity was recorded as 32- 96 % (64%) during the whole duration of the life cycle. During the incubation period, the day temperature and day humidity recorded were 20.75±2.50 °C and 83.75±5.87 % and the night temperature and humidity were6.61±2.92 °C and 47±1.74 %. Hatching of the eggs takes place between 5-8 days; the larva usually enters the young nuts and shoots (Fig. 1 C, D & E).

Description of instars of larva

The larva possesses a brown colored body and dark-brown colored head. All the larval instars have been reported and photographed during the life cycle of the pest. The first instar is light brown in color and ranges in size from 3-5 mm (4±0.81). The second instar is light brown in color but slightly darker than the first instar, with a dark-brown colored head. It ranges in size between 6-8 mm (7±0.81). The third instar intensifies the growth and the rest of the features are the same as in the second instar. It ranges in size between 9-12 mm (10.8±1.22). The

segmentation is clearer than the second instar. It has brown spots with posterior ones larger in size. The body possesses the setae. The fourth instar intensifies the growth and the rest of the features are the same, with more clarity than earlier instars. It is 14-16 mm (15.1±0.87) in length and 2-2.1 mm in width. It has brown spots with clear setae. The fifth instar is a mature larva and all features are well distinct. It is dark brown in color with visible segmentation of the body. The setae are well developed. It ranges in its size between 16-18 mm (17±0.81). Dark brown spots are distinctly present on the body. All the larvae are shown in Fig. 1 F.

Larval duration

The five larval instars have been ascertained during the life cycle of the pest. A total of 12 replicates were used for studying the duration of each stage under laboratory conditions. The duration of 1st, 2nd, 3rd, 4th and 5th larval instars recorded from May to August is shown in tabular form (Table 1). The temperature and humidity play an important role in the longevity of life cycle. During the whole larval phase, the minimum and maximum environmental temperatures were recorded between 3°C and 33°C (18°C) and minimum and maximum humidity were recorded between 91-96% (93.5 %).

Pupa and Pupation

Before pupation, the mature larva leaves the fruit or shoot, for that reason it makes a round exit hole which is much larger than the entry hole and is filled with excrement (easily seen from outside) - the entry hole is much smaller and without excrement (Fig. 2 E & F). The larvae usually pupate in deep cracks in the bark or under loose bark in the butt part of the trunk up to several meters above the soil. The pupa was present inside the dense snow white cocoon tapering on both sides. It measures 9-11 mm (10±0.81) in length and 3.5-5.0 mm (4.25±0.67) in width (Fig. 2 B, C & D). The duration of the pupation lies in the range of 231.5±1.88 days.

Adult emergence

It was observed that as soon as the developing pupa reaches to adult stage, it exerts pressure on the pupal wall that results in its bursting on the anterior side of the pupal covering and emergence of the adult takes place (Fig. 2 A).

