Pollen grains as an additional food for Asian lady beetle, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae).

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Abstract: The multicolored Asian lady beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) has been considered a good candidate for biological control of many insect pests. The non-prey foods as pollen are used by coccinellids to increase survival when prey is scarce, reduce mortality during diapause, fuel migration, and enhance reproductive capacity. We assessed the biological performance of this predator when reared on three different crop pollens (corn, clover, or pulverized bee pollen) as dietary supplements in addition to *Ephestia kuehniella* eggs. Results showed that the duration of pre-imaginal stages was significantly differed among all tested diets. Adult female weights were heavier than males in all treatments. Moreover, pollen type as additive food affect the adult weight and female longivity of *H. axyridis*. Number of egg batches/female were 46.3, 47.2, 22.5 and 34.9; the mean number of eggs/female were 829.5, 1033, 475.5 and 899; hatchability were 86.85, 48.82, 62.84 and 64.61% when fed on clover pollen with *Ephestia* eggs, corn pollen with *Ephestia* eggs, pulverized bee pollen with *Ephestia* eggs and *Ephestia* eggs only, respectively. It can be concluded that a mixture of clover pollen with *Ephestia* eggs is the best suitable diet for rearing of *H. axiridis*.

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1.Introduction:

The multicolored Asian lady beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), is indigenous to central and eastern Asia **[Nalepa *et al*. (1996); Koch (2003)**]. This predator has been considered a good candidate for biological control of many aphid species, scale insects, psyllids and mites **(Choi and Kim (1985); Mogi (1969); McClure (1987)).** and its biology and uses in biological control was extensively reviewed recently by **Koch (2003).**

Non-prey foods are an integral component of the diets of most predaceous coccinellids. At least 39 species of entomophagous coccinellids have been recorded as consuming more than 88 species of pollen under laboratory and field conditions **(Lundgren, 2009a).** They eat pollen for a rich source of protein. In some species, egg predation decreases when sweet corn pollen is available as an alternative food source **(Hukusima and Itoh (1976); Pfannenstiel and Yeargan (2002); Hoheisel and Fleischer (2007)).** Under field conditions, numerous coccinellids consume nectar, honeydew, pollen, fruit, vegetation, and fungus. These non-prey foods are used by coccinellids to increase survival when prey is scarce, reduce mortality during diapause, fuel migration, and enhance reproductive capacity. Each of these non-prey foods has unique nutritional and defensive characteristics that influence its suitability for lady beetles **(Lundgren, 2009b).** Coccinellid populations were four times higher in sweet corn than other crops coincides with **Andow and Risch1985)** observations that ladybugs are often more abundant in areas with high densities of corn pollen and aphids, and food quantity, rather than quality, is a more dominant force driving their biology.

Berkvens *et al.* (2008) examined the potential of three populations of *H. axyridis* to use bee pollen (mixture) as a food to sustain development and reproduction in the absence of insect prey. Other investigators studied using of pollen as alternative food for different predators such as the coccinellid, *Adalia bipunctata* (Linnaeus) (**De Clercq *et al*., 2005)** and *Clitostethus arcuatus* (Rossi) **(Yazdani and Zarabi, 2011),** the pirate bugs *Orius laevigatus* (Fieber) and *Orius albidipennis* (Reuter) (Anthocoridae: Hemiptera) **(Cocuzza *et al*., 1997).**

Moreover, other arthropods is feeding on pollen whereas **Onzo *et al*. (2012)** determined that maize pollen can efficiently sustain *Amblyseius swirskii* (Athias-Henriot) (Mesostigmata: Phytoseiidae) populations when the broad mite, *Polyphagotarsonemus latus* (Banks), densities on plants become low.

Michaud and Grant (2005) compared the nutritive value of various pollen sources for the development of *Coleomegilla maculata* DeGeer under conditions of continuous water availability and simulated drought. When water was continuously available, larval survival was not different from 100% on diets of frozen eggs of the flour moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), corn pollen, sorghum pollen, or pulverized bee pollen, whereas survival of larvae was significantly reduced on the latter three diets in the simulated drought treatment. Pollen of cultivated sunflower, *Helianthus annus* L. proved fatal to both larvae and adults. Moreover**, Lundgren and Wiedenmann (2004)** studied the nutritional suitability of 10 corn hybrids pollen for the facultatively phytophagous predator, *Coleomegilla maculate* lengi. They found that beetles reared on aphids had greater weights, fecundity and a shorter larval duration relative to the pollen-fed beetles.

