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ORIGINAL ARTICLE

Changes in life table parameters and intermediary metabolism of *Cryptolaemus montrouzieri* Mulsant after infection by *Beauveria bassiana*

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Abstract

The effects of two native isolates of *Beauveria bassiana*, AM-118 and BB3, were evaluated on the predatory coccinellid, *Cryptolaemus montrouzieri* by measuring several developmental parameters and intermediary metabolism. Treatment with both isolates significantly increased the length of each developmental stage compared to the control except for the eggs and adults. The preovipositional period in the adults treated with BB3 significantly increased compared to those treated with AM-118 and the control. Other parameters, including longevity, length of oviposition period and fecundity, showed no significant differences between treatments. Although there were no significant differences in the parameters of net reproduction rate (R_0) and gross reproduction rate (*GRR*) between the control and fungal treated *C. montrouzieri*, the intrinsic rate of population increase (r) and finite rate of population (λ) for the control treatments were significantly higher. The activities of both aminotransferases in the larvae and the adults treated with both isolates significantly increased 96 hours post-treatment compared to the control. Although similar results were recorded for acid phosphatase activity, alkaline phosphatase activity showed no significant differences in larvae and adults between the treatments. The amount of protein significantly decreased in the larvae and the adults treated with both isolates after 96 hours, while the amount of triglyceride significantly reduced in the treated larvae compared to control. No significant differences were observed in adults. Our results indicated that both native isolates of *B. bassiana* may affect life fitness of *C. montrouzieri* but isolate AM-118 was more compatible than BB3.

Keywords: biology, entomopathogenic fungi, intermediary metabolism, mealybug ladybird, microbe-predator interaction

Introduction

Tea is one of the oldest and most widely consumed beverages in the world because of its pleasant taste, sedative effect, and the presence of beneficial compounds for humans (Hara 2001). Almost, 32,000 hectares of land in northern Iran are dedicated to tea cultivation with 27,000 tons of production, which ranks Iran as the 11th highest country of tea production globally (FAO 2012). Although different factors reduce the yield in tea plantations, insect pests are the most important. Currently, tea mealybug (*Pseudococcus viburni* Signoret) (Hemiptera: Pseudococcidae), the citrus brown aphid (*Toxoptera aurantii* Boyer de

Fonscolombe) (Hemiptera: Aphididae), red mite (*Brevipalpus obovatus* Donnadieu) (Acari: Tenuipalpidae) and broad yellow mite (*Polyphagotarsonemus latus* Banks) (Acari: Tarsonemidae) are among the most destructive pests of tea in northern Iran (Han *et al*. 2012; Ramzi *et al.* 2019). These pests, in addition to tea, also damage other horticultural and ornamental crops causing quantitative and qualitative losses by feeding on the tea buds. It has been estimated that about eight percent of the economic losses in tea production are caused by these pests (FAO 2012).

Integrated pest management refers to the use of all control methods based on ecological knowledge and understanding the economic concepts of damage (Dent 2000). Considering the disadvantages of the widespread use of chemical compounds in pest control such as resistance, emergence of secondary pests, the presence of chemical residues in crops, environmental pollution and side effects on humans and non-target organisms, integrated pest management aims at efficient control of insects with minimal impact on the environment (Zibaee and Malagoli 2020). One of the most effective methods to control pests is the use of biocontrol agents such as predators, parasitoids and microbes. In some cases, natural enemies alone are not able to reduce the population of pests to the desired level. Therefore, the simultaneous use of several natural enemies in the form of integrated biological control programs can be one way to increase their biological efficiency (Sher and Parella 1996). The combined use of predators and entomopathogenic fungi has been considered in most agricultural ecosystems (Roy and Pell 2000; Jacobson *et al.* 2001; Wang *et al.* 2011). Interactions between natural enemies should be evaluated before being used together. The results of these interactions can be neutral, antagonistic or synergistic, and must be clarified before combining the two biocontrol agents (Roy and Pell 2000). It is important to determine if overall the two agents increase biological control success or does one negatively affect the persistence of another agent, thereby increasing the cost of control.

Both ecological and physiological parameters are of interest to fully clarify insect-microbe interactions. Ecologically, it would be valuable to evaluate the impact of a pathogen on the biological characteristics of a predator by examining life table parameters and food preferences for healthy and contaminated prey. Life tables provide accurate information on survival, growth, reproductive ability, and population growth parameters of a homogeneous group under different conditions (Chi and Liu 1985). In addition to ecological interactions, predatory insects, like herbivores, respond to the presence of entomopathogenic fungi in their bodies through several physiological mechanisms, e.g., immune and antioxidant responses. Our previous study revealed that two native isolates of *Beauveria bassiana* (AM-118 and BB3) significantly affected survival of the larvae and the adults of *Cryptolaemous montrouzieri* Mulsant and induced cellular immunity through elevation of total and differentiated hemocyte counts as well as phenoloxidase activity. Moreover, the isolates considerably increased the activities of antioxidant enzymes in both developmental stages (Aghaeepour *et al.* 2022). To complete our findings on the interactions of these fungi with *C. montrouzieri*, potential changes in life table parameters and immunity were compared after infection with conidia. In recent years, mealybugs and red mites have reduced the quantity and quality of tea products, and the proposed solution is to use *C. montrouzieri*. As a result of severe damages and the rate of pest dispersal on tea plantations, in some years the use of this predator is not effective and applying other control techniques is recommended. Due to lethal effects of *B. bassiana*, and the suitable conditions of tea plantations in terms of moisture and canopy for the spread of entomopathogenic fungi, its application may help control mealybug. However, the combined use of the predatory ladybug and the entomopathogenic fungus requires the study of possible interactions between *C. montrouzieri* and *B. bassiana* that improves our knowledge on the interaction of these two useful biocontrol agents.

Materials and Methods

Insect rearing

Samples of tea mealybug were provided from the National Tea Research Institute, transferred to pumpkin by brush and propagated under laboratory conditions of 26 ± 1°C, relative humidity of 70 ± 5% and a 16 L : 8 D photoperiod. After rearing a sufficient number of mealybugs, adults of *C. montrouzieri* were provided from an insectarium of the Iranian Institute of Plant Protection (third generation cohort), then transferred to the laboratory and fed mealybugs in separate pairs in clear plastic containers 3 × 6 × 7 cm. After three generations of rearing, larvae and adult ladybugs were used for the experiments.

Fungi culture

Conidia of *B. bassiana* BB3 and AM-118 originally isolated from Amol rice fields and soil from the Fashand region of Alborz province, respectively, and whose toxicity had already been investigated on tea mealybug (Maqsoudi *et al.* 2018), were cultured on potato dextrose agar (PDA) using 90 mm diameter plastic Petri dishes. The culture medium was prepared at a ratio of 39 $g \cdot l^{-1}$ of sterilized water and autoclaved at 121°C and

a pressure of 11.5 atmospheres for 20 minutes. After cooling, the culture medium was poured into 90 mm sterile Petri dishes. Prior to culturing the fungi, the culture media were exposed to ultraviolet light for half an hour to kill potential unwanted microorganisms. Petri dishes were inoculated with the above isolates, surrounded by parafilm, and placed in a germinator at 25 ± 1°C for 3 weeks.

