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EX-SITU PREDATION POTENTIAL OF APHIDOPHAGOUS COCCINELLIDS

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Coccinellid beetles are widely recognized biological control agents and are voracious feeder of cereal aphids. Aphid predation by Coccinellid predators is quite imperative for long-term regulation of their population in agricultural crops. The factual role of natural enemies in biological control depends on their capability to devour large number of pests. Therefore, this study was aimed to determine predatory potential of selected Coccinellid beetles in the laboratory as well as to ascertain their hierarchy in aphid preference. On account of that per day feeding efficacy of four Coccinellid species, *Coccinella septempunctata, Coccinella sexmaculata, Coccinella transversalis* and *Hippodamia variegata* as well as their larval instars were determined on aphid species *Myzus persicae, Rhopalosiphum padi, Schizaphis graminum,* and *Sitobion avenae* in order to elucidate their efficiency in choice and no choice feeding assays. *Coccinella sexmaculata* was found to be the most efficient predatory species followed by *H. variegata, C. septempunctata* and *C. transversalis* against aphids. Aphids *M. persicae* and *S. avenae* were preferred diet for adult and larvae of *C. septempunctata* as compared to *M. persicae* and *R. padi* for *C. transversalis.* Maximum consumption per day of adult as well as larval instars of *C. sexmaculata* was observed for *S. graminum.* Adults of *H. variegata* fed more on *S. avenae* while grubs fed more on *S. graminum. M. persicae* was found to be the potential host for all predatory Coccinellids. Highly significant variations ($P<0.001$) were observed for per day feeding efficacy of all selected predators and their larvae. Coccinellid predators appeared to have substantial capacity to consume aphids that evinced their ability as viable control agents of aphids.
Keywords: Biological control, Coccinellids, Feeding efficacy, Hierarchy, Predators.

INTRODUCTION

Coccinellids are of immense practical and scientific importance as natural enemies of phytophagous insect pests (Joydeb *et al.*, 2015). Coccinellids contribute to the development of biological pest control for their trophic characteristics or as bio-indicators in terms of biodiversity (Zahoor *et al*., 2013). Most of the Coccinellid predators and their nymphs are effective against aphids and other small insects (Hoffmann *et al.*, 1993). Various studies have clearly demonstrated the ability of Coccinellid natural enemies as viable biological control agents of different pest species, (Hagler and Naranjo, 2005; Zhang *et al*., 2007). Costamagna *et al*. (2008) and later Sajid *et al*. (2015) proved that lady beetles had major impact on aphid populations than other pests.

There have been numerous studies on the effectiveness of various species of lady beetles as aphid predators such as Doghairi (2004) found *Adonia variegata, Coccinella undecimpunctata* and *Coccinella novemnotata* as biocontrol agents against cereal aphids. Kontodimas and Stathas (2005) proved *H. variegata* and *C. septempunctata* as predators of various aphid species. Greenstone and Shufran (2003) detected anthocorid and *Rhopalosiphum maidis in the feed of* ladybird larvae. Pervez and Omkar (2004) found *C. sexmaculata effective against Myzus persicae whereas Clitostethu sarcuatus effective* against *Trialeurodes vaporariorum aphids.* Saleem *et al*. (2014) found *Menochilus sexmaculatus* as efficient predator against *Macrosiphum rosae.*

A fundamental premise of using predators in biological control depends on their predation potential. Determination of predator's feeding niche is important to understand its potential role in biological control (Chapman *et al*., 2012). In order to evaluate the biological control potential of a predator, it is important to obtain accurate measures of predation frequencies. This can lead to an understanding of those prey species that are preferentially consumed when they become available. Several methods have been adopted by researchers to discern trophic linkages between predator and prey (Weber and Lundren, 2009) and one of them is *EX-SITU* feeding experiments.

Therefore, numerous researchers determined predation frequencies of Coccinellid predators against aphid species during *EX-SITU* trials such as Patel (2003), Evans (2009), Skouras *et al*. (2015), Arif *et al*. (2011), Ahmad *et al.* (2018) and many others.

