Interaction between two species of mealybug predators *Spalgis epius* and *Cryptolaemus montrouzieri* and mealybug attendant ant species

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1. INTRODUCTION

Fundamental question in biological control is how multiple predators interact collectively to suppress the populations of herbivorous pests (Denoth *et al.,* 2002; Symondson *et al.,* 2002; Wilby& Thomas, 2002; Cardinale*et al.,* 2003). Inter- and intraspecific competitions are important interactions among organisms which share the same food. Cannibalism has attracted much attention as these interactions are significant and widespread among many taxa of predatory arthropods. Cannibalism determines the fate of a community (Godfray & Pacala, 1992).

Several different species of mealybugs (Hemiptera: Pseudococcidae)are the major pests of economically important crops in the temperate and tropical regions. Some of the important species i.e. *Planococcus citri*(Risso), *P.lilacinus* (Cockerell.), *Phenacoccus solenopsis* Tinsley, *Paracoccus marginatus* Williams, and Granara de Willink, *Ferrisia virgata* (Cockerell.) and *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae) are serious pests on various crops, for example, coffee, citrus, cocoa, guava, grapes, papaya, cotton, mango, mulberry, and vegetables worldwide (Browning 1992; Franco *et al.*2001). Satisfactory control of any species of mealybugs has not been achieved with insecticides because of their protective wax body coating and hiding nature in bark crevices and other inaccessible parts of plants (Krishnamoorthy and Singh 1987; Joyce *et al*. 2001).

Biological control of mealybugs using parasitoids and predators is the most important control method as chemical control is less effective and environmentally undesirable (Bentley 2002). The predator *Cryptolaemus montrouzieri* (Coleoptera: Coccinellidae) was imported from Australia to control mealybugs in India, and is being used against mealybugs on various crops. Larvae and adults of *C. montrouzieri* prey and consume all stages of mealybugs (Clasusen, 1978). *C .montrouzieri* completes its life cycle in 28.4 days with four larval instars in laboratory conditions (Mani & Thontadarya, 1987).

Moreover, the apefly *Spalgis epius* (Lepidoptera: Lycaenidae) has been recorded as an effective indigenous predator on various species of mealybugs in the field in India (Dinesh & Venkatesha, 2011a, b). Studies on the biology, development, mating and egg laying behaviour, feeding potential and mass rearing of *S. epius* have been conducted (Venkatesha *et al.,* 2004; Venkatesha, 2005; Venkatesha & Shashikumar, 2006; Dinesh *et al.,* 2010; Dinesh &Venkatesha, 2011a, b; Venkatesha & Dinesh, 2011; Dinesh & Venkatesha, 2012; Dinesh &Venkatesha, 2013a, b). *Spalgi epius* completes its life cycle in 23.8 days with four larval instars in laboratory condition (Dinesh *et al*., 2010).

A mass-rearing method for this predator has been developed to exploit this predator as a major biocontrol agent of mealybugs (Dinesh *et al.* 2010; Venkatesha and Dinesh 2011). In the field, mealybug colonies are attended by different species of ants, the interaction between ants, and hemipterans are common and they play an important role in ecology. (Holl-dobler & Wilson 1990; Stadler & Dixon 2005; Styrsky & Eubanks 2007). Hompterians and ants live on the same habitats (Stadler & Dixon 2005); the interaction between homopterans and ants is a common phenomenon (Helms &Vinson 2002; Brightwell & Silverman 2010). Ants can promote hemipteran colonies by giving protection from their natural enemies (Daane *et al*., 2007; Powell & Silverman 2010).In exchange, ants will receive a large amount of honeydew as mutualism(Holland *et al*., 2005) honeydew contains sugar, carbohydrates, and various amino acids(Yao &Akimoto 2002; Helms & Vinson 2008) which is helpful in growth and survival for the ant colony. This type of mutualism is called trophobiosis (Holldobler & Wilson 1990). Thus, several mealybug attendant ant species are known to hinder the activities of parasitoids and insect predators of mealybugs (Le Pelley 1968).

Spalgis epius and *C. montrouzieri* coexist in agricultural fields sharing common prey resources (Mani, 1995). Moreover, both are potential predators of different species of mealybugs, but there is no information is available on ants interaction with mealybug predators and interaction with biocontrol agent i.e., both predators in the presence and absence of prey species. Hence, this study was conducted to know the interaction of ants with predators in the laboratory condition, and to find the competition, cannibalism, and IGP of both the predators and the relationship between them, and finally to check whether the combination of both predators will be a good biological control agents in the management of mealybugs.

In view of the above, an investigation was undertaken with the following objectives:

- **Investigation on intra-specific interaction between mealybug predators, *Spalgis epius* and *Cryptolaemus montrouzieri*.**
- **Investigation on inter-specific interaction between *S. epius* and *C. montrouzieri*.**
- **Investigation on interaction between mealybug attendant ant species and *S. epius*/*C. montrouzieri***.

