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LADYBIRD BEETLES *Adalia bipunctata* L. INTERACTION WITH LEGUME BEAN *Phaseolus* SPECIES IS DEPENDENT ON LIFE NEEDS

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ABSTRACT

Two-spots ladybird beetle (*Adalia bipunctata*) needs support from the plants species of legumes family common bean (*Phaseolus vulgaris*) and lima bean (*P. lunatus*) for completing their life circle. This interaction serves the need in terms of food, shelter, host-plant suitability and mutualism. This study investigated the interaction and different behavioural activity between the domestic ladybird and global plant species. Ladybird interacted with both bean species and showed intensive activity on the plants studied. Dualistic eating habits and their behavioral activity with *Phaseolus* species plants are new finding in this study. Results exhibited that, selected plant species are extremely important for *Adalia bipunctata* in context of their local ecosystems. It is suggested that interaction between domestic ladybird and global bean plants is extremely important for both types of organisms in the context of their local ecosystems.

Key words: behaviour, ecosystem, interaction, ladybird, legumes, mutualism, *Phaseolus vulgaris, Phaseolus lunatus*

INTRODUCTION

One of the most important studied questions in biology and applied sciences is the relationship between insects and plants. These two groups of organisms are distributed on high scale in the global ecosystem and their interactions influence the survival of their subsequent generations. Life of these organisms is related to their events occurring in their locality and climatic conditions, and their developmental phases are crossed in similarity from the point of view of developmental success (Forister *et al.,* 2012). The new developments in the evolution of ecological specialization have provided greater details on the genetic architecture of insects and plants, and their multi-tropic interactions. Insect-plant interactions have been studied very actively during last four decades (1973–2013) with 17660 papers published (data as per using the key words ‘insects’ and ‘plants’) (WoS. 2013). However, the data on the relationship, behaviour and possible communications between insects and plants is insufficient. This relationship is scientifically interesting study not only from the perspectives of ecology and evolution but also from the economic significance. Some ladybirds are considered extremely useful in field productively practices (Banfield-Zanin *et al.,* 2012). Ladybird-plants interactions have been therefore actively studied also from this point of view, with 300 papers published that are yet to be scientifically challenged. Santos *et al.* (2012) studied coccinellid communities associated with olive, chestnut and almond crops in north-eastern Portugal and found that, coccinellid species such as *Coccinella septapunctata* L. and *Adalia decempunctata* L. can be used in eliminating pest management programmed that encourage greater natural enemy biodiversity in agro-ecosystems. Moreover, the interactions between the populations of insect species and plants make the problem of specialization and food preference by ladybirds more complex and difficult to resolve. This behavioural interaction in not known, especially in the cases of plants that are not a part of the natural habitat for ladybirds. Therefore, this study intends to broader the knowledge on this area by synthesizing new developments and collecting basic data and evidences from empirical experiments with ladybird and legume species. In this paper, the ladybird *Adalia bipunctata* L. and legumes are described and their formal interactions are

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documented. The synthesis of previously published data has been provided for the experimental and theoretical understanding of the diverse relationship of these two organisms. The choice of these organisms for the experiments was determined in light of previous studies that suggested their importance (Schoonhoven 1999) and for extending these organisms on the global scale (Kajita and Evans, 2010; Xue *et al.,* 2012). Experiments were established with local populations of ladybirds and two globally known economic beans. The following questions were addressed: (1) is there any real interaction between ladybird and beans?; (2) what kind of behaviour of these organisms occurs and what is the reason of this behaviour? and; (3) what are the sources of interaction between two kinds of organisms? The experimental data has been discussed in the light of the theoretical synthesis and a set of reserved questions finding has been presented as resource for future research.

MATERIALS AND METHODS

Study was conducted at the Research and Teaching Laboratory of Applied Botany, Biological Interactions and Ecological Engineering of the Department of Biology, University of Eastern Finland during 2010–2012. The research plan was established after analyzing of old and new existing data previously published. Analysis of this data material was done and new experiment findings as well as the synthesis of the main knowledge of the ladybird-legume relationships were made. The data on ladybird and legume species relationship was collected experimentally.