Preparation of fungal suspension

Under sterile conditions, the conidia of each isolate were collected separately from the culture surface with a scalpel and transferred to a Falcon tube containing 20 ml of sterile distilled water to which 0.02% Tween-80 was added. To make a uniform solution during shaking, three grams of glass bead were added to the solution and vortexed for 10 minutes. The suspension was passed through Whatman paper No. 1 to separate the mycelium or parts of the culture medium. To count the conidia and determine the concentration, 100 µl of the resulting suspension (10^9 conidia · ml^{-1}) was taken and poured onto a hemocytometer (Chemkind Co., Germany) to be counted.

Life table parameters of *Cryptolaemus montrouzieri* treated with LC_{30} concentration of *Beauveria bassiana* isolates

The current experiment was done with two groups of *C. montrouzieri* individuals treated with the LC_{30} concentration of each isolate (1.3 × 105 and 8.1 × 105 conidia · ml^{-1}) and a third group treated with Tween-80 solution (0.02%) as the control (Aghaeepour *et al.* 2022). Three hundred eggs were separately transferred to rearing containers where they were kept under the afore-mentioned conditions until hatching. The first instar larvae were fed separately in breeding containers containing tea mealybug (topically infected by each isolate and the control larvae were fed mealybugs treated only with Tween-80, 0.02%). This breeding process continued until the larvae developed into adults (Gholamzadeh-Chitgar *et al.* 2017). All developmental stages including embryonic length, larval and pupal stages as well as mortality of each stage were recorded daily until the emergence of adults. The adults were kept in separate pairs (489 pairs) in containers covered with organic mesh. The number of eggs laid, and the length of the preoviposition period of males and females were recorded daily and continued until the death of the adults. Data on biological and reproductive parameters in the treatment and control groups were analyzed based on the theory of two-sex life table assessment (Chi and Su 2006). The standard error of the developmental period, longevity of different developmental stages and two-sex life table parameters were estimated using the bootstrap method with 1000 repetitions (Chi 2008). To determine the significance of the differences between the obtained parameters, the paired bootstrap test was used in Two-Sex-MSchart software (Chi 2008).

Preparation of samples for enzymatic activity

Samples for biochemical analyses were prepared based on the method of Yaroslavtseva *et al.* (2017). The treated insects (from 30 third instar larvae and adults individually in 5 groups of 6) were homogenized in sodium phosphate buffer (0.1 mM, pH 7.2) containing phenyl thiourea (4 mg · ml^{-1}). Then they were centrifuged at 20,000 *g* for 15 minutes at 4°C.

Alanine aminotransferase activity

Alanine aminotransferase activity was measured according to Thomas's method using the Iran Biochem Kit (Thomas 1998). First, 50 µl of reagent B was mixed with 10 µl of reagent D. After 5 minutes, 10 µl of enzyme sample was added to the reaction and incubated at 25°C for 30 min. Then, 50 µl of additional reagent C was added to the samples and incubated for 25 min at 25°C. Finally, 110 µl of 0.4 N sodium hydroxide was added and the absorbance was read at 492 nm after 5 min.

Aspartate aminotransferase activity

The assay was done by the Thomas method (Thomas 1998) using the kit of Biochem Company, Iran. First, 50 µl of reagent A and 10 µl of reagent D were incubated for 5 min, then 10 µl of enzyme sample was added and after 60 min, 50 µl of reagent C was added and the whole mixture was incubated for 20 min. Finally, 110 µl of 0.4 N sodium hydroxide was added and the absorbance was read at 492 nm after 5 min.

Acid- and alkaline phosphatase activity

The method of Bessey *et al.* (1946) was used to determine the activity of these enzymes. To measure acid phosphatase, 50 µl of Tris buffer (20 mM) with pH 5, 30 µl of *p*-nitrophenyl phosphate as substrate and 15 µl of enzyme sample were added and incubated for 5 min before reading the absorbance at 405 nm. To measure alkaline phosphatase, 50 µl of Tris buffer (20 mM) with pH 8, 30 µl of substrate and 15 µl of enzyme sample were added and incubated for 5 min before reading the absorbance at 405 nm.

Total protein

The amount of protein was determined according to the method of Lowry *et al.* (1951) using the kit of Biochem Company, Iran.

Triglyceride measurement

Reagent solution contained phosphate buffer (50 mM, pH 7.2), adenosine triphosphate (2 mM), 4-chlorophenol (4 mM), glycerokinase (0.4 kU · l^{-1}), Mg^{2+} (15 mM), lipoprotein lipase (kU · $L2^{-1}$), peroxidase (0.2 kU · l^{-1}), 4-aminoantipyrine (0.5 mM) and glycerol 3-phosphate-oxidase (0.5 kU · l^{-1}). In detail, 10 µl of the enzyme sample were mixed with 70 µl of reagent solution. The mixture was incubated for 20 min at 25°C, then the absorbance was read at 546 nm. The following equation was used to calculate the amount of triglyceride (Fossati and Prencipe 1982):

$$\text{mg/dl} = (\text{OD of sample} / \text{OD of standard}) \times \times\ 0/01126.$$

Statistical analysis of data

Chi program was used to analyze the data obtained from the life table study. The results of the biochemical tests were analyzed to achieve the least significant difference in a completely randomized design using Tukey's multiple range at probability less than 5% using SAS 9.4 software.

Results

The effects of fungal isolates on the life table parameters of *Cryptolaemus montrouzieri*

Treatment with the native isolates of *B. bassiana* caused significant differences in life table parameters of *C. montrouzieri* compared to the control. The length of each developmental stage in the treatment significantly increased compared to the control, except for the eggs and adults (Table 1). The individuals treated with BB3 had the longest developmental stages from the first instar larvae to prepupae ($Pr > F$: 46.36; df = 5632; $p < 0.0001$, Table 1).

The preoviposition period in the adults treated with BB3 significantly increased compared to those treated with AM-118 and the control ($Pr > F$: 84.12; df = 5621; $p < 0.0001$) while other parameters including longevity ($Pr > F$: 33.16; df = 5236; $p < 2.89$), ovipositional period ($Pr > F$: 2.69; df = 5236; $p < 31.12$) and fecundity ($Pr > F$: 6.87; df = 5842, $p < 32.89$) showed no significant differences between treatments (Table 2). Moreover, there were no significant differences in net reproduction rate (R_0) ($Pr > F$: 2.21; df = 5412; $p < 12.02$) and gross reproduction rate (GRR) ($Pr > F$: 3.75; df = 5423; $p < 2.16$) for the control and fungal treated *C. montrouzieri* (Table 3). In contrast, intrinsic rate of population increase (r) ($Pr > F$: 56.18; df = 5241; $p < 0.0001$) and finite rate of population (λ) ($Pr > F$: 64.31; df = 5412; $p < 0.0001$) for control individuals were significantly higher than fungus treatments. Mean generation time (T) ($Pr > F$: 72.11; df = 5328; $p < 0.0001$) of the treated *C. montrouzieri* by fungal isolates significantly increased compared to the control (Table 3).

The results showed that treatment with fungal isolates increased the age-specific survival rate (Sxj) compared to the control ($Pr > F$: 43.29; df = 4528; $p < 0.0001$; Fig. 1). The age-specific survival rate (S_{xj}) indicates the probability that a newly laid egg will survive to age x and stage j. The highest survival rates in egg stages, first instar, second instar, third instar, fourth instar, prepupae, pupae and adult male and female insects of the control were 1, 0.96, 0.68, 0.52, 0.66, 0.54, 0.92, 0.52 and 0.4 (Fig. 1A), while these were 1, 1, 0.92, 0.78, 0.74, 0.44, 0.94, 0.46 and 0.48 for AM-118 (Fig. 1B) as well as 1, 1, 0.96, 0.82, 0.82, 0.52, 0.92, 0.42 and 0.5 for BB3, respectively (Fig. 1C).

