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FIELD BIOLOGY OF THE GREEN SEMI-LOOPER, *Naranga aenescens* MOORE (LEPIDOPTERA: NOCTUIDAE) AND EFFICIENCY DETERMINATION OF *Beauveria bassiana* isolates

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Abstract

According to the importance of none chemical controls based on integrated pest management in the rice fields of Iran, first the field biology of the rice green semi-looper was determined then some experiments were conducted to find entomopathogenic effect of *Beauveria bassiana* on the larvae of the pest. Cages in dimensions 2×1×1 m were used to rearing of insects in rice fields. It was found from field based studies that 22-23 o'clock is the appropriate time to capture the adults. Adult moth laid their eggs on above of leaves and the eggs hatch after 3-3.5 days. *N. aenescens* has three instar larvae so that each instar needs 3-6 days to complete their development. Pupation takes about 4 days and the insect has three generation per year. Field bioassays were conducted by four local isolates of *B. bassiana* including DEBI001, DEBI003, DEBI007 and DEBI008 on the second instar larvae by using two methods, dipping and spraying. In dipping bioassay, concentration 1×10^7 (spore/ml) of isolate DEBI003 demonstrated the significantly highest mortality about 50.76% in comparison to other isolates. In spraying bioassay,

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isolates DEBI003 had the highest mortality percentage (41.30%) in comparison to others. This isolate (DEBI003) caused 81.03% mortality on egg by concentration of 1×10^7 (as LC_{50} concentration) (spore/ml) in dipping bioassay and caused 52.11% mortality at the same concentration in spraying one. The first and the second instar larvae were more susceptible than third instar larvae to 10^7 (Spore/ml) concentration of isolate DEBI003. Experiments showed that LT_{50} increased as larval instar growing up and decreased by increasing the spore concentration.

Keywords: *Naranga aenescens* , *Beauveria bassiana*, Field efficiency, Bioassay

1. Introduction

The rice green leaf semi-looper, *Naranga aenescens* Moore (Lepidoptera: Noctuidae) is a monophagous pest that has been widely distributed in Eastern Asia and Middle East. That is a non-native pest in north of Iran and has been introduced to the rice fields since 1970. Because of some changes in climatic conditions and agronomical approaches, *N. aenescens* has emerged as one of the most destructive pest in rice fields. In suitable climatic conditions, the pest feeds on rice leaves for a long time (Approximately in all phenologic stages of rice) and causes severe damages to rice plants.

Entomopathogenic microbes may offer an applied strategy as biopesticides (Tafoya et al. 2004). There are over 700 species and about 90 gena of fungi that belong to Deutromycetes and Entomophthorales (Hong, 2003). The most important entomopathogenic fungi are *Beauveria bassiana, Metarhizium anisopliae* and *Paeclomyces fumosoroseus* (Feng et al. 1990; Wraight et al.,1998). Wraight et al. (1998) reviewing entomopathogenics of *P. fumosoroseus, P. farinosus* and *B. bassiana* isolates on silver leaf whitefly showed that all isolates have pathogenicity on this pest. Hatting et al. (2004) showed that *B. bassiana* could control up to 65% of *Duraphis noxia* in field condition. Talaei et al. (2002) tested *B. bassiana* on *Eurigaster integriceps* and showed that it was highly effective especially on the nymphal instars and adults.

Control of *N. aenescens* is based on widely spraying by two chemicals, fenitrothione and Carbaryl. Now days, using of safe and rational procedures are more acceptable due to environmental risk assessment of these chemicals. Due to severe damages of *N. aenescens* to rice in recent years, establishing and developing a logical integrated pest management needs to be considered. The first step to reach a such management, the field based biology is required to find the susceptible stages of the pest life cycle and coordination of its biology to phenologic stages of rice and natural enemies. As far as we know, there is no data on field biology of *N. aenescens* and the current one is the first study at least in the recent years. Meanwhile, control policies trend to use safe procedures such as pathogens, predators and parasitoids. Meanwhile, climatic condition of northern Iran is very suitable to usage of fungi, especially *B. bassiana*. So far, no entomopathogenic fungus has been evaluated for the control of *N. aenescens.* In this study, it was studied the susceptibility of second instar larvae to different isolates of *B. bassiana*

under field condition. It could be the first study in this area on *N. aenescens*. However a biological based control strategy can be used on *N. aenescens*.

2. Materials and Methods

2.1. Insect collection and rearing

2.1. Insect rearing

For field experiments, the cages with dimensions 2×1×1 m were used to rearing of *N. aenescens* where five bush of rice were planted in each. Adults were released in each cage on plants for oviposition and rearing of larvae. The climatic conditions during experimental studies were 28-35 °C and humidity about 87-93%.

