Research Article

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Effects of host plants and substrate colour on the oviposition behaviour of green lacewing *Apertochrysa astur* (Banks) (Neuroptera: Chrysopidae)

Remoniya X. and Jeyarajan Nelson S.*

Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore-641003

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Abstract

The Green lacewing, *Apertochrysa astur* also known as "aphid lion" is an efficient predator of various soft bodied insect pests. A laboratory experiment was conducted in Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu to observe the preference of *A. Astur* on their oviposition behaviour. Egg receiving sheets were pasted with black, amber, ash, parrot green, violet, red, light green, pink, blue, fluorescent yellow, sky blue, yellow, orange and white coloured papers. Among all the colours, females of *A. astur* preferred black colour as a substrate for laying eggs (19.6 per cent of eggs) followed by orange colour substrate (15.5per cent) while white was the least preferred colour (4.0 % eggs). Among the host plants examined, maximum number of eggs was laid in the coconut leaves infested with the invasive whiteflies followed by custard apple and banana. Therefore, whitefly infested coconut leaves and the black coloured substrate can be used in the mass production of *A. astur* in the laboratory.

Keywords: Green lacewing, colour preference, oviposition behaviour, coconut

Introduction

Coconut is an important oilseed crop with a production of 14.63 million metric tons (Statista Research Department, 2021). Globally, India ranks third in the list of countries producing coconut. The incidence of rugose spiralling whitefly (RSW) infestation was reported in 2016 at Pollachi, Coimbatore district, western agro-climatic zone of Tamil Nadu (Sundararaj and Selvaraj, 2017) and that of Bondar's nesting whitefly (BNW) in 2019 in Kerala (Josephrajkumar *et al*., 2019). More than 110 exotic insect species had been reported from India, of which, whiteflies and mealybugs constitute a major invasion. In India, 469 whitefly species belonging to 71 genera are known to feed on many plants of agricultural, horticultural, and forestry importance (Sundararaj *et al*., 2021). These whiteflies suck sap from under surface of the palm leaves, thereby causing stress to the host plant. Farmers prefer chemical-based insecticides to manage the whiteflies infestation. However, frequent applications of insecticides result in the problems of resistance, resurgence and residues. Under these circumstances, managing the whitefly population in the coconut ecosystem through natural enemies is considered the best course of action.

The predators devour the insect pests in their egg, larval/ nymphal, pupal and adult stages and maintain their population below economic threshold level (Ballal and Verghese, 2015). Green

**Corresponding Author:* sjn652003@yahoo.co.in

lacewing (Insecta: Neuroptera) is one of the important predators and 1350 species (86 genera) are known worldwide. They manage the population of soft bodied insects in agricultural and forest ecosystems (Henry *et al*., 2010); with their wide distribution, large population, and extensive range of prey species (Tauber *et al*., 2000). It is estimated that up to one third of the successful biological insect pest control programmes are attributable to the introduction of *C. carnea* (Williamson and Smith, 1994). The grubs of this lacewing are generalist predators, whereas the adults feed on nectar, honeydew, and pollen (Wu, 1995).

As environmental concerns increase, eco-friendly methods of pest management in agriculture have become more crucial. Biological control, which utilizes carefully screened/ selected natural enemies to suppress pest populations, is considered environmentally safe and a viable alternative to pesticides (Chen *et al*., 2014). In an augmentative biocontrol strategy, large quantities of beneficial insects and mites are reared and released onto the crop. However, the preference behaviour of the green lacewings is determined by the substrate. For instance, *Chrysoperla mediterranea* (Holzel, 1982) remain more attracted to pines and *Pseudomallada prasinus* (Burmeister, 1839) to oaks (Monserrat and Marin, 1994 & 2001). The reason for this attraction of adults, includes the volatiles produced by each plant and/or its associated pests, the existence of potential prey for the off-springs (Duelli 1984; Szentkiralyi 2001b). An attempt was made to study the preferred substrate of *A. astur* for egg laying considering its biocontrol potential.

Materials and methods

Mass rearing of *A. astur*

The eggs and grubs of *Apertochrysa astur* were collected in coconut growing areas around Coimbatore (location: 11.0168° N, 76.9558° E) during year 2020-2021. These grubs were allowed for pupation by providing the *Corcyra cephalonica* eggs until their third instar. They undergo a pre-pupal stage and grubs were pupated to round white-coloured silken cocoons. The cocoons were collected thrice in a week. The adults were introduced inside the galvanized iron troughs for the better egg production and for maintaining the healthy culture. Hundred pairs of newly emerged adults were introduced into a galvanized iron container. The trough had a wire mesh at the bottom for observing the emerging adults while the top was covered with muslin cloth (0.15 mm) which was tightly secured using a rubber band. The sides of adult rearing troughs were wrapped with a brown sheet which served as an egg receiving card.

