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Research Article

Intra- and inter-specific interactions between *Coccinella transversalis* and *Cheilomenes sexmaculata* in relation to prey abundance

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ABSTRACT: In the present study, we investigated the feeding rate and the effect of intra- and inter-specific interactions between adults, 3rd and 4th instar larvae of the coccinellids, *Coccinella transversalis* and *Cheilomenes sexmaculata* to its conspecific and heterospecific combinations under laboratory conditions. Cannibalism and the effect of interaction were investigated using the multiplicative risk model. Aphidophagous predators and their instars were kept alone, paired with conspecifics, and heterospecifics combinations in the absence and presence of prey (aphid, *Aphis gossypii*). In the presence of aphids, the combined release of these predator species revealed that except for (3rd × 3rd) and (4th× 4th) intraspecific combination of both predators, the observed consumption was lower than the expected consumption. In the case of interspecific combination, the observed consumption was significantly lower than expected and it indicated the antagonistic effect of both the predators in later instars in the presence of sufficient prey population. In the absence of the prey, intra-specific cannibalism was lowest between adults of both the species and highest among *C. transversalis* 3rd × *C. transversalis* adult and *C. sexmaculata* 3rd × *C. sexmaculata* adult. The interspecific predation was highest between *C. transversalis* adult × *C. sexmaculata* 3rd instar and lowest between adults of both species. The results suggest that *C. transversalis* was more a successful aphid predator, being able to find aphids more quickly and consume more of them compared to *C. sexmaculata*. As there was less intraspecific cannibalism among *C. transversalis* and individual consumption is also high, it is considered to play a significant role as a biocontrol agent in aphid management.

KEYWORDS: *Aphis gossypii*, *Coccinella transversalis*, intra and interspecific interactions, *Cheilomenes sexmaculata*

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INTRODUCTION

Coccinellids exhibit a predatory nature against many soft-bodied insect pests such as aphids, mealybugs, whiteflies, thrips, jassids and psyllids (Ullah *et al*., 2012). In field situations, coccinellids colonize early in the season by feeding on alternative prey and thus increase their populations prior to the arrival of the pest (Landis & Vander, 1997). However, when they share a pest species as their common prey, they might engage in conspecific and heterospecific predation of eggs and larvae (Agarwala & Dixon, 1992). The effects of such interactions may either stabilize prey-predator populations (Hanski, 1981) or adversely affect the performance of individual predators. The interactions among coccinellids may be intra- and inter-specific such as cannibalism and intraguild predation that can affect the development and coexistence of predator populations (Rocca *et al*., 2020). Intraspecific competition occurs within a species, while interspecific competition occurs between species that are competing for a limited resource (Price, 1997). Thus, the outcome of such type of interactions among these predatory species has both negative and positive ecological effects on biodiversity and levels of biological control. The intensity and direction of the interactions among coccinellids are influenced by several factors including body size, aggressiveness, spatial distribution, mobility, and the availability of prey (Lucas *et al*., 1998). Competition among coccinellid species may occur during both immature and adult stages, immatures may compete for food and adults may compete for oviposition sites and food (Bellows & Hassell, 1999).

Aphis gossypii is a polyphagous sap-sucking insect that infests over 900 plant species in the world (Blackman & Eastop, 2000). It was found to be a major pest (in number) associated with Okra yellow vein mosaic disease transmission in okra (Bem *et al*., 2018). In our field study at Experimental Farm, Department of Horticulture, AAU,

Jorhat also observed *Aphis gossypii* as a major pest. Among the natural enemies, *C. transversalis* and *C. sexmaculata*, these two coccinellids were observed as dominant predators of *A. gossypii* in the field situation (Das, 2018). Therefore, a laboratory experiment was undertaken to study the interactions of these two natural enemies and their effect on *A. gossypii* so that one of the appropriate species or both in the right combination could be utilised as biological control agents against this pest.

MATERIALS AND METHODS

The experiment was conducted from June 2018 to October 2018. Aphids (*A. gossypii*) were collected from okra fields at the ICR farm area, Assam Agricultural University, Jorhat, Assam and observed under a microscope in the laboratory at the Dept. of Entomology, AAU, Jorhat for confirmation. *Aphis gossypii* (3rd instar) were then transferred to tender leaves of okra plants by using a soft fine camel hair brush. Before initiating the experiments, adult and larval instars (3rd and 4th) of coccinellids viz. *C. transversalis* and *C. sexmaculata* were reared under insectary (25±2°C and 65±5% RH) on aphids, in separate chambers.