2.1.1. Establishment of field study equipment

To determination of life cycle in natural conditions, 10 cages (2×1×1) were provided and established in rice fields of Rice Research Institute of Iran (RRII). In each cage, seedlings of rice were planted in distance 20 cm from each other. It is imperative to represent that the variety of rice was similar in all cages (Taroum variety of rice).

2.1.2. Life cycle studies

The collected adults (above) in number of 25 were released to each cage. Monitoring of life cycle was made every day from egg to re-emerge of adults in each generation. The experiments were made for all three generation (based on our observations) and repeated three subsequent years.

2.2. Isolates

Four local strains of s *B. bassiana* were used to find the most effective one on *N. aenescens.* The used isolates in bioassay have been listed in Table 1.

2.3. *Beauveria bassiana* Culture

All isolates were cultured on Sabouraud Dextrose Agar (SAD) with Yeast extract and incubated at 27°c, 70% RH for 2-3 week until conidia were produced (Tafoya et al. 2004). Then, they suspended in sterile 0.05% Tween 80. The spores were counted under microscope by using hemocytometer and diluted to desired concentration.

2.4. Bioassay procedure

Bioassays were carried out in the two phases. In phase one, concentration of 1×10^7 spore/ml from four isolates were used on 2nd instar of *N. aenescens* to determine the most virulent isolate. Also, this phase was divided into two bioassay methods: dipping and spraying. After determination of the most virulent isolate, different concentration of the isolate (1×10^5, 10^6, 10^7 and 10^9 spore/ml) were prepared and used on eggs as phase two. All experiments were done twice with three replicates.

In dipping bioassay, *N. aenescens* larvae were dipped in fungus suspension of 1×10^7 spore/ml for 10 second and then put on rice leaves. In spraying bioassay, larvae were sprayed on rice leaves directly by 10

ml of a 1×10^7 spore/ml suspension in a spray tower with constant pressure (Areas et al, 1999). Control insects were treated similarly with 0.05% Tween 80. Each assay was consisted of three replicate, 25 larvae per replicate and assay was repeated twice. Insects were examined daily and dead individuals were transferred to petri dishes lined with moist filter paper to encourage external conditions for the germination of fungal spores. Mortality was recorded daily for 10 days.

2.5. Statistical Analysis

Analysis was performed on percentage mortality after correction for control mortality (Abbott, 1925). Data were analyzed by analysis of variance (Anova) in Tukey test (SYSTAT – 10). LC_{50} AND LT_{50} determined by polo-pc software (Robertson, 2007).

3. Results and Discussion

Investigation on the optimal time of adults capturing showed that the highest amount of captured adults was between 22-23 o'clock in nights of May and June (Figure 1) although, the captured adults were recorded from 8:30 to 5 o'clock. Study on oviposition of adults revealed that they preferred to lay the eggs on the above surface of rice leaves in groups of 3 eggs in two rows (Figure 2 a, b). Duration of embryonic development of laid eggs was 3-3.5 days in three subsequent years with the Lowest and the highest temperature about 22.6 °C to 28.5 °C, respectively and relative humidity 85% (Table 1). Alinia (1990) demonstrated that the embryonic development was about 4-5 days under laboratory condition. The longer embryonic development in laboratory (30 °C) in comparison to field could be attributed to the dependence of temperature and humidity so that higher humidity and the lower temperature make the development faster.

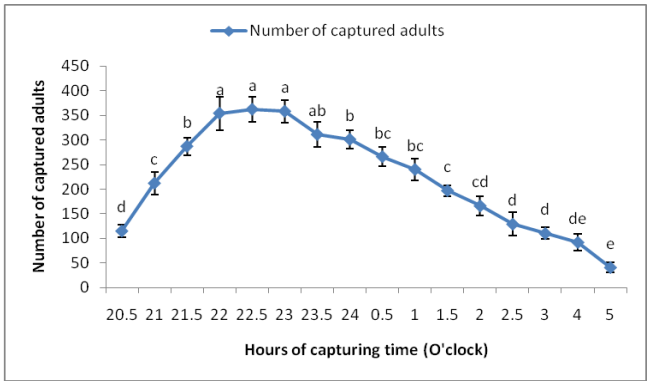


Figure 1. Optimal capturing time of *N. aenescens*. Data are shown as Mean±SE and significant differences have different letters (Tukey test $p\leq0.05$).