Maintenance of culture

Food provision

Standard protein-rich adults diet (Honey: Glucose: Protinex: Yeast: Water v/v @ 1:1:1:1:1) prepared using clean spatula along with a water source in foam sponge (5 cm^2) were provided.

Egg harvesting

Eggs were harvested from the troughs with the help of sharp razor blade. Eggs laid along the side and bottom of the troughs in addition to the muslin cloth were also gently collected. Eggs were harvested daily and the adults were regularly introduced into a fresh pcontainer until their death.

Cleaning of cages

All the cages were cleaned with wet cotton wig and dried gently with the help of tissue paper. The cleaned muslin clothes were used throughout the entire mass production process.

Substrate colour preference for egg laying

An experiment was conducted for understanding the colour preference of the adults for oviposition. In general, the rearing troughs were wrapped with the brown sheets along the sides that act as the egg receiving card. In this experiment, the brown sheet was pasted with black, amber, ash, parrot green, violet, red, light green, pink, blue, fluorescent yellow, sky blue, yellow, orange and white colour of dimension 12.5 × 4 cm. The egg sheets were placed in the rearing troughs in which the freshly emerged 25 pairs of adults were released and covered with the muslin cloth. This experiment was conducted in eight replications. After 24 hrs, the eggs laid by the adults in each colour papers were collected and counted carefully. The observations were recorded until the life span of the released adults.

Influence of host plant on the oviposition behaviour of *A. Astur* (No-choice test)

A laboratory experiment was undertaken to confirm the host preference of the adults of *A. astur* under no-choice condition. The host plants used in the treatments were coconut, banana and custard apple. They were collected freshly and secured with the wet cotton balls. These leaves were then placed inside the conical flasks and maintained inside the oviposition cage of dimension 65cm x 50cm x 65cm. Three pairs of adults were released in each cage. These treatments were replicated four times. The leaves were changed in alternate days and the eggs laid by the adults in each host plants were recorded. This experiment was continued until the life span of the released adults.

Influence of host plant on the oviposition behaviour of *A. astur* (Choice test)

A laboratory experiment was conducted to confirm the host preference by the adults of *A. Astur* under choice test condition. The host plants used in the treatments were coconut, banana and custard apple. They were collected freshly and secured with the wet cotton balls. These leaves were then placed inside the conical flasks and maintained inside the oviposition cage of dimension 65cm x 50cm x 65cm and six pairs of adults were released in each cage. These treatments were replicated eight times. Fresh leaves were provided to the insects on alternate days and the number of eggs laid by the females in each host plants was recorded. This experiment was continued until the life span of the released adults.

Statistical analysis

The data were analysed using analysis of variance (ANOVA) using AGRES 3.01 and AGDATA software. Data in the form of percentages were transformed to arcsine values and those in numbers were transformed to x + 0.5 and analysed. The mean values of the treatments were compared using DMRT at 5 per cent level of significance. The influence of host plants in choice and no-choice condition were analysed by paired t-test ($p>0.05$).

Results and discussion

There are no reports yet describing oviposition behaviour of *A. Astur*. Different colour charts as substrates were used to analyze the preference of the adults (Fig. 1). Numbers of insects attracted to the different colour substrate are significantly different. The adults of *A. astur* females preferred black colour substrate for laying eggs (19.6 per cent) followed by orange colour substrate (15.5 per cent eggs) (Fig.2). Similar to results presented herein, in the laboratory experiment, black coloured substrate was the most preferred and white colour was least preferred for egg laying by the adults of *C. zastrowi sillemi* (Dola *et al*., 2011). Also, black is most preferred colour for oviposition in *C. Carnea* (Anonymous 1994). On the contrary, Elango *et al*. (2020) had reported that *C. z sillemi* had laid more eggs on orange coloured substrate (43.13/female/day) followed by red colour (25.50 eggs/female/day). However, in this study, red coloured substrate was recorded with 14.8 % eggs followed by sky blue coloured substrate (12.4 per cent). Likewise, 10.2, 9.2, 6.5, 6.3 per cent eggs were observed in light green, pink, ash, violet and yellow colour substrate, respectively.

