Short Communication

Laboratory Approach: Alternate Dietary Sources for Coccinellid Ladybird Beetles

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Abstract | Coccinellid beetles are effective predators of soft bodied insect pests. They can be mass reared in laboratories for applied biological control against sucking insect pests. Laboratory rearing of ladybirds often need live host, preferably aphids. Artificial diets prepared from different sources do not prove very successful due to complexity of preparation for coccinellid beetles. Short seasonal availability of aphids hinders the rearing procedures. Therefore, some alternate natural dietary sources are essential to continue the mass rearing of ladybirds. In present study, we compared the longevity and egg laying of two species of ladybirds on alternate natural dietary sources other than natural dietary sources i.e., live aphids. The longevity of seven spotted ladybird *Coccinella septempunctata* was significantly higher on control (live aphids, Mean= 171.5 days) than other treatments including frozen aphids, frozen eggs of Angoumois grain moth and soaked raisins. Among all treatments, longevity was minimum on frozen aphids (Mean= 66.4 days) for seven spotted beetles. Similarly for zigzag beetle, *Menochilus sexmaculatus,* the number of days the beetles survived was significantly higher on live aphids (Mean= 106.54 days) and was minimum on soaked raisins (Mean= 67.21 days). Among different diets, the fecundity of both beetle species was maximum on live aphids (*M. sexmaculatus*, mean = 32.4; *C. septempunctata*, Mean = 42.4). The females of zigzag ladybird produced least number of eggs when given either frozen aphids or frozen eggs and seven spotted did not lay eggs on any other treatment. As an outcome, beetles survived for substantial period on alternate dietary sources (up to six months) and such alternatives can be used until provision of natural host i.e., aphids is made available.

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Introduction

The predacious coccinellids are of great economic importance and have been successfully used in the biological control of many insect pests of agricultural importance (Hayat *et al.*, 2020). Seven spotted aphidophagous ladybird beetle, *Coccinella septempunctata* Linnaeus (Coleoptera; Coccinellidae) is probably the most abundant generalist predator of aphid population and many studies have reported their significance in suppressing aphid abundance (Singh and Singh, 2013). For example, when the coccineelid beetles were not introduced, and aphid infested plants were kept in cages, the population of aphids increased significantly with greater growth rates as compared to the plants where coccinellids were released within cages (Elliott and Kieckhefer, 2000; Brown, 2004). Both adults and larvae are predators and a single ladybug can consume perhaps as many as 3,000 or more aphids in its lifetime and may also feed on scale insects, mites and mealybugs (Ali *et al*., 2012). The zigzag beetle, *Menochilus sexmaculatus* Fabricius (Coleoptera; Coccinellidae) is a coccinellid beetle that feeds upon a wide range of soft bodied insects, primarily aphids (Mari *et al*., 2004). The zigzag beetle is widely distributed in the countries like Pakistan, India, Borneo, Jawa Indonesia, U.K., Philippines, Islands of Bali, France, Sumatra and South Africa and has also shown a significant role in reducing aphid population (Rakhshan and Ahmad, 2015).

Laboratory rearing of predatory ladybird beetles often need a live host particularly aphids. But due to the short seasonal availability of aphids, continuous rearing of ladybird beetles on live aphids becomes temporal and at times when aphids become scarce or unavailable, keeping ladybird beetles alive is a major stumbling block in rearing procedures. Rearing the host further complicates the procedure and exerts extra burden in both monetary and physical terms. Rearing on artificially developed diets is a further challenge in case of predatory insects where specific dietary requirements need to be met to sustain fitness (Youssif and Helaly, 2021). Furthermore, usually coccinellid beetles in general and *C. septempunctata* in particular show diminished biological properties (e.g., fecundity, egg hatching rate, survival rate, etc.) on artificially prepared diets (Cheng *et al*., 2020). Even the cold storage practices and hunger levels can alter the feeding behaviour of this coccinellid species (Suleman *et al*., 2017; Hayat *et al*., 2020). However, addition of juvenile hormone in C. septempunctata significantly lengthened larval development times and reduced pupation and emergence (Cheng *et al*., 2023). Here we compared some natural alternate foods for laboratory rearing of ladybirds at times when aphids are scarce or unavailable with objectives to keep the population alive in laboratory without natural host.

Materials and Methods

Laboratory rearing of adult coccinellids *M. sexmaculatus* (Ms) and *C. septempunctata* (Cs) were conducted on some alternate foods in comparison with their natural host (aphid). Experiment was conducted in Integrated Pest Management Laboratories at Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan. For this experiment, five treatments were used viz., green peach aphid *Myzus persicae* Sulzer (Hemiptera; Aphididae) as aphid species for two treatments (live and frozen; treatment, 1 and 2). Other treatments included, frozen eggs of Angoumois grain moth *Sitotroga cerealella* Olivier (Lepidoptera; Gelechiidae; treatment 3), soaked raisins (soaked and then cut in halves, treatment 4), soaked raisins along with frozen eggs (treatment 5). Ten adults per treatment were used in five replicates (N = 50). All diets were given ad libitum and data were recorded for the longevity and egg production of beetles under controlled laboratory conditions at temperature 24±2°C, humidity 65% and photoperiod of 14 hrs light and 10 hrs darkness.