Rearing of aphids and coccinellids

Young okra seedlings with 4-5 leaves (cv. Parbhani kranti) were transplanted individually in pots (32cm diameter) and placed in different chambers under laboratory conditions. In the first chamber, only fresh and clean plants were grown. In contrast, in the second one, *A. gossypii* was reared on the okra plants and in the third and fourth chambers, the two coccinellid (*C. transversalis* and *C. sexmaculata*) species were reared separately on *A. gossypii* infested okra plants.

Predation efficiency studies of the two coccinellid species were carried out in petri dishes (9 cm diameter and 1.5 cm height) providing aphids. An okra leaf was placed in each petri dish. The periphery of the leaf was covered with a layer of moist cotton to prevent the escape of *A. gossypii* from the leaf. On each leaf, the 3rd instar of *A. gossypii* (150 nos.) was placed gently and left undisturbed for 1hr to settle. Then one grub starved for 24 hrs (3rd, 4th instar) and an adult of each coccinellid species was individually introduced into each dish and counted number of aphids (150 nos.) were provided in each petri dish. Each treatment was replicated 15 times (a dish was a replication).

The petri dishes were kept under laboratory conditions set at 25±2°C and 65±5% relative humidity. After every 24 hours, the predators were removed from the petri dish and the number of aphids consumed in each petri dish was recorded. Feeding potentiality was calculated by counting the number of remaining aphids and subtracting them from the total number of aphids provided. The average consumption rate was calculated by the formula (aphid consumed/aphid offered × 100).

Intraspecific and interspecific interaction between *C. transversalis* and *C. sexmaculata* on *A. gossypii* consumption

The intra and interspecific interaction was studied using 3rd, 4th instars and adult of both the coccinellids. The predators were used singly and in all combinations of the two species together. The predator-prey interaction was investigated by testing each combination both in the absence and presence of aphid prey. For this purpose, okra leaves were placed on petri dishes similarly and in each dish, 3rd instar of aphids was placed and kept undisturbed for 1 hour to settle. In each petri dish, two individuals of the coccinellid (24 hrs starved 3rd, 4th instar and adult) of the same species in different combinations were placed *i.e*. (one each making it two per petri dish). Then, counted numbers of aphids (150 +150 nos.) were offered to each combination. The petri dishes were kept under laboratory conditions (25±2°C and 65±5% RH). After 24 hrs, the predators were removed from the petri dishes and the numbers of total consumed *A. gossypii* in each petri dish were counted. The dead aphids were not considered consumed. The moulted larvae during the experiment were rejected and the treatment was repeated with new larvae. Each treatment was repeated 15 times and the number of unconsumed aphids in each dish was recorded. The average consumption rate was evaluated by formula (aphid consumed/aphid offered × 100).

The different combinations followed for intraspecific interaction were as follows:

- *C. transversalis* (3rd × 3rd)
- *C. transversalis* (4th × 4th)
- *C. transversalis* (adult × adult)
- *C. transversalis* (3rd × 4th)
- *C. transversalis* (adult × 3rd)
- *C. transversalis* (adult × 4th)
- *C. sexmaculata* (3rd × 3rd)
- *C. sexmaculata* (4th × 4th)
- *C. sexmaculata* (adult × adult)
- *C. sexmaculata* (3rd × 4th)
- *C. sexmaculata* (adult × 3rd)
- *C. sexmaculata* (adult × 4th)

For interspecific interaction, in each petri dish (containing 150 nos. of aphids) two individuals of the *C. transversalis* and *C. sexmaculata* (3rd, 4th instar and adult) were placed in different combinations (one each making it two individuals per petri dish). Then, 150 numbers of *A. gossypii* (3rd instar) were fed to each combination. Petri

dishes were kept under laboratory conditions. After 24 hr, the predators were removed from the petri dish and the number of *A. gossypii* consumed in each petri dish was recorded. This experiment was also repeated 15 times (a dish was a replication) and mean consumption was calculated in the same way.