The current study therefore aims to conduct biological activities of this predator when reared on three different crop pollens (corn, clover, or pulverized bee pollen) as dietary supplements in addition to *E. kuehniella* eggs and assessed their biological performance in order to using pollen in the predator mass production with low costs because pollen is cheaper than *E. kuehniella* eggs**.**

2. Material and methods

Predator culture:

Adults of *H. axyridis* were originally provided from Mass Production Laboratory at Faculty of Agriculture, Cairo University, Egypt. All larval instars were reared in Plexiglas boxes at 23°C, 70% R.H. and 16 h day light. The adult stage was placed in Plexiglas cages, where pieces of accordion pleated black canson papers provide a site for egg laying. Both the larvae and adults were fed on the Angoumois grain moth eggs *E. kuehniella* as an alternative prey **(El Arnaouty *et al.*, 2000)**. The eggs were collected and, after hatching, larvae were reared on each diet separately. Four diets were tested, eggs of *E. kuehniella* only, corn pollen with *E. kuehniella* eggs(1:1), clover pollen with *E. kuehniella* eggs(1:1) and pulverized bee pollen with *E. kuehniella* eggs(1:1).

Biological experiments:

Individual larvae were confined in Petri dishes daily. Because of the cannibalistic behavior of larvae on sibling larvae and eggs **(Snyder *et al.*, 2000)**, the larval *H. axiridis* were separated as soon as they became active in Petri dish (7.5×1.5 cm). Each larva was checked for moulting daily till pupation and adults emergence were also observed. There were 50 replicates per treatment. After adult emergence, one pair (male+female) of adults was placed in separate Plexiglas box (7.5X7.5 cm) containing each diet. The number of eggs laid, egg viability and adult mortality were recorded daily until all adults died. Sex ratio was determined in from adults which emerged from reared larvae on each diet using morphological characters to identify the sexes. There were 20 replicates per treatment. All experiments were conducted at 26±2 °C, 60±5% RH and a photoperiod of 16:8 (L:D) h. Newly laid eggs of the predator were removed and transferred to Petri dishes (7.5×1.5 cm). Hatching time of eggs was determined by daily observations.

Statistical analyses:

One way ANOVA followed by Tukey's HSD post hoc test was used to compare biological traits (development of larvae and pupae, sex ratio, fecundity, fertility, longevity) on different diets. Differences were considered significant at Pb0.05. All analyses were conducted using statistical Software (**SPSS, 2002**).

3. Results and Discussion.

The mean duration in days (Mean ±SE) of pre-imaginal stages (Table,1) was significantly differed among all tested diets whereas it was developed slower with feeding on clover pollen with *E. kuehniella* eggs (20.1±0.27) than that of feeding on corn pollen with *E. kuehniella* eggs (15.6±0.23), pulverized bee pollen with *E. kuehniella* eggs (18.7±0.25) and *E. kuehniella* eggs only (12.4±0.14). This finding is according to two reasons: (1) water content of pollen grains types and *E. kuehniella* eggs whereas **Michaud and Grant (2005)** found that Initial water content was highest in corn pollen (36.8%), followed by *Ephestia* eggs (29.2%), sorghum pollen (25.3%), sunflower pollen (8.7%), and bee pollen (4.6%), (2) variation in its components of carbohydrates, proteins and lipids **(Lundgren, 2009a).** Moreover, coccinellids reared solely on pollen invariably require a supplemental source of water **(De Clercq *et al.*, 2005; Michaud and Grant, 2005)**. Finally, pollens vary in their nutrition both intraspecifically and among species (**Lundgren and Wiedenmann (2004)**; **Lundgren (2009a),** and as such the nutritional value of pollen for a coccinellid species can change substantially among testing systems.