Better understanding of natural enemy communities and their feeding relationship with prey is crucial to the provisioning of biological control in agricultural fields but current knowledge on entomophagous beetles feeding niche is insufficient to provide information on how much predation potential these aphidophagous beetles have that maximally benefit biological pest control. The main goal of this study was to investigate the potential role of Coccinellid beetles as natural biological control agents of aphids in agricultural croplands. In order to evaluate the biological control potential of Coleopteran predators, it was necessary to obtain measures of depredation frequencies and to characterize species hierarchy with respect to preferentially consumed prey. Therefore, this study aimed to determine the maximum per day predatory potential of Coccinellid beetles against different aphid species.

MATERIAL AND METHODS

Ex situ study on predation potential of four Coccinellid species was executed against four aphid species in Bio-control Laboratory Department of Zoology, UAF Pakistan. Most abundantly occurring predator species and aphid species were selected for laboratory trials. The whole experiment was divided into two feeding assays as choice and no choice bioassays with three replications. The fertilized eggs of selected species and 1st instar larvae were transferred from fields to bio-control laboratory. Individual predatory species of family Coccinellidae along with an aphid species was kept in a separate labeled Petri dish and then transferred to rearing glass cages with wooden boundaries measuring 32×30×30cm in no choice bioassay whereas, one specimen of each predatory species along with four selected aphid species was kept in separate petri dishes inside rearing cages in the free choice assay (Mushtaq *et al*., 2013)

In the cages leaves and shoots of wheat crop infested with aphids were offered to predators of each species. Aphids were counted before offering. Larvae of each predatory species were kept in different cages and provided with four species of aphids at the same density that was increased as larval stage progressed to check their foraging extent at each life stage. The whole experiment was conducted in the laboratory at almost field like environmental conditions (average temperature 20±5°C, relative humidity 60±5 and day night duration of 16:8 hour during the period of peak aphid population that was in the months of March to April 2016. After 24 hours' cages were cleaned after removing debris containing unconsumed food along with excreta and larvae were given fresh aphids again. The act of cleaning the cages and aphid supply was continued till the conversion of larvae into pupae. Observations were made carefully and continuously to record daily consumption of each developmental stage starting from egg till the emergence of adults. After emergence, adults were fed with four aphid species each day to note their predatory potential per day. Prey preference by larvae of seven Coleopteran predatory species was determined by feeding them four aphid species in the choice experiment whereas predation potential was determined in a choice feeding assay by offering single aphid species.

All aphids that were offered were of same instars. Number of aphids were supplied at different densities and increased with age of larvae and according to requirement. In order to avoid cannibalism, predators were kept in separate dishes along with specific no of aphids. Aphids left over in petri dishes after feeding were recorded after 24 hours and predation rate was determined by deducting the number of preys offered from no of preys consumed by the predators and larval instars.

***Analysis of Variance (ANOVA)*:** Feeding efficacy of each Coccinellid predator and their larvae were compared with each other in *Ex-situ* study for both choice and no choice experiments via Analysis of Variance. Variation in the average per day consumption of each Coccinellid adult and larval stage was computed using ANOVA with Tukey's contrast at 0.05and 0.01 probability level. Predation on aphids with respect to aphid species was investigated and analyzed using three factors ANOVA using SYSTAT 11 for Windows (SYSTAT Software Inc., 2004).

RESULTS

All predators were found voracious feeder on all aphid species. Differential rates of aphid's consumption were observed in no choice and free choice feeding assays.