2. METHODS

Lab rearing of prey and predators:

Initially *Spalgis epius* adults were allowed to mate in the outdoor mating cage and the gravid females were provided mealybug infested pumpkins for egg deposition (Figure 1A,B). Predators *S. epius* and *C. montrouzieri* were cultured in the laboratory by using mealybug *Planococcus citri* (Risso) as a host on pumpkins (*Cucurbita maxima* Duchesne) as described by Serrano & Lapointe (2002). Both predators *S. epius* and *C. montrouzieri* were cultured separately on mealybug infested pumpkins at 28±1°C, 65±5% RH, and photoperiod 12:12 L: D in an environment chamber following the methods of Chacko et al. (1978) and Venkatesha & Dinesh (2011). All experiments were conducted at 28±1°C, 65±5% RH and photoperiod 12:12 L: D in an insect environment chamber (Figure 1C, D & E).

A. **Figure 1**: **A.** Outdoor mating Perspex house for *Spalgis epius* **B.** A *Spalgis epius* female depositing eggs on the mealybug infested pumpkin, **C.** Mass multiplication of *Spalgis epius* on mealybug infested pumpkin, **D**. *Spalgis epius* adults in the rearing cage, **E**. Mass multiplication of *Cryptolaemus montrouzieri* on mealybug infested pumkin

Interaction and feeding potential of predators:

Interaction and prey consumption of *S. epius* and *C. montrouzieri* was studied by using plastic cups (5 cm diameter) with cut-opened bottom fixed on the surface of a pumpkin using melted paraffin wax and this served as an arena for the experiment. Through the open end of the cup, 200 mealybug crawlers (first instar nymphs) were released on the pumpkin and the mouth of the cup was closed using muslin cloth. When nymphs reached the adult stage, the number of adult mealybugs present inside the cup was counted and the first instar larva of *S. epius* and *C. montrouzieri* were released into the cup in three different combinations: a) one larva each of *S. epius* and *C. montrouzieri,* b) two larvae of *C. montrouzieri*, and c) two larvae of *S. epius*. Observations were made on inter- and intraspecific larval interactions and the number of prey consumed in the three combinations. Each experiment was repeated five times.

Inter- and intraspecific interaction in the absence of prey:

All the four larval instars of *S. epius* and *C. montrouzieri* were collected from mealybug- infested pumpkins and kept them individually in Petri dishes (5 cm diameter). These larvae were starved for 12 h to induce a similar level of hunger. In the first set of experiment, intra- and interspecific interaction studies were conducted in Petri dishes in three different combinations: a) one *S. epius* larva and one *C. montrouzieri* larva of similar instars, b) a pair of similar larval instars of *S. epius,* and c) a pair of similar larval instars of *C. montrouzieri*. Thus, there were eight intraspecific and four interspecific combinations of similar instar larval interactions. Each experiment was replicated five times with 10 sets per replication. Cannibalism and predation were recorded, if any after 24 h.

In the second set of experiments, intra- and interspecific interaction studies were conducted in four different combinations: a) younger instar larva of *S. epius vs* one instar older larva of *C. montrouzieri*, b) younger instar larva of *C. montrouzieri vs* one instar older larva of *S. epius*, c) younger instar *vs* one instar older larva of *S. epius,* and d) younger instar *vs* one instar older larva of *C. montrouzieri.* Thus, there were six intraspecific and six interspecific larval combinations. In all experimental combinations one larva from each predator was used. Each experiment was replicated five times with 10 sets per replication. Cannibalism and predation were recorded, if any after 24 h.

In the third set of experiment, intra- and interspecific interaction studies were conducted in five different combinations: a)10 eggs of *S. epius vs* one I/II/III/IV instar larva of *S. epius*, b) 10 eggs of *C. montrouzieri vs* one I/II/III/IV instar larva of *C. montrouzieri*, c) 10 eggs of *S. epius vs* one I/II/III/IV instar larva of *C. montrouzieri*, d) 10 eggs of *C. montrouzieri vs* one I/II/III/IV instar larva of *S. epius,* and e) 10 eggs, one larva each from four larval instars, one prepupa and one pupa of *S. epius* independently *vs* one adult of *C. montrouzieri*. Thus, there were eight intraspecific and 15 interspecific combinations. Each experiment was replicated five times with 10 sets per replication.

Interaction of mealybug attendant ants with mealybug predators:

To study the interaction of mealybug attended ant species with mealybug predators *S. epius/C. montrouzieri.* Mealybug infested pumpkin were placed near the ant colony, ants were attracted to honeydew present on the mealybug colony, then they will establish their colony for food source on mealybug infested pumpkin.