Ladybirds and plant materials – Alive ladybirds were collected from the area of Joensuu city by sweeping mosquito nets and stored in glass bottles for experimental uses. In total, 293 individual ladybirds were collected from mid-May to mid-August. Each ladybird was morphologically studied, and its taxon was identified. Only ladybird with abundant appearance (two-spotted ladybird, *A. bipunctata* L.) was used in the experiment. Ladybirds were translocation back to their living host area after the experiment. Behaviour of the ladybirds was studied on two legume species (common bean, *Phaseolus vulgaris* L. and lima bean, *Phaseolus lunatus* L.) grown for study purpose. The seeds for the experiments were obtained from commercial market Oy Exotic Garden ab, Nämpnäs.

Field experiments – Field experiments were established in 2010 in an open sunny area in the northeastern part of the Botanical Garden (Botania) of University of Eastern Finland covering the area of 16 m^2 divided into four identical plots. The soil was tilled by machines, weeds were pulled out manually, and finally the soil was ready for sowing. Twelve plants seed were put in each plot and allowed to grow for 6 weeks, until approximately 5–7 leaves were grown. The growth of all plants was normal and typical. No damages or pathological phenomena of plants were observed before the experimental contact with ladybirds. The experimental area was protected against rabbits and others animals by metal nets, which were 85 cm high and with 5.5 cm diameter of the holes (Fig. 1a).

Laboratory experiments – The laboratory experiments were established by growing plants (six individuals of both species) in pots similarly to those used in the field experiment. Plants were grown in big glass boxes illuminated to attain a temperature of 21°C (Fig. 1b). All the plants were growing well and not pathological damage was observed before experimental contact with the ladybirds. Newly collected ladybird beetles were stored in glass bottles with legume plants leaves for the laboratory experiments. Young leaves, aphids and moistened cotton were put in the glass bottle as food for the ladybirds (Fig. 1c).

Measurements of plants and ladybirds – All plants and ladybirds were measured before their interaction experiments. The heights of plants were measured using centimeter scale. The size of leaves and ladybird beetles were measured by using the DIGIMATIC scale with electronic reader (accuracy up to 0.00 mm). The ladybird beetles were weighed on SCALTEC scale (accuracy up to 0.000 g).

Measurements of interactions – The ladybirds were placed on plants covered by mosquito nets. Movement of the ladybirds from the base of the stem to the top of the plants was measured by stopwatch. Other behaviour of ladybirds (resting, walking, flying, and trans-locating from the plant to the mosquito net, and changing places) was observed in the field and laboratory by magnifying glass and video camera. Searching and eating behaviour (eating style, activity, and food selection) were measured by using appropriate equipment (magnifying glass, microscope, alternative foods and digimatic scale). Adult ladybirds were kept in glass bottles containing beans leaf and moistened cotton to serve as food, and under 21ºC temperature.

Statistical analyses – Data collected were statistically analyzed using Sigma Plot 11.0 statistical and graph program. Analysis of variance (ANOVA) was applied with (Kruskal-Wallis) One Way analysis of Variance was used. We used Descriptive statistics experimental design and multiple-comparison test with (Holm-Sidak method) for statistical analyses. For photography, Canon (EOS 500D and EOS 450D) camera and microscope camera Leica S8APO were used.

RESULTS AND DISCUSSION

A total of 293 ladybird beetles were collected, 93.3% of them were individuals belong to the species *Adalia bipunctata*. Other individual species were *Adalia quadrimaculata*, *Coccinella septempunctata*, *Propylaea quatuordecimpunctata* and *Hippodami tredecimpunctata* (Fig. 1d). The size and weight of *A. bipunctata* ladybird was measured individually. Experimental ladybird body length was in the field and laboratory from 3.56 to 5.19 mm, wide from 1.20 to 2.16 mm. Same time used adult ladybirds weight was from 0.002 mg to 0.004 mg (Fig. 5). Analytical results showing that ladybird beetles size are significantly different whereas *Adalia bipunctata* body length 4.43 ± 0.51 and wide 1.71 ± 0.27. *Adalia* species of ladybird body length and wide statically significant difference value was P <0,001 in experiment.

