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Effect of Vermicompost on Life History of *Hippodamia variegata* Preying on *Aphis gossypii* Glover

Taiebeh ALIZAMANI[1] Jabraeil RAZMJOU[1*] Bahram NASERI[1]
Mahdi HASSANPOUR[1] Anvar ASADI[2] Christopher KERR[3]

[1]Plant Protection Department, Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil, IRAN
[2]Agriculture and Natural Resources Center, Moghan, Ardabil Province, IRAN
[3]Entomology and Nematology Department, University of Florida, Fl, USA
e-mails: alizamanitaiebeh@yahoo.com, *razmjou@uma.ac.ir, bnaseri@uma.ac.ir, hassanpour@uma.ac.ir, ishbua@ufl.edu

ABSTRACT

The variegated ladybird beetle, *Hippodamia variegata* (Goeze), with worldwide distribution is among the most successful predators of aphids. In this study, the life history of *H. variegata* feeding on *Aphis gossypii* Glover as prey was evaluated on cucumber plants treated with different vermicompost concentrations (0, 15 and 30%) in a growth chamber at 25 ± 2° C, 60 ± 10% RH, and 16:8 (L: D) h. The results showed that demographic parameters of *H. variegata* were affected by vermicompost treatments. Duration of total developmental time of larvae and pupal period of *H. variegata* on the tested vermicompost concentrations were significantly different. No significant differences were found for the adult longevity, life span,fecundity, and oviposition period of *H. variegata* when fed on aphids reared on vermicompost-treated cucumber plants. Based on the results, the differences of intrinsic rate of natural increase (r_m), finite rate of increase (λ), net reproductive rate (R_0) and doubling time (DT) of *H. variegata* among different vermicompost treatments were significant; The values of r_m and R_0 increased as the percentage of vermicompost increased from 0 to 30%. It seems that some population growth parameters of *H. variegata* were positively affected by high rates of vermicompost. Our results showed that the addition of vermicompost to the growing medium of cucumber plants could positively affect some of the demographic parameters of *H. variegata* which would make it as a suitable predator of *A. gossypii* in vermicompost-treated plants.

Key words: Organic fertilizer, population growth, *Hippodamia variegata.*

INTRODUCTION

The greenhouse cucumber is threatened by outbreaks of a variety of pests due to the ideal environmental conditions encountered under greenhouse production. Sucking pests, especially aphids, are the most important pests limiting the production of cucumber. Among aphids, the melon aphid, *Aphis gossypii* Glover (Hom., Aphididae) is the most serious pest (Ebert and Cartwright, 1997; Blackman and Eastop, 2000). It is a polyphagous aphid that attacks a broad range of crops such as cotton, okra, potato, eggplant and cucurbits (Eastop, 1983; van Steenis and El-Khawass, 1995;

Stoetzel *et al*., 1996; Blackman and Eastop, 2000; Razmjou *et al.*, 2006). The aphid produces large populations on the host plants and causes direct and indirect damage to them. Direct damage of aphids by feeding on the phloem sap causes the leaves to curl. Furthermore, a heavy infestation may result in the buildup of large amounts of honeydew secretions on the leaves which increases sooty mold on the host plants. The mold limits available light to the leaves and impairs plant photosynthesis. Indirect damage is via transmission of more than 50 plant viruses to the host plants (Ebert and Cartwright, 1997; Blackman and Eastop, 2000).

Greenhouse cucumber producers mostly use insecticides to suppress the melon aphid population, but the application of chemicals on the crop may result in the development of resistance to the insecticides which would lead to the need for more frequent application of pesticides to obtain similar rates of control (Hardee, 1993; Barber *et al*., 1999; Foster *et al*., 2002). For decreasing the harmful effects of insecticides multiple control tactics such as cultural, physical, and biological control methods are used in combination with insecticides in integrated pest management (IPM) programs. To produce high quality yields, fertilizers are being applied into the growing medium of plants. Organic fertilizers, due to their improvement of soil structure and by providing essential nutrients for plant growth, are being extensively used in agriculture as a cultural method. Vermicompost is an organic fertilizer that has been shown to increase plant growth (Atiyeh *et al.*, 2000; Chaoui *et al*., 2002; Arancon *et al*., 2004, 2006; Razmjou *et al*., 2011). Vermicompost is produced through interaction between earthworms and microorganisms under a non-thermophilic process. This organic amendment increases biodiversity of the soil which is necessary for maintaining soil health and the activity of microorganisms (Zink and Allen, 1998; Arancon *et al*., 2005).