Brown colour substrate attracted 5.6 per cent eggs (Table 1). However, Sattar and Abro (2011) reported that green and brown colours were least preferred by the females of *C. carnea* for oviposition. Around 5.5 % of eggs were laid in fluorescent yellow substrate which was followed by parrot green and amber coloured substrates with 5.4 and 5.3 per cent, respectively. The least preference for oviposition was recorded in white coloured substrate (4.0 per cent) which is in concordance with the report of Elango *et al*., (2020). McEwen *et al*. (1999) had outlined a production system of *C. carnea* and have worked out economics of production. In our study, the galvanised iron trough

Table 1. Influence of colour on oviposition behaviour of green lacewing, *A. astur*

Substrate colour	Number of eggs laid by *A. astur* adults (in days)																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Total	Mean(%)
Black	0.0	0.0	1.0	19.0	12.0	73.0	33.0	44.0	55.0	68.0	31.0	40.0	4.0	0.0	3.0	7.0	390.0	19.6
	(0.707)	(0.707)	(1.225)	(4.416)	(3.536)	(8.573)	(5.788)	(6.671)	(7.450)	(8.276)	(5.612)	(6.364)	(2.121)	(0.707)	(1.871)	(2.739)		
Amber	0.0	0.0	5.0	0.0	8.0	1.0	28.0	7.0	0.0	7.0	0.0	0.0	32.0	11.0	2.0	4.0	105.0	5.3
	(0.707)	(0.707)	(2.345)	(0.707)	(2.915)	(1.225)	(5.339)	(2.739)	(0.707)	(2.739)	(0.707)	(0.707)	(5.701)	(3.391)	(1.581)	(2.121)		
Ash	0.0	0.0	8.0	16.0	20.0	0.0	18.0	0.0	24.0	5.0	11.0	1.0	3.0	14.0	7.0	2.0	129.0	6.5
	(0.707)	(0.707)	(2.915)	(4.062)	(4.528)	(0.707)	(4.301)	(0.707)	(4.950)	(2.345)	(3.391)	(1.225)	(1.871)	(3.808)	(2.739)	(1.581)		
Violet	0.0	0.0	3.0	0.0	3.0	4.0	19.0	29.0	11.0	28.0	18.0	0.0	1.0	6.0	8.0	0.0	130.0	6.5
	(0.707)	(0.707)	(1.871)	(0.707)	(1.871)	(2.121)	(4.416)	(5.431)	(3.391)	(5.339)	(4.301)	(0.707)	(1.225)	(2.550)	(2.915)	(0.707)		
Red	0.0	0.0	2.0	21.0	9.0	27.0	15.0	38.0	63.0	41.0	56.0	10.0	8.0	3.0	1.0	1.0	295.0	14.8
	(0.707)	(0.707)	(1.581)	(4.637)	(3.082)	(5.244)	(3.937)	(6.205)	(7.969)	(6.442)	(7.517)	(3.240)	(2.915)	(1.871)	(1.225)	(1.225)		
Parrot green	0.0	0.0	9.0	7.0	0.0	5.0	4.0	9.0	19.0	26.0	15.0	2.0	0.0	8.0	3.0	0.0	107.0	5.4
	(0.707)	(0.707)	(3.082)	(2.739)	(0.707)	(2.345)	(2.121)	(3.082)	(4.416)	(5.148)	(3.937)	(1.581)	(0.707)	(2.915)	(1.871)	(0.707)		
Light green	0.0	0.0	4.0	8.0	19.0	8.0	14.0	24.0	29.0	34.0	18.0	18.0	17.0	4.0	6.0	0.0	203.0	10.2
	(0.707)	(0.707)	(2.121)	(2.915)	(4.416)	(2.915)	(3.808)	(4.950)	(5.148)	(5.874)	(4.301)	(4.301)	(4.183)	(2.121)	(2.550)	(0.707)		
Pink	0.0	0.0	8.0	13.0	9.0	10.0	4.0	30.0	7.0	62.0	0.0	28.0	0.0	3.0	9.0	0.0	183.0	9.2
	(0.707)	(0.707)	(2.915)	(3.674)	(3.082)	(3.240)	(2.121)	(5.523)	(2.739)	(7.906)	(0.707)	(5.339)	(0.707)	(1.871)	(3.082)	(0.707)		
White	0.0	0.0	5.0	0.0	8.0	1.0	16.0	24.0	4.0	0.0	12.0	3.0	3.0	4.0	0.0	0.0	80.0	4.0
	(0.707)	(0.707)	(2.345)	(0.707)	(2.915)	(1.225)	(4.062)	(4.950)	(2.121)	(0.707)	(3.536)	(1.871)	(1.871)	(2.121)	(0.707)	(0.707)		