Experimental legume plants were grown well in both the field [height from 17.5 to 42.5 cm (*Phaseolus vulgaris*) and from 9.5 to 41.5 cm (*Phaseolus lunatus*)] and in the laboratory experiment for winter condition [height from 35 to 57 cm (*Phaseolus vulgaris*) and from 19 to 41.5 cm (*Phaseolus lunatus*)] (Table 1). Leaf size was on the field [length from 9.3 to 12 cm (*Phaseolus vulgaris*) and from 6.8 to 9.3 cm (*Phaseolus lunatus*)] and in the laboratory [length from 5.6 to 10.2 cm (*Phaseolus vulgaris*) and from 3.9 to 7.4 cm (*Phaseolus lunatus*)]. Same time in the field leaf [wide from 5.3 to 6.9 cm (*Phaseolus vulgaris*) and from 4.8 to 6.7 cm (*Phaseolus lunatus*)] and the laboratory leaf [wide from 3.2 to 6.1 cm (*Phaseolus vulgaris*) and from 3.5 to 5.4 cm (*Phaseolus lunatus*)] (Table 2). Statically we analyses all the experimental plants in both places and we found significant different with them. The result showed

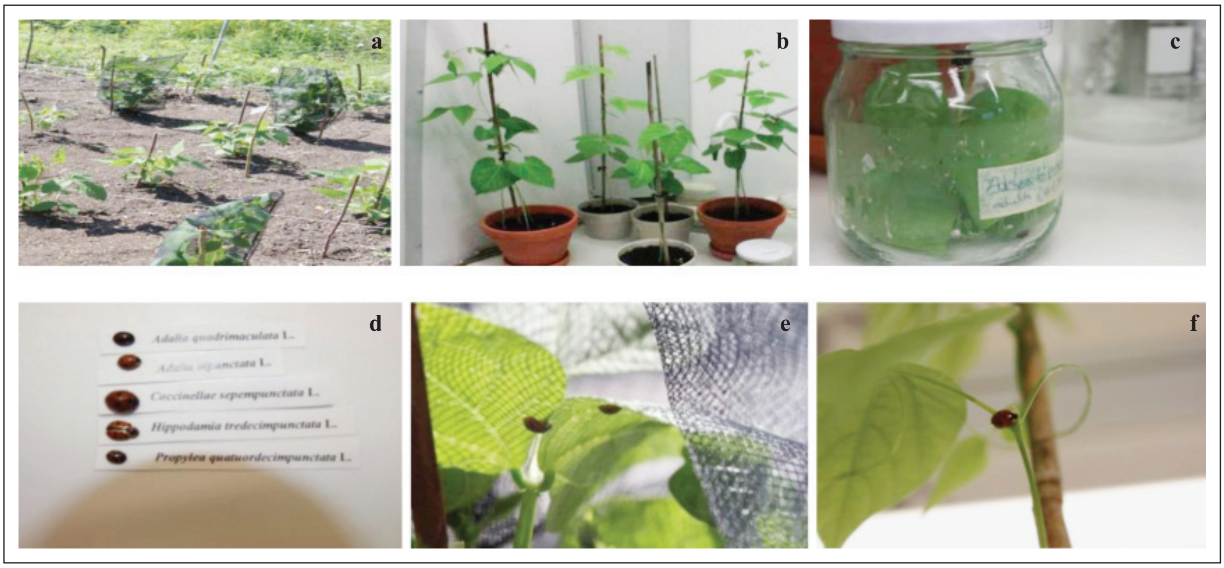

Fig. 1: Overview of experiments established. **a**. *Phaseolus vulgaris* and *Phaseolus lunatus* in the field experiment established in Botania with mosquito net; **b.** *Phaseolus vulgaris* and *Phaseolus lunatus* growing in the laboratory for behaviour experiments; **c.** Feeding process are ladybirds for behaviour experiments; **d.** Collected five different species of ladybird beetles in the Joensuu area; **e**. Resting behaviours of *Adalia bipunctata* L. under the leaves of *Phaseolus* spp; **f**. *Adalia bipunctata* L. eating nectar or leaf buds in unavailability of aphids food.