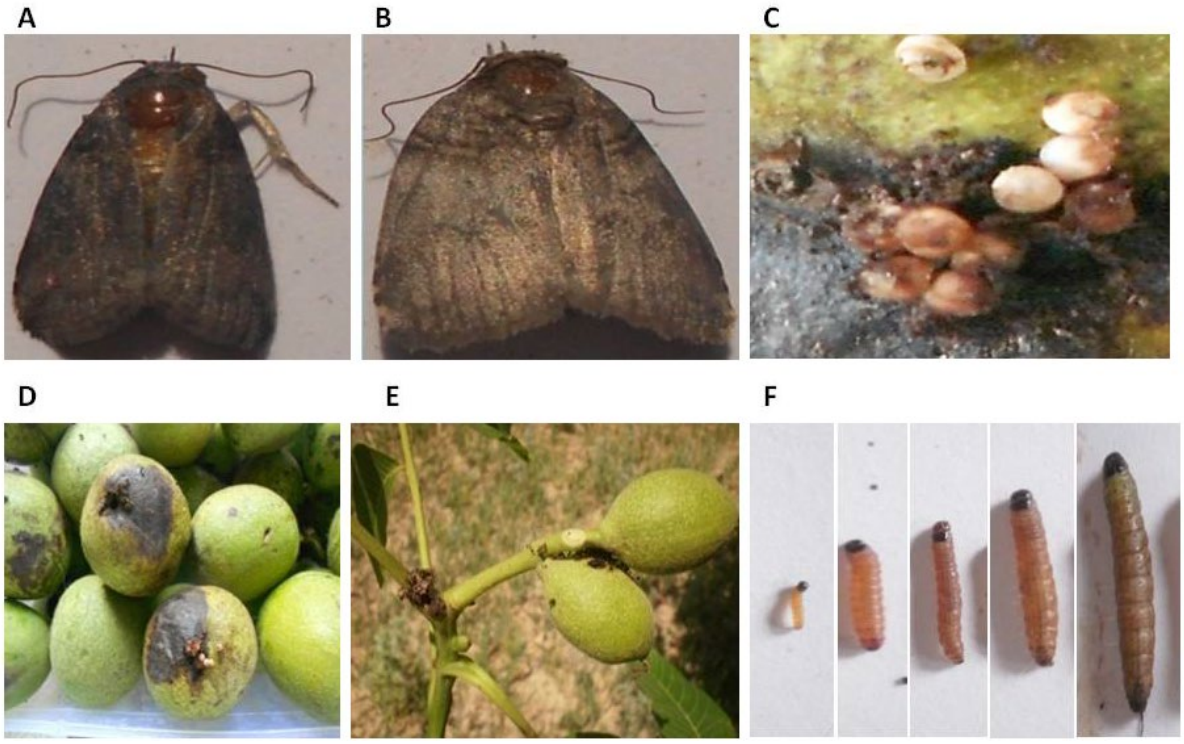

Fig.1. General Habitus of *Garella masculana* (Lepidoptera: Nolidae), their eggs and the initial stage of activity, damage on common walnut in Kashmir Himalaya with their larval instars. (A) Male, (B) Female, (C) Eggs, (D, E) Damage of first instar larvae after penetrating to fruit and shoot and (F) their different larval stages