Several species of Coccinellidae are used in both fields and greenhouses as biological control agents. The variegated ladybird beetle, *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae), is a polyphagous and cosmopolitan coccinellid species (Krafsur *et al*., 1996; Wheeler and Stoops, 1996). Both adults and larvae of this species are voracious predators of aphids, mealybugs, scales, whiteflies, cicadellids, curculionid larvae as well as mites (Singh *et al.*, 1991; Sadeghi and Esmailli, 1992; Obrycki and Kring, 1998). Feeding on 12 different aphid species (Franzmann, 2002) *H*. *variegata* has been reported as an effective natural enemy of aphids on various host plants in different countries, for example: on pepper in Bulgaria (Natskova, 1973), maize in Ukraine (Gumovskaya, 1985), shrubs in Italy (Nicoli *et al*., 1995), grain in India (Hammed *et al*., 1975) and cotton in Turkmenistan (Belicova and Kosaev, 1985). It has also been shown to control melon aphid infestations on related host plants (Kavallieratos *et al.*, 2002).

Most reports in the literature indicate that vermicompost can decrease pest populations through bottom-up effects (Razmjou *et al.*, 2011, 2012). For example, Arancon *et al.* (2006) found that vermicompost can negatively affect establishment of arthropod pest populations such as two spotted spider mites, mealy bugs and aphids on the host plants. Edwards *et al.* (2010) reported similar results for cucumber beetles and tobacco hornworms. Furthermore, it has been reported that adding fertilizers into the soil may affect the strength of top-down effects by the natural enemies (Hosseini *et al.*, 2010; Veromann *et al*., 2013). For example, Pope *et al.* (2012) reported that *Brassica oleracea* L. plants grown with different fertilizer treatments had significant differences in the percentage of *Brevicoryne brassicae* L. parasitized by *Diaeretiella rapae* (M'Intosh). Fox *et al.* (1996) reported that the parasitoid *Diadegma insulare* Cress preferred diamondback moths, *Plutella xylostella* (L.), on fertilized plants compared to those on unfertilized plants. Since there are few reports (Duchovskiene *et al.,* 2012) on the effect of vermicompost on the third trophic level, we tried to evaluate the life table and population growth parameters of *H. variegata* preying on *A. gossypii* to test the hypothesis that these parameters are influenced by the application of vermicompost into the growing medium of cucumber plants.

MATERIALS AND METHODS

This study was conducted during 2012 in the greenhouse and laboratory of Plant Protection Department, Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil, Iran. The cow manure vermicompost used in this research was obtained from Anooshe Aaraab Co. Ltd., Tehran, Iran.

Plant rearing

Cucumber seeds, *C. sativus* cv. Royal, were planted in plastic pots (16 cm diameter × 12.5 cm height) filled with field soil mixed with three different rates of vermicompost (0 (as a control), 15 and 30%). The plants were kept in a greenhouse at 19-28 °C, 40-50% RH, and the natural photoperiod before using in experiments. Cucumber plants at the six- to eight- leaf stages were used for experiments.

Insect culture

The melon aphid population used in this experiment was collected from infested cucumber plants in greenhouses of Moghan region, Ardabil province in spring of 2012. The aphid colony was separately reared on cucumber seedlings grown at the mentioned vermicompost rates for about 7-8 generations prior to experiments. Every week, some apterous aphids were transferred from infested plants to non-infested young seedlings of cucumber to maintain the aphid colony. Adults of *H. variegata* were collected by sweep-netting from the wheat fields near the city of Ardabil, Iran. Males and females of *H. variegata* were paired and kept in separate plastic Petri dishes (9 cm diameter and 1.5 cm height) lined with wet filter papers (Wu *et al.,* 2010). A hole, 2 cm in diameter, was cut in the lid of each Petri dish and covered with a fine mesh cloth for ventilation. *H. variegata* was daily fed with different life stages of *A. gossypii* on cucumber leaf disks. The eggs produced by females were collected and transferred to new Petri dishes every day. The larvae of *H. variegata* were individually reared in the mentioned Petri dishes and fed with *A. gossypii.* The insect rearing was conducted in a growth chamber at 25 ± 2°C, 60 ± 10% RH, and a photoperiod of 16:8h (L: D).

