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Predatory insects as biological control agents against walnut aphids in Kashmir, India

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Abstract: Management of pests by biological control agents is a natural and environmental friendly method. It is an important part of integrated pest management programs where biological predators play a significant role in controlling various pests. During the present study, different insect pests were observed infesting walnut trees and fruits causing significant damage to the walnut orchards. Among these pests, sap suckers were found to cause notable damage out of which aphids were most dominating pests**.** High population of walnut aphids causes leaf drop, reduced tree vigour, nut quality and size. In addition to this, some insect predators were also observed on these walnut aphids. Six species of predaceous coccinellid beetles were found on walnut leaves in interaction with walnut aphids and they were identified belonging to sub family Coccinellinae. Among which *Calvia punctata* (Mulsant, 1853) and *Harmonia dimidiata* (Fabricius, 1781) were the most encountered species. Besides, one species from syrphidae family *Metasyrphus latifasciatus* (Macquart, 1859) was also observed. Moreover, extreme feeding potential was noted among these predators.

Key words: pest management, sap suckers; coccinellids, walnut aphids, *Calvia punctata*, *Harmonia dimidiata*, *Metasyrphus latifasciatus*, predatory potential

Plenilske žuželke kot biološki nadzor listnih uši na navadnem orehu v Kašmirju, Indija

Izvleček: Upravljanje škodljivcev z biološkimi sredstvi je naravna in okolju prijazna metoda. Je pomemben del integriranega varstva rastlin, v katerem imajo plenilski organizmi pomembno vlogo pri nadzorovanju različnih škodljivcev. V tej raziskavi so bile ugotovljene različne škodljive žuželke, ki napadajo navadni oreh in povzročajo znatno škodo v njegovih nasadih. Med temi škodljivci povzročajo znatno škodo sesajoče žuželke, med katerimi prevladujejo listne uši. Velike populacije listnih uši povzročajo na orehu odpadanje listja, zmanjšujejo vitalnost dreves, velikost in kakovost orehov. V raziskavi so bile opažene tudi plenilske žuželke, ki se hranijo z listnimi ušmi. Na listju navadnega oreha je bilo ugotovljeno šest vrst plenilskih polonic, ki se hranijo z listnimi ušmi in pripadajo poddružini Coccinellinae. Med njimi sta bili najbolj pogosti vrsti *Calvia punctata* (Mulsant, 1853) in *Harmonia dimidiata* (Fabricius, 1781). Med temi plenilskimi žuželkami je bila najdena tudi vrsta muh iz družine trepetalk, vrsta *Metasyrphus latifasciatus* (Macquart, 1859). Pri vseh teh plenilskih žuželkah je bil ugotovljen velik potencial hranjenja z listnimi ušmi.

Ključne besede: upravljanje s škodljivci**,** sesajoče žuželke, polonice, listne uši navadnega oreha, *Calvia punctata*, *Harmonia dimidiate*, *Metasyrphus latifasciatus*, plenilski potenciala

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1 INTRODUCTION

Kashmiri walnut known as *Juglans regia* L. is one of the prime industry of the valley with numerous cultivars of about 199 indigenous and exotic (Verma et al., 2009). After apple industry, it is the second major fruit covering an area of 26.91 % and is important source of state economy. Walnut has enormous health benefits as it is rich in vitamin B, omega-3, omega-6, essential fatty acids, sterols and phenolic substance (Davis et al., 2007; Vigneshwara, 2011). Irrespective of these health benefits walnut is highly prone to pest attacks and harbours diversity of pests which cause deterioration in its quality and huge losses occur every year. Among the different pests, aphids act as the major pests (Sharma et al., 2012). Aphids deteriorate the plant health by changing the nutrient amount leading to reduced photosynthetic capacity resulting in leaf yellowing, premature death and spotting (Dedryver et al., 2010). Incessant feeding of walnut aphid leads to reduced tree vigour, quality, size of nut and mostly the yield (Aliniazee & Hagen, 1995; Cecilio & Illharco, 1997). However, the overall population is restrained due to the impact of natural enemies mostly predators and parasitoids (Gaston & Lawton,1988; Storck-Weyhermüller,1988). Different biological agents feed on these aphids and acted as management tool for their control to reduce outburst of population. Some of the important group of predators belong to the family Coccinellidae, Syrphidae, Mantidae and Chrysopidae. Coccinellid beetles are most abundant biological control agents against aphids. They are of great economic importance as predators both in their larval and adult stages on various important crop pests such as aphids, coccids and other soft bodied insects (Hippa et al., 1978; Kringet et al., 1985). The coccinellid beetles are considered to be of great economic importance in agro-ecosystem due to their successful use in the biological control of many injurious insects (Agarwala & Dixon, 1992). After coccinellids, syrphidae family are also active group of predators. The present work is the attempt to find the different species of predators feeding on walnut aphids and the feeding potential of dominating species so as to develop biological control for aphids feeding on sap of walnut leaves.