In this study, both predators (*S. epius/C. montrouzieri*) larval culture was maintained separately from which required larval instar were selected based on the body size and day of ecdysis to find the same age larva. This method is fallowed for all the 4 larval instars of both the predators for further experimental use. Five larvae from the same instar (Ist, IInd, IIIrd, IVth) were selected and released on the ant colony established on mealybug infested pumpkin to study the interaction of ants with mealybug predators. The behaviour of ant was also observed. The same experiment was repeated for all other larval instars of both predators (*S. epius*/*C. montrouzieri*). In this investigation, the behaviour of five different species of ants (Ghost ant, *Tapinoma melanocephalum* (Fabricius) (Hymmenoptera: Formicidae), Carpenter ant *Camponotus variegates* (Hawaiian), Tropical fire ant *Solenopsis geminata* (Fabricius), *Monomorium latinode* (mayr), and *Crematogaster* sp. against predator larvae was recorded (Figure 2A,B,C,D,E).

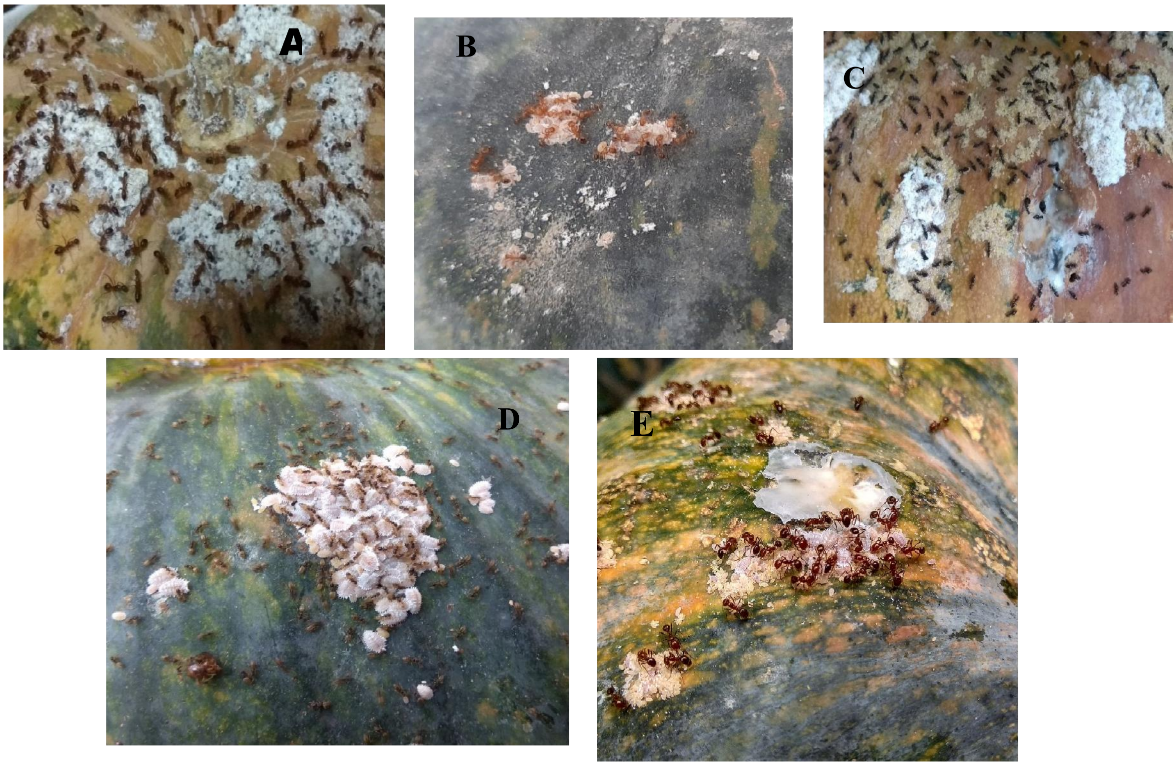

Figure 2: Different mealybug attendant ant species. **A**. *Camponotus variegates,* **B**. *Monomorium latinode,* **C**. *Crematogaster* sp.*,* **D**. *Tapinoma melanocephalum,* **E**. *Solenopsis geminata*

3. RESULTS

In Inter and intraspecific interaction between the combination of two predator *S. epius* and *C. montrouzieri* larva from first to fourth-instar larvae presented in table 1.

Table 1: Interspecific interaction between *Spalgis epius*(Se) and *Cryptolaemus montrouzieri* (CM)

Larval instar (species)	*S. epius* acts as prey	*S. epius* acts as predator
I instar Se vs. I instar Cm	15	5
II instar Se vs. II instar Cm	25	5
III instar Se vs. III instar Cm	15	10
IV instar Se vs. IV instar Cm	5	5
	Younger *S. epius* acts as prey	younger *S. epius* acts as predator
I instar Se vs. II instar Cm	35	0
II instar Se vs. III instar Cm	16	0
III instar Se vs. IV instar Cm	11	6
	Younger *S. epius* acts as prey	younger *S. epius* acts as predator
I instar Cm vs. II instar Se	10	5
II instar Cm vs. III instar Se	30	6
III instar Cm vs IV instar Se	6	0

N=50 for each pairing

Interaction between the same larval instar of two predators, third instar larva of *S. epius* predated maximum on *C. montrouzieri* larva compared to other larval instars, whereas the second instar larva of *C. montrouzieri* predated maximum on *S. epius* compared to other instar larvae. Between the combinations of the same larval instar, *S. epius* third instar larva showed maximum IGP (cannibalism) compared to other larval instars whereas in *C. montrouzieri* first instar larva showed more IGP compared to other larval instar (Table 2).