Table 1. Height of bean plants measured in the both field and laboratory experiments

Plant height (cm)			
Field		Laboratory	
Phaseolus vulgaris	*Phaseolus lunatus*	*Phaseolus vulgaris*	*Phaseolus lunatus*
29.5	10.0	35.0	56.0
17.5	13.0	56.0	62.0
42.5	14.5	46.0	19.0
38.5	41.5	57.0	59.0
31.5	9.5	41.0	27.0

Table 2. Adult leaves parameters of experimental plants

Leaf size (cm)							
Field				Laboratory			
Phaseolus vulgaris		*Phaseolus lunatus*		*Phaseolus vulgaris*		*Phaseolus lunatus*	
Ll	Lw	Ll	Lw	Ll	Lw	Ll	Lw
12.0	6.8	9.3	6.5	9.5	3.7	7.4	4.7
10.4	5.8	8.7	6.7	10.2	4.8	5.7	3.1
9.5	6.4	7.1	5.0	7.3	6.1	4.3	5.1
9.3	5.3	6.8	4.8	5.6	5.3	6.6	3.5
10.9	6.9	7.0	5.2	8.4	3.2	3.9	4.6

Abbreviations: Ll = Leaf length, Lw = Leaf wide

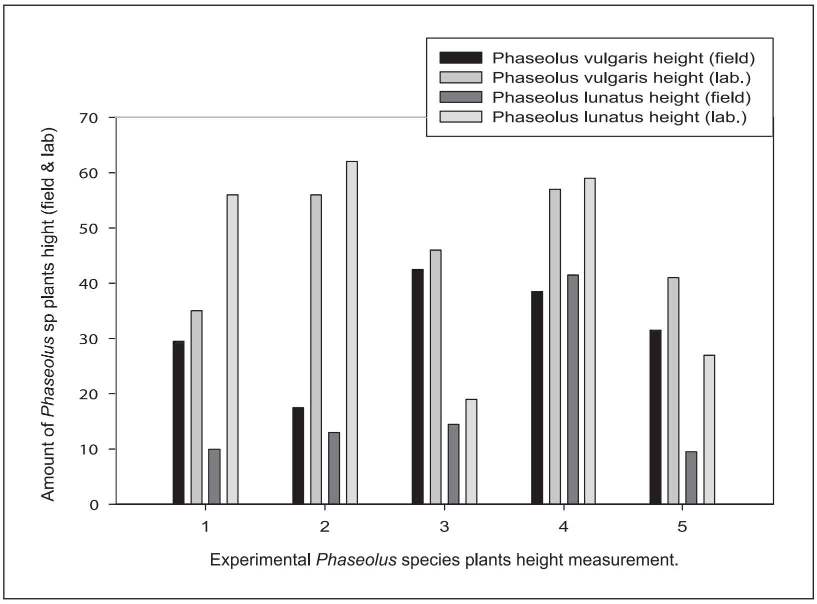

Fig. 2. A measurement of the *Phaseolus* species plants height.

in ANOVA analysis in the field *P. vulgaris* height was 31.9 ± 9.6 where as in the laboratory height 47.0 ± 9.5, same time in the *P. lunatus* height was 17.7 ± 13.4 where as in the laboratory height 44.6 ± 20.03. Field and laboratory grown *Phaseolus* species plants statistically significant difference was P<0,015 (Fig. 2). On the other hand *Phaseolus* species of plants leaves size has significantly different with behavioral activities. Leaves size depending on aphid density and availability in both places plants. Our analytical result showed that, in the field *P. vulgaris* leaf length was 10.42 ± 1.09 where as in the laboratory leaf length 8.20 ± 1.82, same time in the field *P. lunatus* leaf length was 7.78 ± 1.13 where as in the laboratory leaf length 5.58 ± 1.48. And the field grown *P. vulgaris* leaf wide was 6.24 ± 0.68 where as in laboratory leaf wide was 4.62 ± 1.17, same time in the field grown *P. lunatus* leaf wide was 5.64 ± 0.89 where as in laboratory leaf wide was 4.20 ± 0.85. Both places grown *Phaseolus* plant leaves length were statistically significant difference value was P<0,001 and wide value was P<0,011 (Fig. 3 & 4).

Experimental ladybird beetles and legume plant behavior activities were fully significant. Behavioral observation results showed that, *Adalia bipunctata* ladybird moving activity in the *Phaseolus* species plants were 16.60 ± 4.80 and their walking activity was in plant or plant parts 18.26 ± 2.90. But ladybird beetles resting activity was different in same species plants. In the *P. vulgaris* plant *A. bipunctata* resting time was 18.41 ± 3.31, whereas *P. lunatus* plant resting time was 10.75 ± 2.86. They have statically behavioral significant with all above activities and their significant different value P < 0,001 (Fig. 6 & 7). Observation data showed that, the ladybird's body sizes and weights was affected for food searching in the different stages on the host-plant. Most of ladybirds were active in the *Phaseolus* spp. plants in search of aphids for food.