***Consumption as recorded per-day*:** Aphid consumption on average by adult *C. septempunctata* recorded per day was greater on *M. persicae* as 47.00±2.18 samples and rather less on *S. graminum* 27.68±0.96 in no choice feeding assay (Table 1a). Aphid consumption of adult *C. sexmaculata* per-day on average was observed maximum 58.30±1.01 for *S. graminum* and relatively lesser number of *R. padi* 30.68±1.00 were consumed (Table 1b). The predatory efficacy of *C. transversalis* was recorded less compared to other Coccinellid predators. Average per-day feeding of adult *C. transversalis* was greater on *M. persicae* with an average of 41.90±1.56 specimens and least on *S. graminum* 33.70±1.48 while a close competition occurred between consumption of *M. persicae* and *R. padi* (Table 1c). Adults of *H. variegata* voraciously fed more on *S. avenae* and their feeding rate was found to vary differently for all aphid species. Average per-day consumption of *H. variegata* was maximum 50.60±2.40 on *S. avenae*, 48.70±3.01 on *S. graminum*, 46.30±2.14 on *M. persicae* and relatively a smaller number of *R. padi* 27.80±0.98 were consumed (Table 1d). From the results, highly significant difference was recorded for average per-day consumption of all predatory Coccinellids at $P<0.01$ (Tables 2).

***Prey preference*:** In the free choice feeding assay differential potential of aphid consumption were recorded. Adult *C.*

Table 1a. 1b, 1c, 1d. Mean per day consumption by Predators (adult) and larval instars for different aphid species.

(a) *C. septempunctata.*

Life stage	Prey species				Average consumption/day
	M. persicae	***R. padi***	***S. graminum***	***S. avenae***	
1st instar	9.70±0.80h	7.60±1.23hi	3.37±1.72j	9.00±0.99hi	7.42±0.91E
2nd instar	18.30±0.79g	15.30±0.50g	6.16±0.90ij	17.30±0.46g	14.27±1.48D
3rd instar	34.40±0.46d	24.70±0.42f	17.80±1.48g	29.30±1.03e	26.55±1.89C
4th instar	52.90±1.21a	47.80±0.39bc	26.27±1.10ef	49.70±0.76ab	44.17±3.19A
Adult	47.00±2.18bc	45.90±0.91c	27.68±0.96ef	45.80±2.52c	41.60±2.54B

(b) *C. sexmaculata*

Life stage	Prey species				Average consumption/day
	M. persicae	***R. padi***	***S. graminum***	***S. avenae***	
1st Instar	8.80±1.00g	3.46±1.14h	8.00±2.06g	7.60±0.43g	6.97±0.83E
2nd Instar	17.10±0.91f	6.19±1.48gh	17.30±0.77f	15.30±1.60f	13.97±1.47D
3rd Instar	28.70±1.71d	18.90±0.84f	29.30±1.26d	24.70±1.00e	25.40±1.36C
4th Instar	60.30±1.56a	28.13±1.56de	59.70±1.38a	47.80±1.49b	48.98±3.98A
Adult	56.80±2.50a	30.68±1.00d	58.30±1.01a	41.90±1.51c	46.92±3.49B

(c) *C. transversalis*

Life stage	Prey species				Average consumption/day
	M. persicae	***R. padi***	***S. graminum***	***S. avenae***	
1st Instar	7.00±1.21klm	6.10±0.77lm	3.60±1.50m	4.10±1.10m	5.20±0.65D
2nd Instar	14.30±0.98h	13.60±1.25hi	9.10±0.99jkl	10.20±1.21ijk	11.80±0.82C
3rd Instar	27.10±1.73fg	25.80±0.53g	16.50±1.40h	12.70±1.96hij	20.53±1.95B
4th Instar	47.40±1.91a	43.20±0.79b	31.90±1.89e	30.50±1.53ef	38.25±2.28A
Adult	41.90±1.56b	39.70±0.33bc	33.70±1.48de	36.30±1.83cd	37.90±1.12A

(d) *H. variegata*

Life stage	Prey species				Average Cosumption/day
	M. persicae	***R. padi***	***S. graminum***	***S. avenae***	
1st Instar	5.00±1.33jk	2.60±0.81k	5.70±0.52jk	4.40±1.14k	4.43±0.55E
2nd Instar	13.10±0.81hi	5.90±0.35jk	14.30±1.39h	9.10±1.58ij	10.60±1.11D
3rd Instar	25.40±0.92ef	11.90±0.57hi	27.20±0.62ef	20.90±0.84g	21.35±1.81C
4th Instar	53.60±1.67b	23.30±2.19fg	60.90±1.50a	54.30±1.56b	48.03±4.45A
Adult	46.30±2.14d	27.80±0.98e	48.70±3.01cd	50.60±2.40bc	43.35±2.91B