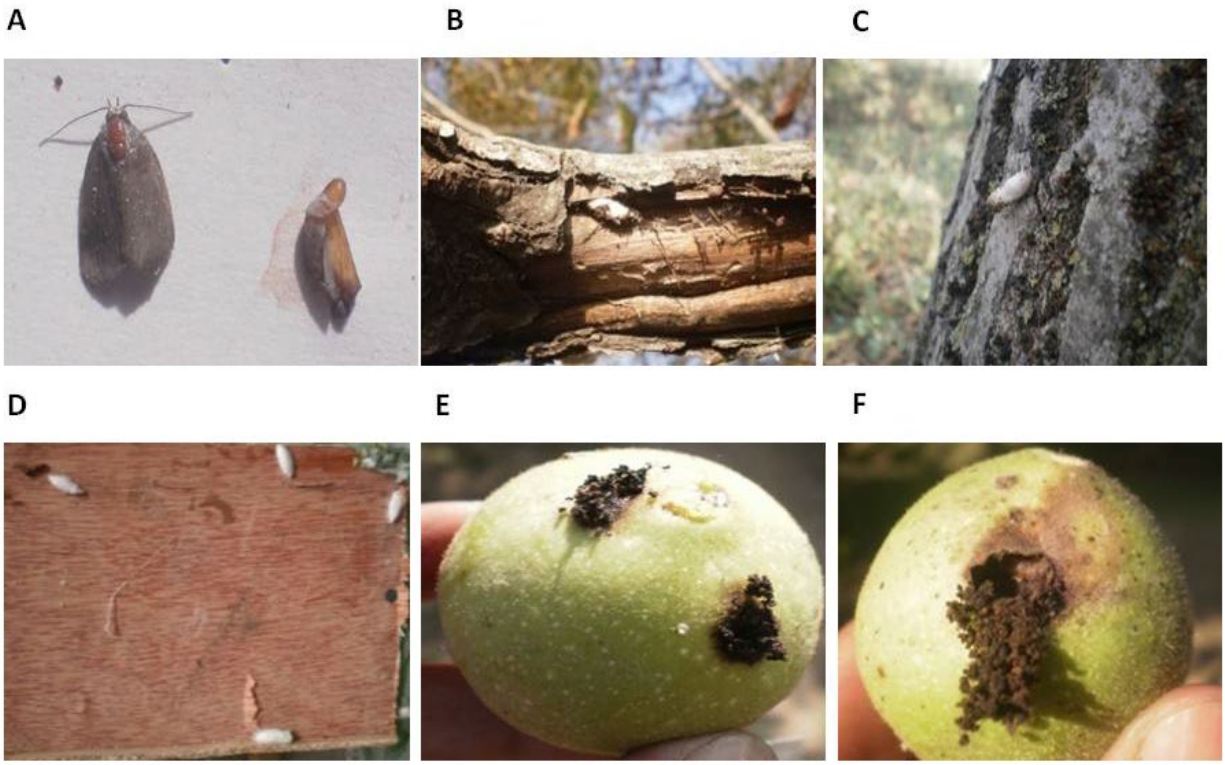

Fig.2. Emergence and pupation of *Garella masculana* (Lepidoptera: Nolidae) (A) Emergence of adult moth from its pupa (B, C, D) Pupation (white pupa) in cracks and under loose bark and (E, F) Entrance and exit holes of larvae on nut

Time Line of *Garella musculana*

The emergence, egg laying, development of larval instars, and pupation take place from May to August. The pupa, formed in August, undergoes overwintering to tide over the harsh conditions (rain, snow, freezing temperature, and frost) of the study area from October to April. The adults emerge from the overwintering pupae in April-May when suitable climatic factors set in and resume the new life cycle. A pictorial representation of the life cycle of *G. musculana* is shown in Fig. 3. The total life cycle duration was recorded between 275-320 days (297.5±4.45). Timeline is shown in Table 2.

Table1. Time line of *Garella musculana*

Life cycle stages	Time line Month	Average duration (days)	Range of size (mm)	Mean Temp.	Mean Humidity
Emergence	May	-	-	03-33ºC	91-96%
Egg Laying	May	-	-		
Incubation	May	6.5	-		
Larva Ist Instar	May	10.5	3-5		
Larva 2nd Instar	May	12.5	6-8		
Larva 3rd Instar	June	10.5	9-12		
Larva 4th Instar	June-July	14	14-16		
Larva 5th Instar	July-August	18.5	16-18		
Pupa	Sept. to April	231.5	9-11		
Total life cycle		297.5			

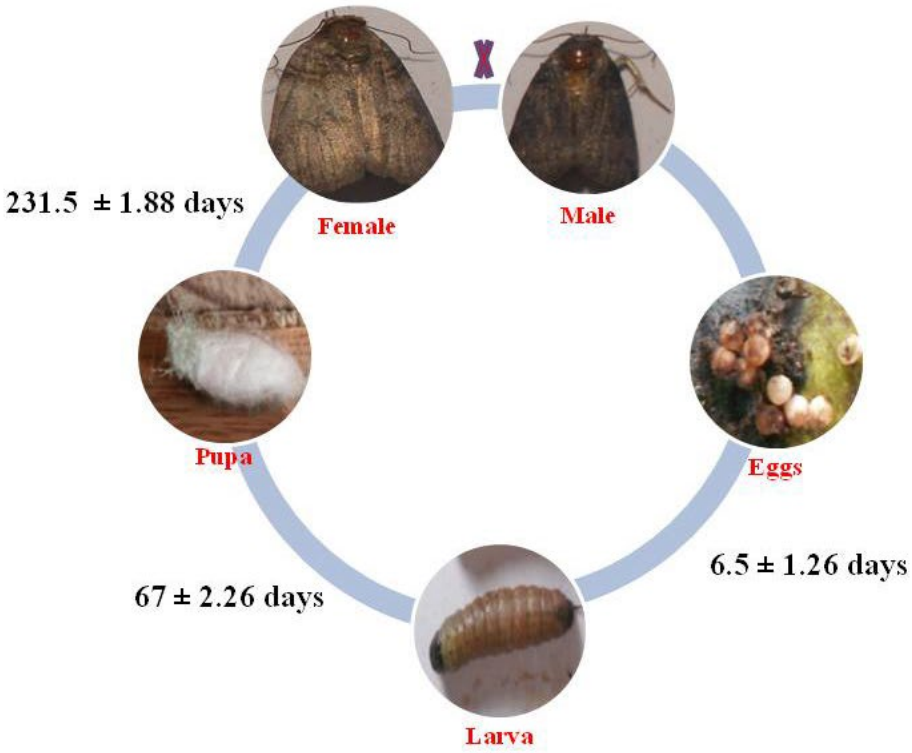

Fig.3. Life cycle of *Garella masculana* (Lepidoptera: Nolidae) on common walnut *(Juglans regia)* in Kashmir Himalaya