Age-specific survival rate (l_x) indicates the survival probability of an egg up to the age of x. In these

Table 1. Mean length of growth periods in different developmental stages of *Cryptolaemus montrouzieri* after treatment by isolates of *Beauveria bassiana*

Stage	Mean developmental time ± SE [days]		
	control	BB3	AM-118
Egg	4.34 ± 0.097 a	4.5 ± 0.071 a	4.42 ± 0.081 a
First instar	3.7 ± 0.112 b	4.02 ± 0.105 a	3.88 ± 0.097 ab
Second instar	2.77 ± 0.116 c	3.94 ± 0.107 a	3.62 ± 0.094 b
Third instar	2.02 ± 0.113 c	3.51 ± 0.091 a	3.18 ± 0.086 b
Fourth instar	2.83 ± 0.119 c	3.98 ± 0.089 a	3.54 ± 0.079 b
Prepupa	1.85 ± 0.08 b	2.04 ± 0.086 a	2 ± 0.061 ab
Pupa	9.38 ± 0.127 b	10.39 ± 0.126 a	10.23 ± 0.111 a
Adult	58.15 ± 2.65 a	52.25 ± 3.211 a	54.95 ± 2.821 a

Different letters in each row show statistical difference (Tukey test, $p \leq 0.05$)

Table 2. Some biological traits of *Cryptolaemus montrouzieri* after treatment by isolates of *Beauveria bassiana*

Biological parameters	Control [mean ± SE]	BB3 [mean ± SE]	AM-118 [mean ± SE]
Adult longevity (days)	80.62 ± 3.526 a	78.94 ± 4.05 a	81.7 ± 3.54 a
Pre-oviposition period (days)	30.08 ± 0.416 c	35.76 ± 0.33 a	34.3 ± 0.35 b
Oviposition period (days)	46.24 ± 2.37 a	45.38 ± 3.04 a	44.78 ± 2.64 a
Fecundity (eggs/female)	118.07 ± 10.01 a	116.57 ± 9.11 a	114.39 ± 7.72 a

Different letters in each row show statistical difference (Tukey test, $p \leq 0.05$)

Table 3. Life table parameters of *Cryptolaemus montrouzieri* after treatment by isolates of *Beauveria bassiana*

Life table parameters	Control [mean ± SE]	BB3 [mean ± SE]	AM-118 [mean ± SE]
Net reproduction rate (R_0) (offspring/female)	63.708 ± 9.845 a	48.94 ± 8.95 a	52.79 ± 8.70 a
Intrinsic rate of population increase (r) (days^{-1})	0.89 ± 0.003 a	0.072 ± 0.003 b	0.076 ± 0.003 b
Mean generation time (T) (days)	46.39 ± 0.702 b	53.54 ± 0.935 a	51.40 ± 0.931 a
Finite rate of increase (λ) (days^{-1})	1.094 ± 0.004 a	1.075 ± 0.004 b	1.079 ± 0.003 b
Gross reproduction rate (GRR) (offspring/female)	77.26 ± 11.366 a	70.05 ± 11.51 a	98.77 ± 10.611 a

Different letters in each row show statistical difference (Tukey test, $p \leq 0.05$)

graphs, the survival is a uniform descending function in which mortality occurs slowly throughout life but it is more severe in old age. The individuals treated by fungal isolates had a higher survival rate than controls where the controls survived for 9 days ($Pr > F$: 21.57; df = 5863; $p < 0.0001$, Fig. 2A), while the individuals treated by AM-118 and BB3 survived for 13 and 10 days (Fig. 2B, C). Moreover, Figure 2 represents the age-specific fertility curve (mx) of control and treated individuals. Fertility in the controls started from day 25 (Fig. 2A) but only from days 30 and 31 for AM-118 and BB3 treated individuals, respectively (Fig. 2B, C). The peak of fertility in the controls was recorded on day 41, while it was found on days 71 and 83 for AM-118 and BB3 treatments (Fig. 2B, C).

Figure 3 presents a comparison of the life expectancy (e_{xj}) of *C. montrouzieri* treated by the fungal isolates versus control. Life expectancy refers to the length of time an individual is expected to survive to age x and stage j. Life expectancy changes are the opposite of the mortality rate, so that in the first days of life, when the mortality rate is at its lowest value, life expectancy is at its highest level.

The age-specific fertility curve (V_{xj}) is the number of offspring expected to be produced in the future by any female at age x and developmental stage j. Reproductive values indicate the relative participation of each age group to help future generations. Reproduction rates of *C. montrouzieri* in the controls and treatments are shown in Figure 4. The highest participation of individuals in the controls is on day 34 (by each female, Fig. 4A) while the highest participation of individuals treated with AM-118 and BB3 were observed on day 36 (Fig. 4B) and on day 37, respectively (Fig. 4C).

The effects of fungal isolates on the intermediary metabolism of *Cryptolaemus montrouzieri*

Aminotransferases

The activities of both aminotransferases in the larvae and the adults treated by fungal isolates significantly increased after 96 hours post-infection compared to the control. The highest activity of aspartate aminotransferases in the larvae was found after BB3 treatment while both isolates caused the highest enzymatic activity in the adults (Fig. 5A, B). Also, the activity of alanine aminotransferase increased significantly 96 hours post-infection although the highest enzymatic activity of the larvae was recorded after treatment by both isolates. BB3 was the only isolate which led to a high activity in the adults (Fig. 6A, B).

Acid- and alkaline phosphatases

ACP activity in the larvae and the adults treated by fungal isolates significantly increased at 96 h post-treatment compared to the control (Fig. 7A, B) while ALP activity showed no significant differences in both larvae and adults between the treatments (Fig. 8A, B).

Storage macromolecules

The amount of protein significantly decreased in the larvae and the adults treated by fungal isolates after 96 hours (Fig. 9A, B). In addition, the amount of triglyceride significantly reduced in the treated larvae compared to the controls, while no significant differences were observed in adults (Fig. 10A, B).