Different combinations followed for interspcific interactions are as follows:

- *C. transversalis* (3rd) × *C. sexmaculata* (3rd)
- *C. transversalis* (4th) × *C. sexmaculata* (4th)
- *C. transversalis* (adult) × *C. sexmaculata* (adult)
- *C. transversalis* (3rd) × *C. sexmaculata* (4th)
- *C. transversalis* (4th) × *C. sexmaculata* (3rd)
- *C. transversalis* (3rd) × *C. sexmaculata* (adult)
- *C. transversalis* (4th) × *C. sexmaculata* (adult)
- *C. sexmaculata* (3rd) × *C. transversalis* (adult)
- *C. sexmaculata* (4th) × *C. transversalis* (adult)

Intraspecific cannibalism and interspecific predation among coccinellids

This was done to investigate intraspecific cannibalism and interspecific predation among coccinellids (*C. transversalis* and *C. sexmaculata*) in the presence and absence of *A. gossypii*. In the presence of *A. gossypii*, the same methodology was used. In the absence of *A. gossypii,* an okra leaf was placed in petri dishes and the sides of the leaf were covered by moist cotton. A 24-hour starved grub (3rd, 4th instars) and adult of *C. transversalis* were introduced together with another 24-hour starved grub (3rd, 4th instars) and adult of *C. transversalis* (intra) and with *C. sexmaculata* (inter) in a petri dish. Each treatment was replicated 15 times. Petri dishes were kept under laboratory conditions (25±2°C and 65±5% RH). After 24 hrs, mortality which occurred in the petri dish due to intra (cannibalism) and inter-specific competition was recorded.

The different combinations followed for intraspecific cannibalism were as follows:

- *C. transversalis* (3rd ×3rd)
- *C. transversalis* (4th × 4th)
- *C. transversalis* (adult × adult)
- *C. transversalis* (3rd × 4th)
- *C. transversalis* (adult × 3rd)
- *C. transversalis* (adult × 4th)
- *C. sexmaculata* (3rd ×3rd)
- *C. sexmaculata* (4th × 4th)
- *C. sexmaculata* (adult × adult)
- *C. sexmaculata* (3rd × 4th)
- *C. sexmaculata* (3rd × adult)
- *C. sexmaculata* (4th × adult)

The different combinations followed for interspecific predation among the coccinellids were as follows:

- *C. transversalis* (3rd) × *C. sexmaculata* (3rd)
- *C. transversalis* (4th) × *C. sexmaculata* (4th)
- *C. transversalis* (adult) × *C. sexmaculata* (adult)
- *C. transversalis* (3rd) × *C. sexmaculata* (4th)
- *C. transversalis* (4th) × *C. sexmaculata* (3rd)
- *C. transversalis* (adult) × *C. sexmaculata* (3rd)
- *C. transversalis* (adult) × *C. sexmaculata* (4th)
- *C. transversalis* (adult) × *C. sexmaculata* (3rd)
- *C. transversalis* (adult) × *C. sexmaculata* (4th)
- *C. transversalis* (3rd) × *C. sexmaculata* (3rd)

Statistical analysis

The difference in predatory potential of *C. transversalis* and *C. sexmaculata* (3rd, 4th instar and adult individually) were statistically analysed by using one-way Analysis Of Variance (ANOVA). The effect of intra and inter-specific interactions among the predators on aphid prey when used individually and in combinations were assessed by using the multiplicative risk model (Soluk & Collins, 1988; Soluk, 1993; Sih *et al*., 1998). This model was considered to account for prey removal because a prey individual eaten by one predator was no longer available to other predators. If predators had independent effects, then the proportion of prey that survives both predators should be:

$$E(P1 + P2) = P1 + P2 - (P1 \times P2) \qquad (A)$$

where, E (p1+p2) is the expected proportion consumed in the two-predator treatments. P1 is prey mortality when exposed to predator (1) alone and P2 is prey mortality when exposed to predator (2) alone.

The observed predation rates were used as the response variable. These values were compared to the expected derived from the model to test for emergent multiple predator effects. The expected portion consumed in the two predator treatments was P1 + P2 – (P1 × P2). The analysis was performed with one-way ANOVA. Analysis was conducted with the statistical packa9ge JMP 10.0 (SAS Institute, 2012).

RESULTS AND DISCUSSION

Intraspecific interaction among *Coccinella transversalis* in the presence of aphid

When the 3rd instar of *A. gossypii* (150 numbers) was provided to 24 hours starved *C. transversalis* (3rd, 4th instar and adult) individually in petri dish, it was found that the mean consumption of *A. gossypii* by the *C. transversalis* 3rd instar grub was 50.4±0.91, 4th instar 76.06±1.61 and adult 101.26±1.49. In case of different combinations, when

grubs (3rd, 4th instar) and adult of *C. transversalis* were released together into each petri dish with aphid (150 nos.) it was observed that the mean consumption was significantly highest in *C. transversalis* (adult) × *C. transversalis* (adult) 134.13±1.56 and lowest in *C. transversalis* (3rd) × *C. transversalis* (3rd) 80.13±1.01 which was significantly lower than the individual consumption of adult and at par with individual consumption of 4th instar (Table 1). The rate of consumption was significantly different from each other.