*Mean of eight replications; figures in parentheses are square root transformed values

Table 2: Effect of host plants on the oviposition behaviour of *A. astur* (No- choice test)

Host plants	Number of eggs laid by adults of *A. astur*								
	2 days	**4 days**	**6 days**	**8 days**	**10 days**	**12 days**	**14 days**	**16 days**	**Total**
Coconut Infested	0.0 (0.707)	6.0 (2.550)	69.0 (8.337)	156.0 (12.510)	183.0 (13.546)	108.0 (10.416)	48.0 (6.964)	9.0 (3.082)	579
Coconut Un-infested	0.0 (0.707)	12.0 (3.536)	90.0 (9.513)	123.0 (11.113)	150.0 (12.268)	73.0 (8.573)	47.0 (6.892)	11.0 (3.391)	506
Custard apple Infested	0.0 (0.707)	9.0 (3.082)	72.0 (8.515)	138.0 (11.769)	183.0 (13.546)	105.0 (10.271)	39.0 (6.285)	6.0 (2.550)	552
Custard apple Un-infested	0.0 (0.707)	18.0 (4.301)	63.0 (7.969)	120.0 (10.977)	162.0 (12.748)	72.0 (8.515)	45.0 (6.745)	3.0 (1.871)	483
Banana Infested	0.0 (0.707)	9.0 (3.082)	69.0 (8.337)	123.0 (11.113)	159.0 (12.629)	93.0 (9.670)	50.0 (7.106)	12.0 (3.536)	515
Banana Un-infested	0.0 (0.707)	9.0 (3.082)	60.0 (7.778)	105.0 (10.271)	153.0 (12.390)	90.0 (9.513)	30.0 (5.523)	21.0 (4.637)	468

*Mean of four replications; Figures in parentheses are square root transformed values

Table 3: Effect of host plants on the oviposition behaviour of *A. astur* (Choice test)

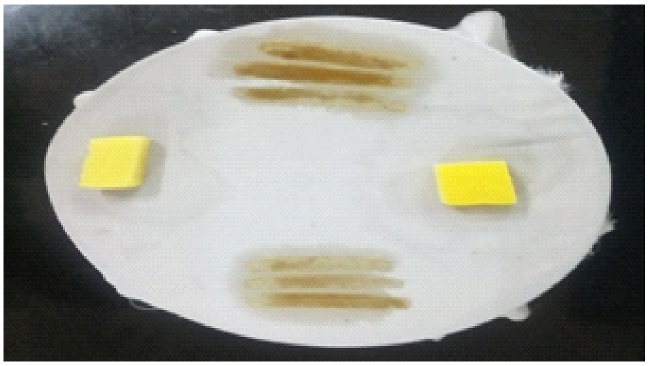
Host plants	Number of eggs laid by adults of *A. astur*								
	2 days	**4 days**	**6 days**	**8 days**	**10 days**	**12 days**	**14 days**	**16 days**	**Total**
Coconut Infested	0.0 (0.707)	19.0 (4.416)	55.0 (7.450)	99.0 (9.975)	169.0 (13.019)	85.0 (9.247)	43.0 (6.595)	3.0 (1.871)	473
Coconut Un-infested	0.0 (0.707)	8.0 (2.915)	0.0 (0.707)	38.0 (6.205)	0.0 (0.707)	0.0 (0.707)	10.0 (3.240)	4.0 (2.121)	60
Custard apple Infested	0.0 (0.707)	12.0 (3.536)	41.0 (6.442)	72.0 (8.515)	135.0 (11.640)	51.0 (7.176)	15.0 (3.937)	3.0 (1.871)	329
Custard apple Un-infested	0.0 (0.707)	5.0 (2.345)	0.0 (0.707)	0.0 (0.707)	35.0 (5.958)	0.0 (0.707)	0.0 (0.707)	3.0 (1.871)	43
Banana Infested	0.0 (0.707)	3.0 (1.871)	12.0 (3.536)	49.0 (7.036)	95.0 (9.772)	31.0 (5.612)	11.0 (3.391)	6.0 (2.550)	207
Banana Un-infested	0.0 (0.707)	0.0 (0.707)	2.0 (1.581)	8.0 (2.915)	0.0 (0.707)	12.0 (3.536)	0.0 (0.707)	1.0 (1.225)	23