Table 2. Results of Analysis of variance for per day predation of aphids by *C. septempunctata, C.sexmaculata, C. transversalis* and *H. variegata* life stages

Specie Name	Life stage	Prey	Life stage x Prey
C. septempunctata	754.85**	187.56**	10.74**
C. sexmaculata	746.72**	166.46**	17.21**
C. transversalis	477.21**	54.47**	5.05**
H. variegate	685.02**	132.71**	18.60**

NS = Non-significance (P>0.05), *= for Significant (P<0.05), **= highly significant (P<0.01)

septempunctata preferred *M. persicae* 17.00±1.21 followed by *S. avenae* 15.30±0.95, *R. padi* 10.50±1.85 and *S. graminum* 7.20±0.69 (Figure 1a). *C. sexmaculata* adult consumed highest 18.50±0.68 number of *M. persicae* compared to *S. graminum*17.78±1.00, *S. avenae* 11.90±0.87 and *R. padi* 8.90±0.64. Figure 1b shows difference in prey choice of *C. sexmaculata* divulged by prey consumption. Figure 1c revealed difference in prey choice of *C. transversalis* adults against four aphid species and consumption was recorded maximum 15.70±1.21 on *M. persicae* followed by 10.50±1.27 on *R. padi*, 8.20±0.69 on *S. graminum*, and minimum 6.80±1.67 on *S. avenae* (Figure 1c). *H. variegata* preferred *S. graminum* by consuming 19.60±0.98 aphids compared to *M. persicae* 15.70±0.82, *S. avenae* 13.90±0.40 and *R. padi* 9.80±0.93 (Table 1d). From the finding highly significant variations (P<0.01) in predatory efficacy of *C. septempunctata, C. sexmaculata, C. transversalis* and *H. variegata* were concordant (Table 3).

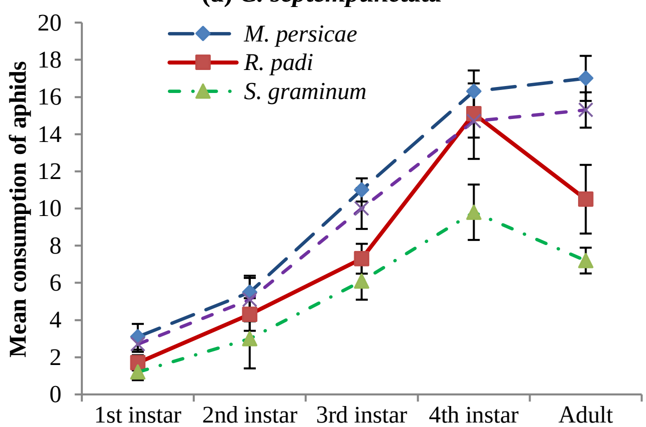
Consumption at Different Larval Instars: Present study revealed that prey consumption at instars levels of all

Table 3. Analysis of variance for prey preference of *C. septempunctata, C. sexmaculata, C. transversalis* and *H. variegate* (adult) and larval instars for different aphid species in free choice feeding assay.

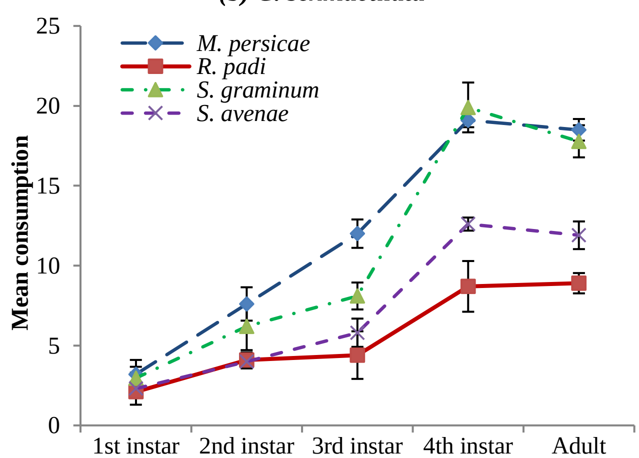
Predator Species	Life stage	Prey	Life stage x Prey
C. septempunctata	80.87**	19.96**	2.04*
C. sexmaculata	127.79**	49.05**	5.00**
C. transversalis	47.16**	21.63**	2.15*
H. variegate	305.50**	77.16**	6.23**