Intraspecific interaction among *Cheilomenes sexmaculata* in the presence of aphid

When *A. gossypii* (150 numbers) were provided to 24 hours starved 3rd, 4th instars and adult of *C. sexmaculata* individually in petridish, it was observed that adults consumed significantly highest numbers of aphids (69.67±1.57) followed by 4th (52.46±1.004) and 3rd (36.33±0.64) instar grubs (Table 2). When *C. sexmaculata* was used in different combinations with aphid (150+150) nos. for each combination), the highest mean consumption was recorded in *C. sexmaculata* (adult) × *C. sexmaculata* (adult) 96.67±1.55 which was followed by *C. sexmaculata* (adult) × *C. sexmaculata* (4th) 82.40±1.25. This was significantly lower than the adult × adult combination. The consumption of *C. sexmaculata* (adult) × *C. sexmaculata* (3rd) and *C. sexmaculata* (4th) × *C. sexmaculata* (4th) were 78.87±0.92, 81.67±1.38, respectively. However, *C. sexmaculata* (3rd) × *C. sexmaculata* (4th) consumed 67.06±1.25 which was at par with individual consumption of adults (69.67±1.57). Among the all combinations, the lowest was recorded in *C. sexmaculata* (3rd) × *C. sexmaculata* (3rd) at 58.00±1.70 which was significantly lower than individual adult consumption (69.67±1.57).

Table 1. Consumption of *A. gossypii* by *C. transversalis* larval instars (3rd, 4th instar) and adult individually and their different combinations

Treatments	Consumption (Mean ± SE)
C. transversalis (3rd)	50.4 ± 0.91^{A}
C. transversalis (4th)	76.06 ± 1.61^{B}
C. transversalis (adult)	101.26 ± 1.49^{DE}
C. transversalis (3rd x 3rd)	80.13 ± 1.01^{B}
C. transversalis (4th x 4th)	110.26 ± 1.10^{F}
C. transversalis (3rd x 4th)	85.20 ± 0.59^{C}
C. transversalis (adult x adult)	134.13 ± 1.56^{G}
C. transversalis (adult x 3rd)	99.80 ± 1.74^{D}
C. transversalis (adult x 4th)	105.06 ± 1.47^{E}
P<value	<.001*
F value	187.20
Df	8,134

Data represents the mean of 15 observations, different letter-sharing values are significant.

Table 2. Consumption of *A. gossypii* by *C. sexmaculata* larval instars (3rd, 4th) and adult individually and their different combination

Treatments	Consumption (Mean ± SE)
C. sexmaculata (3rd)	36.33 ± 0.64^{A}
C. sexmaculata (4th)	52.46 ± 1.004^{B}
C. sexmaculata (adult)	69.67 ± 1.57^{D}
C. sexmaculata (3rd x 3rd)	58.00 ± 1.70^{C}
C. sexmaculata (4th x 4th)	81.67 ± 1.38^{E}
C. sexmaculata (3rd x 4th)	67.06 ± 1.25^{D}
C. sexmaculata (adult x adult)	96.67 ± 1.55^{F}
C. sexmaculata (adult x 3rd)	78.87 ± 0.92^{E}
C. sexmaculata (adultx 4th)	82.40 ± 1.25^{E}
P<value	<.0001*
F value	162.80
Df	8,134

Data represents the mean of 15 observations

Interspecific interaction between coccinellids in the presence of aphid

When a different combination of grub (3rd, 4th instar) and adult of *C. transversalis* and *C. sexmaculata* were released together on petri dishes with aphid (150 nos. for each treatment), it was observed that mean consumption was highest on *C. transversalis* (adult) × *C. sexmaculata* (adult) 109.60±1.41 and lowest in *C. transversalis* (3rd) × *C. sexmaculata* (3rd) 69.53±1.16. However, no significant difference was observed in the case of *C. transversalis* (adult) × *C. sexmaculata* (3rd) 89.26±1.84, *C. transversalis* (3rd) × *C. sexmaculata* (adult) 86.80±1.10 and *C. transversalis* (4th) × *C. sexmaculata* (3rd) 86.33±0.57.