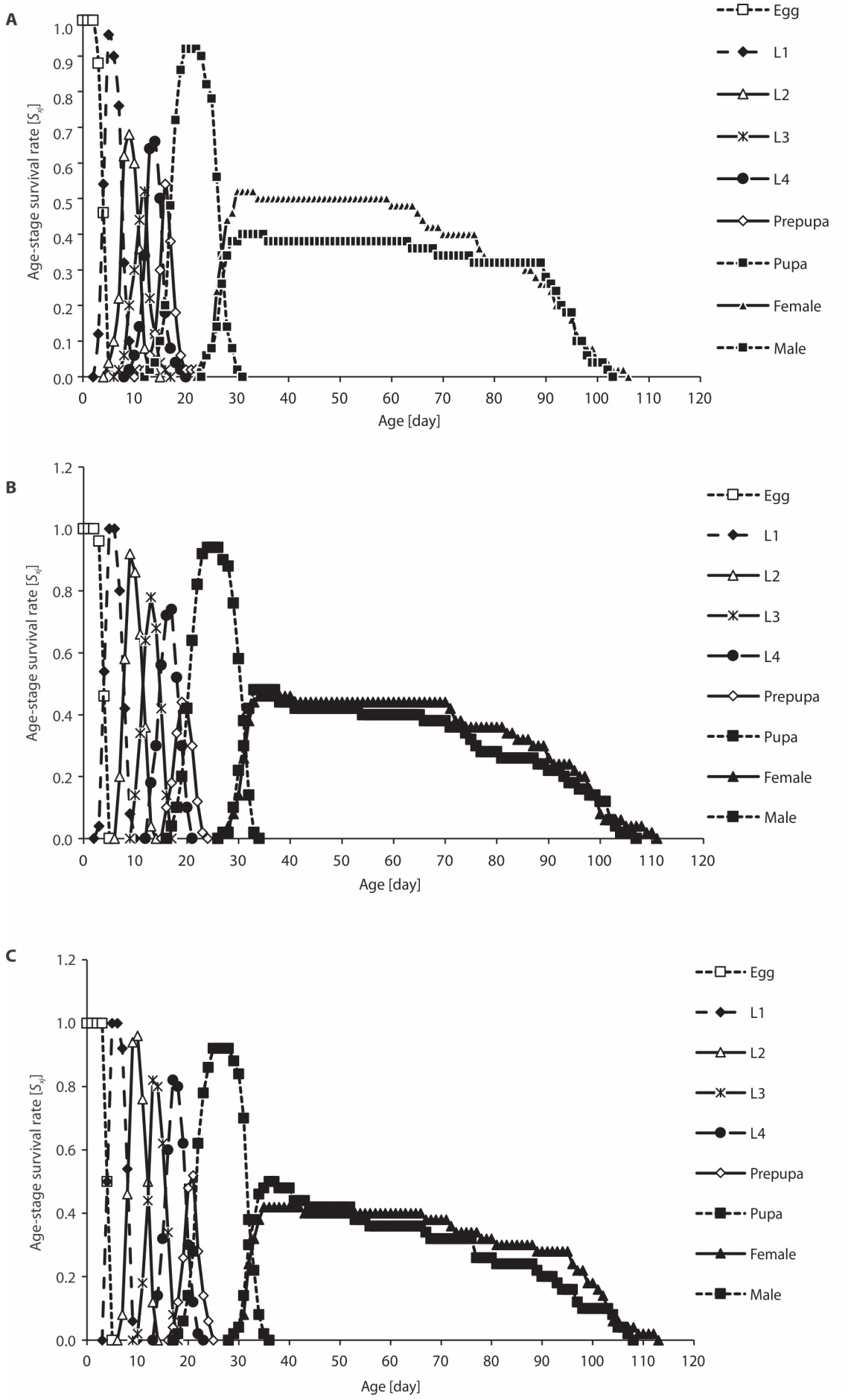

Fig. 1. Age-stage survival rate (S_{xj}) of *Cryptolaemus montrouzieri* treated by fungal isolates. A – control, B – treated by AM-118, C – treated by BB3

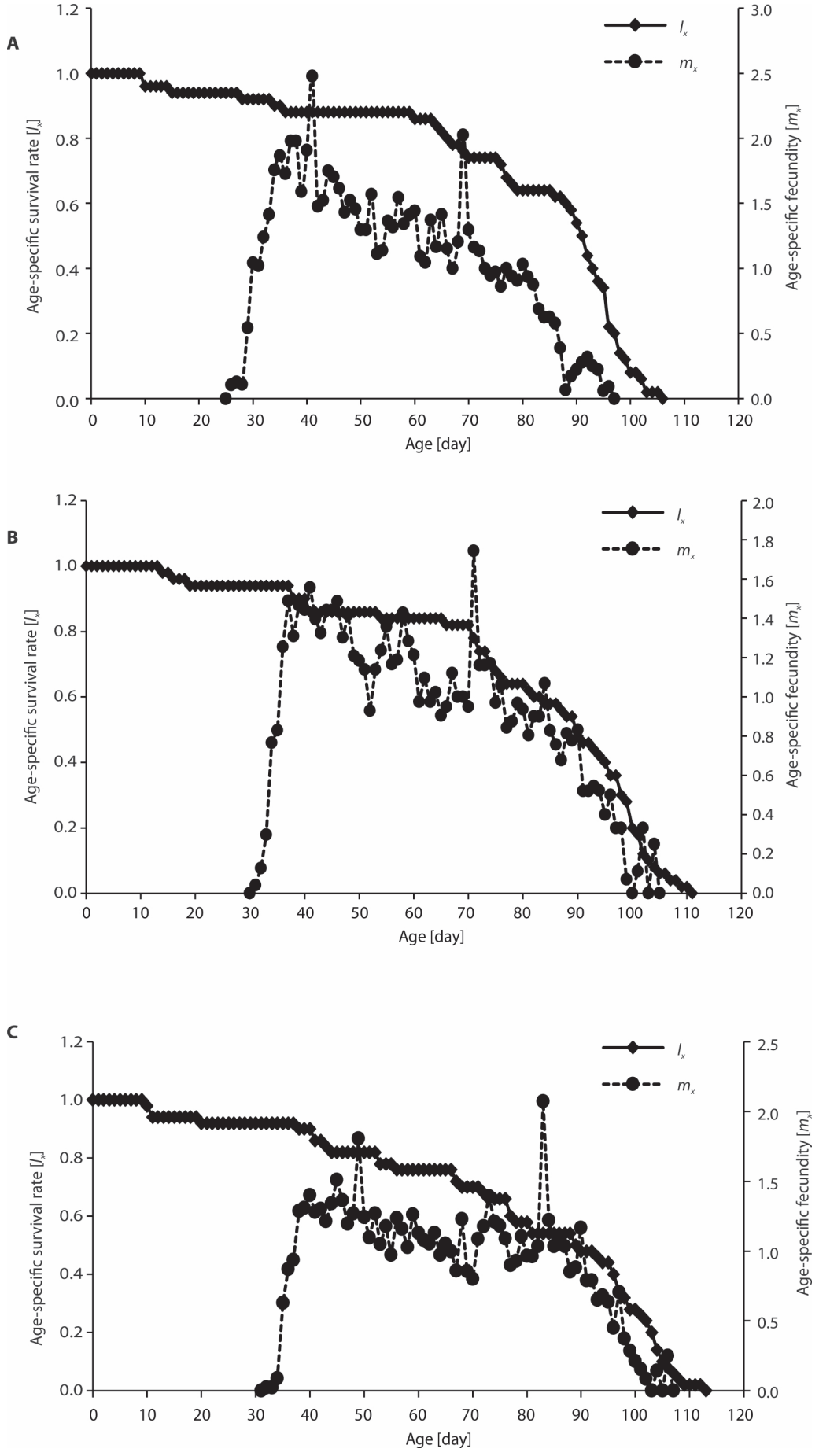

Fig. 2. Age-specific survival rate (l_x) and age-specific fecundity (m_x) rate of *Cryptolaemus montrouzieri* treated by fungal isolates. A – control, B – treated by AM-118, C – treated by BB3