predators increased steadily from 1st instar to 4thinstar for all aphid species. Aphid voracity increased till pre-pupal stage and least aphidophagy was noted at the end of 4th instar. It was seen that 4th instar larvae of *C. septempunctata* consumed highest no of aphids of each aphid species compared to other life stages and *M. persicae* was the most used aphid species on the whole while *S. graminum* remained the least consumed aphid species. An average of 52.90±1.21 *M. persicae* were consumed per day by 4th instar in no choice feeding assay followed by 34.40±0.46, 18.30±0.79, 9.70±0.80 by 3rd, 2nd and 1stinstars, respectively (Table 1a). In free choice experiment it was evident that *M. persicae* was the most preferred prey compared to other aphid species. The order of prey preference was *M. prsicae*>*S. avenae*>*R. padi*> and *S. graminum* (Figure 1a).

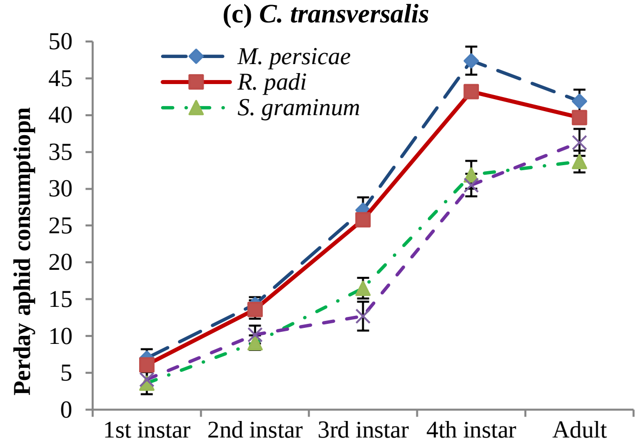
(a) *C. septempunctata*



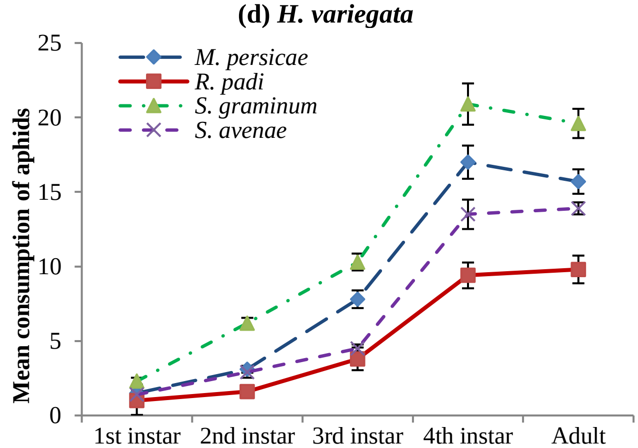
(b) C. sexmaculata



(c) *C. transversalis*



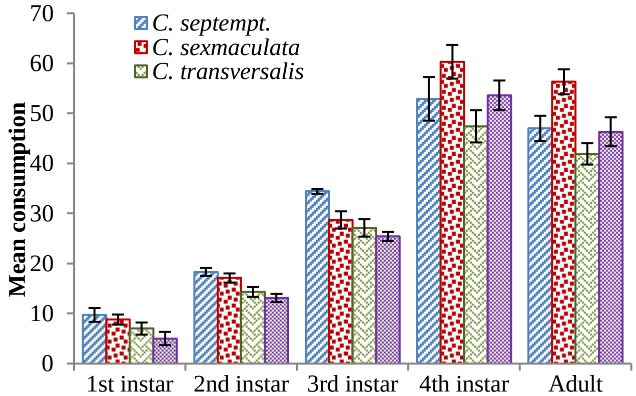
(d) *H. variegata*



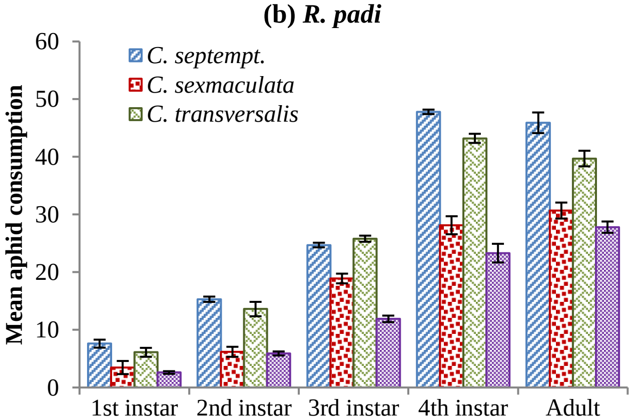
Figures 1a, 1b, 1c, 1d. Prey preference of *C. septempunctata, C. sexmaculata, C. transversalis* and *H. variegata* (adult) and larval instars for different aphid species in free choice feeding assay