2 MATERIALS AND METHODS

2.1 STUDY AREA

Studies were carried out in three different districts of Kashmir namely Ganderbal (34° 13' 39.11" N, 74° 46' 19.78" E), Anantnag (33.7311° N, 75.1487° E) and Kupwara (34° 32′ 14.85″ N 74° 13′ 50.81″ E). In each district one site was monitored throughout the year 2018. The three sites include Kangan from district Ganderbal, Kelam from Anantnag and Hatmulla from Kupwara district. In each site two orchards were selected.

2.2 SAMPLING METHODS

Each site was repeatedly sampled after two week interval for three hours throughout the year 2018. Sampling method for collection of predatory insects involved collection by one man one hour swept net collection method from ten trees selected randomly from each walnut orchard (Khairmode, 2014). For active fliers sampling was based on sweeping with insect collecting net which was repeated after a gap of 10 minutes with 10 sweeps each time. However, some beetles were collected by net sweeping method and hand picking method (Jonathan, 1995). The collected specimens were kept in collecting jars than transferred to vials containing 70 % alcohol for wet preservation and number was counted. The specimens were then brought to entomological laboratory for further studies.The branches harbouring insects were randomly selected for quantification and maintenance of record. The pests were also collected from different walnut orchards by using the various research equipment's like hand lens, forceps, soft camel hair brush, pair of scissors, vials (5 × 3 cm), polythene bags, specimen tubes containing 90 % alcohol as a preservative, insect killing bottles and Insect collecting net.

2.3 IDENTIFICATION

The collected specimens of each species were carefully studied for all details under binocular microscope and were identified by running keys and taxonomic work of different workers. The different taxonomic keys used to identify these species include Kapur (1958, 1956b); Kuznetsov (1997); Gordon (1970b, 1970e, 1985, 1987); Ahmad and Ghani (1966); Poorani (2002a, 2002b); Slipinski (2007); Slipinski et al. (2005); Slipinski and Giorgi (2006). Some specimens which were not identified were sent to Zoological survey of India (ZSI), Kolkata for taxonomic characterization.

2.4 FEEDING POTENTIAL

For evalutation of the feeding potential of predators feeding on walnut aphids, adults from field were collected and reared under laboratory conditions (26 ± 2 ˚C and 65 ± 5 % R.H.). Newly emerged adults and immature stages were used for the study. Different predatory species were kept individually in separate petri dishes (9 cm in diameter). They were provided with known number of adult walnut aphids (*Chromaphis juglandicola* (Kaltenbach, 1843)*)* and covered with muslin cloth. In order to record consumption rate, the number of aphids left out after 24 hr were counted. The number of aphids consumed during 24 hr was recorded and whole experiment was repeated three times but with increased number of known aphids. The data obtained during the present study was tabulated and presented as per the required statistical methods. One-way ANOVA at 0.05 % level of significance (Ducans test) was done. Analysis was done by using SPSS (Version 16.00) software.

3 RESULTS AND DISCUSSION

The results attained during the present study showed several insect pests infesting walnut trees causing significant damage. Sap suckers were observed to cause notable damage out of which aphids were most dominating pests viz., *Chromaphis juglandicola* (Kaltenbach, 1843) and *Panaphis juglandis* (Goeze, 1778). *C. juglandicola* is commonly called as walnut aphid and *P. juglandis* is called as dusky -veined aphid. Among these aphid species, infestation caused by *C. juglandicola* was observed very high on walnut trees. Both the species are sap suckers and are serious pests, feeding on phloem. The infestation starts from April and remains till October with the peak infestation in the month of June. Mir & Wani (2005) also reported that aphids attack walnut during April–October, causing stunted growth in nursery plantation. Each pest has different distribution on leaves. *C. juglandicola* was found scattered on the underside of leaves while *P. juglandis* was present on the upper side of leaves feeding near the mid rib of leaves although it is a minor pest in comparison to *C. juglandicola*. Both aphid species maintained distance and never fed on same leaf and strongly reinforces the Gause's Competition Exclusive Principle, which states that no two species having same ecological niche can coexist together (Wani & Ahmad, 2014). *C. juglandicola* and *P. juglandis* were never seen feeding on same leaf during the study period as *C.juglandicola* is important factor for limiting the population of latter as it excretes acidic honeydew (Olson 1974).