Table 2: IGP between *Spalgis epius* (Se) and *Cryptolaemus montrouzieri* (Cm) larvae in the absence of prey

Larval stages	Number of trails	Younger Se acts as predator	Younger Se acts as prey	Number of IGP
L1	50	5	15	30
L2	50	6	26	18
L3	50	12	13	25
L4	50	4	6	40

Combination of younger and older larval instars of *S. epius* and *C. montrouzieri,* older *S. epius* larval predation on younger *C. montrouzieri* larva and younger *S. epius* larval predation on older *C. montrouzieri* was not much difference between the different larval combination whereas in *C. montrouzierei* second instar larva predated more on first instar larva. Whereas in *S. epius* third instar larva predated more than other larval instars (Table 3).

Table 3: Percentage of predation and IGP between younger *Spalgis epius* (Se) and older *Cryptolaemus montrouzieri* (Cm) larvae

Laraval stages	Number of trails	Younger Se acts as predator	Younger Se acts as prey	Number of IGP
L1	50	0	35	15
L2	50	0	14	36
L3	50	4	17	33

The combination between the same species of young and one instar old larva, the third instar larva of *S. epius* shows more cannibalistic on both second and fourth larva than other larval instars in combination. Whereas in *C. montrouzierei* second instar larva was more cannibalistic on first instar larva than other larval instars in the combination. Cannibalism was less in younger larva on older *C. montrouzieri* and there was no much difference between different larval combinations (Table 4).

Table 4: Percentage of predation and IGP between older *Spalgis epius* (Se) and younger *Cryptolaemus montrouzieri* (Cm) larvae

Larval stages	Number of trails	Older Se acts as predator	Older Se acts as prey	Number of IGP
L2	50	3	11	36
L3	50	6	31	13
L4	50	0	4	46

Cannibalism within the same larval instar of *S. epius* and *C. montrouzierei*, in *S. epius* third instar larva, showed maximum cannibalism when compared to another larval instar (Table 5a). Whereas in *C. montrouzierei* second larval instar showed maximum cannibalism than other larval instars within the species (Table 5b).

Table 5a: Mean of cannibalism in the same larval instar of *Spalgis epius* in absence of prey

Larval stages	Percentage of cannibalism
L1	0.08
L2	0.38
L3	0.58
L4	0.18

Table 5b: Mean of cannibalism in the same larval instar of *Cryptolaemus montrouzieri* in absence of prey

Larval stages	Percentage of cannibalism
L1	0.51
L2	0.59
L3	0.07
L4	0.12

Overall predation of *S. epius* larva by *C. montrouzieri* larva was more than that of *C. montrouzieri* by *S. epius*. Overall cannibalism was more in *C. montrouzieri* than that in *S. epius* larva (Table 6).

Table 6: Mean percentage of cannibalism and predation in *Spalgis epius* (Se) *Cryptolaemus montrouzieri* (Cm) in absence of prey

Larval combination	**Percentage of predation/cannibalism**
Se predated by Se	0.3
Cm predated by Cm	0.42
Cm predated by Se	0.11
Se predated by Cm	0.6

Interaction between ants and mealybug predators:

Ant species foraging on mealybug infested pumpkin was more from day one of the experimental setup and reached its maximum foraging on the third day in *Monomorium latinode* and *Tapinoma melanocephalum*, whereas in *Solenopsis geminata* and *Crematogaster* sp. they took five days. In *Camponotus variegates* (Hawaiian) it was noticed on the sixth day to reach maximum forging.The number of ants foraging was more in smaller body-sized ant species i.e, *M. latinode* and *T. melanocephalum* than S*. geminata*, *Crematogaster* sp*.,* and *C. variegates* on mealybug infested pumpkin and the number of ants also depends on the honeydew producing hemipterans.

Interaction between mealybug predators Spalgis epius, Cryptolaemus montrouzieri and Monomorium latinode:

Monomorium latinode is the smaller body-sized ants, which were active and they showed good olfaction in the present study. When different larval instar of predators was released on ant foraging mealybug colony with fourth instar larva of *S. epius* attended by the maximum number of ants and time taken for the identification of larva was less compare to other larval stages (I,II, and III instar) (Table 7a). Whereas in *C. montrouzieri* adults were attended by maximum number of ants followed by IV, III, II, and I instar larvae and the time taken by ants to kill the different stages of predators was noticed more in adults followed by II,IV,I,III instar larva. (Table 7b).