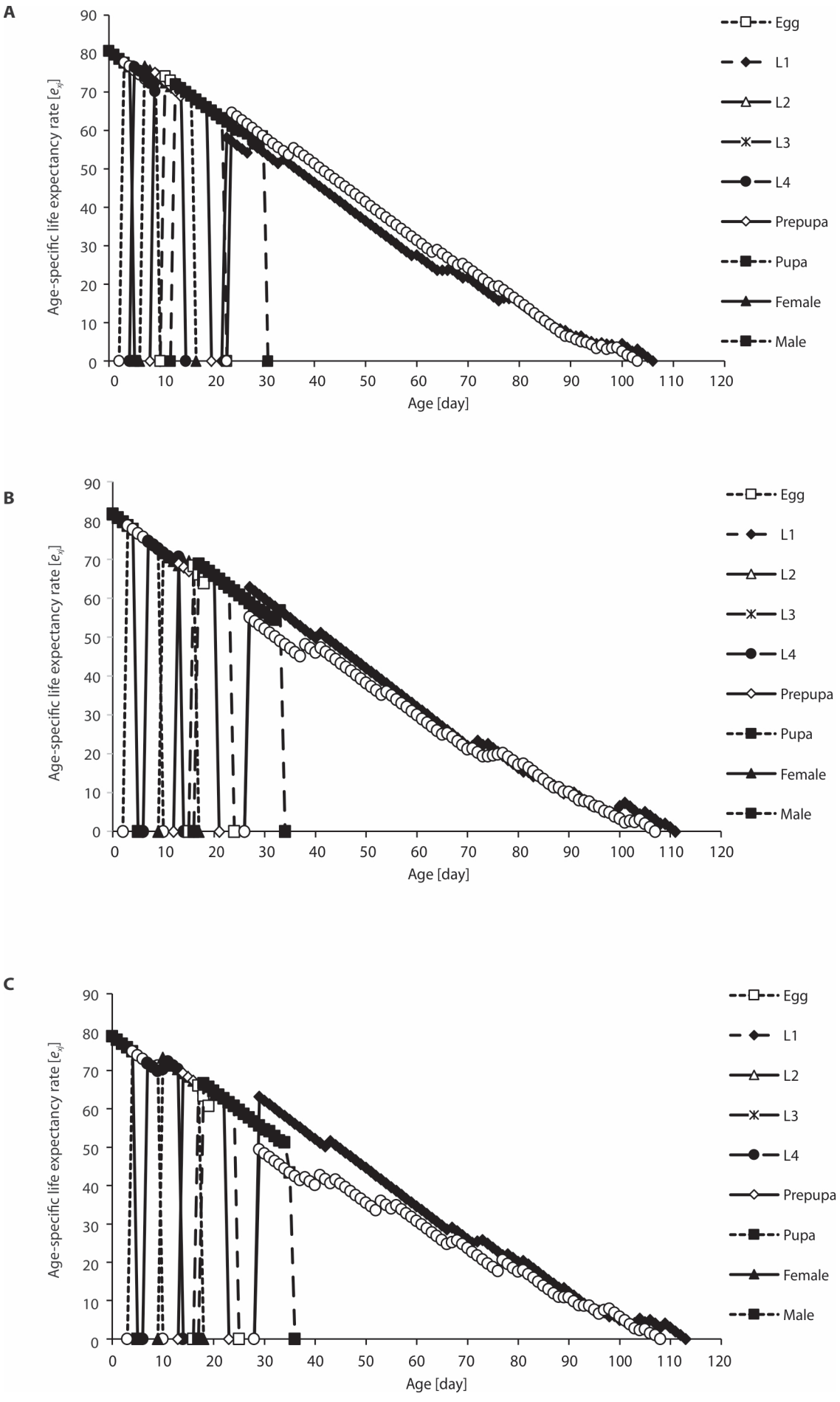

Fig. 3. Age-stage life expectancy (e_{xj}) of *Cryptolaemus montrouzieri* treated by fungal isolates. A – control, B – treated by AM-118, C – treated by BB3

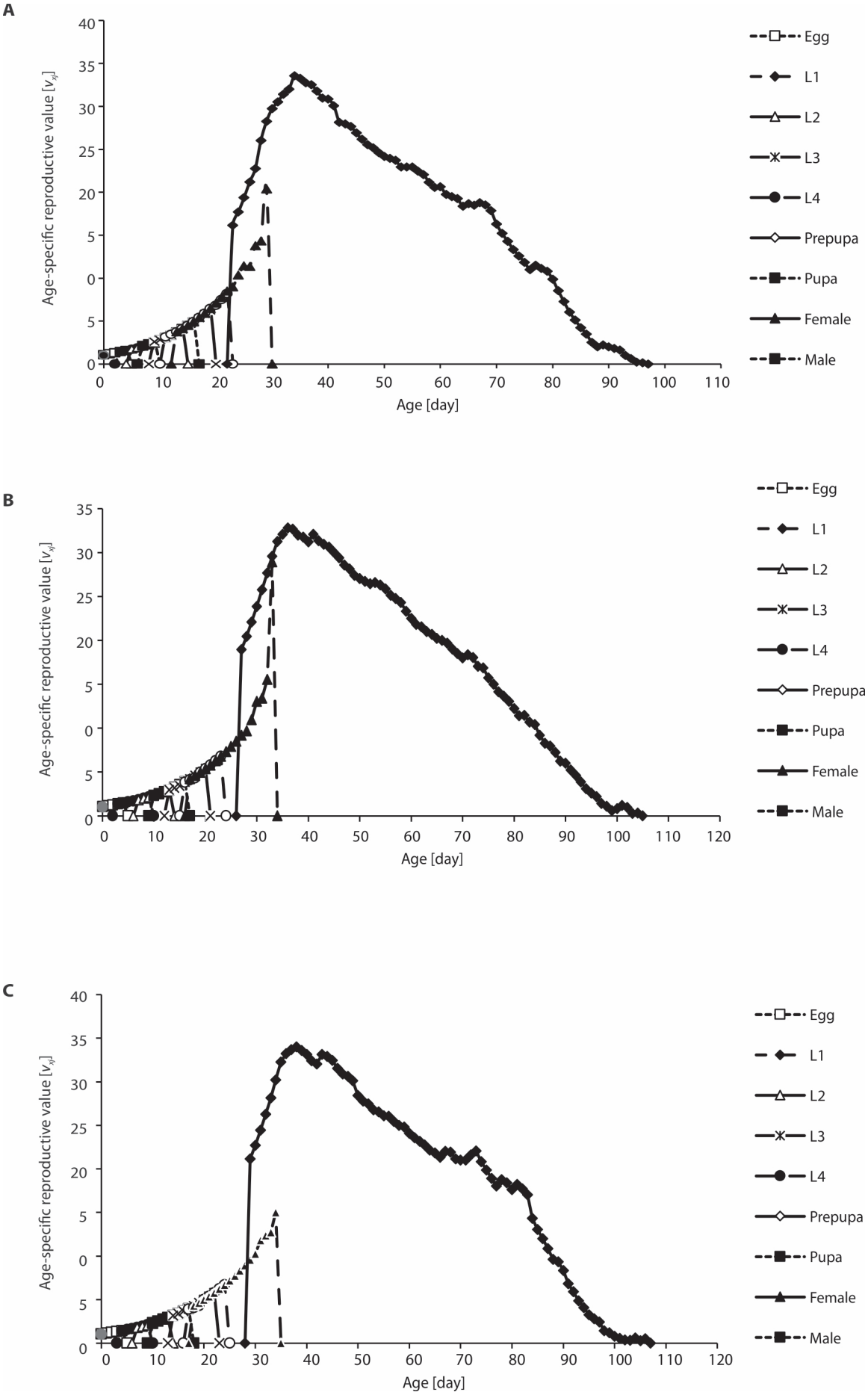

Fig. 4. Age-stage specific reproductive value (v_{xj}) of *Cryptolaemus montrouzieri* treated by fungal isolates. A – control, B – treated by AM-118, C – treated by BB3