Extent of damage

Under present observation, the immature walnut fruits were observed with brown excrement accumulated in the exit holes by the caterpillars in the fruits and in the shoots. The fruits and shoots were observed with entry holes near the stalk end of the fruit and node of the shoot, respectively. The shoots, when opened, show the empty mid portion with excrement. There is yellowing and wilting of the infested shoots and black coloration of the fruits (Fig. 4 A-H). The first instar of the larva feeds usually during the green period of the fruit and impedes normal fruit development. They usually enter the fruit early during the watery stage and complete the development within the fruit resulting in malnutrition of the fruit. As a result, under natural conditions, the affected fruits fall off before they reach maturity. After finishing one nut, a caterpillar passes into another and continues to feed. The last stages of the caterpillars were found to feed on the green outer pericarp that renders the walnuts unmarketable as the aesthetic value of hard husk and nutritive qualities of kernels are badly affected and the people engaged with walnut cultivation in Kashmir experience their economic loss.

Discussion

Garella masculana is a serious insect pest of walnuts reported in some Eurasian nations. In J&K, walnut trees are attacked by this insect pest. An understanding of egg incubation period is also important for correct timing of insecticide applications to target the early instar of *G. masculana* prior to entering the fruit. Regarding the biological investigations, our observation revealed the developmental period of the larva as 68.5 days and, as per the study conducted by Yildiz *et al.* (2018), the duration of the larva lies in the range between 25-40 days. The extended larval life in our observation may be the quiescent nature at lower temperatures. A study by Battisti *et al.* (2005) and Toffolo *et al.* (2006) revealed that higher winter temperature had increased the larval survival of the pine processionary moth, *Thaumetopoea pityocampa* (Schiff) (Lepidoptera: Notodontidae), allowing its northern expansion in its range. In our investigation of *G. masculana* univoltine- one generation per year was observed which is not complementary to the study conducted by Bostanci *et al.* (2019) in Bartin, Turkey and EPPO (2005) where two to four generations were found.