Determination of larval instars by observing exuviae was very difficult because new emerged larvae fed on their exuviae after ecdysis. Anyway, daily observations revealed the time of ecdysis in 7:30 to 11 o' clock every day and being just two exuviae represented three instar larvae for *N. aenescens*. It was found that the duration of three instar larvae was 3-3.8 days for first instar, 4.4-5 days for second instar and 5.5-6.1 days for third instar (Table 1). The obtained results were made in three subsequent years. In a laboratory study, the average of larval duration was 17.3 days for all larval instars (Alinia, 1990).

Table 1. Duration of several growth stages of *N. aenescens* under field condition.

Growth Stages	First year	Second year	Third year
Embryonic	3.5±0.034	3.1±0.1	3±0.18
First instar larva	3±0.34	3.2±0.55	3.8±0.19
Second instar larva	5±0.44	4.4±0.18	4.7±0.76
Third instar larva	5.5±0.48	6.1±0.34	5.5±0.98
Pupa	4.7±0.67	4.17±0.33	4.87±0.5
Adults	5.8±0.79	7.3±0.44	6.23±0.74

[*]. In the period of study the temperature was 22.6 °C to 28.5 °C and the relative activity was 85%.

[×]. The study was carried out for three year.

[+]. Data are represented as day.

Determination of larval instars was made by using Dyar constant (Dyar, 1990). To calculate the width of head capsule following equations were considered:

$$\text{Increasing rate of head capsule} = \frac{\text{Width of head capsule in the last instar larva}}{\text{Width of head capsule in previous instar larva}}$$

Results showed that the average of head capsule in three instar larvae were 0.29, 0.54 and 0.92 cm, respectively (Table 2). In field condition, last instar larvae make the tip of rice leaves in a curve-shape and pupated but some larvae pupate near the junction of leaves and stems (Figure 2 d, e, f). It was observed that the non-diapausing pupae are green and smaller but the diapausing pupae are brown and larger. Duration of pupal stage was obtained 4.17 to 4.87 days in three subsequent years (Table 1). Also, Duration of adults was measured about 5.8 to 7.3 days in three subsequent years. Pupal and adult development took 5.1 and 2-4 days under laboratory conditions that was lower than our results (Alinia, 1991). Also the weight, length and width size of pupae have been shown in Table 3 as male and female, respectively.

Table 2. The width of larval head capsule based on Dyar's constant.

Larval stages	Head capsule width (cm)	Increasing Rate of larval head capsule
First nstar larvae	0.29±0.036	-
Second instar larvae	0.54±0.081	1.7
Third instar larvae	0.92±0.039	-

Table 3. Size and weight (mean±SE) of male and female pupa of *N. aenescens*.

Parameter	Female	Male	df	*p*
Weight	0.021±0.003	0.014±0.002	34	0.017≤0.05
Lenght	9.2±0.87	7.4±1.2	37	0.008≤0.05
Width	3.9±0.67	2.48±0.34	32	0.005≤0.05

Different instar larvae of *N. aenescens* can move from one rice plant to another by producing silk (Figure 3). This behavior is more prevalence in the first instars in comparison to last ones. Larvae hang leaves by producing silk and reach themselves to the nearest rice plant. Also, our observations showed that the first instar larvae depended to humidity for development so that they active in rice parts near the water under the bush but the third instar fed on the tip of leaves (Figure 3).

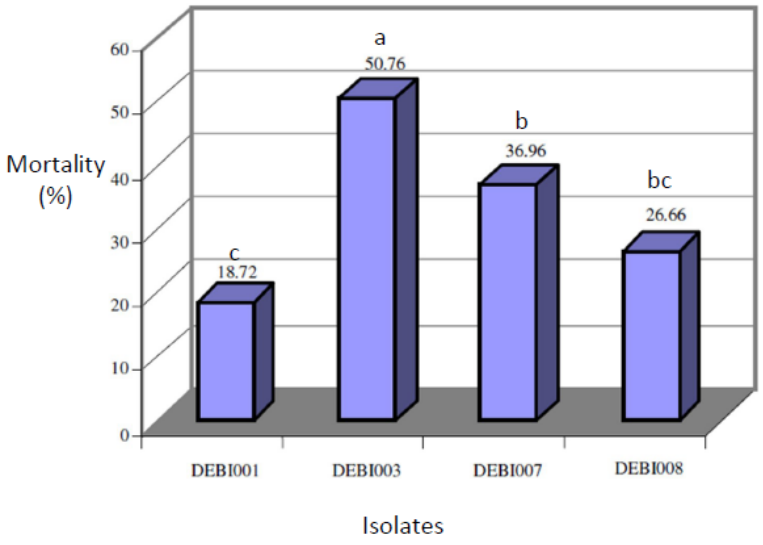
Results of two bioassay procedures (dipping and spraying) on rice green semi looper larvae showed that all four isolates of *B. bassiana* caused statistical pathogenicity on larvae of *N. aenescens* (Figure 4). Results of dipping bioassay showed significant differences in treatment so that isolate DEBI003 had the most pathogenicity on *N. aenescens* larvae (Figure 4). Results of spraying bioassay were similar to dipping method and showed the isolate DEBI003 as the most virulent isolate on *N. aenescens* larvae (Figure 4).