*Mean of eight replications; figures in parentheses are square root transformed values

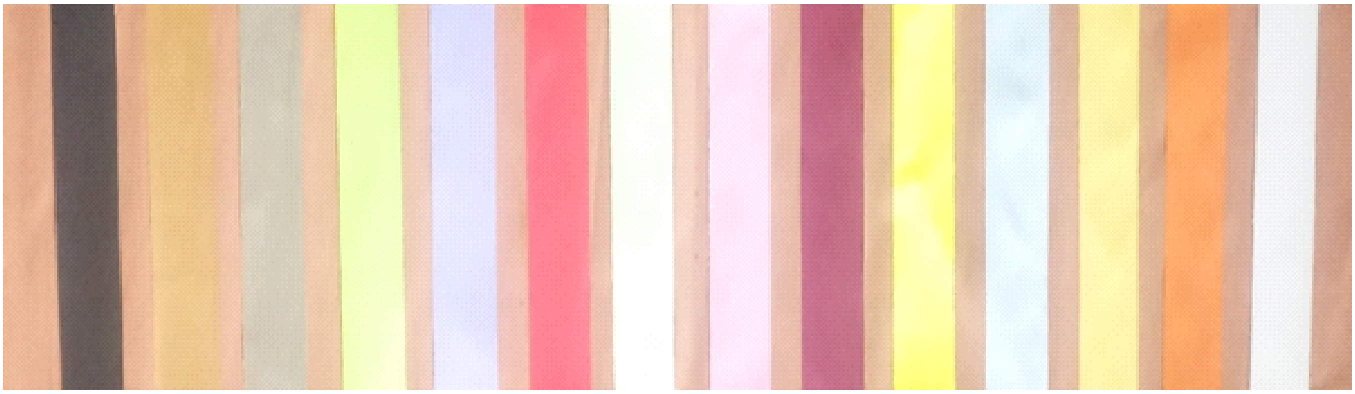
was found to be better for the mass production strategy yet, Tulisalo and Korpela (1973) had reported that higher number of eggs was laid in cylindrical plastic cages by the adults of *C. carnea* with 80-83% hatching percentage.

The influence of host plants on the oviposition behaviour of *A. astur*, revealed that the females were observed to lay their eggs more in the infested than the uninfested leaves of the host plants. Under no-choice condition, when 3 pairs of adults were released, 579 numbers of eggs were found on the infested and 506 eggs ($t = 2.54$, $df = 27$, $p = 0.008$) over the un infested leaves of the coconut (Table 2). Similarly, 552 and 483 eggs ($t = 3.55$, $df = 27$, $p = 0.000$) were observed in the infested and un infested leaves of the custard apple. The least number of 515 and 468 eggs ($t = 3.56$, $df = 27$, $p = 0.000$) were recorded in the infested and un infested leaves of the banana.

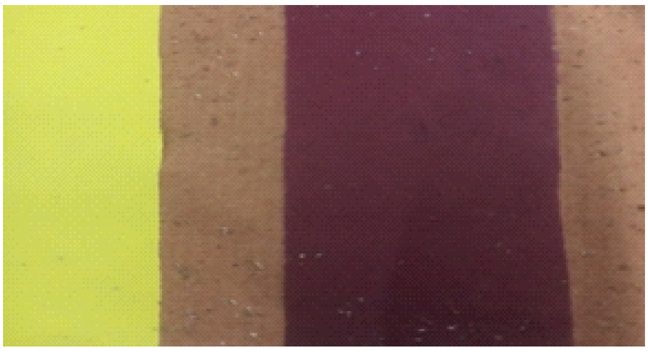
Colour charts wrapped along the galvanised iron trough



Adults released inside trough for oviposition



Different colour substrates used for studying the oviposition behaviour



Eggs laid by adults of *A. astur*

Fig. 1 Influence of colour on oviposition behaviour in green lacewing *A. astur*

In choice test condition, the females laid a maximum of 473 eggs in the infested and 60 eggs ($t = 7.67$, $df = 63$, $p = 0.000$) in the uninfested leaves of the coconut (Table 3). Likewise, 329 and 43 eggs ($t = 8.15$, $df = 63$, $p = 0.000$) were observed in the infested and un infested leaves of the custard apple. The least numbers of 207 and 23 eggs ($t = 6.01$, $df = 63$, $p = 0.000$) were recorded in the infested and un-infested leaves of the banana.