Also, our results showed that the duration of larval instars (Table, 1) was prolonged gradually from the first to fourth instar in all treatments except the third instar (1.3±0.08) with feeding on *E. kuehniella* eggs only was shorter than second instar (1.74±0.08).This result is in agreement with **LaMana and Miller (1998)** with the exception of the first instar duration was longer than second instar duration.

Adult female weights(mg) were heavier than males in all treatments (Table, 2). Males raised from reared larvae on *E. kuehniella* eggs only were heavier (31.58±0.8) than all other, but females raised from reared larvae on *E. kuehniella* eggs only (35.76±0.9) did not significantly differed with others except those raised from reared larvae on pulverized bee pollen with *E. kuehniella* eggs(21.6±0.58). It is clear that pollen type as alternative or additive food affect the adult weight of coccinellids whereas our results are in parallel with **Berkvens *et al.* (2008)** who recorded that adult body weight of *H. axiridis* was reduced by 37–68% with a diet of pollen alone, compared to individuals offered the diets containing *E. kuehniella* eggs. Moreover, **Tsaganou *et al*.(2004)** recorded that the mean weight of *H. axiridis* adults raised from larvae was significantly different when reared on different food types. Also, Michaud and Grant (2005) demonstrated that the adult weight of *C. maculata* were not decreased of either males or females on either the *Ephestia* egg or corn pollen diets but it reduced female weight on the sorghum pollen diet and the weight of both males and females on the bee pollen diet.

Female longevity in days (mean±SE) (Table, 2) when they was fed on eggs of *E. kuehniella* only (47.93 ±2.38) was significantly increased than when they was fed on clover pollen with *E. kuehniella* eggs (34.73 ±2.9) or pulverized bee pollen with *E. kuehniella* eggs (35.2±3.44) but not significantly differed when they was fed on corn pollen with *E. kuehniella* eggs (41.40±2.08)

Number of egg batches/female (mean±SE) (Table, 2) when they was fed on pulverized bee pollen with *E. kuehniella* eggs (22.5 ±3.9) was significantly decreased than when they was fed on clover pollen with *E. kuehniella* eggs (46.3±4.3) or corn pollen with *E. kuehniella* eggs (47.2±4.3) but not significantly differed when they was fed on eggs of *E. kuehniella* only (34.9 ±5.4) but the mean number or eggs/female was highly decreased with feeding on pulverized bee pollen with *E. kuehniella* eggs (475.5 ±79.1) than with feeding on all other tested diets. Moreover, hatchability(%) with feeding on clover pollen with *E. kuehniella* eggs (86.85±2.14) was significantly increased than with feeding on all other tested diets. Our results is in accordance with **De Clercq *et al.* (2005)** who demonstrated that when a diet of *E. kuehniella* eggs was supplemented with frozen moist bee pollen, egg hatch of *Adalia bipunctata* was better than that on *E. kuehniella* eggs only. Moreover, pollen is important in spermatogenesis **(Hemptinne and Naisse, 1987)** and, particularly when mixed with prey, may help to promote reproduction in some species of coccinellids **(Hemptinne and Desprets (1986); Michaud (2000); De Clercq *et al*. (2005); Omkar (2006) and Berkvens *et al*. (2008)).** On the contrary, **Michaud and Grant (2005)** demonstrated that reproductive adult females of *C. maculata* that received corn or sorghum pollen as a supplement to *Ephestia* eggs did not differ in fecundity or fertility from those fed only *Ephestia* eggs.

Table (1): Comparing pre-imaginal developments in days of *H. axiridis* on different diets.