Observed and expected consumption of aphids by coccinellids in intraspecific interaction

When the individual consumption of aphids and combined consumption were observed, it was found that the individual consumption was comparatively more than the combinations. Therefore, to confirm their interaction (negative, positive and neutral) Multiplicative Risk Model (MRM) was used. From the MRM study (Table 4) it was found that in the case of *C. transversalis* (3rd x 3rd) and *C transversalis* (4th x 4th), the observed and expected consumption were 80.13±1.01; 83.79 ±1.21 and 91.07±1.10; 94.34±0.49 respectively which did not differ significantly from each other. It indicated that there was no negative

Table 3. Consumption of *A. gossypii* when applied the coccinellids in their different interspecific combinations

Treatments	Consumption (Mean ± SE)
C. transversalis (3rd) x *C. sexmaculata* (3rd)	69.53 ± 1.16^{A}
C. transversalis (4th) x *C. sexmaculata* (4th)	94.53 ± 0.96^{E}
C. transversalis (adult) x *C. sexmaculata* (adult)	109.60 ± 1.41^{F}
C. transversalis (3rd) x *C. sexmaculata* (4th)	75.93 ± 0.82^{B}
C. transversalis (4th) x *C. sexmaculata* (3rd)	86.33 ± 0.57^{C}
C. transversalis (3rd) x *C. sexmaculata* (adult)	86.80 ± 1.10^{C}
C. transversalis (4th) x *C. sexmaculata* (adult)	90.80 ± 0.79^{D}
C. transversalis (adult) x *C. sexmaculata* (3rd)	89.26 ± 1.84^{CD}
C. transversalis (adult) x *C. sexmaculata* (4th)	95.00 ± 1.52^{E}
P<value	<.0001*
F value	87
Df	8,134

Data represents the mean of 15 observations

Table 4. Observed and expected Consumption of *A. gossypii* by coccinellids in their intra-specific interactions

Treatments	Observed (Mean ± SE)	Expected (Mean ± SE)	P	F
C. transversalis (3rd x 3rd)	80.13 ± 1.01^{A}	83.79 ± 1.21^{A}	0.068	0.901
C. transversalis (4th x 4th)	91.07 ± 1.10^{A}	94.34 ± 0.49^{A}	0.071	14.05
C. transversalis (adult x adult)	120.40 ± 1.56^{A}	126.22 ± 1.17^{B}	0.006	0.161
C. transversalis (adult x 3rd)	99.80 ± 1.74^{A}	110.48 ± 1.11^{B}	0.0001	2.43
C. transversalis (adult x 4th)	105.06 ± 1.47^{A}	120.67 ± 0.84^{B}	0.0001	2.42
C. transversalis (3rd x 4th)	85.02 ± 0.59^{A}	100.92 ± 1.06^{B}	0.0001	2.48
C. sexmaculata (3rd x 3rd)	58.00 ± 1.70^{A}	63.83 ± 0.97^{A}	0.06	6.13
C. sexmaculata (4th x 4th)	81.67 ± 1.38^{A}	86.49 ± 1.31^{A}	0.058	0.032
C. sexmaculata (adult x adult)	96.67 ± 1.55^{A}	106.75 ± 1.70^{B}	0.0001	0.592
C. sexmaculata (adult x 3rd)	78.87 ± 0.92^{A}	89.13 ± 1.22^{B}	0.0001	2.50
C. sexmaculata (adult x 4th)	82.40 ± 1.25^{A}	97.78 ± 1.12^{B}	0.0001	0.801
C. sexmaculata (3rd x 4th)	67.06 ± 1.25^{A}	76.08± 0.94^{B}	0.0001	2.46

Data represents the mean of 15 observations

interaction between the 3rd and 3rd instars and 4th and 4th instars of *C. transversalis*. In the case of *C. transversalis* (adult) x *C. transversalis* (adult) the observed value (120.40±1.56) was significantly lower than the expected value (126.22±1.17). In the case of *C. transversalis* (3rd) x *C. transversalis* (adult), *C. transversalis* (4th) x *C. transversalis* (adult), *C. transversalis* (3rd) x *C. transversalis* (4th), and observed consumption were significantly lower than the expected consumption which indicated that there was a negative interaction of higher and lower instars coccinellids. Similarly, in the case of *C. sexmaculata* (3rd) x *C. sexmaculata* (3rd), and *C. sexmaculata* (4th) x *C. sexmaculata* (4th) the observed values (58.00±1.70 and 81.67±1.38) were non-significant from expected values (63.83±0.97 and86.49±1.31), which indicated that there was no competition among the similar instars. In other combinations of *C. sexmaculata* the observed consumption values were significantly lower than the expected values (Table 4). This indicated that there was a competition among the older and younger instar's combinations for food.