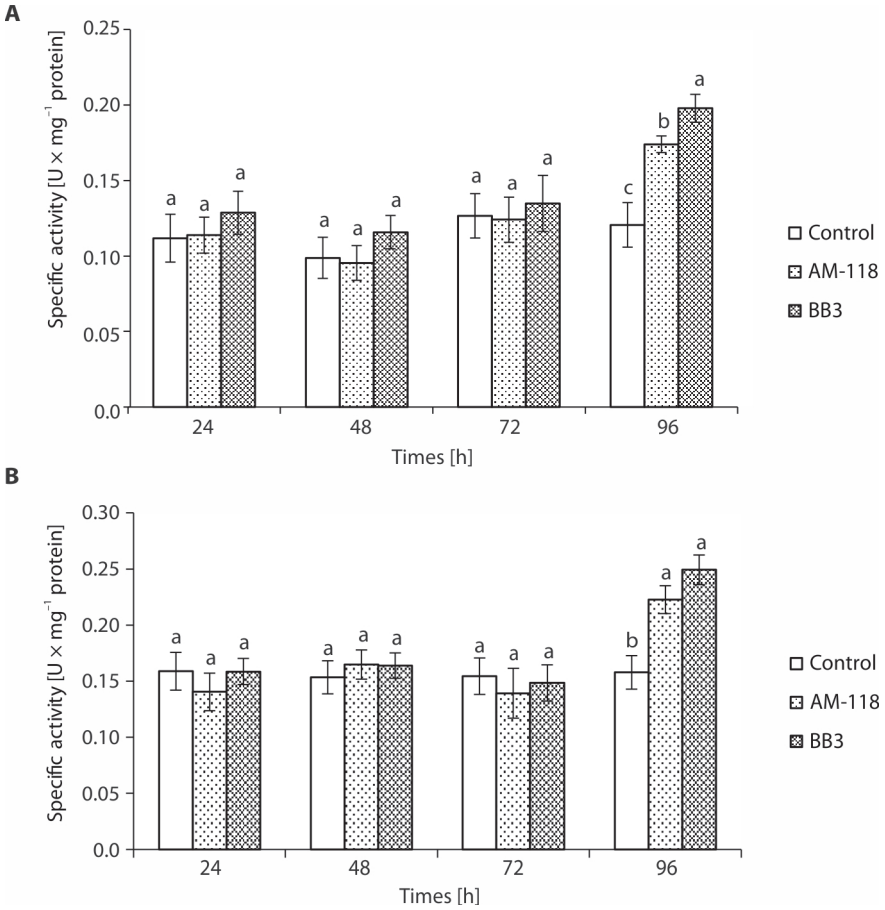

Fig. 5. Changes in aspartate aminotransferase activity of the larvae (A) and adults (B) of *Cryptolaemus montrouzieri*. Statistical differences have been shown with different letters (Tukey test, $p \leq 0.05$)

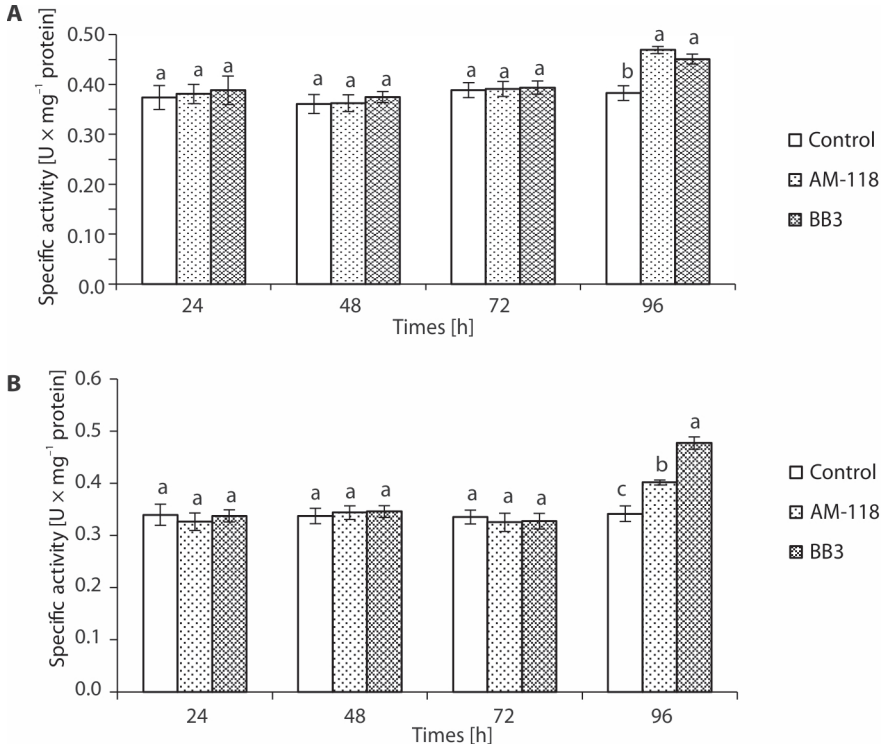

Fig. 6. Changes in alanine aminotransferase activity of the larvae (A) and adults (B) of *Cryptolaemus montrouzieri*. Statistical differences have been shown with different letters (Tukey test, $p \leq 0.05$)

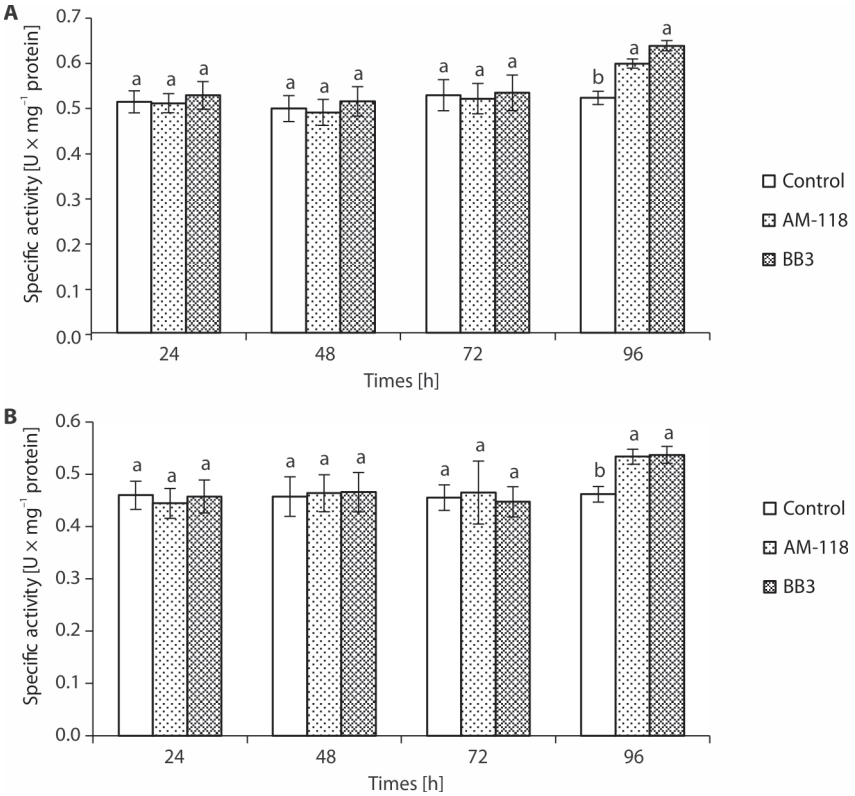

Fig. 7. Changes in acid phosphatase activity of the larvae (A) and adults (B) of *Cryptolaemus montrouzieri*. Statistical differences have been shown with different letters (Tukey test, $p \leq 0.05$)