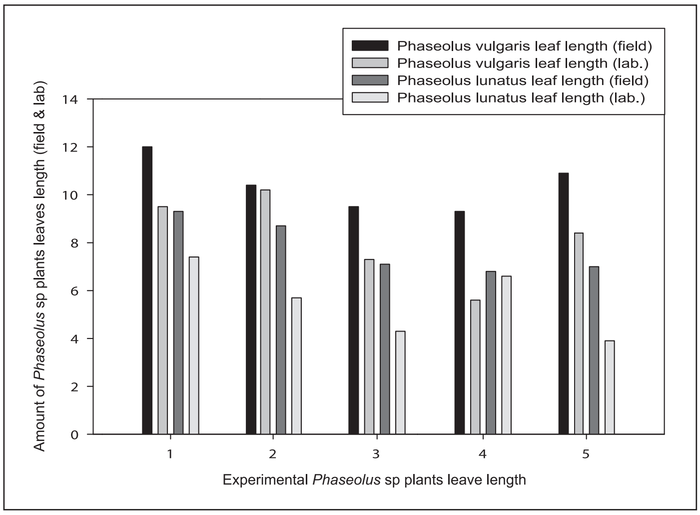

Fig. 3. A measurement of the *Phaseolus* species plants leaves length.

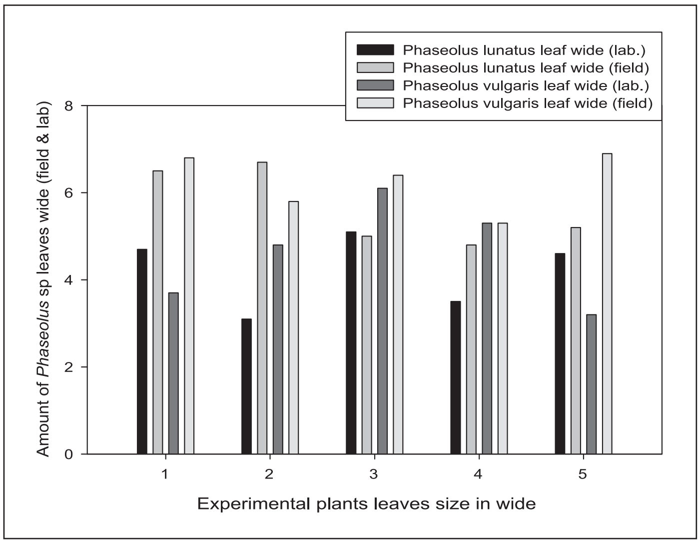

Fig. 4. A measurement of the *Phaseolus* species plants leaves.

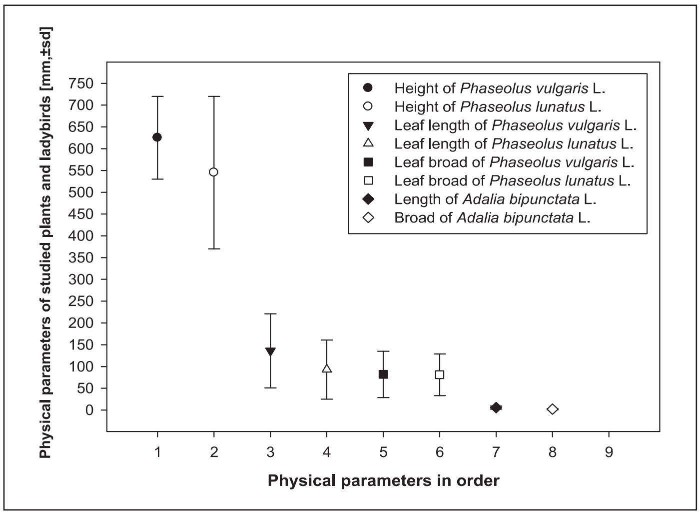

Fig. 5. Plants and ladybird beetles measurements.

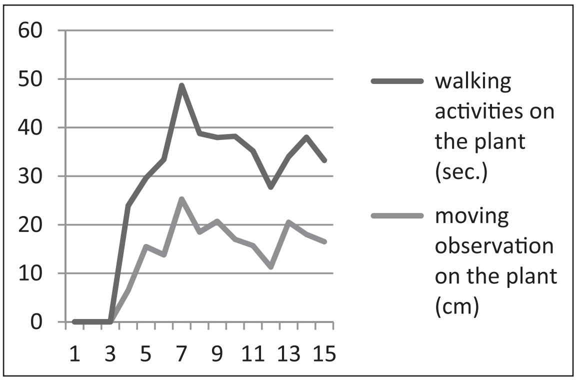

Fig. 6. *Adalia bipunctata's* walking and moving activities on the plants.