Observed and expected consumption of aphids by coccinellids in interspecific interaction

In the case of interspecific interaction, in all combinations, the observed consumption was significantly lower than the expected consumption which indicated that there was a negative interaction among the different combinations of *C. transversalis* and *C. sexmaculata* (Table 5). This lower consumption of aphids might be due to the interspecific antagonistic interaction, which indicated some harmful orantagonistic effects among them when both predators released together.

Intraspecific cannibalism among different instar of coccinellids in the absence of aphids

The result obtained from intraspecific cannibalism among different instars and adults of *C. transversalis* and *C. sexmaculata*, indicated that in the absence of aphids, there was no cannibalism among the *C. transversalis* (adult) x *C. transversalis* (adult) (0%) and *C. sexmaculata* (adult) x *C. sexmaculata* (adult) (0%). The highest cannibalism was found in *C. sexmaculata* (adult) x *C. sexmaculata* (3rd) (26.68%) and the lowest was found in the case of *C. sexmaculata* (3rd) x *C. sexmaculata* (3rd) (10%) and *C. transversalis* (3rd) x *C. transversalis* (3rd) (10%). In the present study, less per cent cannibalism (<30%) among all the combinations of the same species was observed, which indicated that there was less competition or less amount of antagonistic effect between intraspecific interactions (Table 6).

Table 5. Observed and expected Consumption of *A. gossypii* by coccinellids in interspecific interactions

Treatments	Observed (Mean± SE)	Expected (Mean ± SE)	P	F
C. transversalis (3rd) x *C. sexmaculata* (3rd)	69.53 ± 1.16^{A}	74.53 ± 0.80^{B}	0.001	4.12
C. transversalis (4th) x *C. sexmaculata* (4th)	94.53 ± 0.96^{A}	101.92 ± 0.70^{B}	0.0001	3.12
C. transversalis (adult) x *C. sexmaculata* (adult)	109.60±1.41^{A}	118.19 ± 0.94^{B}	0.0001	1.42
C. transversalis (3rd) x *C. sexmaculata* (4th)	75.93 ± 0.82^{A}	83.50 ± 2.48^{B}	0.007	1.84
C. transversalis (4th) x *C. sexmaculata* (3rd)	93.98 ± 0.57^{A}	86.33 ± 1.07^{B}	0.001	2.37
C. transversalis (3rd) x *C. sexmaculata* (adult)	86.80 ± 1.10^{A}	96.73 ± 0.88^{B}	0.0001	0.448
C. transversalis (4th) x *C. sexmaculata* (adult)	90.80 ± 0.79^{A}	110.35 ± 1.03^{B}	0.001	2.49
C. transversalis (adult) x *C. sexmaculata* (3rd)	89.27 ± 1.84^{A}	104.96 ± 1.08^{B}	0.001	5.85
C. transversalis (adult) x *C. sexmaculata* (4th)	95.00 ± 1.52^{A}	111.30 ± 1.11^{B}	0.001	1.06

Data represent the mean of 15 observations

Table 6. Intraspecific cannibalism among different combinations of larval instars (3rd, 4th) and adult coccinellids in the absence of prey

Treatments	Per cent cannibalism
C. transversalis (3rd) x *C. transversalis* (3rd)	10 %
C. transversalis (4th) x *C. transversalis* (4th)	15 %
C. transversalis (adult x adult)	0 %
C. transversalis (3rd x 4th)	20 %
C. transversalis (adult x 3rd)	26.65 %
C. transversalis (adult x 4th)	20 %
C. sexmaculata (3rd x 3rd)	10 %
C. sexmaculata (4th x 4th)	15 %
C. sexmaculata (adult x adult)	0 %
C. sexmaculata (3rd x 4th)	20 %
C. sexmaculata (adult x 3rd)	26.68 %
C. sexmaculata (adult x 4th)	%

Data represents the mean of 15 observations

Interspecific predation among different instars of coccinellids in the absence of aphid