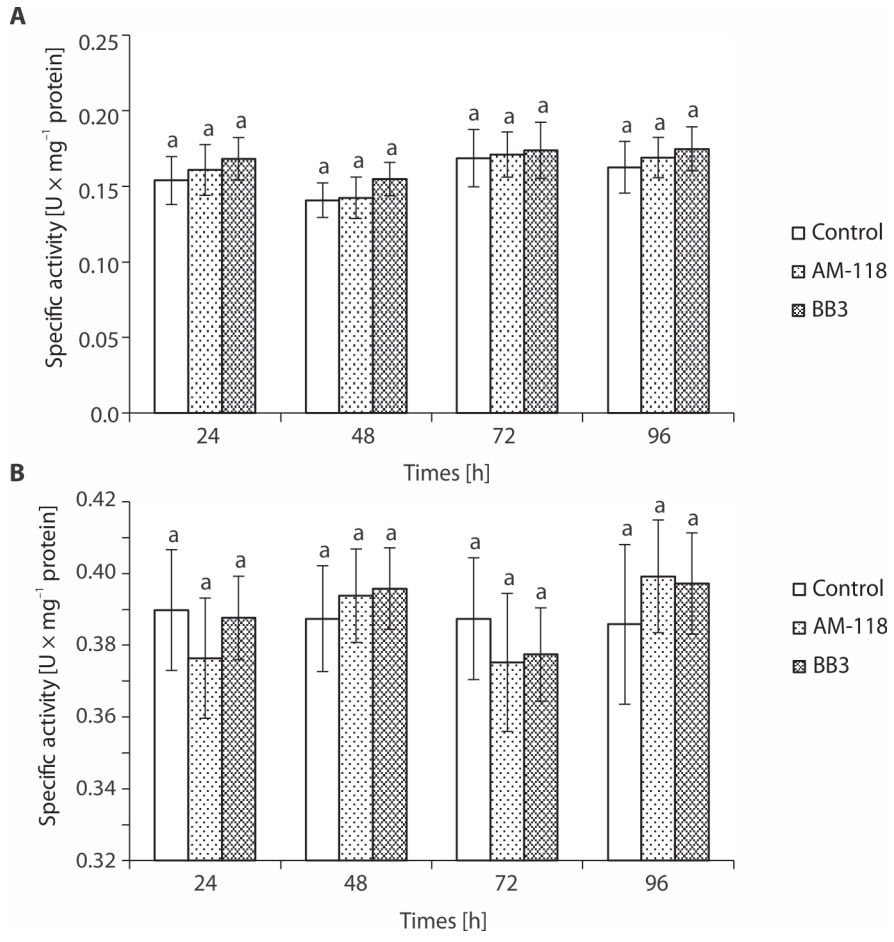

Fig. 8. Changes in alkaline phosphatase activity of the larvae (A) and adults (B) of *Cryptolaemus montrouzieri*. Statistical differences have been shown with different letters (Tukey test, $p \leq 0.05$)

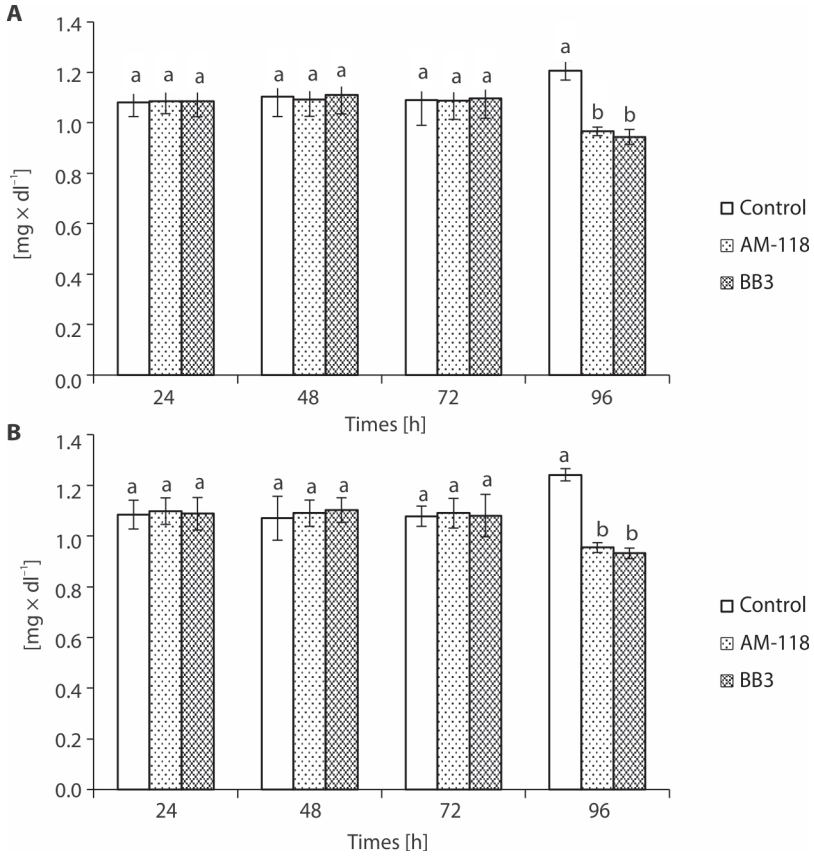

Fig. 9. Changes in protein contents of the larvae (A) and adults (B) of *Cryptolaemus montrouzieri*. Statistical differences have been shown with different letters (Tukey test, $p \leq 0.05$)