Experiments

To study the effects of feeding on aphids reared at different vermicompost concentrations on the survival and pre-adult period of *H. variegata*, egg clusters less than 24 h old were selected and held in Petri dishes (at above-mentioned conditions) inside a growth chamber at 25 ± 2°C, 60 ± 10% RH, and 16L: 8D. The hatch rate and incubation period were recorded daily. The newly hatched larvae of *H. variegata* were individually transferred to Petri dishes by a fine brush and fed daily on *A. gossypii*. The aphids were daily provided for larvae on cucumber leaf discs (2 cm in diameter) grown at different vermicompost rates until pupation. The larvae were monitored daily for molting, survivorship and duration of the larval and pupal periods.

Newly emerged adults of *H. variegata* were paired and placed in individual Petri dishes containing aphids infested leaf discs for subsequent mating and oviposition. The Petri dishes were checked daily for possible egg clutches. During the experiments numbers of eggs laid by each female were recorded and removed from Petri dishes. Observations continued until the death of last adult. By monitoring the Petri dishes, pre-oviposition period, oviposition period, post-oviposition period, adult longevity and fecundity of *H. variegata* were recorded.

Statistical analysis

All data of survivorship, duration of immature stages, ovipositon period, pre-and post- oviposition period, adult longevity and fecundity of *H. variegata* were evaluated for each treatment by analysis of variance (ANOVA) using the MINITAB-16 statistical software (Minitab Inc., 2010). When differences among treatments were significant, comparison among means were determined using *Tukey's* test at $P = 0.05$. Differences in population growth parameters including intrinsic rate of increase (r_m), net reproductive rate (R_0), mean generation time (T), doubling time (DT), and finite rate of increase (λ) values and their standard errors were tested by Jackknife procedure (Maia *et al.*, 2000) using the SAS system software v 6.12 (SAS Institute, 1989). Their mean values were compared by Tukey's test at $P = 0.05$.

RESULTS

Development of *H. variegata*

Effects of feeding on aphids reared on cucumber plants amended with different vermicompost ratios on the development of *H. variegata* are listed in Tables 1 and 2. Based on the results, there were no significant differences for the egg incubation period ($F = 1.03$; $df = 2, 168$; $P = 0.359$), the duration of first ($F = 0.73$; $df = 2,138$; $P = 0.484$), second ($F = 2.01$; $df = 2,138$; $P = 0.138$), third ($F = 1.39$; $df = 2,138$; $P = 0.252$) larval instars and immature stages ($F = 0.13$; $df = 2, 138$; $P = 0.878$) of *H. variegata* on various treatments. However, the duration of fourth instar larvae of *H. variegata* significantly differed on the tested treatments ($F = 13.18$; $df = 2, 138$; $P = 0.01$). The shortest value of this parameter was recorded for the *H. variegata* when feeding on aphids reared at 30% vermicompost treatment (Table 1). Eggs from 30%

vermicompost treatment had higher hatch rate (64%) than those at control (51%) or 15% (60%) vermicompost concentrations. Furthermore, significant differences were observed for the total developmental time of larvae (F = 8.20; df = 2, 138; P = 0.01) and pupal period (F = 8.91; df = 2, 138; P = 0.01) of *H. variegata* feeding on aphids reared on the tested vermicompost-treated plants. Total developmental time of larvae was longest at control and 15% vermicompost rates and shortest at 30% vermicompost rate. The same trend was observed for pupal period of *H. variegata* (Table 1).

Table 1. Development of immature stages of *H. variegata* feeding on *A. gossypii* reared on cucumber plants amended with three vermicompost concentrations.