When *C. transversalis* (3rd and 4th instar and adult) x *C. sexmaculata (*3rd, 4th instar and adult) were released together in pair-wise combinations without aphids, it was found that more or less each species attack one another (Table 7). However, adults of *C. transversalis* showed more aggressiveness towards the 3rd and 4th instar of *C. sexmaculata*. Among the all stages, maximum larval predation of *C. sexmaculata,* 3rd instar, *i.e.,* (45 %) followed by 4th instar (30 %) was observed by an adult of *C. transversalis*. In the case of *C. transversalis* (4th) x *C. sexmaculata* (3rd) and *C. transversalis* (4th) x *C. sexmaculata* (4th) combinations, *C. transversalis* predated 20 % of *C. sexmaculata*. However, *C. sexmaculata* also predated on *C. transversalis* but their predation was comparatively lower than the *C. transversalis*. The adult *C. sexmaculata* cannibalised 20 per cent *of C. transversalis* 3rd instar and 10 per cent of *C. transversalis* (4th) instar. There was no predation between the adults of both species. From these data, it was revealed that among the *C. transversalis* and *C. sexmaculata* in older instars, there was less or no predation in the absence of aphid prey and younger and older instars combinations had more interspecific predation was observed.

The difference in predatory potential of the two coccinellids presented in Tables 1 and 2 revealed that *C. transversalis* consumed more aphids than *C. sexmaculata*, possibly because they are larger. Factors such as differences in size, age and speed of searching between the predators can influence the outcome of competition between predators (Stevens 1992; Polis & Holt 1992). Hence, to control the aphid population (*A. gossypii*) in the okra ecosystem, *C. transversalis* is preferred over *C. sexmaculata*. When 3rd, 4th and adult of *C. transversalis* were released together, maximum consumption was found among *C. transversalis* (adult) × *C. transversalis* (adult) compared to other combinations and minimum was found among *C. transversalis* (3rd) × *C. transversalis* (3rd) combination. Similarly, *C. sexmaculata* when released together in different combinations, the highest consumption was observed among *C. sexmaculata* (adult) × *C. sexmaculata* (adult) and the lowest was found among *C. sexmaculata* (3rd) × *C. sexmaculata* (3rd). This might be due to their varied dietary requirements (Muhammad *et al*., 2014).

The outcome of intraspecific interaction studies indicated that older instars and combinations of older instars of both coccinellids feed more aphids compared to younger or smaller ones. Here, the fourth instar showed better predation activity than the third larval instar and the adult consumed more aphids as compared to the 3rd and 4th instar grubs. Possibly because they have higher energy requirements (Ahlawat *et al*., 2008; Mishra *et al*., 2012) and store energy for future development and metamorphosis (Ferran & Larroque, 1977). Earlier studies reported that older instar required more food than the previous instar due to bigger size, longer duration and accumulated nutrients for the pupal period (Pandi *et al*., 2012; Işikber, 2005; Prabhakar & Roy, 2010). According to Sabelis (1992), the size of prey attacked generally increased with the size and age of the predators. In general, insects with larger body sizes can delay satiation time and have faster digestive ability (Mills 1982; Omkar & Pervez, 2004).

In the case of interspecific interaction, both predators showed an antagonistic effect in the presence of sufficient *A. gossypii* population (Table 3). Therefore it is recommended not to release these two coccinellids together in protected cultivations or when aphid populations are low. For control of aphids (*A. gossypii*), any one of the predators can be released. The present findings revealed that interspecific consumption was comparatively less than intraspecific combination. This might be due to their competition for food. However, much recent work on predator ecology suggested that increasing natural enemy diversity increases the risk of intraguild predation among them, such that herbivore suppression

Table 7. Interspecific interaction among different combinations of larval instars (3rd, 4th) and adult coccinellids in the absence of prey

Treatments	Per cent cannibalism by	
	C. transversalis	***C. sexmaculata***
C. transversalis (3rd) x *C. sexmaculata* (3rd)	10 %	0 %
C. transversalis (4th) x *C. sexmaculata* (4th)	20 %	5 %
C. transversalis (adult) x *C. sexmaculata* (adult)	0 %	0 %
C. transversalis (3rd) x *C. sexmaculata* (4th)	15 %	5 %
C. transversalis (4th) x *C. sexmaculata* (3rd)	20 %	0 %
C. transversalis (adult) x *C. sexmaculata* (3rd)	45 %	0 %
C. transversalis (adult) x *C. sexmaculata* (4th)	30 %	10 %
C. sexmaculata (adult) x *C. transversalis* (3rd)	0 %	20 %
C. sexmaculata (adult) x *C. transversalis* (4th)	0%	5 %

