

Evaluation the Effects of the Entomopathogenic Fungus *Beauveria Bassiana* (Ascomycota: Hypocrales) on some Histological and Physiological Parameters for the Green Bug *Nezara Viridula* (L.) (Hemiptera: Pentatomidae)

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ABSTRACT

The present study aimed to determine the effect of the entomopathogenic fungus *Beauveria bassiana* on some histological changes in the mid gut (mesenteron) of *Nezara viridula* adults after 24, 48, and 72 hours of treatment. Also, its effect on some physiological parameters as α -amylase, lactate dehydrogenase (LDH) activities. The contents of some minerals (calcium, inorganic phosphorous, and magnesium) has been studied. The histological studies showed several malformations in the treated *N. viridula* adult's midgut. Fungal infection caused damage in different sites of the midgut after 48 and 72h of treatment. The α -amylase activity, increased significantly, in the infected adult. The highest increase in the α -amylase activity was exhibited after 24h of infection and then gradually decreased after 48 and 72h of treatment. On contrary, the LDH activity was significantly reduced in the infected adults; after 24h of treatment. With regard to the minerals, all tested minerals content were decreased significantly, in the infected adults, irrespective of the time after fungal treatment. *Beauveria bassiana* could be considered as a promising model for biological control of the green bug *N. viridula*.

Key words: Entomopathogenic fungus, *Beauveria bassiana*, *Nezara viridula*, histological study, enzymes activities.

INTRODUCTION

Nezara viridula (L.) (Hemiptera, Pentatomidae) is considered as one of the most important pest species among the family Pentatomidae. It prevails in the majority of tropical and subtropical territories in Asia, Africa, and America. It has a large host range includes 145 plant species classified in 32 families worldwide, ranging from herbs to vegetables (Noda and Kamano, 2002; Todd, 2003 ; Panizzi, 2015).

N. viridula (adults & nymphs) pierce plant tissues to absorb large amounts of plant sap and inject digestive enzymes. It feeds through different parts of the host, including leaf veins, stems, developing shoots, flowers, unripe fruits and even seeds (Meglič *et al.*, 2001),

resulting in tissues deformation, premature seeds abortion, and reduced germination of developed seeds.

Medrano *et al.* (2009) reported that *N. viridula* can transmit the pathogenic bacterium, *Pantoea agglomerans* (Ewing and Fife) into green cotton bolls resulting in plant infection. The universal anamorphic fungus *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocrales) is an environmentally friendly entomopathogenic fungus that causes insects' mortality (Mirhaghparast *et al.*, 2013; Petlamul *et al.*, 2019 ; Gad and Nada, 2020).

B. bassiana species have been isolated from a wide range of insects all over the world. The efficacy of *B. bassiana* against *N. viridula* was documented in several previous studies (Sosa-Gómez and Moscardi, 1998; Nada, 2015 ; Raafat *et al.*, 2015).

Benzina *et al.* (2018) demonstrated that treatment of the 4th larval instar of *Culex pipiens* with *B. bassiana* induced disturbances in all parts of the body, including structure of the cuticle, adipose tissue, haemolymph and intestine. Furthermore, Intodia *et al.* (2019) observed that treated termite workers by *B. bassiana* lysed the cells of midgut and brush border to gut lumen.

Insects have several enzymatic proteins as phenoloxidases, hydrolases, peroxidases, acetyl choline esterases, glucosidases and esterases (Arimura *et al.*, 2005).

N. viridula have different enzymes than their host plant. Some bio-insecticides effects on the activities of transaminase enzymes (GOT & GPT) and carbohydrate hydrolyzing enzymes (invertase and amylase) (Mead, 2000).

α -Amylase (α -1,4-glucan 4-glucanohydrolase, EC 3.2.1.1) is one of the des-polymerases that catalyses the dissociation of 1,4-glucan linkages in starch and glycogen (Terra and Ferreira, 2012). The structural integrity and activity of insect α -amylases are reliant on calcium and chloride ions. In physiological approach, α -amylases enhance digestive performance of insects

DOI: 10.21608/asejaiqsae.2022.239209

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Received April 10, 2022, Accepted, May 25, 2022.

allowing them to survive in a variety of environments and improving their biological fitness (Kaur *et al.*, 2014).