Table 7a: Interaction between *Spalgis epius* and *Monomorium latinode*

Larval instar	No. of larvae used	Mean number of ants attended	Time taken to identify larva (in seconds)	Time taken to take out waxy coat (in seconds)	Time taken to kill the predator (in seconds)
I instar	50	2.34	86.02	0	111.38
II instar	50	3.86	66.7	102.12	115.48
III instar	50	5.22	50.16	67.96	80.54
IV instar	50	7.04	18.6	40.54	63.82

Table 7b: Interaction of between *Cryptolaemus montrouzieri* and *Monomorium latinode*

Larval instar	No. of larvae used	Mean number of ants attended	Time taken to identify larva (in seconds)	Time taken to take out waxy coat (in seconds)	Time taken to kill the predator (in seconds)
I instar	50	2.4	64.16	0	77
II instar	50	3.58	48.92	58.28	71.36
III instar	50	5.58	37.2	57.46	79.22
IV instar	50	6.78	26.24	48.38	74.34
Adult	50	7.36	16.18	0	57.26

Interaction between mealybug predators Spalgis epius, Cryptolaemus montrouzieri and Camponotus variegates:

Camponotus variegates is the largest ant in its body size compared to other ants observed in the study. This ant species showed its aggressiveness towards predator *S. epius* fourth instar larvae, which were attended by a maximum number of ants than other instars (III, II, I instars) and also fourth instar larva was identified and killed by ants than other larval instars (Table 8a). Similarly, in *C. montrouzieri,* maximum number of ants attended the fourth instar larvae and *C. montrouzieri* adults were identified and killed easily than other larval stages (Table 8b).

Table 8a: Interaction between *Spalgis epius* and *Camponotus variegates*

Larval instar	No. of larvae used	Mean number of ants attended	Time taken to identify larva (in seconds)	Time taken to take out waxy coat (in seconds)	Time taken to kill the predator (in seconds)
I instar	50	1	119.1	0	128.74
II instar	50	1.2	93.9	107.18	116.2
III instar	50	2.84	73.595	85.59	97.375
IV instar	50	3.24	58.18	70	83.6

Table 8b: Interaction between *Cryptolaemus montrouzieri* and *Camponotus variegates*

Larval instar	No. of larvae used	Mean number of ants attended	Time taken to identify larva (in seconds)	Time taken to take out waxy coat (in seconds)	Time taken to kill the predator (in seconds)
I instar	50	1	76.82	0	83.64
II instar	50	1.22	58.172	65.884	73.636
III instar	50	1.78	46.9	59.56	75.52
IV instar	50	2.6	29.46	42.84	58.76
Adult	50	2.58	19.42	0	41.54

Interaction between mealybug predators Spalgis epius, Cryptolaemus montrouzieri and Tapinoma melanocephalum:

Tapinomamelano cephalum is highly competitive for sugar sources where more number of ants forage on the mealybug colony when predator larva released on ant foraging mealybug colony, fourth instar larva of *S. epius* was attended by more number of ants, and also identified and killed the fourth instar easily than other larval instars (Table 9a). Adult of *C. montrouzieri* was noticed by ants than other larval instars and took less time to kill adults than other stages (Table 9b).

Table 9a: Interaction of *Spalgis epius* and *Tapinoma melanocephalum*

Larval instar	**No. of larvae used**	**Mean number of ants attended**	**Time taken to identify larva (in seconds)**	**Time taken to take out waxy coat (in seconds)**	**Time taken to kill the predator (in seconds)**
I instar	50	3	96	0	108.9
II instar	50	3.82	79.6	97.64	124.54
III instar	50	4.5	63.06	88.5	114.28
IV instar	50	5.8	48.88	72.78	103.6

Table 9b: Interaction of *Cryptolaemus montrouzieri* and *Tapinoma melanocephalum*

Larval instar	**No. of larvae used**	**Mean number of ants attended**	**Time taken to identify larva (in seconds)**	**Time taken to take out waxy coat (in seconds)**	**Time taken to kill the predator (in seconds)**
I instar	50	2.76	76.4	0	91.12
II instar	50	4.4	60.04	81.26	101.46
III instar	50	5.4	51.08	73.46	99.54
IV instar	50	6.44	38.86	62.82	91.18
Adult	50	7	31.22	0	77.5

Interaction between mealybug predators Spalgis epius, Cryptolaemus montrouzieri and Crematogaster sp.:

Crematogaster sp. shows its aggression on mealybug predator when *S. epius* larva was released on ant tending colony- maximum ants attacked and identified the fourth instar larva fallowed by III, II, and I instar, time duration to kill the larva was minimum in the fourth instar (Table 10a). More number of ants attacked *Cryptolaemus montrouzieri adult* and time taken to identify and kill the same was less in adults compared to other larval stages (Table 10b).

Table 10a: Interaction between *Spalgis epius* and *Crematogaster* sp.