Parameter	Vermicompost 0%	Vermicompost 15%	Vermicompost 30%
Egg incubation	2.48±0.50a	2.51±0.54a	2.61±0.49a
First instar	1.63±0.63a	1.56±0.54a	1.71±0.74a
Second instar	1.59±0.57a	1.85±0.65a	1.79±0.45a
Third instar	1.85±0.36a	1.77±0.52a	1.67±0.56a
Fourth instar	2.07±0.38a	1.92±0.40a	1.55±0.61b
Total developmental time of larva	7.15±0.53a	7.10±0.59a	6.71±0.63b
Pupal period	3.78±0.42a	3.81±0.44a	4.18±0.63b
Immature stages	13.48±0.85a	13.44±0.71a	13.51±0.84a
Survival rate%	41	57.14	59

Differences among different vermicompost concentrations were determined by Tukey's test. In columns, mean values followed by the same letter are not significantly different (P > 0.05).

In the current study, no significant differences were observed for the female longevity (F = 2.03; df = 2, 33; P = 0.148), life span (F = 1.71; df = 2, 33; P = 0.196) and fecundity (F = 1.81; df = 2, 33; P = 0.179) of *H. variegata* feeding on aphids treated at vermicompost amended plants (Table 2). Furthermore, although pre-oviposition (F = 0.4; df = 2, 33; P = 0.671), oviposition (F = 0.38; df = 2, 33; P = 0.684), and post-oviposition period (F = 3.18; df = 2, 33; P = 0.055) of *H. variegata* did not significantly differ on the tested treatments, increasing the dosage of vermicompost in the soil resulted in decreasing of the pre-oviposition and post-oviposition periods of *H. variegata* (Table 2).

Population growth parameters

According to the data, population growth parameters of *H. variegata* were significantly affected when feeding on aphids reared at cucumber plants amended with various vermicompost rates. The intrinsic rate of natural increase (r_m) (F = 20.07; df = 2, 33; P = 0.01), finite rate of increase (λ) (F = 18.10; df = 2, 33; P = 0.01), and net reproductive rate (R_0) (F = 9.05; df = 2, 33; P = 0.001) of *H. variegata* were significantly increased by raising the vermicompost concentration from 0 (control) to 30%. Furthermore, *H. variegata* had significantly shorter doubling time (DT) when fed on aphids reared on plants that had received 15 and 30% vermicompost (F = 18.30; df = 2, 33; P = 0.01). The mean generation time (T) of *H. variegata* was not significantly influenced by vermicompost rates (F = 3.09; df = 2, 33; P = 0.059) (Table 3).

Table 2. Longevity, hatching rate (%), life span and fecundity (Mean±SD) of *H. variegata* feeding on *A. gossypii* reared on cucumber plants amended with three vermicompost concentrations.

Parameter	Vermicompost 0%	Vermicompost 15%	Vermicompost 30%
Female longevity	49.90±5.507a	43.30±10.531a	44.39±7.148a
Pre-oviposition	7.90±6.887a	7.00±1.528a	6.47±1.450a
Oviposition	30.60±7.090a	28.84±4.580a	30.61±5.801a
Post-oviposition	11.40±3.596a	7.61±5.606a	7.30±2.750a
Fecundity	188.40±71.37a	238.69±69.10a	236.85±69.18a
Life span	62.60±5.481a	56.84±10.605a	57.15±6.866a
Hatching rate%	51	60	64

Differences among different vermicompost concentrations were determined by Tukey's test. Means followed by the same letters in columns are not significantly different ($P > 0.05$).

Table 3. Population growth parameters and sex ratio (%) of *H. variegata* feeding on *A. gossypii* reared on cucumber plants amended with three vermicompost concentrations.

Parameter	Vermicompost 0%	Vermicompost 15%	Vermicompost 30%
R_0	39.40±14.94a	63.15±17.70b	71.28±20.82b
r_m	0.120±0.01a	0.138±0.01b	0.152±0.01c
T	30.59±01.82a	30.01±3.18a	28.01±2.63a
DT	5.76±0.66a	5.015±0.40b	4.56±0.34b
λ	1.128±0.02a	1.147±0.01b	1.164±0.01c
Sex ratio%	51.85	45.83	51.51

Differences among vermicompost treatments were determined by Tukey's test, based on Jackknife method. Within columns, means followed by different letters are significantly different ($P > 0.05$).