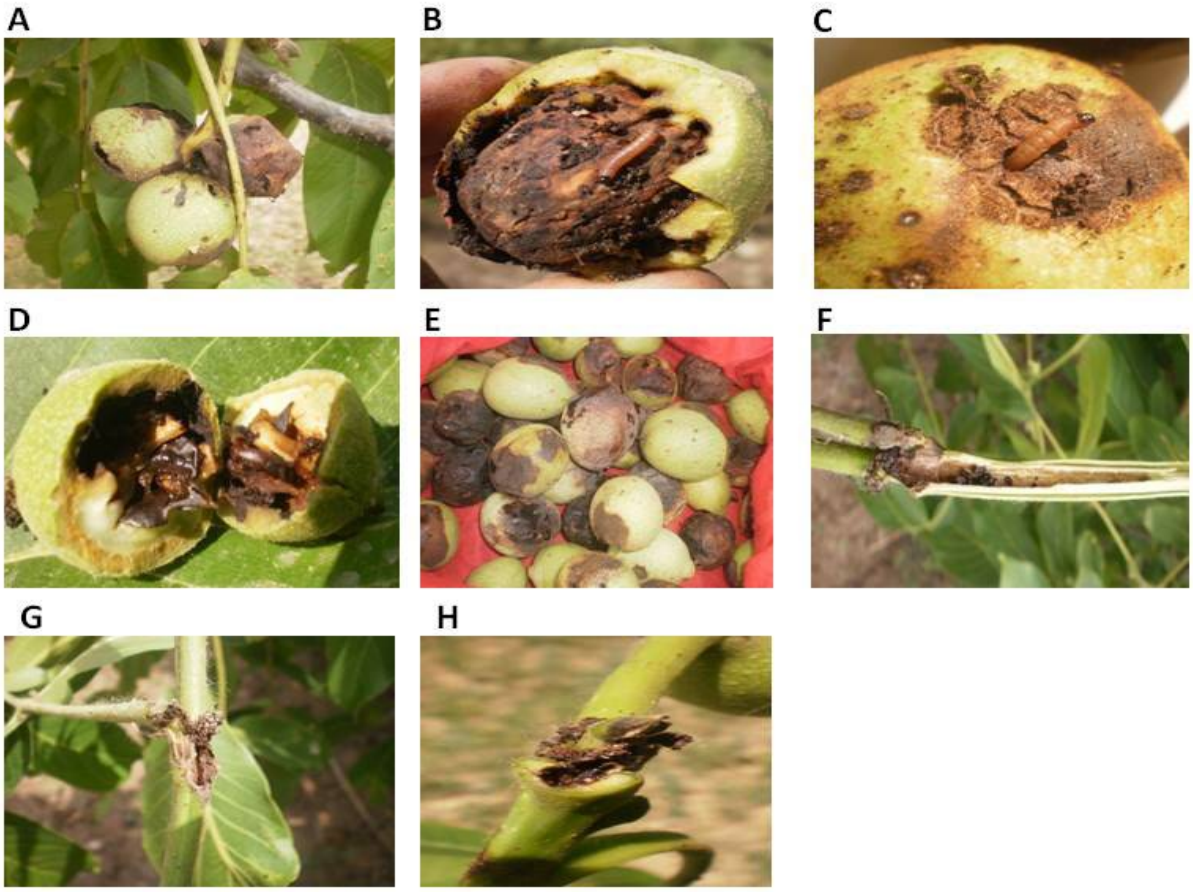

Fig.4. Walnut damage by the larvae of *Garella masculana* (Lepidoptera: Nolidae) (A, B, C, D, E) show fruit damage while (F, G, H) show shoot damage

The reason could be the altitudinal gradient and climatic factors. Temperature has a foremost attribute in development, fecundity, population growth and survival of insects (Howe, 1967; Dean, 1974; Deutsch *et al.*, 2008; Wang *et al.*, 2021). In our observation, we have found that its development undergoes five larval instars and four moultings which is also supported by the study carried out by the European and Mediterranean Plant Protection Organization (EPPO, 2005). After completing their development in nuts and stalk we have observed that the larvae leave the host tree *J. regia* in a typical head-to-tail procession to look for a suitable pupation site within its vicinity. The larva leaves the plant only to look for a place to pupate (EPPO, 2005). Usually, cocoon formation takes place in deep cracks or loose bark. The cocoon is a casing spun of silk by a lot of moths and caterpillars (Darby, 1958). We also found that the pupa overwinters (white cocoons) to avoid harsh atmospheric conditions, which is supported by the study of Yildiz *et al.* (2018) and EPPO (2005) with the exception that its pupae complete its development in roughly 10 days.

Table 2. Duration of egg and larval instars

S. No.	Parameters	Mean duration (in days ±SD)	Range in days
1	Incubation period	6.5±1.26	5-8
2	Larval period Instar I	10.5±0.51	10-11
3	Instar II	12.5±1.15	11-14
4	Instar III	10.5±1.19	9-12
5	Instar IV	14±0.69	13-15
6	Instar V	18.5±1.07	17-20
7	Total larval period	67.1±2.26	65-72

The reason of extended pupation could be the nutritional status of larva before pupation and to evade adverse conditions of winter temperature that plays an important role for the long-lasting diapauses in moths across their geographic range (Salman *et al.*, 2019). We observed pupae being present singly as well as in pairs (one generation per year) under loose bark whereas large aggregations of cocoons (round about 170 specimens) under loose bark of the trees (two to three generations per year) have also been observed as per the study of Vassiliev (1912) and Degtyareva (1964). This is supported by the fact that at optimum temperature of insects there is an increase in their biological metabolism and hence their activity (Jaworski *et al.*, 2013; Juroszek *et al.*, 2020). In general, the critical life cycle stages of insect pests are more or less directly influenced by temperature, humidity and photoperiods.