The number of aphids devoured by each instar larvae per day of *C. sexmaculata* showed difference in predation extent for four aphid species. Prey consumption by the 1st instar larvae diverged with an average of 6.97±0.83 while *M. persicae* was the most consumed prey with an average of 8.80±1.00 aphids. The 2nd instar consumed an average of 13.97±1.47 aphids. *S. graminum* was the most consumed prey with maximum consumption 17.30 ±0.77 aphids per day. The 3rd instar larvae exhibited better predatory activity and faster response and consumed on average 25.40±1.36 aphids. *S. graminum* were devoured more 29.30±1.26 compared to others. The 4th and final instar consumed a maximum of 62 aphids with an average of 48.98±3.98 aphids per day (Table 1a). Maximum predation potential per day of 4th instar was observed for *M. persicae* 60.30 ±1.56 and minimum for *R. padi* 28.13±1.56 ((Table 1b). In the free choice xperiment larvae of 1st, 2nd and 3rd instar showed prey preference for *M. persicae* whereas 4th instar for *S. graminum* followed by *M. persicae*, *S. avenae* while minimum in case of *R. padi* (Figure 1b).

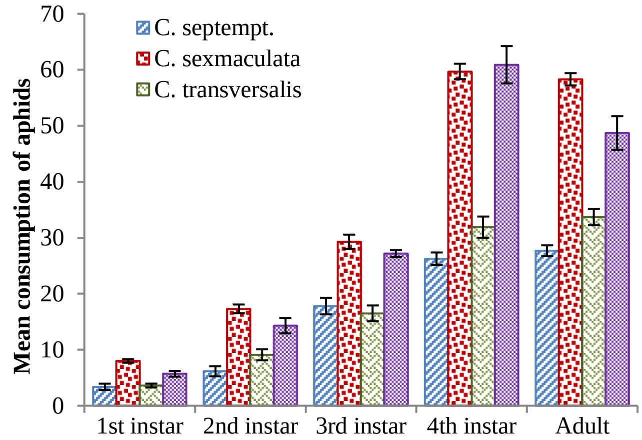
(a) *M. persicae*



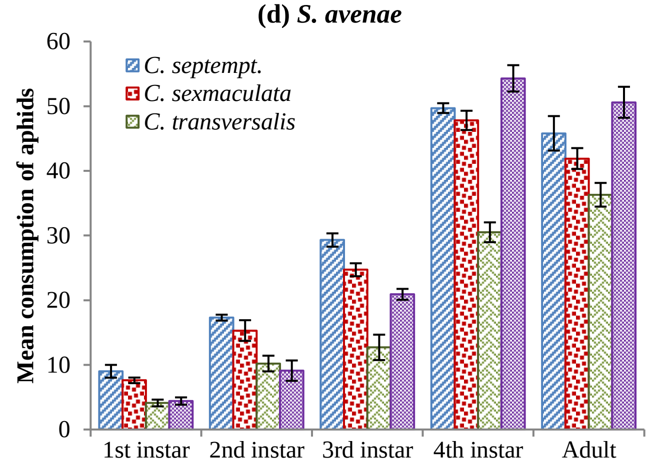
(b) *R. padi*



(c) *S. graminum*



(d) *S. avenae*



Figures 2a, 2b, 2c, 2d. Comparative efficacy of adult as well as larval instars of different predators for *M. persicae*, *R. padi*, *S. graminum* and *S. avenae*