Susceptibility of *N. aenescens* eggs to isolates DEBI003 was investigated by dipping and spraying procedures in different prepared concentrations. Mortality percentage were presented as hatched and un-hatched eggs and obtained 80.03I unhatched by dipping bioassay and 52.11% unhatched by spraying one (concentration of LC_{50}) (Figure 5).

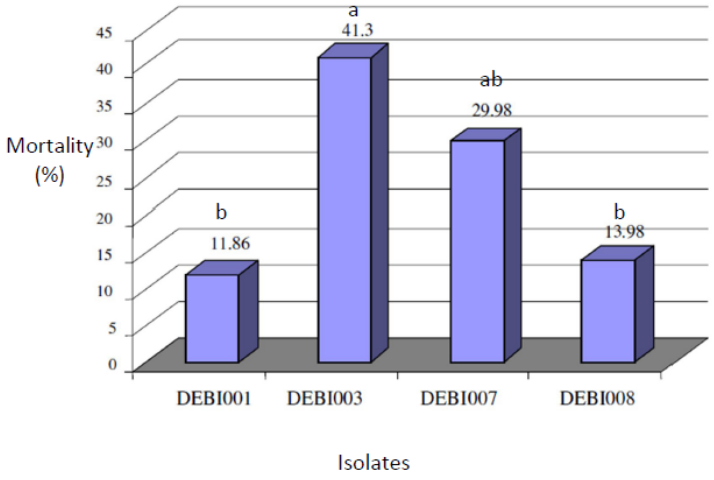
Susceptibility of different instar larvae to isolate DEBI003 was significantly different and obtained 83.51, 51.40 and 12.2% for first, second and third instar larvae, respectively (Table 4). LT_{50} values regarding effect of different concentrations of DEBI003 were analyzed and obtained 4.89, 5.01 and 7.07 days on one, second and third instar larvae, respectively (Table 5).

Figure 2. Different developmental stages of *N. aenescens*.

Figure 3. Damages and Silk production of *N. aenescens* larvae.



(a)



(b)

Figure 4. Comparison of mortality in second instar larvae of rice green semi looper caused by different isolates of *B. bassiana* in two treatment procedure; (a) dipping and (b) spraying. Different letters show significant differences among values (Tukey's test; $p<0.05$).

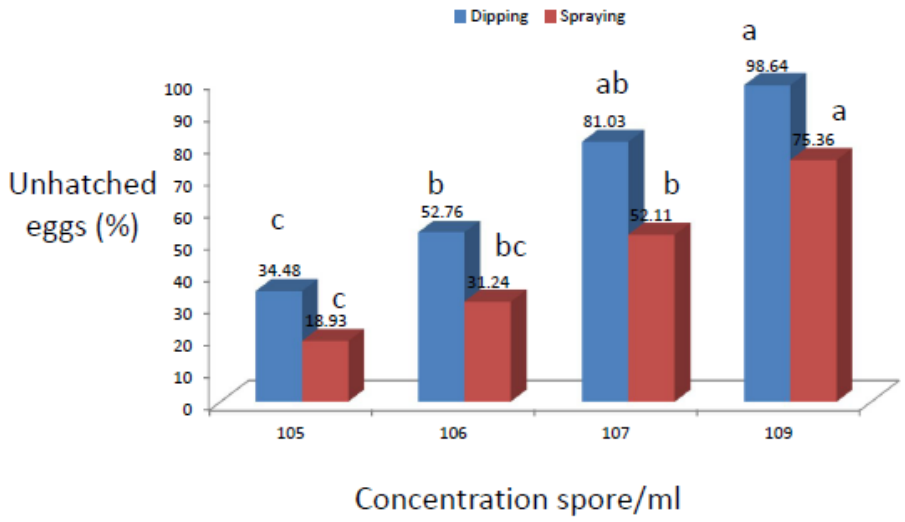


Figure 5. Effect of *B. bassiana* isolate DEBI003 on egg hatching of rice green semi-looper. Different letters show significant differences among values (Tukey's test; $p<0.05$).