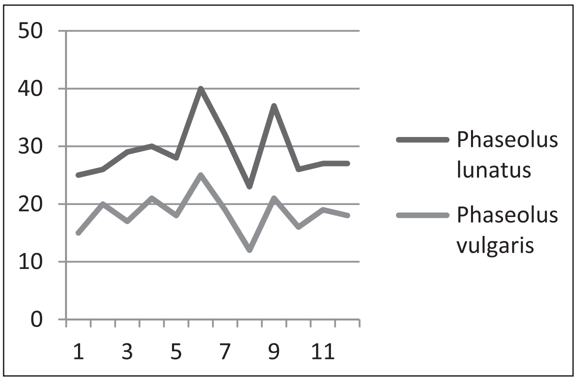

Fig. 7. *Adalia bipunctata's* resting behaviors in the experimental plants (min).

Table 3. *Adalia bipunctata* L. size and weight measured for behavioural activity with *Phaseolus* sp. plants

Adalia bipunctata length (mm)	*Adalia bipunctata* wide (mm)	*Adalia bipunctata* L weight (mg)	*A. bipunctata* moving on plants (cm)	*A. bipunctata* resting on *P. vulgaris* (min)	*A. bipunctata* resting on *P. lunatus* (min)	Walking activities (*Phaseolus* sp. plants) (sec.)
4.62	1.64	0.004	6.50	15.00	10.00	17.14
4.48	1.80	0.003	15.50	20.00	6.00	14.14
4.46	1.83	0.003	13.80	17.00	12.00	19.63
5.07	1.89	0.004	25.30	21.00	9.00	23.37
4.21	1.62	0.002	18.50	18.00	10.00	20.30
3.82	1.35	0.003	20.70	25.00	15.00	17.25
4.32	1.64	0.004	17.00	19.00	13.00	21.20
4.21	1.69	0.003	15.70	12.00	11.00	19.50
3.56	1.20	0.002	11.30	21.00	16.00	16.45
4.12	1.61	0.003	20.50	16.00	10.00	13.50
5.15	2.16	0.002	18.00	19.00	8.00	20.00
5.19	2.09	0.004	16.50	18.00	9.00	16.75

In the field observation we found, most of ladybirds have eaten four to ten pieces of aphid in each day from their host-plants. The observation results showed that the medium and big adults of *A. bipunctata* were active in walking over the plants and took longer rests than the smaller *A. bipunctata* (Fig. 1e). The length, breadth and height of the leaves of the experimented plants of *P. vulgaris* and *P. lunatus* were calculated for graphical structure. *Adalia bipunctata* body lengths, breadth and their activities with *Phaseolus* spp plants were calculated from expected results (Table 3). Results showed that, the ladybirds were more interested in attacking and staying for longer periods in legume plant with aphids. The main reasons for this interaction behaviour with legumes is not only to for aphids as food, but also for resting, taking shelter and obtaining alternative food sources like juice, leaf buds, nectar and young plant leaf parts. In the experiment, we measured of the moving speed of the ladybirds in their food searching behavioural activity. When foods were nearer or available in the host-plant, they moved slowly with simultaneous movement of their antenna around those spaces (average moving speed $16.60 \pm 6.50_{cm/min}$). The walking activity was also observed and measured for interpreting the behavioral interaction. A new finding in this observation was that they first walked in the plant or plant parts for food searching, for finding other food source from the host-plants or to take shelter in the host-plant. Their average walking speed was $18.26 \pm 13.50_{cm/min}$. The results showed that, most of time walking speed activity depended on the plants height, because food or food source availability in the upper part of the plant varied in comparison to those in the lower parts. The eating style of *A. bipunctata* beetles was also observed in the experiment. Hungry *Adalia* spp. ladybird ate aphids very actively and quickly, but their feeding activity was slower when they eating leaf buds, juice or leaves of the plants. Resting inside the mosquito net of the *Phaseolus* spp plants, ladybird beetle took rest for different time in different parts of the plant like the steam, leaves, at the ground level of plants, in leaf buds and occasionally over the mosquito nets. Their average resting period was 12.00 ± 18.4_{min} with *P. vulgaris* and 6.00 ± 10.75_{min} with *P. lunatus*. The observation result showed that, the ladybird took more rest in *P. vulgaris* than in *P. lunatus* plants or plant parts; probably its happened cause of aphids was more available in *P. lunatus* plant.