From the results, it is evident that coconut is the most preferred host with a maximum number of *A. astur* eggs followed by custard apple and banana under both choice and no-choice experiments. Herrera *et al*. (2019) stated that almond is the preferred host for the oviposition by the different species of chrysopids. The females of the *A. astur* lay single eggs to avoid the level of predation. The continuous mobility of the green lacewing during reproduction resulted in better spread of the eggs on the host surface. On the other hand, the spacing between the eggs has reduced the chances for the evading sibling cannibalism or parasitism.

The eggs were found mainly on the lower surface of the leaves of host plants along the whitefly infested area. This is because the female chrysopids require suitable architectural complexity and vegetal structures/abundant food for their own and their progeny. Hence both these factors are considered crucial for determining oviposition behaviour (Canard *et al*. 1984, Duelli 1984, Nakamura *et al*., 2000). On the contrary, (Herrera *et al*., 2019) had observed that the eggs were found abundant on the edge and the upper side of the leaves (spiny in Mediterranean vegetation) with a very fewer eggs over on the pedicels of the leaves, twigs, fruit, and flower buds. The host plants

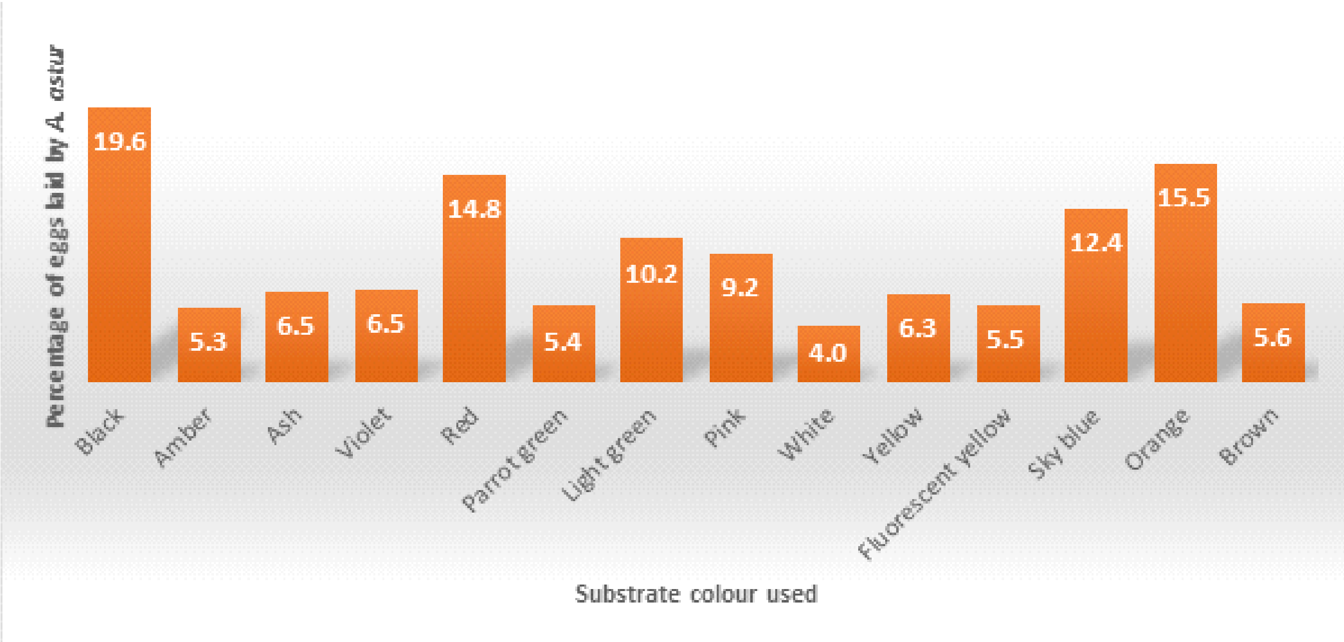


Fig. 2 Percentage of egg laid by adults of *A.astur* in different colour substrate

used for the study were devoid of hairy structures however, lacewings apparently prefer to lay eggs on hairy plant organs (Szentkiralyi, 2001b and c).

Conclusion

Invasive whiteflies in the coconut leaves significantly influenced the oviposition behaviour of *A. astur* compared to the other host plants like banana and custard apple. From the findings it can be concluded that the coconut infested with the whiteflies and black substrate can be utilised in the mass production of the green lacewings under laboratory conditions.

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