In this study, we determined the effect of four isolates of *B. bassiana* on *N. aenescens* in field condition. The result showed that isolate DEBI003 had the highest effect on larvae and decreased the half of *N. anescens* population between 4-7 days. The influences of environmental conditions as abiotic factors on biocontrol agents are complex (Haraprasad et al., 2001). There are several reports regarding the influence of climatic factors on the fungal colonization. Fungal epizootics have been considered as very much dependent upon weather conditions, particularly on high RH. A high RH is an essential factor in the development of fungal propagules in the field (Haraprasad et al., 2001). In the present study, 90% RH was found to cause significant semi-looper mortality. Dipping bioassay of isolate DEBI003 caused almost complete mortality in the laid eggs of *N. aenescens*. *N. aenescens* females usually lay eggs more quickly so the timing of when females drop of their eggs onto fungi-contaminated leaves, and the point at which egg laying is reduced, may be of critical importance in terms of reducing the tick population.

Table 4. Effect of isolate DEBI003 of *B. bassiana* on mortality of different instars of rice green semi-looper larvae.

Larval instar	**Mortality (%)**	**Statistical grouping**
1st instar	83.51	a
2nd instar	51.40	b
3rd instar	12.21	c

*. Different letters show significant differences among values (Tukey's test; $p<0.05$).

Table 5. LT_{50} values of different instar larvae of green semi-looper treated by isolate DEBI003 *B. bassiana*.

Larval instar		Confidence limit 99%		Confidence limit 95%	
	LT_{50}	Upper limit	Lower limit	Upper limit	Lower limit
1st instar	4.86	7.05	2.72	6.64	4.89
2nd instar	5.01	7.17	2.84	6.76	3.25
3rd instar	7.07	9.23	4.09	8.82	5.31

1. Time in days

2. Values calculated by POLO-PC software. Confidence limits based on Robertson (2007).

Entomopathogenic fungus, *B. bassiana* could be used against crop pests as an efficient biological agent. This fungus uses against pests such as White Flies, Thrips, Lepidoptera, Bugs and etc (Westwood et al. 2005). On *N. aenescens*, four isolates of *B. bassiana* were virulent on eggs and larvae instars so that mortality of larvae was acceptable in the field condition. Hicks et al. (2001) demonstrated different isolates of *B. bassiana* caused a high mortality on *Panolis flammea.* Similar results were obtained when *B. bassiana* used on *Lymantria xylina.* Majidi et al. (2002) identified soma isolates of *B. bassiana* on *Chilo suppressalis* in rice fields of Iran had a suitable effect on this major pest rice. The LT_{50} of *B. bassiana* isolate DEBI003 to first, second and third instar larvae was 4.89, 5.01 and 7.07 days, respectively. By the concentration of 10^7 spore/ml, younger larvae are more susceptible to fungus than older one, which could be due to their cuticle and week immune system. This phenomenon is common in insect-pathogen relationship (Bextine and Thorvilson, 2002).

Concerns on using chemical insecticides caused a greet trend to using safe procedures to decrease pest populations. The application of biological agents is elevating due to environment awareness and food safety in addition to the failure of conventional chemicals because of pest resurgence, appearing of secondary pests and pest resistance to chemical (Dent, 1993; Zibaee and Bandani, 2009; Zibaee et al., 2009). *B. bassiana* is a widely distributed fungal pathogen of insects. It has been used against red imported ant in the USA, migratory grasshopper, predatory insects, rice stem borer and coffee berry borer in many parts of the world (Tafoya et al., 2003). It has also been used for the control of many important pests of various crops around the world and tested on different target insects (Zibaee et al., 2009). By Also, knowing the field biology of *N. aenescens*, achieving to such management must be easier. Meanwhile, the paddy field is not only the habitat of rice arthropods, but it is also an alternative habitat for many endangered aquatic insects associated with the vanishing natural wetlands. Therefore, the rice management strategy should strike a balance between IPM and conservation, this is the prerequisite to develop the sustainable agriculture system are wide in paddy ecosystems of Iran.

The present results indicate considerable potential for utilization of entomopathogenic fungi to control of rice green semi looper in rice fields of Iran. It may represent a practical, safe and cost effective method of controlling of this pest. Further research will be conducted in field trials to evaluate the effectiveness of *B. bassiana* on other pests of rice field by compatibility of other controlling practices especially chemical control and using biological agent such as *Trichogramma spp.*

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