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Simultaneous Use of Entomopathogenic Fungus *Lecanicillium* and Predatory Ladybird *Hippodamia Variegata* in Control of Pea Aphid, *Acyrthosiphon Pisum*

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Abstract **- Pea aphid, *Acyrthosiphon pisum* is an important pest in most parts of the world. The main damage is caused by transmission of viral diseases. *Hippodamia variegata* Goeze, is a predator with very broad host range in Palearctic areas and from there has extended to Nearctic areas. *Lecanicillium longisporum* Petch has a wide host range and its isolates can infect aphids, scale, whiteflies, thrips and plant pathogens. The main purpose of this study was to evaluate simultaneous use of entomopathogenic fungus *L. longisporum* and the ladybird *H. variegata* in biological control of pea aphids. Third instar larvae and adults of *H. variegata* were inoculated by sub-lethal, LC_{50} and lethal concentrations (estimated values on *A. pisum*) of *L. longisporum*. Lab experiments evaluating interactions between fungus and ladybird showed no significant effect of the fungus on survival of the predator when sprayed directly on third instar larvae and adults. Also feeding on infected aphids in the interval of 24, 48 and 72 h after fungud treatment, no significant effect was observed on survival of coccinellids. On the basis of our results, it could be suggested that *H. variegata* and *L. longisporum* are compatible biocontrol agents in integrated management of pea aphid population.**

Keywords **– Biological Control, *Hippodamia Variegata, Lecanicillium Longisporum*, Pathogen, Predator**.

I. Introduction

Pea aphid, *Acyrthosiphon pisum* found in most parts of the world. Adults and nymphs feed on sap from stems, leaves and buds of host plants and causes yellowing and wilting. The main damage is caused by transmission of viral diseases [1]. Ladybird are agents useful in agricultural ecosystems that play an important role in the create state of balance and natural control of aphids, psylla, whiteflies, leafhoppers, mites, moth eggs and larvae of insects [2]. *Hippodamia variegata* Goeze, is a predator with very broad host range in Palearctic areas and from there extends to Nearctic areas [3]. Among the entomopathogenic agents, fungi are the most promising of factors in the regulate pest populations [4]. *Lecanicillium longisporum* (Petch) Zare and Gams is a well-known entomopathogenic fungus which has a wide host range, including insects, mites, spiders, nematodes, and phytopathogenic fungi [5]and has been registered as a biological control agent of many pests [6]. The most efficient biological control of any pest is achieved when the agents involved in the program are compatible [7]. Despite the antagonistic and synergistic interactions between the biocontrol agents, It is quite normal that the synergistic interaction increases performance of Control and antagonistic interaction reduces performance of control [8].The main purpose of this study was to evaluate simultaneous use of entomopathogenic fungus *L. longisporum* and ladybird *H. variegata* in biological control of pea aphids.

II. Materials and Methods

A. Plant cultures

Broad bean, *Vicia faba* seeds were planted in PVC pots (17cm diameter, 16 cm high) containing sawdust. The pots were kept in greenhouse conditions (25±5℃, 50±20% RH). Fourteen days after cultivation, plants were used for rearing of aphids.

B. Aphid cultures

A stock culture of *Acyrthosiphon pisum* were maintained on broad bean, in growth chamber conditions at 25±1℃ and 70±10% R.H. and a photoperiod of 16 h light:8 h dark for several generations. Approximately 25 adults were placed on each bean plants and allowed to produce progeny. Following 12 h, adults were removed. Neonate nymphs were then reared as a synchronous cohort until they reached second instar age.

C. Ladybird culture

The ladybird eggs were provided by the Population Ecology lab, University of Tehran. They were kept in octagonal dishes (11cm diameter: 4 cm high). After hatching, larvae were fed with *Acyrthosiphon pisum* and *Aphis fabae*. The coccinellids were paired and transferred in plastic Petri dishes (9cm). The culture was incubated at a temperature of 25±1℃ and humidity of 68±5% RH. Females were allowed to lay eggs. Hatched larvae were transferred into plastic Petri dishes having substrate food (a broad bean leaf with aphids). Third instar larvae and 1-5 day-old adults were used in bioassays.