Larval instar	No. of larvae used	Mean number of ants attended	Time taken to identify larva (in seconds)	Time taken to take out waxy coat (in seconds)	Time taken to kill the predator (in seconds)
I instar	50	1.82	113.04	0	125.18
II instar	50	2.26	83.68	97.04	114.78
III instar	50	3.38	63.14	76.9	98.6
IV instar	50	3.66	51.68	75.44	91.14

Table 10b: Interaction between *Cryptolaemus montrouzieri* and *Crematogaster* sp.

Larval instar	No. of larvae used	Mean number of ants attended	Time taken to identify larva (in seconds)	Time taken to take out waxy coat (in seconds)	Time taken to kill the predator (in seconds)
I instar	50	1.225	68.345	0	77.11
II instar	50	2.42	49.88	62.38	75.9
III instar	50	3.24	37.96	51.58	60.98
IV instar	50	3.4	23.58	37.14	49
Adult	50	5.12	17.86	0	48.82

Table 11a: Interaction between *Spalgis epius* and *Solenopsis geminata*

Larval instar	No. of larvae used	Mean number of ants attended	Time taken to identify larva (in seconds)	Time taken to take out waxy coat (in seconds)	Time taken to kill the predator (in seconds)
I instar	50	1.4	69.46	0	83.94
II instar	50	2.6	54.59942	71.6544	84.21734
III instar	50	4.94	45.28	70.03	97.64
IV instar	50	6.4	28.9	62.04	89.46

Table 11b: Interaction between *Cryptolaemus montrouzieri* and *Solenopsis geminata*

Larval instar	No. of larvae used	Mean number of ants attended	Time taken to identify larva (in seconds)	Time taken to take out waxy coat (in seconds)	Time taken to kill the predator (in seconds)
I instar	50	1.18	56.84	0	66.94
II instar	50	2.24	39.76	52.3	65.7
III instar	50	3.52	30.16	43.2	57.78
IV instar	50	5.4	12.74	33.34	47.28
Adult	50	4.58	13.64	0	36.52

Interaction between mealybug predators Spalgis epius, Cryptolaemus montrouzieri and Solenopsis geminate:

Solenopsis geminate is known for its aggression when *S. epius* fourth instar larva released on ant foraging mealybug - maximum number of ants attended and also less time taken to identify the larva than other larval instars (II, I, III instar). Ants than other larval stages (Table 11a) killed the first instar larva easily. In *C. montrouzieri* fourth instar larva attended by a maximum number of ants followed by other instars (adult, III, II, I instar) where fourth instar larva was identified and killed by ants than other larval stages (Table 11b).

4. DISCUSSION

From this study, it demonstrates that no cannibalism and reciprocal predation exist in both predators *S. epius* and *C. montrouzieri* in the presence of prey. The maximum number of eggs was deposited by *S. epius* on the mealybug infested pumpkin (Dinesh &Venkatesha 2013b), which is because of the absence of cannibalism in egg /larva. In the combination of two *S. epius* larva consumed the maximum number of prey, which may be due to continuous and voracious feeding behaviour. So *S. epius* may be considered as a potential predator of mealybug. Also, biocontrol of mealybug may be useful in a combination of *S. epius* and *C. montrouzieri* larva as they can reduce the prey population successfully than *C. montrouzieri* larvae alone. *S. epius* larvae are known for their voracious feeding of mealybug mass, whereas *C .montrouzieri* larvae clear half-eaten adults of mealybug, eggs, and nymphs from the prey colony. This feeding behaviour of both the predators may help in put down the pest population. *C. montrouzieri* and *S. epius* larvae are known to share the same food resources and reducing the prey population in agriculture fields (Mani 1995).

Cannibalism and IGP are the two important factors in predators this is the managing mechanism of population growth performing through negative density-dependent feedback. In the success of biological control, IGP is considered as an important mechanism (Grez *et al.* 2012). Predation of *S. epius* larvae by *C. montrouzieri* larvae in absence of prey may be sluggish nature of *S. epius* larvae may be liable to interspecific attack. *S. epius* larvae mimic a mealybug colony by carrying debris on their back (Venkatesha *et al*. 2004) and thus they escape from mealybug attendant ants (Venkatesha *et al*. 2004). However, in absences of mealybug, *S. epius* larvae are liable to interspecific attack by *C. montrouzieri* larvae. Similarly, lepidopterous larvae feed by coccinellid aphid predator *Harmonia axyridis* (Pallas) in absence of prey (Koch *et al*. 2003; Kim *et al*. 1968; Shu and Yu 1985; Hoogendoorn and Heimel 2003).