Statistical analysis

Statistical programme SPSS ver. 16.0 was used for all analysis. Normality of the data was checked by One-sample Kolmogorov-Smirnov test. Non normal data were log transformed. Normal data were subjected to parametric variance test. Analysis of variance ANOVA was performed. LSD test was used for the differences between variables.

Results and Discussion

Longevity of *C. septempunctata* was significantly different for different feeding sources (ANOVA, $F_{(4, 49)}$= 24.19, P= 0.03). It was significantly higher on live aphids followed by soaked raisins along with frozen *S. cerealella* eggs (Figure 1). Their longevity on soaked raisins and frozen aphids alone was not different significantly. Longevity on frozen eggs alone was slightly higher than on frozen aphids and soaked raisins alone but again was significantly not different from these two treatments and the treatment of soaked raisins + frozen eggs (Figure 1).

Similar food treatments when were given to *M. sexmaculatus*, again there was significant difference for different feeding regimes (ANOVA, $F_{(4, 49)}$=14.34, P= 0.02). The longevity of adults was significantly higher on live aphids followed by soaked raisins and frozen eggs treatment (Figure 1, Table 1). Least length of longevity was observed on soaked raisins alone. Diets of frozen eggs and frozen aphids alone were

Table 1: *Mean adult longevity and fecundity of two coccinellid ladybird beetles on alternate dietary sources.*

Dietary Sources	Adult longevity (Mean ±SE)		Fecundity (Mean ±SE)	
	C. septempunctata	*M. sexmaculatus*	*C. septempunctata*	*M. sexmaculatus*
Frozen eggs	95.34±1.23BC	70.34±1.10BC	0±0	8.5±0.57C
Frozen aphids	66.4±1.59C	73.78±1.67BC	9.4±0.45B	7.7±0.32C
Soaked raisins	68.65±1.22C	67.21±1.34C	0±0	0±0
Soaked raisins + Frozen eggs	127.34±1.21B	83,21±1.07B	0±0	10.8±0.48B
Aphids	171.5±1.09A	106.54±1.35A	42.4±1.02A	32.4±0.89A

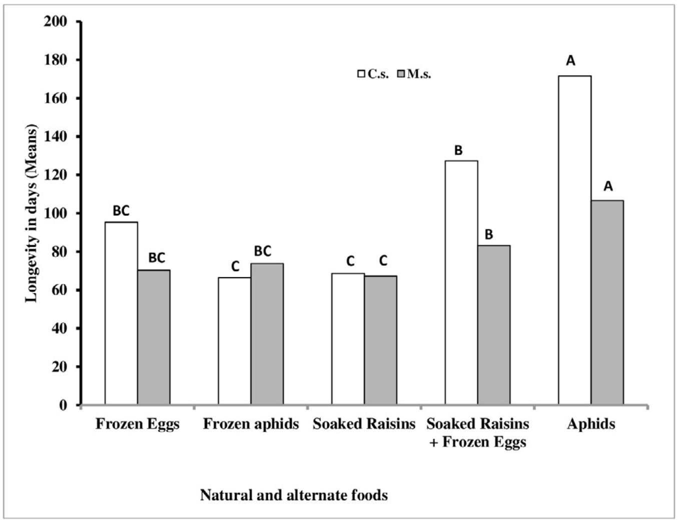

Figure 1: *Adult longevity of C. septempunctata (open bars) and M. sexmaculatus (closed bars) on natural and alternate foods under laboratory conditions (N = 50) (Means followed by the same letter were significantly not different within species (P> 0.05).*

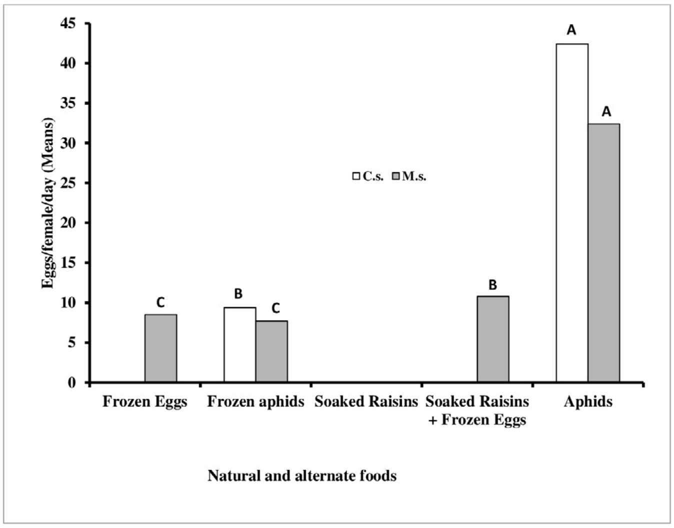

Figure 2: *Fecundity of C. septempunctata (open bars) and M. sexmaculatus (closed bars) on natural and alternate foods under laboratory conditions (Means followed by the same letter were significantly not different within species (P> 0.05).*

statistically at par and did not differ significantly from the treatments of soaked raisins and soaked raisins + frozen eggs (Figure 1, Table 1).

