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Development of artificial diets for rearing of adult lady bird beetle *Coccinella septumpunctata* in laboratory

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ABSTRACT

Adult lady bird beetle, *Coccinella septumpunctata* was reared on semi-solid and liquid artificial diets in the laboratory at 25±1°C temperature and 70±5% relative humidity during 2011-12 and 2012-13. In first effort ten combinations of artificial diet in semi-solid form comprising 3 basic ingredients, *i.e.* protinex, home food plus and nutripet in different combinations along with other ingredients like agar powder, yeast powder, honey, vitamin B-complex and vitamin E were evaluated against adult of *C. septumpunctata*. Methyl paraben and formaldehyde were used as diet preservatives. The adult of *C. septumpunctata* survived maximum period of 41.3 days on home food plus (100%) based diet while minimum period (15.7 days) on nutripet (100%) based diet. The survival pattern in other diets was recorded from 24.0 days to 39.7 days. Further efforts were made to improve the quality of diets and again 10 combination of artificial diets in liquid form based on five basic ingredients *i.e.* protinex, cerelac (baby feed), honey, home food plus and sucrose alone and with other food supplements like vitamin B-complex, vitamin E, ascorbic acid and yeast powder were prepared using methyl paraben and formaldehyde as diet preservatives. The diets devoid of other food supplements proved very excellent by adult longevity and per cent adult survival at different time interval. Longest adult survival (302 days) was recorded in cerelac based diet. However adult longevity on protinex, honey, home food plus and sucrose based diets was recorded as 296, 207, 258 and 296 days respectively.

Key words: Adult longevity, Artificial diets, *Coccinella septumpunctata*, Lady bird beetle, Predator, Rearing

Lady bird beetle, *Coccinella septumpunctata* L. (Coleoptera: Coccinellidae) is a predator of mustard aphid, *Lipaphis erysimi* (Kaltenbach) (Homoptera: Aphididae) as well as several other soft bodied insect-pests like whiteflies, jassids, thrips, small lepidopterous larvae etc. Despite their preference for aphid and other soft bodied arthropods, *C. septumpunctata* will readily consume a wide range of food items like flower nectar, pollen, water, fungal spores and honeydew (sugary excretion of piercing-sucking insects) such as aphids and whiteflies (Hodek 1996). Mustard aphid, *L. erysimi* is a key pest of rapeseed-mustard crops in India (Mandal *et al*. 1994) and found to be distributed throughout the world on all *Brassica* crops (Yue and Liu 2000). In India, the pest is responsible to cause yield loss ranging from 65-96% (Bakhetia 1984) and 15% oil reduction (Verma and Singh 1987). This pest is also responsible to transmit single-stranded RNA luteoviruses while feeding, which cause disease in the crop (Banerjee *et al*. 2004). Both nymphs and adults suck the cell sap from different parts of the plant, i.e. inflorescence, leaf, stem, twig and pods. In heavy infestation, plants are stunted and dried up, resulting in no pod formation. Insect secretes honeydew, which attracts black fungus upon it called "Sooty mould" leading to hinder the photosynthesis. *C. septumpunctata* is an excellent predator of *L. erysimi*. Grub of *C. septumpunctata* is capable to eat upon 40-50 individuals of *L. erysimi* per day while adult may devour 70-75 aphids per day. Ability of *C. septumpunctata* to persist in the environment prior to the arrival of aphid pests, or during period of low temperature and low pest densities, may enhance their suppressive impact on aphid pests (Harwood and Obrycki 2005). Despite of all these favours, the role of *C. septumpunctata* remains restricted in biological control programme of mustard aphid, mainly in want of suitable artificial diet for their mass multiplication in the laboratory. Similarly on the other side,

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lack of reasonable source of resistance against *L. erysimi* among the cultivated *Brassicas*, forced the farmers to use only hazardous chemical pesticides to contain this dreaded pest. Environmental pollution, hazards to (human beings, domestic animals and pollinators), development of resistance in insect-pests, increase in cost of production of new chemical pesticides and build up of insecticide residue in oil and cake beyond the permissible limit are several disadvantages associated with the use of chemical pesticides. To strengthen the biological control programme of mustard aphid and lessen our dependency on chemical pesticides, several artificial diets were prepared in different form and evaluated against the adult lady bird beetle, *C. septumpunctata* in the laboratory.