Both the aphids over winter as egg stage hatch out in early spring and produce young ones without mating. Thus, have many generations per year resulting in development of many colonies in summer season. In case of dusky-veined aphid (*P. juglandis*), winged males and wingless females appear in the month of September and cause general blackening of leaves while walnut aphid (*C. juglandicola*) cause early leaf drop and reduced nut size. During the present investigation, high infestation of walnut aphid population per leaflet was observed which showed close congruity with the results of UCIPM (2011) who had evaluated population of walnut aphid beyond 15 aphids per leaflet can reduce nut yield in terms of quality and quantity. Our results are in accordance with the studies carried out by Ginzel (2010) who reported both these aphid species are contributing factor for reduced tree vigour, nut size and yield.

Besides the pests on walnut trees different predators were also observed. A total of 7 predatory species were observed on the walnut leaves in interaction with walnut aphids. Six species were identified belonging to the family Coccinellidae and one from Syrphidae family. The species belonging to Coccinellidae include *Coccinella septempunctata* (Linnaeus, 1758) *Calvia punctata* (Mulsant, 1853), *Harmonia dimidiata* (Fabricius, 1781), *Oenopia conglobata* (Linnaeus, 1758), *Macroilleis hauseri* (Mader, 1930) and *Adalia tetraspilota* (Hope, 1831). These findings are in accordance with Khan et al. (2009, 2017) and Sluss (1967). From Syrphidae family only one species, *Metasyrphus latifasciatus* (Macquart, 1859) was observed. The relative abundance of these collected predators was observed in all the three districts and is shown in Table 1. *C. punctata* was the most abundant predator prevailing in all walnut orchards. It showed 19.83 % relative abundance at district Anantnag, followed by 18.3 % in district Ganderbal and 14.29 % in district Kupwara. The lowest occurrence was of *H. eucharis* (5.11 %) in district Ganderbal although, it was absent in district Kupwara. *M. latifasciatus* showed it's the highest occurrence in district Ganderbal with a relative abundance of 17.87 %. Likewise, low population of predators was observed in early March but later it gradually increased during the month of June, July and August (Fig.1).

3.1 FEEDING POTENTIAL OF *Calvia punctata* AND *Harmonia dimidiata*

The feeding potential of *Calvia punctata* and *Harmonia dimidiata* on adults of walnut aphid*, Chromaphis juglandicola* under laboratory conditions was observed. On analyzing the data, results revealed that the adults of *C.punctata* consumed on an average of 12.00 (± 1.0), 29.33 (± 1.52), 45.66 (± 2.51) and 69.00 (± 20) aphids when fed with 20, 40, 60 and 80 aphids respectively (Table 2). The consumption ranged from 60 to 86.25 % compared to *H. dimidiata* which on an average consumed 8.00 (± 1.0), 23.33 (± 4.0), 36.00 (± 3.0) and 47.33 (± 3.7) aphids respectively, with a consumption percentage of 40 to 59.16 % (Table 3). Both beetles fed on aphids

Table 1: Percentage relative abundance and Mean (± SE) of predatory insects collected in walnut orchards of three districts of Kashmir valley during the year 2018