In no choice assay, prey consumption by the 1st instar larvae of *C. transversalis* diversed with an average of 5.20±0.65. The 2nd instar consumed a maximum of 14 aphids with an average of 11.80±0.82. The 3rd instars larvae devoured efficiently on average 20.53±1.95 aphids. An average of 38.25±2.25 aphids per day were devoured at 4th stage. Maximum mean feeding rate per day for all larval instars were recorded for *M. persicae* as 7.00±1.70 was recorded for 1st, 14.30±0.98 for 2nd, 27.10±1.73 for 3rd and 47.40±1.91 for 4th instar in preference to other aphid species followed by *R. padi* while *S. graminum* and *S. avenae* consumption was almost same (Table 1c). It was evident from the findings that *M. persicae* was the preferred prey. The order of preference overall in the choice assay was *M. persicae*<, *R. padi*<, *S. graminum*< and *S. avenae* (Figure 1c).

Each larval instar of *H. variegata* devoured highest mean number 5.70±0.52, 14.30±1.39h, 27.20±0.62 and 60.90±1.50 of *S. graminium* per day by 1st, 2nd, 3rd and 4th instars, respectively in choice feeding assay while least consumption was noted for *R. padi*. Significantly higher consumption rate was observed by the 3rd and 4th larval instars for *S. graminium* and *M. persicae* **(**Table 1d). *S. graminum* was the preferred prey for 1st, 2nd, 3rd and 4th instars both in choice and no choice feeding assays surveyed by *M. persicae, S. avenae* and *R. padi* (Figure 1d). The above findings revealed highly significant difference (P<0.01) in predatory efficiency among all larval instars of four predators as well as prey species observed in both feeding assays (Tables 2 and 3).

DISCUSSION

Predatory potential of different selected Coccinellid predators were tested in the laboratory in order to predict that generalist predators show hierarchy in aphid preference. The extent of predation and prey preference of adults as well as larvae was determined in choice and no choice feeding assays conducted in laboratory. *C. sexmaculata* was found to be the most efficient predatory species as maximum per day predation potential 58.30±1.01a was observed for this predator followed by *H. variegata* 50.60±2.40, *C. septempunctata* 47.00±2.18 and least 41.90±1.56 of *C. transversalis* against aphids. Maximum per day predation potential of *C. septempunctata* and *C. tranversalis* was observed for *M. persicae, C. sexmaculata* for *S. graminum* and *H. variegate* for *S. avenae.* Omkar *et al.* (2003) found maximum 45.3 per day consumption of *C. septempunctata* on mustard aphid and Suhail *et al*. (1999) found about 40-173aphids consumption daily both for young ones (grubs) and adults of *C. septempunctata*.

Significantly higher feeding efficacy of *C. sexmaculata* and *H. variegata* in evaluation to *C. septempunctata* and *C. transversalis* was observed in the present study. The lower consumption rate of *C. septempunctata* and *C. transversalis* might be due to their sluggish behavior. Omakar (2005) observed *C. sexmaculata* as best killer for the administration of aphid species due to its efficiency in searching and handling of prey than other predators. In another study of Koul (2003), *H. variegata* was proved as a significant natural enemy of aphids in a variety of crops due to its non-specificity, thereby enhancing its biotic potential in addition to its higher fecundity. *H. variegata* proved as an efficient biological control agent against *M. persicae* and *M. rosae* whereas *C. septempunctata* was found more efficient aphidophagus species than *B. suturalis*, *C. sexmaculata* and *Menochilus sexmaculatus*. In contrast to these results, the present research findings suggest that *C. sexmaculata* had great potential against aphids. Moreover, different stages also had significant effect on consumption rate.