Lactate dehydrogenase (LDH) is a vital glycolytic enzyme found in almost all tissues (Kaplan and Pesce, 1996). It plays a role in carbohydrate metabolism and has been used as a characteristic criterion for chemical stress exposure (Diamantino *et al.*, 2001). LDH is also a toxicological and clinical chemistry parameter that is used to diagnose cell, tissue, and organ damage (Ribeiro *et al.*, 1999; Senthil *et al.*, 2006).

The present investigation aims to evaluate the biocontrol potential of *B. bassiana* against *N. viridula* adults and investigate the interaction between them as a pathogen and host through some histological studies as well as its effects on some host's enzymes and mineral content, for the first time.

MATERIALS AND METHODS

Insect colony

Adults of *N. viridula* were collected from green bean (*Phaseolus vulgaris* L.) fields in the Faculty of Agriculture, Experimental station, Giza, Egypt. Green bug individuals were moved to the laboratory and maintained on potted broad beans (*Vicia faba* L.), which were covered with cylindrical glass 5cm in diameter, 22 cm in length and covered with muslin at 28 ± 2 °C, 75–90 %RH and 16 h light: 8 h dark photo-cycle.

The deposited egg masses were collected and placed in Petri dishes containing a piece of moistened cotton wool and fresh green beans until hatching. Finally, adult were collected and used for the needed experiments (Nada, 2006).

Entomological fungus isolates

B. bassiana (Hypocreales: Cordycipitaceae) strain was originally isolated from soil of Dakahalia Governorate, Egypt according to the method described by Nada (2006). The fungus was cultured on autoclaved Sabourad dextrose yeast agar (SDAY), containing 1% peptone, 0.2% yeast extracts, 4% dextrose and, 1.5% agar dissolved in 1L distilled water and incubated for two weeks at 25 ± 1 °C.

Bioassay procedure

Spores were harvested by rinsing with sterilized aqueous solution of 0.02% Tween 80, and then filtered through cheese cloth to reduce mycelium clumping. Spores were counted in the suspension using a haemocytometer (Neubauer improved HBG, Germany 0.100 mm x 0.0025 mm²). A concentration of 1×10^7 spores/ml of isolated spores was prepared. An aqueous solution of 0.02% Tween 80 was used as the control treatment. Adults of *N. viridula* were dipped for 10 seconds in flasks containing 10 ml conidial suspension.

This procedure was replicated three times. Thereafter, treated adults were maintained in Petri dishes with ventilation on the cover and prepared with saturated filter paper and fresh green bean was supplemented as food (Gad and Nada, 2020).

Histological study

After 24, 48, 72 h of treatment, treated and untreated adults of *N. viridula* were dissected ($n = 5$ per each treatment time and control) and the mid gut was immediately fixed in 10% buffered formalin for 24 h, then dehydrated in an ethanol-xylene series and embedded in paraffin wax and cut on 4 μ m. Sections, then deparaffinized, rehydrated, and stained by Haematoxylin and Eosin for histopathological examination under an optical microscope to determine the various anomalies that may have appeared in the mid gut of the treated *N. viridula* adults (Bancroft and Stevens, 1996).

Biochemical analysis

Adults of *N. viridula* were immersed in 1×10^7 spores/ml conidial suspension of *B. bassiana* for 10 seconds and then excess suspension was removed using a filter paper. Aqueous solution of 0.02% Tween 80 was used as a control. Treated adults were maintained in Petri dishes with ventilation on the cover and prepared with saturated filter paper and fresh green bean was supplemented as food (Nada, 2015). The treated adults were used for the enzyme activity determination according to (Gholamzadeh-Chitgar *et al.*, 2015).

Sample preparation

The treated adults of *N. viridula* were collected after 24, 48 and 72h of infection ($n = 20$ per treatment for each enzyme). The samples were homogenized separately in 250 μ l of 0.2 M phosphate buffer (pH 7.0) containing 0.05 % Triton X-100 using a plastic pestle. The homogenate was, then, centrifuged at 12000g for 10 min at 4°C. The supernatant was used as an enzyme solution for assessing the α -Amylase and Lactate dehydrogenase. And also, for assessing some mineral contents such as calcium, Magnesium and Phosphorus.