Regarding the extent of damage, the present investigation has revealed that the inflicted damage on host plants is in the form of yellowing and wilting of infested shoots and black coloration with multiple entry and exit holes. Both the young shoot and fruit of the plant are damaged by caterpillar of insect pest supported by a similar kind of damage observed by Bostanci *et al.* (2021). This similar observation was also observed by authors Vassilie, 1912 and Degtyareva, 1964.

Conclusion

Severe damage is caused by *Garella masculana* to *Juglans regia* in Kashmir Valley of Himalaya. The young shoots of walnut trees were badly affected in districts Budgam and Kupwara. This study provides the basis for decision-making about managing *G. musculana*, to avoid further economic damage. Moreover, another host *Juglans nigra* has also been found to be infested by this insect pest (Bostanci *et al.*, 2019; EPPO, 2020), so it is important to prevent and limit the spread of this pest through trade and regulate the movement through phytosanitary measures.

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زیست شناسی و خسارت شب‌پره آسیایی گردو، *Garella musculana* (Lepidoptera: Nolidae) ، آفت مهم گردو در کشمیر هیمالیا

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چکیده

در طول دو دهه گذشته، خسارت شب‌پره آسیایی گردو *Garella musculana* (Erschoff, 1874) (Lepidoptera: Nolidae) در اوراسیا افزایش یافته و آسیب‌های فراوانی روی گردو *Juglans regia* ایجاد کرده است. لارو آفت، آسیب زیادی به میوه جوان و شاخه درختان کشت شده و طبیعی گردو (*J. regia*) وارد می‌کند. لاروها از نیمه اردیبهشت تا نیمه مرداد ماه در حال فعالیت و تغذیه از میوه دیده می‌شوند. در اواخر مرداد ماه، لاروها با خروج از میوه وارد مرحله شفیرگی شده تا چرخه زندگی را در سال آینده ادامه دهند. محل تشکیل شفیره، زیر پوستک‌ها و در داخل شکاف درختان گردو، درون پیله‌های سفید می‌باشد. در این مطالعه، چرخه زندگی و میزان خسارت آفت، مورد بررسی قرار گرفت. لارو آفت، پنج سن لاروی داشت و حدود ۲/۲۶ ± ۶۷/۱ روز طول کشید. مرحله شفیرگی در پیله‌ای سفید و متراکم تشکیل شده که زمستان گذرانی می‌کند و پس از مساعد شدن هوا در بهار، حشرات بالغ ظاهر می‌شوند. میانگین مدت زندگی حشره، ۴/۴۵ ± ۲۹۷/۵ روز بود. حشره، تک نسلی است و لارو آن از پریکارپ گردوی جوان تغذیه می‌کند که در نتیجه، ظاهر میوه، تغییر شکل یافته و بدین ترتیب، فعالیت آفت باعث کاهش عملکرد قابل توجهی در اوایل پاییز می‌شود. برجای ماندن فضولات لارو روی پوسته، منجر به افت کیفیت گردو و در نتیجه کاهش ارزش اقتصادی آن می‌شود. شناخت چرخه زندگی حشرات آفت، در طراحی استراتژی مدیریت، بسیار مهم است. در این خصوص، گزینه های مدیریت این آفت، محدود است زیرا لاروهای خسارت‌زای آفت، زندگی مخفی در پوسته میوه دارند. از نکات مهم در مدیریت این آفت، زمان ظهور حشرات بالغ و تخم گذاری آنهاست. کاربرد دشمنان طبیعی به عنوان رویکردی سازگار با محیط زیست تحت عنوان کنترل زیستی، به عنوان روشی مناسب جهت استفاده در مدیریت این آفت در این منطقه خاص را تسهیل می‌کند. همچنین ترویج توسعه ارقام جدید گردو با بازده بالقوه بالا و پوشش مقاوم تر میوه در باغات گردو تازه کاشته شده نیز حائز اهمیت است.

کلمات کلیدی: شب پره آسیایی گردو، مدیریت آفت، چرخه زندگی، پرورش، گردو

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