In absence of prey, lesser predation of *C.montrouzieri* by *S. epius* in the first, second, and fourth larval instar when compared to third instar larva this may be because of active movement of first and second instar larvae and early pupation of fourth instar larvae of *C.mountrouzieri*. In the third instar larva of *C. mountrouzieri,* which has a long body, filaments are dangerous to predation. Less predation of *S.epius* by *C.mountrouzieri*in the first, third and fourth larval instar maybe because of the delicate mouthparts of the first instar larva of *C.mountrouzieri*and early pupation of the third and fourth larval instar of *S.epius.* Whereas, the third instar of *C. montrouzieri* rarely pupates. IGP among predatory coccinellids in natural situations havebeen documented when their prey becomes scarce (Hironori and Katsuhiro 1997; Musser and Shelton 2003; Schellhorn and Andow 1999).

Whereas in the similar larval stages, cannibalism was more in the first and second larval instar of *C.montrouzieri* this may be because of active feeding behaviour. Maximum cannibalism by third instar larvae of *S.epius* may be due to voracious feeding behaviour (Vinod kumar*et al*. 2008a) when a combination of a younger and older larva of the same species, third instar larva of *S.epius* was more cannibalistic as it attacked both the smaller second and sluggish fourth instar larva. Predation of the same species larva in Lepidoptera with average food supply or no food is common and more strong individuals (Dethier 1937) usually attack the smallest, less healthy, or less active larvae. In absence of prey, the existence of cannibalism in *C.montrouzieri*is similar to other coccinellids *Propylea dissecta* (Mulsant) and *Coccinella transversalis* Fabricius (Omkar et al. 2005), and *H. axyridis* (Snyder et al. 2004). Similarly, cannibalism in *S.epius* in the absence of prey species is reported in phytophagous lepidopterans *Apante stsarge* Drury (Detheir 1937), *Antho charisscolymus* (L.) (Kinoshita 1998), and *Spodoptera frugiperda* (Smith) (Chapman et al. 2000).Hence, cannibalism in the first and second larva instar of *C.montrouzieri* and third instar larva of *S. epius* could be grater in the field whenever there is a more larval accumulation with a declining prey population.

All larval instars of *C. montrouzieri* potentially predated on all larval instars of *S.epius* in the absence of prey. The aggressive behaviour of hungry larvae of *C. montrouzieri* towards *S.epius* larvae was common. The cannibalistic nature of older larval instar of *C.montrouzieri* on the first instar larva may be due to their difference in their body size. Ladybird species older larvae move faster than young larvae (Ng 1988) thus fast-moving older larval instars of *C. montrouzieri* easily attack and consume the younger larval instars. The older larvae feed more and convert less prey biomass into predator biomass because of the high metabolic cost (Baumgartner *et al*. 1987). Hence, the requirement of more food intake in older larval instars of *C.montrouzieri* may drive them to increasingly indulge in cannibalism as well as IGP.

The studies showed that different ant species have different methods for protecting hemipterans against natural enemies. Ant attendance by all five species significantly reduced the number and percentage of predation on the mealybug colony. However, most of the ant species showed more aggression towards the predator larva. Fire ants *S. invicta* protects *P.solenopsis* from its predator and parasitoids (Zhou *et al*. 2013). Honeydew produced by hemipterans act as a major food resource for ants species. Food derived from animal sources is essential for colony growth of *S. invicta* (Helms & Vinson 2008). Many studies showed that honeydew of hemipterans could support the colony growth (Porter 1989; Davidson et al.2004; Abbott & Green 2007). Results showed that ant foraging on the mealybug colony maintains hygienic conditions. Ants tending can help in population growth of hemipterans, not only reducing the predation and parasitism from natural enemies but also reducing the risk of fungal infection (Way 1963; Stadler & Dixon 1998; Helms & Vison 2003; Daane *et al*.2007).

Both predators *C. montrouzieri* larvae and *S.epius* larvae were predated by all the five species of ants with different aggressive behaviour in different time intervals. All the larval instars and adult *C.montrouzieri* was predated, but *S.epius* larvae less attacked by predators due to its sluggish and mimic nature by placing the mealybug debris on the back (Venkatesha *et al*. 2004). Thus they are less attacked by mealybug attendant ants (Venkatesha *et al*. 2004). The third instar larva of *C. mountrozieri* was noticed easily by ants due to its long body filaments and easily predated. A lower number of *C.montrouzieri* larvae were found in the presence of ants, ants killed the predatory larvae were (Rmansour *et al*. 2011).

Results showed that in all five ants species number of ant attendance is depend on the predator larval size (larval instar), waxy coating present on them, and time duration to kill the individual predator larva vary with different ant species. Whereas the bigger body size ant (*Camponotus variegates, Solenopsis geminate, Crematogaster* sp.) were less attended compare to smaller body size ants (*Tapinoma melanocephalum and Monomorium latinode)* in the present study.The time taken to notice the predator larva varies according to their activity of ants species as mentioned in the above results. *C.montrouzieri* adults were attacked by all the ant species than *S.epius* adult. *S.epius* adult oviposition behaviour was different than *C. montrouzieri* adult. Whereas all stages including the adult of *C. montrouzieri* consume all the stages of mealybug (Clausen 1978).