α -Amylase activity

Digestive enzymes were determined according to the modifications of Amin (1998) to the method described by Ishaaya and Swirski (1976). 20 μ l of diluted enzyme solution was incubated for 10 min at 30 °c with 250 μ l 1 % starch (soluble potato starch, Lintner grade, Sigma Chemical Co.) in 50 mM acetate buffer pH 5.0 containing 20mM NaCl and 0.1mM CaCl₂. The reaction was stopped by adding 250 μ l DNS reagent to each tube in boiling water for 5 min. Samples were cooled, diluted with 2.5 ml H₂O, and read at 550 nm on Spectronic 1201 (Beckman, USA).

Glucose was used as a standard. Appropriate dilutions of enzyme supernatant were used to obtain a linear production of glucose equivalents. The enzyme activity was expressed as μg glucose released /min/gm body weight.

Lactate dehydrogenase activity (LDH):

The activity of LDH was assayed by the procedure described by the German Society for clinical chemistry (Chemie,1972). Lactate dehydrogenase catalyzes the conversion of pyruvate to lactate, NADH is oxidized to NAD in the process. The rate of decrease in NADH is directly proportional to the LDH activity and is determined photo metrically.

The reaction mixture consisted of phosphate buffer; 68mmol/L, pH 7.5, pyruvate 0.73 mmol/L, and 1.1 mmol/L NADH. Hundred microliters sample were mixed with 2.5ml of the reaction mixture that preincubated at 37 °C. Then they were poured into spectrophotometer cuvette and the initial absorbance was read. Timer was started simultaneously, and the absorbance was read again after 1,2, and 3 min. Zero adjustment was against air. LDH activity was calculated according to the following equation:

LDH activity = Factor X ΔA / 340nm/min, where: Factor = 4468 (as recommended by the used kit; Randox, United Kingdom), ΔA = the change in absorbance/min. LDH activity was expressed as μU ; the amount of enzyme that oxidized 1.0 μmol of NADH /min/gm body weight at pH 8.8 at 37° C.

Mineral assay

Calcium (Ca):

Calcium ion was determined using Bio-analytics kit (email: bioanlab@bellsouth.net. Palm city, USA). Calcium reacts with cresophthalein in an alkaline medium to form a coloured complex. The colour developed has a maximum absorbance at 570 nm and is proportional to the calcium concentration in the sample. Measurement was against reagent blank and compared to calcium standard (10 mg /dl) (Amin, 1998).

Inorganic Phosphorus

The phosphate ion was detected using a commercial kit of Quimica Clinica Aplicada S. A. kit (Spain). Phosphorus reacts with molybdate to produce phosphor-molybdate, which is finally reduced to a molybdenum blue which is photometrically measured at 650 nm. Zero adjustment was against reagent blank, and results obtained after comparison with a reference standard (conc.4 mg %) (Amin, 1998).

Magnesium (Mg):

Magnesium was determined by the xylidyl blue method followed using Quimica Clinica Aplicada S.A. kit (Spain). In an alkaline medium, the magnesium ions of the sample will produce a colored complex with xylidyl blue . Color intensity is directly proportional to the magnesium ions concentration. Ten microliters of the sample were added to 1 ml reagent (0.1 mM xylidyl blue; 0.3 mM Tris buffer pH 11 ; 50 μM Glycoetherdiamine -N,N,N N - tetra acetic acid as a chelating agent for 10 min at room temperature (20-25 °C). The colour produced was read at 520 nm against standard (4 mg /dl) (Amin, 1998).

All assays were performed in duplicate and each assay was repeated at least three times.

Statistical analysis

Statistical analysis of data was performed using analysis of variance (ANOVA) and differences among the means were determined for significance at 0.01 using LSD test (Steel and Torrie 1980).