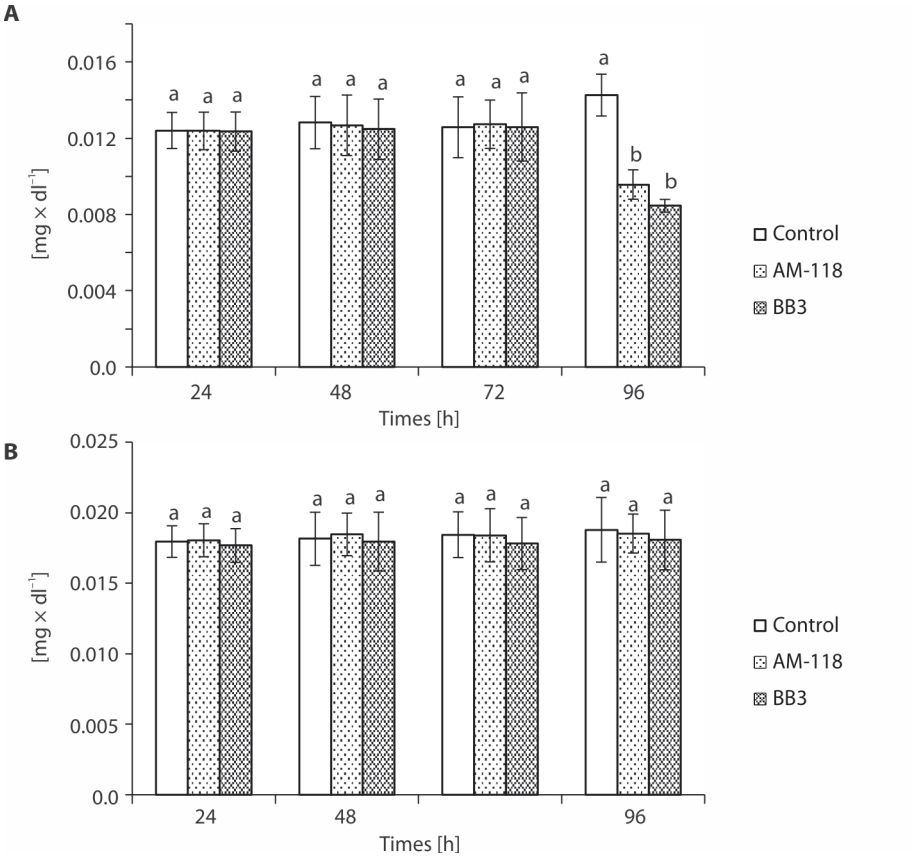

Fig. 10. Changes in triglyceride contents of the larvae (A) and adults (B) of *Cryptolaemus montrouzieri*. Statistical differences have been shown with different letters (Tukey test, $p \leq 0.05$)

Discussion

The current study revealed that the native isolates of *B. bassiana* caused changes in the development, biological parameters and life table parameters of *C. montrouzieri*. The increase in the average length of developmental stages compared to the control can be attributed to a depletion of insect energy to remove the foreign agent (fungus) rather than this energy being used for growth or development. In our study, fungal treatments increased the length of larval and pupal stages of *C. montrouzieri* eminently highlighted by the higher value of mean generation time, but they did not affect fertility and ovipositional period nor net reproduction rate and gross reproduction rate. Moreover, the intrinsic rate of population increase (r) and the finite rate of increase (λ) as the two main parameters in the life table of insects significantly decreased in *C. montrouzieri* treated by fungal isolates. These two factors demonstrate the amount of insect adaptability to different environmental and nutritional conditions (Southwood and Henderson 2000; Liu *et al*. 2004). The lower values indicate stress or physiological pressure on individuals affecting their normal development or reproduction because the individuals should provide the energy requirements for immune responses to kill fungal conidia as reported in our previous study (Aghaeepour *et al*. 2022). There are a few reports on life table parameters of biocontrol agents after exposure to entomopathogenic fungi. Gholamzadeh-Chitgar *et al.* (2017) evaluated the effects of *B. bassiana* on the predatory bug of *Andrallus spinidens* Fabricius (Hemiptera: Pentatomidae). No statistical differences on adult longevity, fertility and ovipositional period in the treated individuals compared to the control were recorded. In another study, the effects of Egyptian isolates of *B. bassiana* and *M. anisopliae* were determined on the population parameters of *C. montrouzieri* (Mohamed 2019). Net reproduction rate, intrinsic rate of population growth and finite rate of population increase reduced in the treated coccinellids while the mean generation time significantly increased compared to the control. Portilla *et al*. (2020) reported that *B. bassiana* isolate NI8 significantly affected all population parameters of *Chrysoperla rufilabris* Burmeister (Neuroptera: Chrysopidae). The intrinsic and finite rates of population growth, gross and net rates of reproduction in the treated adults also significantly decreased compared to the control.

Although these results imply negative effects of entomopathogenic fungi on ecological fitness of *C. montrouzieri*, the isolate AM-118 showed fewer moderate effects than BB3. Origin of collection, pathological properties, e.g., spore membrane composition, potential of extracellular enzyme production and capability of immune responses of hosts are among the main factors affecting virulence of entomopathogenic fungi against insects. Based on our earlier study (Aghaeepour *et al.* 2022), *C. montrouzieri* showed higher immune responses to AM-118 than BB3 which is a possible reason of less susceptibility to Am-118.

Aspartate- (AST) and alanine aminotransferases (ALT) are two important enzymes in transamination which are active in the hemolymph and fat bodies of insects. These enzymes provide the energy needed for different biochemical reactions, converting different amino acids to each other and transferring amino groups from amino acids to keto acids to be entered into the Krebs cycle (Klowden 2007). The activities of both aminotransferases in the larvae and the adults treated by fungal isolates significantly increased after 96 hours of treatment compared to the controls. Such an elevation indicates a protein deficiency in the insect's body, which may be due to the differentiation of homocytes, the detoxification of fungal secondary metabolites or tissue regeneration following fungal infection (Senthil-Nathan *et al.* 2006). Any changes in protein availability for tissue development and energy demands would definitely affect development, reproduction and the potential of spreading throughout an ecosystem. Moreover, protein deficiency or processing may make insects vulnerable to environmental stress such as temperature and infection by microorganisms. Therefore, such a deficiency may affect life table parameters, mainly developmental time of stages, because these individuals need more time to acquire the required amounts of proteins.

Acid- (ACP) and alkaline (ALP) phosphatase are the hydrolyzing enzymes in insect hemolymph, gastrointestinal tissue and fat bodies which are involved in energy production by breaking down phospholipids under acidic or alkaline conditions (Senthil-Nathan 2006). In the present study, ACP activity significantly increased in the treated larvae and adults however, no significant difference was recorded in ALP activity. Our findings on ACP were consistent with Mirhaghparast *et al.* (2013) who found that *B. bassiana* and *M. anisopliae* conidia significantly increased ACP activity. In another study, Karthi *et al.* (2018) showed that the activity of ACP and ALP enzymes in *Spodoptera littura* Fabricius (Lepidoptera: Noctuidae) larvae increased after exposure to *Aspergillus flavus* conidia. Increased ACP activity is related to the induction of immune responses reported previously (Aghaeepour *et al.* 2022) and possible affliction of tissues due to fungal infection in accordance with increased activity of aminotransferases.

Insects receive the energy they need for biological functions from food sources such as triglycerides, glycogen, and protein. Fat bodies play a major role in storing and supplying energy to insects (Klowden 2007; Nation 2008). Triglyceride, glycogen and protein

are the macromolecules that serve as a storage form of energy in insects (Nation 2008). The amount of food reserves including protein and triglycerides significantly decreased in the treated *C. montrouzieri* which indicates a disorder in the physiology of digestion and intermediate metabolism following fungal infection. Induction of immune responses and antioxidant enzymes as well as alterations in the activities of aminotransferases and phosphatasesin requires biochemical molecules and efficient energy that has been provided by triglycerides and proteins.

Conclusions

The current study was the final part of a project assessing the synchronous application of *C. montrouzieri* and *B. bassiana* as biocontrol agents against tea mealybugs in the tea plantations of Iran. It was found that the coccinellid may trigger its immune responses against fungal conidia to survive, but this involves some discrepencies in physiological processes mainly intermediary metabolism and antioxidant system. Although both fungal isolates had some similar results on the studied life table parameters, fertility and reproductive rates of *C. montrouzieri* after fungal infections were not affected. Therefore, the coccinellid may survive on plantations also infected by *B. bassiana*. It is important to implement the joint application of *C. montrouzieri* and *B. bassiana* to efficiently suppress mealybug and mite outbreaks in tea plantations and produce pesticide-free green leaves. Such an action will increase the efficiency of a biocontrol program to significantly suppress mealybug populations in seasons suffering outbreaks.The combined use of these two agents may increase the efficiency of the biological control against tea mealybug and it will be worthwhile to investigate control persistence in tea orchards.

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