Diets No.	Larva				Pre-pupa+pupa	Total
	1st instar	2nd instar	3rd instar	4th instar		
1	2.15^{a} ±0.062 (2-4)	2.13^{ab} ±0.059 (2-4)	3.45^{a} ±0.168 (2-6)	7.05^{a} ±0.147 (5-9)	4.95^{ab} ±0.092 (4-6)	20.1^{a} ±0.266 (18-24)
2	2.06^{a} ±0.061 (2-3)	2.36^{a} ±0.325 (1-4)	2.64^{ab} ±0.133 (2-3)	4.21^{b} ±0.114 (4-5)	5.29^{a} ±0.125 (5-6)	15.6^{b} ±0.228 (14-17)
3	1.52^{b} ±0.082 (1-3)	2.29^{ab} ±0.168 (1-5)	2.45^{ab} ±0.137 (1-5)	7.23^{a} ±0.179 (5-11)	5.28^{a} ±0.096 (4-6)	18.7^{c} ±0.254 (16-23)
4	1.07^{c} ±0.037 (1-2)	1.74^{b} ±0.082 (1-3)	1.30^{b} ±0.085 (1-3)	3.67^{b} ±0.119 (2-5)	4.57^{b} ±0.114 (3-7)	12.4^{d} ±0.141 (11-14)
F value	56.706	3.186	43.950	136.557	10.070	241.02
df	3,155	3,148	3,142	3,141	3,133	3,133
P	.000	.011	.000	.000	.000	.000

Diets No.: 1, Clover pollen with *E. kuehniella* eggs; 2, Corn pollen with *E. kuehniella* eggs; 3, Pulverized bee pollen with *E. kuehniella* eggs; 4, *E. kuehniella* eggs only. All means followed by ± SE and (minimum-maximum). Means followed by similar letters are not significantly different at the same column ($P \leq 0.05$).

Table (2): Comparing egg and adult parameters of *H. axiridis* on different diets.

Diets No.	Male weight (mg)	Female weight (mg)	Female longevity	No. of batches /Female	No. of eggs /Female	Hatchability
1	22.82^{ab} ±0.90 (19.4-29)	28.08^{ab} ±2.98 (18.4-29.1)	34.73^{a} ±2.9 (14-54)	46.27^{a} ±4.29 (22-72)	829.5^{a} ±98.4 (298-1725)	86.85^{a} ±2.14 (74.2-93.2)
2	25.60^{a} ±2.06 (19-30)	30.44^{a} ±0.93 (27-36)	41.40^{ab} ±2.08 (27-53)	47.20^{a} ±4.31 (14-76)	1033^{a} ±66.4 (588-1439)	48.82^{b} ±2.45 (36.9-58.8)
3	19.20^{b} ±0.60 (14.7-24)	21.60^{b} ±0.58 (15.4-26.7)	35.2^{a} ±3.44 (15-56)	22.53^{b} ±3.89 (2-54)	475.5^{b} ±79.1 (61-983)	62.84^{b} ±4.31 (48.1-83.2)
4	31.58^{c} ±0.80 (18.6-39.9)	35.76^{a} ±0.90 (28.1-40.3)	47.93^{b} ±2.38 (29-61)	34.93^{ab} ±5.40 (8-80)	899^{a} ±114.6 (156-1644)	64.61^{b} ±6.40 (41.9-96.4)
F value	48.003	8.885	5.106	6.589	6.767	14.115
df	3,58	3,67	3,56	3,56	3,56	3,36
P	.000	.000	.003	.001	.001	.000

Diets No.: 1, Clover pollen with *E. kuehniella* eggs; 2, Corn pollen with *E. kuehniella* eggs; 3, Pulverized bee pollen with *E. kuehniella* eggs; 4, *E. kuehniella* eggs only. All means followed by ± SE and (minimum-maximum). Means followed by similar letters are not significantly different at the same column ($P \leq 0.05$).

4. Conclusion

Mixing of clover pollen with *E. kuehniella* eggs is the best suitable diet among the tested diets for rearing of *H. axiridis* which coincided with the meta-analysis of published literature on that matter. However, it seems that the suitability of essential foods is usually improved when they are mixed with other foods for coccinellids (Lundgren, 2009a). Moreover, pollen grains are a significant food source for numerous insects **(Cottrell and Yeargan, (1998); Lundgren *et al*. (2005); Hoheisel and Fleischer (2007)).** Our findings indicate that *H. axiridis* is able to compensate for a suboptimal diet of animal prey by supplementary feeding on flower pollen. It can be a crucial trait of this predator for its mass production and use in biological control programmes.

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