Fecundity of both species of ladybird beetles was affected on diets other than their natural host (ANOVA, $F_{(1, 29)}$= 17.52, P = 0.12 for fecundity *C. septempunctata* and $F_{(3, 59)}$ = 12.67, P = 0.21 for *M. sexmaculatus*). For *M. sexmaculatus*, maximum number of eggs was laid when they were fed live aphids, followed by the treatment of soaked raisins and frozen eggs (Figure 2, Table 1), whereas, females produced least number of eggs when given either frozen aphids or frozen eggs (Figure 2, Table 1). On the other hand, females of *C. septempunctata* did not produce any eggs when given frozen eggs, soaked raisins alone, or in combination with frozen eggs (Figure 2, Table 1). Maximum number of eggs was produced on diet of live aphids and fewer eggs were laid on the frozen aphid diet (Figure 2, Table 1).

Laboratory studies for the period under report have shown that ladybird beetles can survive for substantial time periods on alternate foods like frozen eggs, frozen aphids, soaked raisins or combination of these but the fecundity is affected. The natural diet comprising of live aphid host proved significantly superior to the alternate diets. So until a suitable artificial diet is prepared or arranged, we can keep the coccinellid beetles alive by providing some sugary or sweet source (like soaked raisins etc.) or frozen and previously stored eggs or aphids along with adequate water. Previous studies have shown that other than live insect host, frozen alternate dietary sources can be used. For example, frozen moist bee pollen was a suitable alternative diet for coccinellid *Harmonia axyridis* (Berkvens *et al*., 2008). In a separate study, longevity of *M. sexmaculatus* on frozen aphids was reported as 63 days and egg laying as 6.4/ day (Khan and Khan, 2002). According to our findings, longevity of *M. sexmaculatus* on frozen aphids was even higher (73.78) and so were the eggs per day (7.7). Whereas, *C. septempunctata* produced eggs on live aphids and fecundity was greatly reduced on frozen aphids.

Adults of the genus *Coccinella* may consume other hosts but females become reproductively inactive when these prey serve as the sole diet (Richards and Evans, 1998). The number of eggs produced by a female *C. septempunctata* depended primarily on the number of aphids consumed (Evans *et al.*, 2004). Facultative reproductive diapause has also been reported in this species when limited aphids or non-aphid foods are offered (Suleman, 2015). Aphids may possess specific nutrients required for *C. septempunctata* reproduction that are lacking or present only in small amounts in alternative prey or diet sources (Hagen, 1987). This ladybird may feed on a variety of non-adequate foods like small insects, insect larvae, pollen, honeydew, and fungi in the diet, in particular outside the reproductive season (Triltsch, 1999). Few studies deal with the role of alternative prey for ladybird performance. Considerable reduction in fecundity was reported in ladybirds on non-aphid prey (Evans and Gunther, 2005). Increased fecundity in two ladybird species was reported when a limited diet of aphids was supplemented with abundant alternative prey (weevil larvae), which alone could not support reproduction (Evans *et al.*, 1999). In addition to difficulty of rearing ladybeetles on artificial diets, the consequences on the fitness, such as, weaker progeny, poor searching efficiency and a weaker response to aphids (Abbas *et al.*, 2023), further challenges the basic aim of their rearing.

Present study showed that alternate foods like soaked raisins and frozen eggs proved good dietary sources for keeping the beetles alive for substantial time periods but no egg laying was observed in case of seven spotted ladybird. The information presented here is of particular value for assessing the use of alternative dietary sources for the mass rearing of ladybird beetles in the slack periods of natural host availability. Such alternate foods can be used as a supplement when aphids are scarce or limited in number to keep the adults alive until next source of aphids is available. Artificial rearing of aphids on different host plants or artificial diet (Emden and Wild, 2020; Li *et al.*, 2021) can also be established where resources are available for an uninterrupted supply of live host. As the alternate dietary sources mentioned in this study and simple, relatively easy to prepare with results showing prolonged survival and fitness, these must be incorporated in the rearing techniques of coccinellid beetles until the supply of live host is established. These laboratory reared predatory beetles could be released as a component of integrated pest management (IPM) of various agricultural crops, especially in fruits and vegetables. This rearing methodology could be helpful in reducing the mortality of adult beetles in mass rearing facilities and further ensures their timely availability for field application strategies.

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Novelty Statement

This article is introduction of a new perspective that adds to the existing knowledge of artificial diets of predators in IPM studies.

Author's Contribution

Nazia Suleman and Asia Riaz were involved in conception or design, acquisition, statistical analysis, interpretation of data, obtaining funding, coordinating the research project and drafting of the manuscript.

Conflict of interest
The authors have declared no conflict of interest.

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