RESULTS AND DISCUSSION

RESULTS

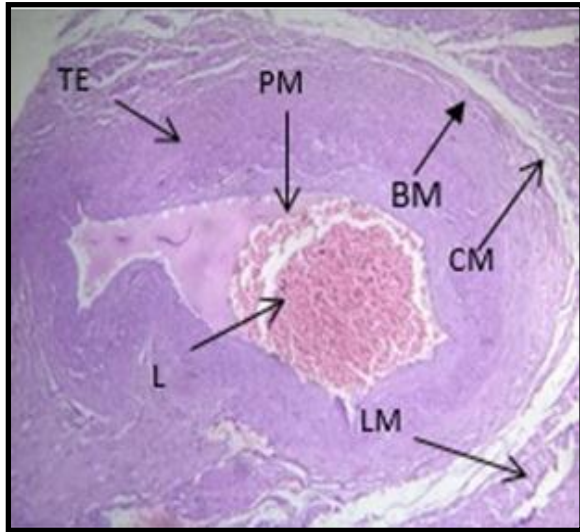
Histological Studies of *N. viridula* Adult:

The untreated and treated *N. viridula* adults were histologically examined after 24, 48 and 72 h by light microscope.

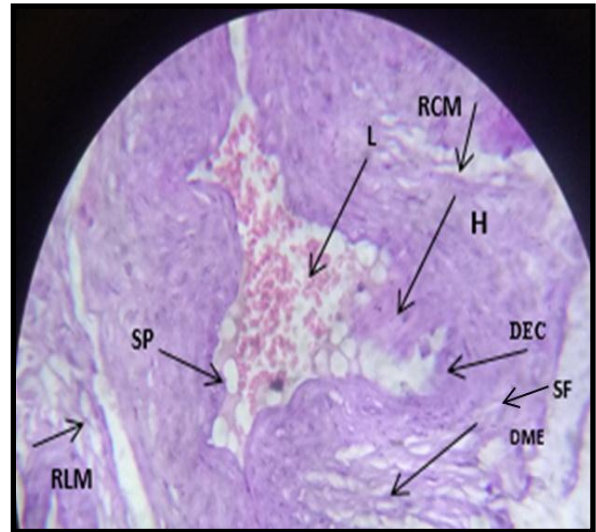
The microscopic examination of *N. viridula* adults in this study revealed that the *B. bassiana* spore suspension brought about massive disintegration and deformation of the adult of *N. viridula* midgut tissue. Also, it revealed the development and the colonization of the fungus inside the insect.

The untreated adults of *N. viridula* (Fig. 1a) showed the normal structure of the adult's mid-gut with epithelial layer, basement membrane and its muscles. On the other hand, the treated individuals showed many abnormalities in the insect's mid-gut after 48 and 72h of treatment.

The cross section in the mid-gut showed the effect of the fungus on the muscular layers, which caused a change in the thickness of these layers compared with those of the controls, as the disappearance of the supports of the muscle layer (Fig. 2 c,d).

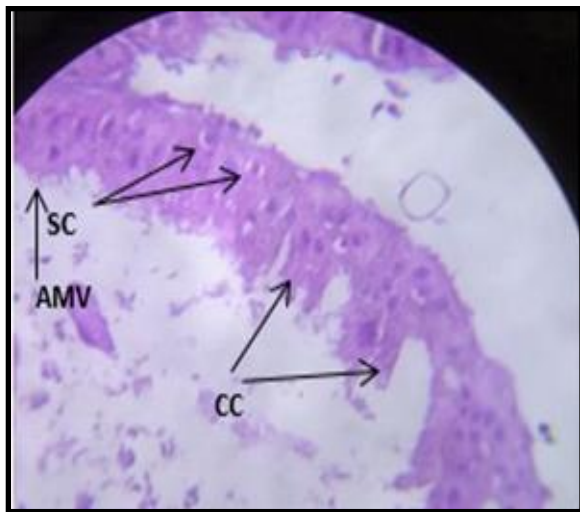


a: Transverse section of *N. viridula* adult's mid-gut region showing different layers. Circular muscles (CM), Basement Membrane (BM), Longitudinal muscles (LM), Thin peritrophic membrane (PM), Transitional Epithelial cells (TE) and Gut Lumen (L)



b.: Transverse section of *N. viridula* adult's mid-gut after 48h. of fungal infection showing many abnormalities of midgut layers. Reduced Longitudinal Muscles (RLM), Reduced Circular Muscles (RCM), Disintegrating Epithelial Cells (DEC), Space Formation between layers (SF), Hyphae (H), Degenerated Midgut Epithelial cells (DME), Gut Lumen (L), blastospores (SP)