MATERIALS AND METHODS

Ten different combinations of artificial diet in semi-solid form comprising 3 basic commodities like protinex (trade name of a diet supplement), home food plus and nutripet (ready made dog food with meat extract) along with supplements, i.e. agar powder (Hi-Media), yeast powder (Hi-Media), honey (Dabur), vitamin B-complex (Sigma-Aldrich) and vitamin E (Sigma-Aldrich) were evaluated against adult of *C. septumpunctata* during 2011-12. Methyl paraben and formaldehyde were used as diet preservatives (Table 1). However, ten combinations of artificial diets in two sets were prepared during 2012-13. In one set, protinex, cerelac, honey, home food plus and sucrose were taken as base commodities and mixed well in required quantity of distilled water. Methyl paraben and formaldehyde were added as diet preservatives. However, in another set protinex, cerelac, honey, home food plus and sucrose were used as base commodities along with other diet supplements, i.e. vitamin B-complex, vitamin E, yeast powder and ascorbic acid while formaldehyde 10% and methyl paraben were used as diet preservatives (Table 2). Agar powder was used to solidify the diets during 2011-12 only while liquid diets were prepared during 2012-13. Liquid diets were provided to the adult with the help of soaked cotton balls. Each treatment (diet) was replicated thrice and ten adult of *C. septumpunctata* were reared in each replication using plastic petri plates of 10 cm diameter. Observations were recorded on every day for survival of adult in days. The experiment was conducted at 25±1° C temperature and 70±5% relative humidity in BOD incubator at Directorate of Rapeseed-Mustard Research, Bharatpur (India). The data were analyzed using complete randomized block design.

RESULTS AND DISCUSSION

The results from the present study revealed that semi-solid and liquid form of artificial diets significantly affected the development of *C. septumpunctata*. The liquid form of artificial diet has proved excellent in rearing of adult predatory beetle, *C. septumpunctata* over the semi-solid form. The survival pattern of adult predator on all the ten combinations of artificial diets in semi solid form revealed that insect survived on all the diets with different longevity (Table 1). Maximum adult longevity (41.3 days) was obtained in the home food plus (100%) based diet whereas minimum (15.7 days) in the diet where nutripet (100%) was used as base commodity. Next in the order was, protinex and home food plus (50:50) based diet where adult survived till 39.7 days. Protinex (100%) based diet supported the adult survival up to 33.0 days. The survival pattern in other

Table 1 Longevity of adult lady bird beetle, *Coccinella septumpunctata* on different artificial diets (semi-solid) in laboratory during 2011-12

Name of diets used (combinations of ingredients)	Adult longevity (days)
Protinex (25 g) + agar powder (2.5 g) + honey (2.5 ml) + vitamin B-complex (150 mg) + vitamin E (400 mg) + formaldehyde 10% (0.2 ml) + distilled water (69.65 ml)	33.0
Home food plus (25 g) + agar powder (2.5 g) + honey (2.5 ml) + vitamin B-complex (150 mg) + vitamin E (400 mg) + formaldehyde 10% (0.2 ml) + distilled water (69.65 ml)	41.3
Nutripet (25 g) + agar powder (2.5 g) + honey (2.5 ml) + vitamin B-complex (150 mg) + vitamin E (400 mg) + formaldehyde 10% (0.2 ml) + distilled water (69.65 ml)	15.7
Protinex (12.5 g) + home food plus (12.5 g) + agar powder (2.5 g) + honey (2.5 ml) + vitamin B-complex (150 mg) + vitamin E (400 mg) +formaldehyde 10% (0.2 ml) + distilled water (69.65 ml)	39.7
Protinex (12.5 g) + nutripet (12.5 g) + agar powder (2.5 g) + honey (2.5 ml) + vitamin B-complex (150 mg) + vitamin E (400 mg) + formaldehyde 10% (0.2 ml) + distilled water (69.65 ml)	29.7
Home food plus (12.5 g) + nutripet (12.5 g) + agar powder (2.5 g) + honey (2.5 ml) + vitamin B-complex (150 mg) + vitamin E (400 mg) + formaldehyde 10% (0.2 ml) + distilled water (69.65 ml)	32.0
Protinex (8.33 g) + home food plus (8.33 g) + nutripet (8.33 g) + agar powder (2.5 g) + honey (2.5 ml) + vitamin B-complex (150 mg) + vitamin E (400 mg) + formaldehyde 10% (0.2 ml) + distilled water (69.65 ml)	30.3
Protinex (25 g) + agar powder (2.5 g) + honey (2.5 ml) + yeast powder (3 g) + methyl paraben (0.5 g) + formaldehyde 10% (0.2 ml) + distilled water (66.3 ml)	30.7
Home food plus (25 g) + agar powder (2.5 g) + honey (2.5 ml) + yeast powder (3 g) + methyl paraben (0.5 g) + formaldehyde 10% (0.2 ml) + distilled water (66.3 ml)	31.3
Nutripet (25 g) + agar powder (2.5 g) + honey (2.5 ml) + yeast powder (3 g) + methyl paraben (0.5 g) + formaldehyde 10% (0.2 ml) + distilled water (66.3 ml)	24.0