Ant species showed aggression when both the predators larvae released on the ants foraging mealybug colony. Aggressive behaviour was noticed in all five different ant species and more attention was found in

S.germinate followed by the other four species. *T. melanocephalum* workers effectively utilize their pygidial gland secretion as an alarm-defense system during aggressive encounters with other invaders (Tomalski*et al.* 1987).

5. CONCLUSION

This study provided the information on inter and intra-specific interaction between the two predators in the different larval combinations of predators and ant interaction with predator stages. Both the predator *S. epius* and *C. mountrouzieri* combination can effectively suppress the prey population. In absence of prey, there was no cannibalism and IGP in both the predators, which can be employed together in biological control of mealybug. Interaction of ants with mealybug predators indicated the beneficial relationship between ants and homopterans. Ant species harmed both the predators and thus predation performance was significantly reduced.

6. SUMMARY

Spalgis epius and *Cryptolaemus montrouzieri* are the two potential predators of different species of mealybugs. In *S. epius* only larval stages feed on different stages of mealybugs whereas adult feeds on the nectar source. But in *C. montrouzieri* all the stages including adult feeds on various stages of mealybug. Mutualism between ants and honeydew-producing hemipterans is a well-known phenomenon in ecosystems, the interaction between ants and hemipterans have been extensively studied. Honeydew producing hemipterans shows a mutualistic relationship with ants. Ants receive a large amount of honeydew as nutritional resources from hemipterans, which are essential for colony growth. In return, they provide various benefits, mainly they protect mealybugs from predators and parasitoids and maintain colony hygiene by reducing the fungal growth. In this study, investigation was conducted on intra and interspecific interaction, cannibalism, and inter guild predation (IGP) between both predators in the presence and absence of prey.

Investigation was conducted on the interaction between mealybug attendant ants with mealybug predators, when predators were introduced into the mealybug *Planococcus citri* (Risso) (Homoptera: Pseudococcidae) culture in the presence of mealybug attended ants. Cannibalism and predation were not seen in both *S. epius* and *C. montrouzieri* larvae in the presence of prey. A significant number of mealybugs were consumed by a pair of *S. epius* larvae than one *S. epius*/*C. montrouzieri* larva or a pair of *C. montrouzieri* larvae. *C. montrouzieri* larva showed significant predation on *S. epius* larva than the predation of *C. montrouzieri* by *S. epius.* Cannibalism in *C. montrouzieri* was more compared to that in *S. epius.* This study supports using *C. montrouzieri* larvae as an additive predator along with voracious *S. epius* larvae under an abundant prey population. It was also noted asymmetric IGP between the two predators in the absence of prey. This study indicated that both *S. epius* and *C. montrouzieri* larvae could maintain stable coexistence when there is abundant prey, whereas *C. montrouzieri* dominate the guild in the absence of prey.

The results of this study indicate that when ants were foraging on mealybug colonies, ant species easily recognize the mealybug Predator *C. montrouzieri*, than *S. epius* because of mimicking behavior of *S. epius.* The behaviour of individual ants was noted with both the predator larval stages separately. Ants attack the predator larva Ist, IInd, IIIrd, and IVth instar by aggressive behaviour, *Solenopsis geminata* was more aggressive compared to the other four species of ants i.e., *Crematogaster* sp., *Camponotus variegates, Monomorium latinode*, and *Tapinoma melanocephalum*. Ant species can easily distinguish the older instar larva than the younger instar larvae of both predators (IVth to Ist) and the number of ants to strike the predator larvae and the time taken to kill the larva differs for both predators with different ant species while praying on the mealybugs. Ants attack showed a negative impact on both the predators and predation performance was significantly reduced. A mutual beneficial relationship exists between ants and invasive mealybug under the presence of predators. Therefore, the interaction between predators and ants may facilitate the invasion of mealybug species. *S. epius and C. montrouzieri* can maintain stable coexistence in abundant prey populations and at the time of prey scarcity. However, *C. montrouzieri* may dominate the guild and becomes a threat to *S. epius* larvae under the situation of a total absence of prey. This information will be helpful in the biological control of mealybugs.

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8. **PAPER PRESENTED IN SYMPOSIUMS AND CONFERENCES**

National conference on Future India: Science and Technologyheld at Bangalore, organized by Oxford College, Bengaluru, on 10th and 11th October 2018. Presented the paper "Interaction between two mealybug attendant ant species Ghost ant, *Tapinoma melanocephalum* and Carpenter ant, *Camponotus variegatus* on Citrus mealybug, *Planococcus citri" .*

International Conference on Recent trends in Zoology, Biodiversity, Genetics and Environmental Sciences organized by Department of Zoology, Bangalore University, Bengaluru, held on 4th and 5th of December 2018. Presented the paper "Interaction of mealybugs attendant ant species *Solenopsis geminata* (Fabricius) (Hymmenoptera: Formicidae) in presence of mealybug predator *Cryptolaemus montrouzieri* (Mulstant) (Coleoptera:Coccinellidae)".

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