Districts	Kangan (Ganderbal)		Kelam (Anantnag)		Hatmulla (Kupwara)	
Predators	Relative Abundance %	Mean (± SE)	Relative Abundance %	Mean (± SE)	Relative Abundance %	Mean (± SE)
Coccinella septempunctata	11.06	2.17 (± 0.76)	12.81	2.58 (± 0.85)	8.76	1.58 (± 0.50)
Calvia punctata	18.30	3.58 (± 1.23)	19.83	4.00 (± 1.28)	14.29	2.58 (± 0.98)
Adalia tetraspilota	7.23	1.42 (± 0.60)	10.33	2.08 (± 0.73)	6.45	1.17 (± 0.41)
Oenopia conglobata	6.81	1.33 (± 0.53)	8.26	1.67 (± 0.69)	10.14	1.83 (± 0.72)
Harmonia dimidiata	12.34	2.42 (± 0.96)	14.88	3.00 (± 1.09)	19.82	3.58 (± 1.36)
Harmonia eucharis	5.11	1.00 (± 0.41)	4.55	0.92 (± 0.42)	0.00	0.00 (± 0.00)
Metasyrphus latifasciatus	17.87	3.50 (± 1.23)	17.77	3.58 (± 1.20)	8.76	3.33 (± 1.29)

but *C. punctata* devoured more number of aphids in all four given treatments. On comparing the mean consumption rate of both the beetles by one-way ANOVA, data revealed that all the four treatments were significantly different from each other at $p < 0.05$ (Fig.2), with a high consumption rate (40-60 %) during the first few hours, followed by an appreciable reduction in later stages. Significant differences in mean consumption rate of aphids were also observed between the two coccinellid beetles in terms of mean consumption rate of aphids. Similarly percent mean aphid consumption under host density of 20 aphids was 40 % and 60 % for *H. dimidiata* and *C. punctata* respectively while it increased to 58.32 % in *H. dimidiata* and 73.32 % in *C. punctata* when host density was 40. The data showed an increasing trend with a host density of 60 and 80 with a percent mean consumption of 76.1 % and 86.25 % in *C. punctata* while 60 % and 59.16 % in *H. dimidiata* respectively. Overall results depicted that host density played a significant role in the aphid consumption rate (Table 2 & 3).

The results showed both the coccinellid beetles are voracious feeders on walnut aphids. These findings are in corroboration with Khan et al. (2007) who also reported *C. punctata* feeding on aphids and scale insects on walnut trees and other wild vegetation. Khamashon et al. (2018) who studied feeding behavior and consumption rate of *C. punctata* found that the consumption rate of adult females and males ranged from 86.9-72.9 % respectively. Sathe and Bhosale (2001) who reported around 21 species of coccinellid beetles feeding on aphids and other soft-bodied pests feeding on agricultural and forest plants.

Table 2: Feeding potential of *Calvia punctata* on *Chromaphis juglandicola* under laboratory conditions

Treatments	No. of Aphids offered	Aphids consumed* (Mean ± SD)	Mean Consumption (%)	95 % confidence limits	
				Lower	Upper
I	20	12.00 ^{d} (± 1.0)	60	9.51	14.48
II	40	29.33 ^{c} (± 1.52)	73.32	25.53	33.12
III	60	45.66 ^{b} (± 2.51)	76.10	39.41	51.91
IV	80	69.00 ^{a} (± 2.0)	86.25	64.03	73.96

*Mean of 3 replications/treatment; figures in parenthesis are standard deviation; means followed by different letters indicate that the means are significantly different ($p < 0.05$) by Duncan's test