p-value =0.01

Table 2 Survival of adult lady bird beetle, *Coccinella septumpunctata* on different artificial diets (liquid) in laboratory during 2012-13

Name of diets used (combinations of ingredients)	% adult survival (Days after feeding)					
	50	100	150	200	250	300
Protinex (15 g) + methyl paraben (0.5g) + formaldehyde 10% (0.5 ml) + distilled water (84 ml)	80	67	60	23	3 (296)	0
Cerelac (15 g) + methyl paraben (0.5g) + formaldehyde 10% (0.5 ml) + distilled water (84 ml)	90	87	83	17	13	7(302)
Honey (15 ml) + methyl paraben (0.5g) + formaldehyde 10% (0.5 ml) + distilled water (84 ml)	70	60	53	7(207)	0	0
Home food plus (15 g) + methyl paraben (0.5g) + formaldehyde 10% (0.5 ml) + distilled water (84 ml)	70	47	27	7	3(258)	0
Sucrose (15 g) + Methyl paraben (0.5g) + Formaldehyde 10% (0.5 ml) + Distilled water (84 ml)	87	83	63	13	3(296)	0
Protinex (15 g) + vitamin B-complex (150 mg)+ vitamin E (400 mg) + yeast powder (3 g) + ascorbic acid (0.5g) + methyl paraben (0.5g) + formaldehyde 10% (0.5 ml) + distilled water (81 ml)	90	67	30	7(223)	0	0
Cerelac (15 g) + vitamin B-complex (150 mg)+ vitamin E (400 mg) + yeast powder (3 g) + ascorbic acid (0.5g) + methyl paraben (0.5g) + formaldehyde 10% (0.5 ml) + distilled water (81 ml)	33	07(134)	0	0	0	0
Honey (15 g) + vitamin B-complex (150 mg)+ vitamin E (400 mg) + yeast powder (3 g) + ascorbic acid (0.5g) + methyl paraben (0.5g) + formaldehyde 10% (0.5 ml) + distilled water (81 ml)	57	40	27	13(238)	0	0
Home food plus (15 g) + vitamin B-complex (150 mg)+ vitamin E (400 mg) + yeast powder (3 g) + ascorbic acid (0.5g) + methyl paraben (0.5g) + formaldehyde 10% (0.5 ml) + distilled water (81 ml)	57	13	3(165)	0	0	0
Sucrose + vitamin B-complex (150 mg)+ vitamin E (400 mg) + yeast powder (3 g) + ascorbic acid (0.5g) + methyl paraben (0.5g) + formaldehyde 10% (0.5 ml) + distilled water (81 ml)	77	23	20	3	3(278)	0

Figure in parentheses indicates last day of survival, p-values (diets $\leq$ 0.01, stages $\leq$ 0.01, interaction $\leq$ 0.01)

diets combinations was recorded from 24.0 to 32.0 days respectively. It was interesting to note that when yeast powder (3g per 100g diet) was added to protinex (100%) and home food plus (100%) based diet instead of vitamin B-complex and vitamin E, the longevity of adult decreased to 30.7 and 31.3 days from 33.0 and 41.3 days respectively while the longevity of adult increased significantly in case of nutripet (100%) having yeast powder instead of vitamin B-complex and vitamin E.