D. Fungus isolate and culture

Three different entomogenous fungus isolates used in the study were *L. longisporum* LRC190 isolated from Chrysanthemum aphid, *Macrosiphoniella sanborni* (Hem.: Aphididae) in UK, *Lecanicillium* LRC216 isolated from Codling moth, *Cydia pomonella* (Lep.: Tortricidae) in Quebec (Canada) and *L. longisporum* LRC229 (Koppert Canada Ltd.). *Lecanicillium* isolates were cultured on potato dextrose agar (PDA) and incubated for 12-14 days. Conidia were harvested with distilled water containing 0.02% Tween 80 and filtered through filter paper into

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sterile vials. Concentrations of conidial suspensions were estimated under a compound microscope using a hemocytometer. Spore viability was determined before each experiment by spreading 0.2 ml of a 1×10^4 conidia/ml suspension on PDA and estimating the number of germinated conidia under a compound microscope after 24 hour of incubation at room temperature. The viability of conidia was estimated [9] to be more than 95% for all experiments.

III. Bioassays

A. Laboratory evaluation of isolates Lecanicillium longisporum against Acyrthosiphon pisum

Virulence of each isolate was determined using a range of spore concentrations (10^3, 10^4, 10^5, 10^6 and 10^7 conidia/ml) on *A. pisum* nymphs. Rounded 5 cm petri dishes, with a meshed hole (200 mm) in the lid, were filled with a 0.5 cm thick layer of 1.5% water agar. Freshly excised broad bean leaf discs (two-leaf stage) were placed upside down onto the water agar. Then, 15 second-instar cohort Pea aphid nymphs were placed on the broad bean discs in each Petri dish. Experiments were performed in three replicates. Two milliliters of each conidial concentration were applied using a potter spray tower (Burkard, Uxberge, UK) with a nozzle size of 0.25 mm diameter and 0.7 kg/cm^2 pressure. For control units, nymphs were treated with sterile distilled water only. Following treatment, the aphids were air dried to remove excess suspension. Petri dishes were incubated at a temperature of 25±1℃ and humidity of 68±5% RH. Mortality of aphids was daily assessed for 7 days. Dead aphids were collected and after sterilizing, placed in a Petri dish with damp filter paper and placed in a humid chamber (100% RH). Only aphids that exhibited fungal sporulation were considered to have died from the fungi.

B. Direct effect of the virulent fungal isolate on third instar larvae and adults of H. variegata

Conidial suspensions of *L. longisporum* LRC216, were prepared at 10^3, 6.8×10^4 (equivalent to LC_{50} for aphids) and 10^7 conidia/ml. Twenty third-instar cohort larvae were placed in each Petri dish. In the case of adults, beetles weresprayed in groups of 20 individuals (10 males, 10 females). Experiments were performed in three replicates. Two ml of each conidial concentration were applied using a potter spray tower with 0.7 kg/cm^2 pressure. For control units, nymphs were treated with sterile distilled water only. Because of cannibalism, each larva was placed into separate Petri dishes individually. Petri dishes were incubated at 25±1℃ and 68±5% RH. During the experiment, live larvae were fed with aphids. Mortality of larvae was daily recorded for 7 days. Dead larvae were assessed for mycosis.

C. Indirect effect of the virulent fungal isolate on male and female adults of H. variegata

After inoculation of aphid nymphs with each conidial concentration (6.8×10^4 and 10^7 conidia/ml) and control (sterile distilled water), the predator was allowed to prey in each experimental unit for 24 h at different treatments including 24, 48 and 72 h post-inoculation of nymphs. Experiments were performed in three replicates. Each replication included groups of 10 adults (5 males, 5 females). Petri dishes were incubated at 25±1℃ and 68±5% RH. Mortality of adults was assessed daily for 7 days. After 24 h, during the experiment, beetles were fed with healthy aphids.

IV. Statistical Analysis

The entire set of all bioassays was replicated three times. Mean mortalities were determined within replications for each treatment. Treated aphid mortality was corrected for control mortality. Data were analyzed by the General Linear Model (SYSTAT 2000). Fisher's Least Significant Difference (F-LSD) test was applied for mean comparisons among treatments. The median lethal dose (LC_{50}) was estimated using the POLO-PC software.

V. Results and Discussion

A. Laboratory evaluation of isolates Lecanicillium longisporum against Acyrthosiphon pisum

There were significant differences among isolates ($F_{2,30}$=8.1, P<0.05). Comparison of the means showed that there were no significant difference in virulence between LRC216 and LRC229 (P=0.625) but there were significant difference between both isolates (LRC216, LRC229) with LRC190 (P<0.05). Mean of total mortalities, at the highest concentration (10^7 conidia/ml) for LRC216, LRC229 and LRC190, were 75.6, 62.2 and 55.5%, respectively. The mortality of nymphs increased when the concentration of spores increased (Fig I).

Median lethal concentration (LC_{50}) values were 3.4×10^6, 2.04×10^5 and 6.8×10^4, respectively (Table I). According to the LC_{50} values, LRC216 was considered as the virulent isolate Median lethal time (LT_{50}) values for isolates LRC190, LRC229 and LRC216 were 7.67, 6.02 and 6.17 days and. Aphid mortality was started from 4th day post-inoculation of fungus and increased rapidly there after (Fig Π).