DISCUSSION

Host plants are known to be one of the important bottom-up factors which could influence the top-down ones (i.e., predators and parasitoids) (Schädler *et al.*, 2010). Changing the nutritional qualities of host plants by addition of fertilizers to the soil may affect the quality of herbivores. Insects can usually distinguish high quality host plants from those of low quality. For example, herbivores generally have better performance on well fertilized host plants (Wang *et al.*, 2006) and parasitoids lay more eggs on plants with high leaf nitrogen contents (Fox *et al.*, 1990).

Based on the obtained data, both the total developmental time and the pupal period of *H. variegata* fed on *A. gossypii* were significantly affected by the vermicompost treatments. The total developmental time of *H. variegata* at 30% vermicompost treatment was 6.71 ± 0.63 d, which was shorter than those developing at control and 15% vermicompost treatments. In this study, no significant difference was found for the life span of *H. variegata*. However, it had shorter life span on vermicompost-treated cucumber plants compared to non-treated ones (i.e., control). According to Kindlmann and Dixon (1999), short-lived species are able to complete more generations and predators with shorter developmental times can deplete the prey more quickly than a species with a relatively long developmental time. Thus, in our study *H. variegata* could produce more generations when feeding on the melon aphid populations reared on plants with higher vermicompost rates compared to the control plants.

Raising the amount of vermicompost from 0 to 30% resulted in increasing of the intrinsic rate of natural increase (r_m) and the net reproductive rate (R_0) of *H. variegata* confirming the importance of plant quality on the predator. The highest r_m value at the 30% vermicompost treatment indicates that *H. variegata* has a greater reproductive potential at this treatment than the other treatments. The finite rate of increase (λ) of *H. variegata* showed a similar trend being significantly highest at 30% vermicompost treatment. Interestingly, the intrinsic rate of natural increase reported in our study (0.152 day^{-1}) for *H. variegata*, is comparable with the value reported by Davoodi Dehkordi *et al.* (2013) for the same species feeding on *A. gossypii* on *Chrysanthemum indicum* Kitan (0.155 day^{-1}). However, the value for intrinsic rate of natural increase in our study is different from the one reported (0.114 day^{-1}) by Lanzoni *et al.* (2004) when *H. variegata* fed on *Myzus persicae* Sulzer. The difference could be related to the different prey species and plant materials.

In the present study, the doubling time (*DT*) was significantly affected by vermicompost rates. The shortest values of *DT* were obtained when *H. variegata* fed on aphids reared on high rates of vermicompost (15 and 30%). As a result, *H. variegata* needed less time to double its size on these treatments. Other studies have shown that modifying the quality of host plants through soil fertility managements affects the natural enemies. For example, Fallahpour *et al.* (2015) reported that fertilization of canola significantly affected the performance of the gall midge, *Aphidoletes aphidimyza* Rondani, as a predator for the mustard aphid, *Lipaphis erysimi* (Kaltenbach). According to the findings of Suryawana and Reyes (2007), the level of parasitism by different parasitoids on pea leafminer (*Liriomyza huidobrensis* Blanchard) was greater on vermicompost treatments in a potato field. Similar observations were made by Ponti *et al.* (2007), who reported that in compost-fertilized broccoli the parasitism rates of *Brevicoryne brassicae* L. by *Diaeretiella rapae* M'Intosh significantly increased. Furthermore, in cow manure amended white cabbage coccinellids settled better in aphid colonies compared to synthetically fertilized plants (Duchovskiene *et al.*, 2012). It seems that biological traits of the third tropic level are influenced by changing the uptake of special elements by plants via fertilization and, for the case of our study, it could be attributed to the slow and continuous uptake of mineral nutrients from vermicompost-amended substrate.

Our results showed that, despite the fact that some life history parameters of *H. variegata* were not significantly influenced by vermicompost rates, other parameters like intrinsic rate of natural increase were significantly affected by this organic fertilizer. An important result however, is that *H. variegata* does not seem to be negatively affected by vermicompost when different concentrations were used. This study offered opportunities for better understanding the relationship between organic fertilization and the predator demographic potential which would be useful for both organic growers and pest management programmers. However, further investigation on the relationship between the application of vermicompost and the predation capacity of *H. variegata* under natural conditions are recommended to obtain optimal biological control.

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