Data represents the mean of 15 observation

might decline as predator diversity increases (Rosenheim *et al*., 1993; Snyder & Ives, 2001; Snyder & Wise, 2001). Earlier, Rohman (2017) studied the interspecific interaction between *C. transversalis* and *Coccinella septempunctata* in the presence of *Myzus persicae* and found that under intraspecific combination coccinellids were more voracious than their interspecific combination. In a field experiment, Connell (1983) found that intraspecific competition is generally more intense than interspecific competition. Ajvad *et al*. (2014) studied the effect of intraguild predation among the five predator combinations including 2nd and 3rd instars grub of *Hippodamia variegata* and 3rd instar grub of *Episyrphus balteatus* and found that in most combinations the 3rd instar grub of *E. balteatus* alone reduced *A. gossypii* population more efficiently than ladybird beetle grub and their combinations.

In our study, the outcome of joint interaction (negative, positive and neutral) of both coccinellids was assessed by using the Multiplicative Risk Model. From the MRM study it was found that when both the predators were released together and their combined effect was assessed, the result showed that in the case of (3rd × 3rd) and (4th× 4th) intraspecific combination of both predators, the observed and expected consumption did not differ significantly from each other. It indicated that no negative interaction between the 3rd x 3rd instars and 4th x 4th instars of both coccinellids in their intraspecific combination. However, in other combinations, the observed value was significantly lower than the expected value. This indicated that there was a competition among the older and younger instar's combinations for food. When predators were released together at a time in interspecific combination, the expected consumption was significantly higher than the observed consumption and it indicated the negative effect of both the predators in the presence of sufficient *A. gossypii* population. This outcome revealed that prey consumption was reduced when both predators were present. In the presence of aphid prey, no cannibalism was found among the different combinations of *C. transversalis* and *C. sexmaculata* in their intraspecific combinations. Therefore it may be suggested that in the presence of a sufficient number of aphids, both species can be used together as biocontrol agents in the okra ecosystem. In the absence of aphid, intra-specific cannibalism was lowest between adults of both the species (0%) and highest among *C. transversalis* 3rd × *C. transversalis* adult and *C. sexmaculata* 3rd x *C. sexmaculata* adult (26.65 % and 26.68 %, respectively). In this experiment, less per cent cannibalism (<30%) among all the combinations of the same species was observed, which indicated that there was less competition or less amount of antagonistic effect between intraspecific interactions without aphids (Table 6). When both the coccinellid species were released together without aphid in inter-specific combinations, *C. transversalis* revealed high aggressiveness towards *C. sexmaculata* and 3rd and 4th larval instars of *C. sexmaculata* suffered more from interspecific predation by the adult of *C. transversalis* (45 % and 30 %). The reason behind this could be the fact that the sessile and low-mobility predators are generally vulnerable to strong interspecific competitors. There was no interspecific predation between the adults of both species. From these data, it was revealed that among the *C. transversalis* and *C. sexmaculata* in older instars, there was less or no predation in the absence of aphid prey and in younger and older instars combinations more predation was observed.

The study showed that low density or scarcity of prey was the main cause of cannibalism in coccinellids. Many researchers reported that cannibalistic behaviour in coccinellid adults and older larvae like feeding on eggs, younger or same-stage larvae, and prepupae/pupae is a widespread phenomenon (Takahashi 1987; Agarwala & Dixon 1992). The eggs and younger larvae appeared to be more susceptible to predation by older larvae and adults and were easily devoured (Dimetry 1974; Nakamura *et al*., 2006). Similar findings were also reported by Lucas *et al*. (1998), who reported that smaller individuals were more vulnerable to predation by larger predators. As the adults were more active and their nutritional requirement was generally high, they might have cannibalized in the absence of prey. A similar trend was also observed in a study by Agarwala *et al*. (2003) who reported that larvae and adults of *C. sexmaculata* were smaller than *C. transversalis* and starving adults and larvae of *C. sexmaculata* had slower searching speed than that of *C. transversalis*. Moreover, it is suggested that eggs and new-born larvae of *C. sexmaculata* are vulnerable to attack from other large-sized species, *C. transversalis* (Agarwala & Bardhanroy 1997; Agarwala *et al*., 1998). It can be concluded from the above study that to control the aphid population in the okra ecosystem, *C. transversalis* is the preferred predator over *C. sexmaculata*. In the case of interspecific interaction, both predators showed antagonistic effects. Therefore it is recommended not to release these two coccinellids together in protected cultivations or when aphid populations are low. For control of *A. gossypii,* any one of the predators can be released.

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