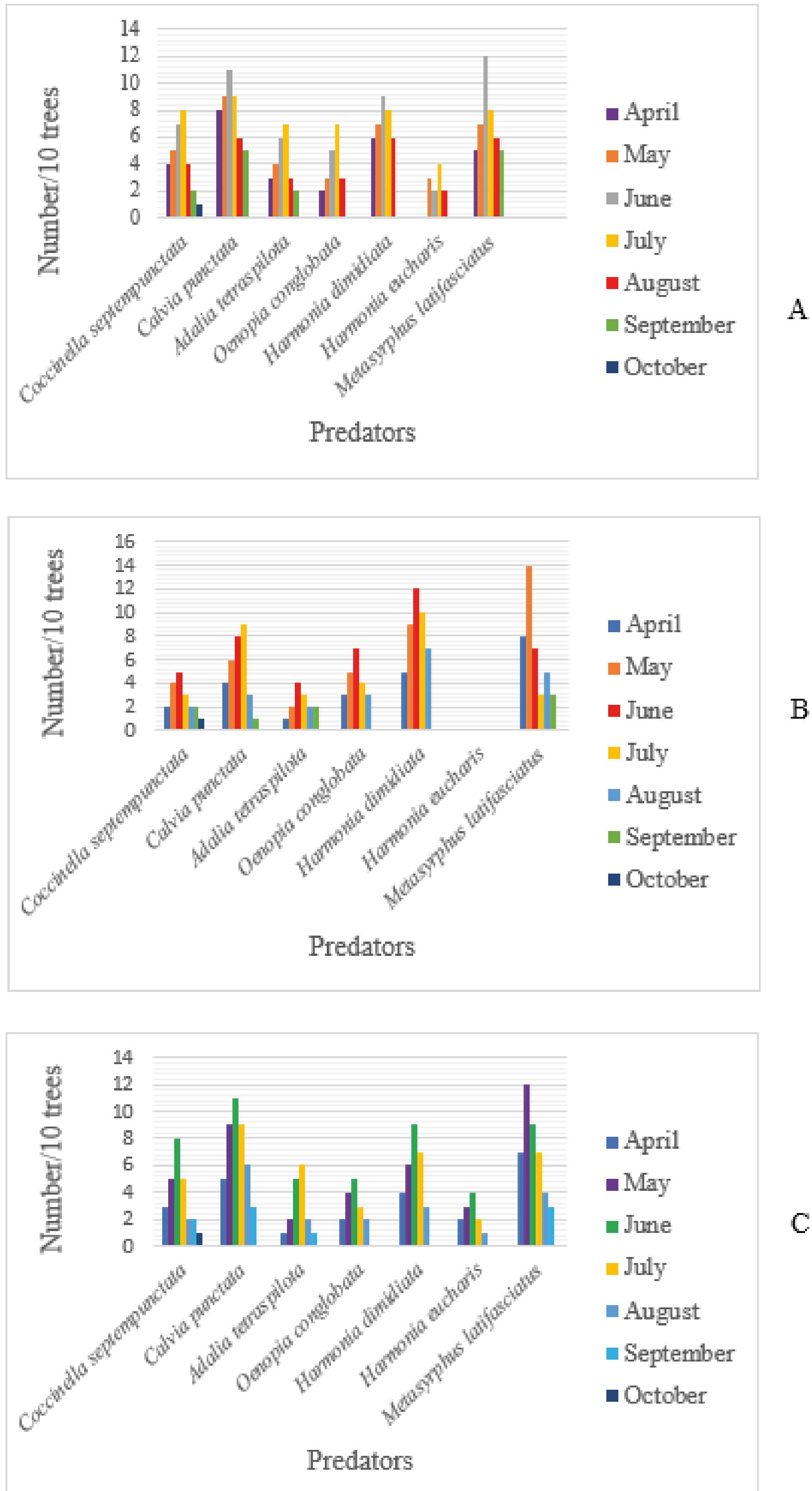

Figure 1: Monthly variation of predators throughout the sampling period at different sites. (A) Kelam (B) Hatmulla and (C) Kangan

Table 3: Feeding potential of *Harmonia dimidiata* on *Chromaphis juglandicola* under laboratory conditions

Treatments	No. of Aphids offered	Aphids consumed* (Mean ± SD)	Mean Consumption (%)	95 % confidence limits	
				Lower	Upper
I	20	8.00^{d} (± 1.0)	40	5.51	10.48
II	40	23.33^{c} (± 4.0)	58.32	13.29	33.37
III	60	36.00^{b} (± 3.0)	60	28.54	43.45
IV	80	47.33^{a} (± 3.7)	59.16	37.92	56.73

*Mean of 3 replications/treatment; in parenthesis are standard deviation; means followed by different letters indicate that the means are significantly different ($p < 0.05$) by Duncan's test

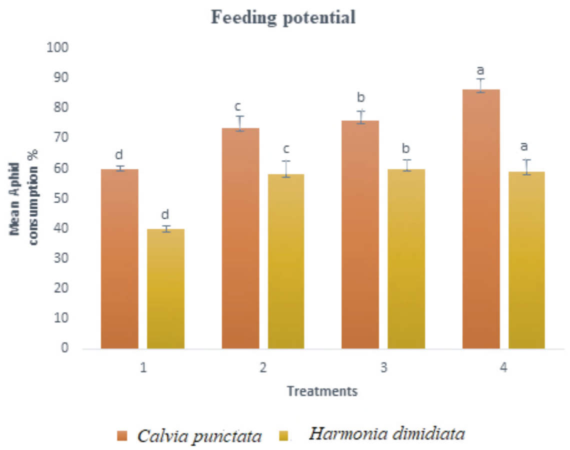

Figure 2: Graph depicting feeding potential of two coccinellid beetles on walnut aphid