B. Direct effect of the fungus isolate on third instar larvae and adults of H. variegata

There were no significant difference among concentrations for the effect on third instar larvae ($F_{3,8}$=1.9, P>0.05) and adults ($F_{3,8}$ = 2.88, P>0.05) of *H. variegata*. At 10^3, 6.8×10^4 and 10^7co./ml, the percentages of pupation of ladybirds were 100, 98.1 and 96%, respectively. Survival rate of adults was 96.7, 96.7 and 94.7% in the direct treatment by 10^3, 6.8×10^4 and 10^7 conidia/ml, respectively.

C. Indirect effect of the fungus isolate on adults of H. variegata

There were no significant difference among different time treatments including 24, 48 and 72 h post-inoculation of aphids for their effect on ladybird survival ($F_{2,18}$=2.4, P>0.05). Survival rates of adult ladybirds were 100, 100 and 96.66% when they were fed with 24, 48 and 72 h infected aphids with 6.8×10^4 co./ml, respectively. These values were 96.66, 96.66 and 93.33% when 10^7 conidia/ml were applied, respectively (Fig Ш).

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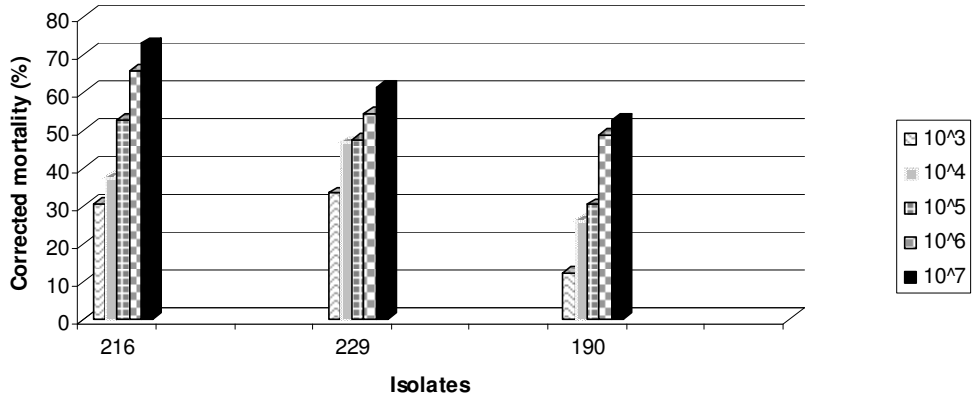


Fig.I. Corrected mortality of aphids following treatment of 2end instars with conidial suspensions of three different isolates of L. longisporum

Table I: The LC_{50} of three isolates of the fungus *L. longisporum* on second nymphal aphids

Degrees of Freedom	X^2	Confidence Interval (95%)	LC_{50} (Co./ml)	Fungus Isolate	Slope±SE
3	0.5152	9.4×10^3- 3.3×10^5	6.8×10^4	LRC216	0.069 ±0.309
3	0.3049	2.5×10^4- 1.2×10^6	2.04×10^5	LRC229	0.064 ±0.157
3	0.8715	5.2×10^5- 1.05×10^8	3.4×10^6	LRC190	0.075 ±0.293