The adult survival and longevity was significantly increased on all the combinations of liquid diet when was compared with the solid form of diet. A record longevity (302 days) of adult *C. septumpunctata* was recorded in liquid form of artificial diet (cerelac based) when compared to solid form of diet, i.e. 41.3 days in home food plus (100%) based diet (Table 2). Per cent adult survival at 50, 100, 150, 200, 250 and 300 days in protinex (15g), cerelac (15g), honey (15 ml), home food plus (15g) and sucrose (15g) based liquid artificial diets devoid of vitamin B-complex (150 mg), vitamin E (400 mg), ascorbic acid (0.5g) and yeast powder (3g) was significantly superior over the artificial diets based on protinex (15g), cerelac (15g), honey (15 ml), home food plus (15g) and sucrose (15g) along with supplements, i.e. vitamin B-complex (150mg), vitamin. E (400mg), ascorbic acid (0.5g) and yeast powder (3g). The adult longevity on protinex, cerelac, honey, home food plus and sucrose based diets devoid of supplements, was recorded as 296, 302, 207, 258 and 296 days respectively. The adult longevity on protinex, cerelac, honey, home food plus and sucrose based diets prepared with addition of other supplements was recorded as 223, 134, 238, 165 and 278 days respectively. This clearly indicates that addition of vitamin B-complex (150mg), vitamin. E (400mg), ascorbic acid (0.5g) and yeast powder (3g) in protinex, cerelac, honey, home food plus and sucrose based artificial diets played a negative role in per cent adult survival at 50, 100, 150, 200, 250 and 300 days after feeding (Table 2). However, their role was recorded positive for adult longevity point of view only in case of honey and sucrose based liquid artificial diets (Table 2). Therefore, liquid artificial diets based on protinex, cerelac, honey, home food plus and sucrose devoid of vitamin B-complex (150mg), vitamin E (400mg), ascorbic acid (0.5g) and yeast powder (3g) proved very excellent by adult survival and longevity point of view.

In the past a number of researchers have tried many

media using a number of ingredients and natural hosts against lady bird beetle but none of them have reported the longevity as long as reported in the present study. Samalo (1976) reported that adult of *Menochilus sexmaculatus* could live for 28 days on sugar syrup and water alone. Khursheed *et al*. (2006) reported that adult longevity of male and female *C. septumpunctata* was 29 and 37 days respectively on its preferred host, i.e. *L. erysimi*. Ashraf *et al*. (2010) reported that adult longevity of *C. septumpunctata* on honey syrup, sugar syrup and plain water was 27.67, 36.00 and 41.67 days respectively at 28° C in laboratory. Sarvar and Saquib (2010) reported that the development of larvae and adult of *C. septumpunctata*, was superior on the artificial diet containing agar, honey, protein hydrolyzate and alfalfa flour yeast over the diet comprising yolk, sucrose and honey. Although, these diets supported only few larvae and adults to survive. Angus *et al*. (2013) reported that adult could live up to 14 days when fed upon artificial diet made of pulverized drone larvae of *Apis cerana* while 71.3 days adult longevity was obtained when adult was fed upon artificial diet plus 10% sugar solution in sponge. Varma *et al*. (2013) found that adult longevity of male and female *C. septumpunctata* was 28.8 ± 4.92, 34.5 ± 5.25 days respectively on brinjal aphid, *Aphis gossypii*. In the past many studies revealed that diet comprising aphid as natural host proved excellent for rearing of coccinellids, but if artificial diet and aphid was given simultaneously, development becomes faster and lady beetle could be reared more successfully.

Natural hosts and supplementary artificial diets used simultaneously or jointly have been successfully reported from some other laboratories. Richards and Evans (1998) fed coccinellids on a variety of prey in addition to preferred aphids. These alternative foods served only to maintain the predator but did not permit immature growth or adult reproduction; females produced very few eggs, and held eggs of very small size in their ovaries, when provided only sucrose (15%). Henderson *et al*. (1992) evaluated three artificial diets based on bee brood, wasp brood and pupae of light brown apple moth, *Epiphyas postvittana* with various additives against larvae of three species of ladybird. A freeze-dried wasp brood-based diet was found to be the most successful for rearing the larvae of these ladybirds. Akram *et al*. (1996) reported that egg stage and larval instars of *C. septempunctata* occupied a total of 9.91 days. The pupal stage lasted for 4.66 days and the adults survived for 6-8 days. Sato and Dixon (2004) studied survival and development of hatchling larvae of three aphidophagous ladybirds, when fed their own and the other species eggs. In all three species, the larvae survived when fed co-specific eggs. In general, larvae were reluctant to eat the eggs of other species. In the present study, liquid artificial diets based on protinex (15g), cerelac (15g), honey (15 ml), home food plus (15g) and sucrose (15g) devoid of vitamin B-complex (150mg), vitamin E (400mg), ascorbic acid (0.5g) and yeast powder (3g) proved very excellent by adult survival and longevity point of view. No study in the past revealed the adult longivity as long as reported in this study on the artificial diets. Same time in these diets only ingredients available in the market round the year were used and no insects or their derivatives were used. This would certainly reduce our dependency to use natural host/ or their derivatives in the artificial diets of natural enemies.

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