3.2 FEEDING POTENTIAL OF SYRPHID FLY, *Metasyrphus latifasciatus*

Similarly, the feeding potential of different larval instars of syrphid fly, *Metasyrphus latifasciatus* was observed on adult walnut aphid, *Chromaphis juglandicola* under laboratory conditions. Feeding potential of syrphid fly on walnut aphid at different larval instars was recorded, although each larval instar tended to reduce the aphid population. The experimental data showed that as the number of instars upsurge, feeding potential also enhances. The mean consumption of first instar larvae was 23.25 %, second instar larvae was 48.25 % and the highest was in third instar larvae reaching 75.83 %. When the data was subjected to one-way ANOVA, it was depicted that the feeding potential of all three larval instars was significantly different with each other at $p < 0.05$ (Fig.3). Among all treatments, 3rd larval instar was found to be voracious feeder and devoured more number of aphids with a mean value of 30.3 (± 3.05). Thus, it can be concluded that the maximum predation rate was for the third larval instar followed by the second larval instar and first larval instar. The feeding rate of each larval instars of *M. latifasciatus* is presented in Table 4. Larval instars showed a different rate of feeding during the experimental conditions. The rate of aphid consumption increased with each developing stage. The present findings are in line with the observations carried by Baskaran et al. (2009) who recorded that the third larval instar consumed the highest number of aphids and had high feeding potential compared to first and second larval instars. The observed results, showed that feeding potential increased with an increase in larval stages from first to third which was in corroboration with the study carried by Ankersmit et al. (1986) who found that the age of larvae played a significant role in the consumption of aphids and high activity was observed at the end of larval development. Pascual-Villalobas et al. (2006) while working on aphids feeding on lettuce found that the syrphid population showed high predation on aphid colonies having 1.1 to 1.9 syrphid larvae/head. Hasken and Poehling, (1994) also observed that the aphid population was controlled by syrphids in all farming systems. Ghorpade (1981) also

Table 4: Feeding potential of syrphid fly larvae on walnut aphid under laboratory conditions

Larval Instar	No. of Aphids offered	Aphids consumed* (Mean ± SD)	Mean Consumption (%)	95% confidence limits	
				Lower	Upper
I	40	9.33^{c} (± 2.08)	23.25	4.16	14.50
II	40	19.6^{b} (± 3.51)	48.25	10.94	28.39
III	40	30.3^{a} (± 3.05)	75.83	22.74	37.92

*Mean of 3 replications/treatment; figures in parenthesis are standard deviation; means followed by different letters indicate that the means are significantly different ($p < 0.05$) by Duncan's test

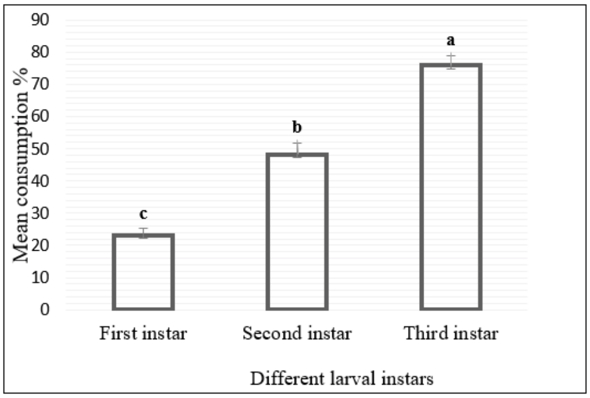

Figure 3: Graph depicting mean percentage of aphid consumption by different larval instars of syrphid fly

observed that syrphids act as one of the major bio-control agents of aphids.

4 CONCLUSIONS

On concluding the present results, it can be enumerated that these predators voraciously feed on aphids and can play a significant role in controlling the walnut aphid, *C. juglandicola* in field conditions. These predators proved their efficiency as an important bio control agent against walnut aphids. Due to their adeptness of having wide range of habitats and diversity, they can be successfully used for mass rearing and then its establishment in pest prevalent regions. Also the aphid density greatly affected the feeding potential. At higher density, predation was higher and at lower density the rate of predation was also observed low. This seems to be a good quality of the predator to feed more at higher prey density and less at lower prey density. This quality of predator not only allows the predators to survive at low prey density but also helps in reducing pest population at higher density.

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