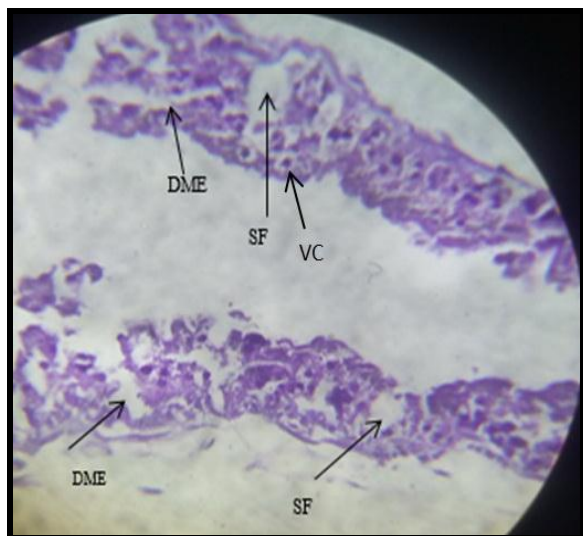
Fig.1. Histoarchitecture of *N. viridula* adult through midgut showing untreated adult (a) compared with 48h treated adult with *B. bassiana* (b) showing blastospores in the digestive tract of *N. viridula* adult



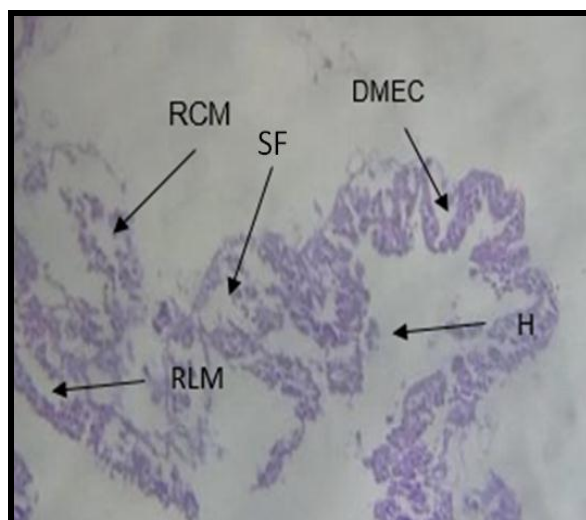
a: Transverse section of *N. viridula* adult's midgut showing Columnar epithelial Cell (CC), Apical Microvilli (AMV), Secretory Cells (SC)



b: Transverse section of *N. viridula* adult's midgut showing Apical Vesicle (AV) from columnar cells, Shredded Vesicles (SV) into Lumen, Degenerated Midgut Epithelial cells (DME) and Space Formation (SF) after 24h. of fungal infection



c: Transverse section of *N. viridula* adult's midgut showing cells, Degenerated Midgut Epithelial cells (DME), Space Formation (SF) and vacuolated cell (VC) after 48h fungal infection



d: Transverse section of *N. viridula* adult's midgut showing attack of fungus on layers of midgut. Reduced Longitudinal Muscles (RLM), Reduced Circular Muscles (RCM), Diffused Midgut Epithelial Cells (DMEC), Space Formation (SF) and Hyphae (H) in Lumen after 72h fungal infection

Fig. 2. Histoarchitecture of different parts of mid gut of *N. viridula* adult comparing control (a) with treated adult with *B. bassiana* after 24h (b), 48h (c) and 72h (d) (G10x40)