In the predation selectivity experiments aphid species *M. persicae* were found preferred diet of *C. septempunctata* as 17.00±1.21 per day *M. persicae* were consumed by this predator while *S. graminum*7.20±0.6 was devoured least. Predation selectivity experiment of Maolin and Hao, (2004) also revealed that adult *C. septempunctata* preferred *M. persicae* among three preys. Moreover, *C. sexmaculata* and *C. transversalis* also preferred *M. persicae* (18.50±0.68a, 15.70±1.21ab) most whereas *S. graminum* was observed as the preferred prey for *H. variegata* with maximum mean aphid consumption 19.60±0.98 in comparison to other species. The phenomenon by which predators seek, locate and recognize their suitable palatable prey is still unknown. This difference in predation might be due to recognition of most suitable prey by its respective morphological (Dixon, 2000), physiological and chemical variations (Omkar *et al*., 2004).

From the findings of present study, it was proved that aphid species *M. persicae* was the potential host for almost all adult Coccinellid predators that might be due to its high nutritional value. Overall, a hierarchy in feeding efficacy was observed in the present research yielding an order of prey preference in general as *M. persicae> S. avenae> R. padi> S. graminum* whereas the research findings of Jindal and Malik (2006) as well as Bilashini and Singh(2009) revealed hierarchy in predation potential for different aphids and yielded the following order of preference: pea aphid > coriander aphid > spinach aphid > cabbage aphid. Moreover, Prabhakar and Roy, (2010) found differential consumption rates of aphids in Coccinellidae predators.

Feeding rate was found to increase from 1st instar to 4th instars. A steady increase in consumption rate was also observed with successive developmental stages. The results of Singh and Singh (2013) are in conformity with ours that aphid voracity of Coccinellid predators increased with the age. Mishra *et al.* (2012) also observed the same trend in ladybird and found 4th instars more rapacious than 3rd, 2nd and 1stinstars. In the present study, number of aphids engrossed by the 3rd and 4th instars larvae showed significant difference while number of aphids engrossed by 1st and 2nd instars was found to be at par. The 4th and final instars were most energetic up to pre-pupal stage as they required more amount of diet than the previous instars due to bigger size, longer durations of larvae and might be food testimony for total pupal period. At this stage they consumed maximum aphids. Ghadam and Yousafpour (2012) observed that 4th instar had highest feeding rates compared to other larval instars. Inayat *et al.* (2011) reported that predation period as well as prey density affect significantly on the predation rate of *C. septempunctata* as both factors increase prey consumption. Moreover, they also found maximum predation rate during initial days of all stages.

In the no choice feeding assay during present study, feeding efficacy of 1st, 2nd and 3rdinstasr was recorded maximum 9.70±0.80h, 18.30±0.79g, and 34.40±0.46 respectively for *C. septempunctata* on *M. persicae* while per day predation potential of 4thinstar was noted maximum 60.90±1.50 for *H. variegata* on *S. graminum*. However, minimum feeding rate was noted of1st and 2nd instar for *H. variegata* as 2.60±0.81 and 5.90±0.35 aphids respectively on *R.padi* whereas minimum predation potential of 3rd instar was 12.70±1.96 recorded for *C. transversalis* on *S. avenae* and of 4thinstar as

23.30±2.19 for *H. Variegate* on *R. padi.* Highly significant interspecific and intraspecific variations occurred in predation potential of all selected predators' larval stages for four aphid species. Predation rate of Coccinellid 4th instar larvae was found higher than adults indicating their stronger impact on pest populations than adult and larvae of 4thinstars proved as voracious feeder. The result findings of Inayat *et al*. (2011) strongly support findings of present study. The contrasting studies of Singh and Singh (2013) as well as Ali and Rizvi, (2009) proved that adults consumed more aphids than grubs.

Conclusion: This study clearly established the ability of Coccinellids as feasible biological control agents of aphids being efficient predators. The study also characterized species hierarchy with respect to their depredating efficiencies. The data of this study might be powerful for studying feeding niche of these predators that may contribute to the growth and multi-objective assessment of biological control program for aphids in order to maximize ecosystem service.

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