The common source of the interaction behaviour between the ladybird beetles *A. bipunctata* and legume plants *P. vulgaris* and *P. lunatus* was food. However, at the same time, we found the ladybirds ate other food sources like young leaves, nectar, and plant juice when the common food aphid was unavailable (Fig. 1f). Aphids were available in the experimental time with *Phaseolus* plants in the field observation. Black leaf aphids *Aphis* spp were the pest on the *Phaseolus* plants. Most of aphids' body was wingless, but some had wings with black or green-brown coloration and wax pollination. They were mostly present under the plants' young leaves or leaf steam or buds. Experimental period we found aphids density was always higher under the young green leaves or leaf buds than the other parts of *Phaseolus* spp plants. The results showed that ladybird beetle *A. bipunctata* are relatively abundant in the study locations and they interaction with *Phaseolus* plant species. Other ladybird species occur also occasionally but with a small population made impossible to their use in the experiment. Legume plants and *Adalia bipunctata* ladybird has significantly interaction relation mainly food source for ecological life. Our experimental data fully indicate that *Adalia* bipunctata ladybird beetle are interacting with *Phaseolus* species plants for aphid and plant-part foods their life needs. Legume plant species of *Phaseolus vulgaris* and *Phaseolus lunatus* have mutualism relation with *Adalia bipunctata*. In the life stage *of Adalia* spp ladybird beetles need interaction relation with *Phaseolus* spp plants for food security and search of a host plant for completion of the life circle. In this case, the experimental behaviour indicated that plants height depends on the available aphids density, which is inviting ladybird for food source, and field experiment indicates that aphids density was more in the higher level of plants' leaves than in the lower level of plants leaves. Previously published studies also reported the importance of interaction behavior of two-spots *A. bipunctata* ladybird with different plants. The results indicate that, selected ladybirds (*A. bipunctata*) and legume plants (*Phaseolus* spp) has closely interaction relation. These behaviours are depended on the different characteristics of plants and ladybirds such as sizes and interests. The interest for the ladybird may include food security and searching for a potential host, and for plants, possible reductions of herbivore bio-agents as food for the ladybirds and leave vibration and cleaning of dust particle. Results indicated that, plants height is important for ladybird invitation for food sources. Especially, *Phaseolus* spp plant leaves at higher level were more attractive to the ladybirds than those at the lower stage leaves. Moreover, physical parameter of the plants and the ladybird studied are important for interaction. The gradual prey suitability and specialization on one aphid species by *A. bipunctata* for generations has an evolutionary significance in its establishment in different zoogeographical habitats. It is also known that food have significant influence on *A. bipunctata's* searching behaviour (Kalushkov 1999). Ladybirds' *A. bipunctata* are known in some countries as one of the most common coccinellid predators, for example, in an Iranian study on fruit and nut crops, this ladybird was very active in the pistachio gardens (Mehrnejad *et al.,* 2010). The low nutritional value or food limitation often expedites their development, resulting in smaller size of an adult ladybird (Frances *et al.,* 2000). After all investigation, the experimental and final results indicate that, *A. bipunctata* and legume plants species of *P. vulgaris* and *P. lunatus* have strong interaction relation in the ecology. Finally, ladybird beetles need internally or externally supports from the legume plants in the natural condition for complete their successful life circle. Insect-plants interactions are very important part of biological sciences in the world and their relations are more important for agriculturist and farmers. Dualistic behavior of local ladybird beetle *Adalia bipunctata* is fully new finding with *Phaseolus* spp plants. Their mutualism and dualistic eating behavior with Aphid eating ladybird beetle *Adalia bipunctata* and selected *Phaseolus* spp will make an important role in the developmental of biological control for economical crop protection. Possible dualistic behavior of ladybirds on crops can pose a problem in biological cropping. In this case, we can say that ladybird beetles *Adalia* spp are eating plant or plant parts for their digestion or body demand of chemical compound which need future study.

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