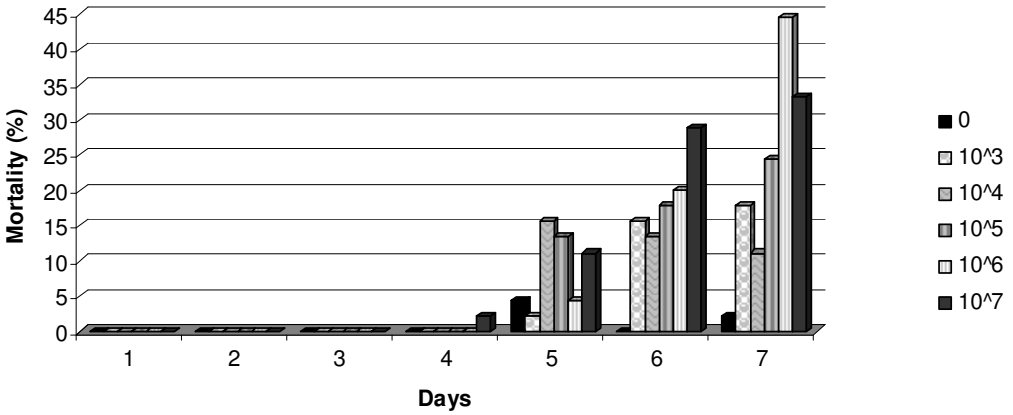
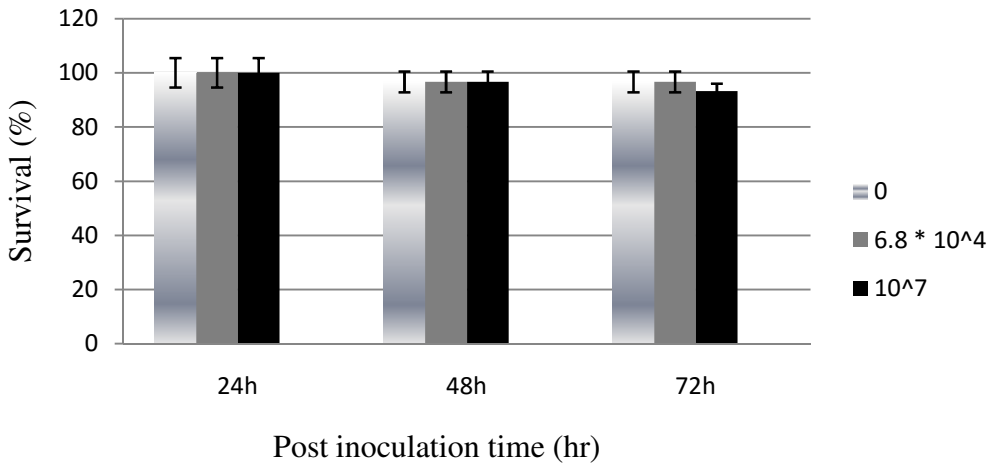


Fig.II. Pea aphid mortality rates at different concentrations of fungal *L. longisporum* LRC216, days after treatment



Post inoculation time (hr)

Fig.III. Survival of adult at different intervals after feeding of infected aphids

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Our observations showed that all three isolates of the fungus *L. longisporum* infected pea aphid. The lowest and highest virulences were recorded for isolates LRC190 and LRC216, respectively. Previous studies demonstrated that conidia of the fungus *L. longisporum* (*V. lecanii*) were highly pathogenic against aphids [Refs. 10-24]. Abd El-Salam & El-Hawary [27] have studied lethal and pathogenic effects of *Beauveria bassiana* and *Lecanicillium lecanii* on the adult and nymph of *Aphis craccivora* Koch and their results indicated that *L. lecanii* was more effective than *B. bassiana* in reduction of the number of young borne produced by the adult stage. Kim& Kim [26] studied two isolates of *Beauveria bassiana*, three isolates of *Paecilomyces* spp. and one isolate of *Lecanicillium attenuatum*, to select for highly virulent isolate against *Aphis gossypii*. An isolate of *L. attenuatum* CS625, had the highest virulence against *A. gossypii* when the host was treated with either conidia or blastospores of the fungus. Value of LC_{50} for LRC216 (our virulent isolate) was 6.8×10^4 conidia/ml that showed high pathogenicity on the pea aphid which was in similar with Safari's [23] research that showed high potency of *Verticillium* in infecting the pea aphid. Isolate *L. longisporum* LRC216 was originally isolated from a lepidopteran species (Codling moth) and it was found to be more virulent to *A. pisum* than *L. longisporum* LRC190 which had been originally isolated from an aphid (Chrysanthemum aphid). It has been revealed in some previous studies similar to our research, pathogenicity was not always related to host and geographical origin of fungus isolate [27, 28]. Some studies have showed that isolates originally from an aphid were more pathogenic than isolates from other insects [26]. Aphid mortality was first observed 4 days post-inoculation and increased rapidly there after and our results confirmed Askary *et al* [10] and Arzanian *et al* [13] results. It seems that fungus need to these days after the inoculation, to provide stages of pathogenicity including adhere to cuticle, spore germination and germ tube production, penetration into the cuticle, infecting hemolymph and different tissues [29]. Lab experiments evaluating interaction between fungus and ladybird showed no significant effect of the fungus on survival of the predator when sprayed directly on third instar larvae and adult. Also feeding on infected aphids in the interval of 24, 48 and 72 h after fungus treatment, no significant effect was observed on survival of ladybirds. A few studies have showed that most of fungus isolates have no effect on the coccinellid predators [Refs. 30-34]. Of course, some studies have showed that isolates of fungus were pathogenic to ladybirds [13 and 35-41]. Here, we observed very low reduction of oviposition in *H. variegata* females when the fed with fungus-treated aphids. But it could be suggested that *H. variegata* and *L. longisporum* are compatible biocontrol agents in integrated management of pea aphid population.

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