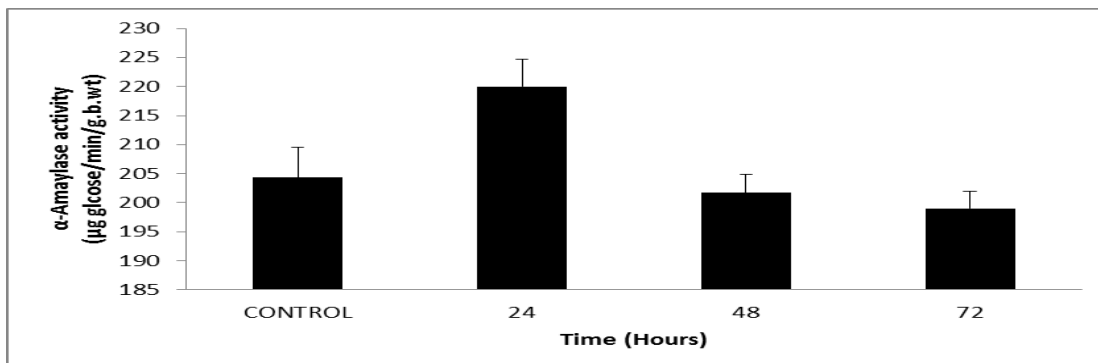


Fig. 3. The α -amylase activity of *N. viridula* adults treated with *B. bassiana*

After 48h of treatment, the midgut's epithelial cells of treated adults were disintegrated, reduced longitudinal muscles and reduced circular muscles (Fig. 1b&2c) and the blastospores appeared in the adults and gradually filled their digestive tract. (Fig.1b).

Furthermore, after 72h of treatment the midgut layers were separated, the fungal hyphae colonized in the muscle of gut and after that permeated through the epithelial cells into the gut lumen (Fig. 2d).

Fungal infection caused many vacuoles appeared in section of the gut epithelium cells, the cells were completely dissolved and separated from the basement membrane. The epithelial midgut cells started decreasing gradually. The adjacent cell adhesion was

lost slowly. The gut cells were atrophied and disorganized especially 72h after fungal treatment (Fig. 2b, c, d).

Effect of *B. bassiana* on digestive enzymes in *N. viridula* adult:

α - Amylase activity

The α -Amylases activity increased sharply after 24h of fungal treatment. It showed a significant increase of 220 $\mu\text{g glucose}/\text{min}/\text{g.b.wt}$ compared with 204.36 $\mu\text{g glucose}/\text{min}/\text{g.b.wt}$ in control (7.6% increase in enzyme activity). Then it gradually decreased after 48 and 72h (201.7 and 199 $\mu\text{g glucose}/\text{min}/\text{g.b.wt}$, respectively) (Fig. 3).

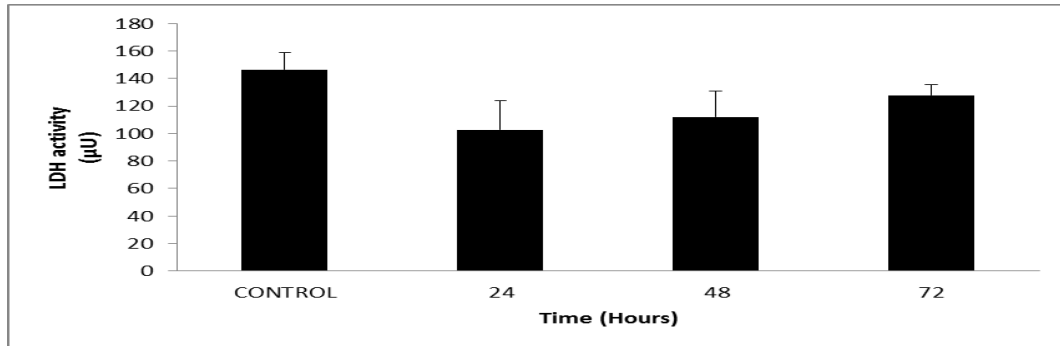


Fig. 4. The lactate dehydrogenase (LDH) activity of *N. viridula* adults treated with *B. bassiana*

Lactate dehydrogenase (LDH) activity

The obtained results showed that the activities of LDH in *N. viridula* adults were, affected with *B. bassiana* treatment. The enzyme activity was decreased significantly, after 24h of fungal infection. It reached 102.67 µU which is a 29.8% decrease in activity compared with control (146.33 µU). Then, it slightly increased after 48 and 72h (112 µU and 127.67 µU 10^3 /g.b.wt), respectively but remained significantly lower than control (Fig. 4).

Effect of *B. bassiana* infection on some mineral content in the adult of *N. viridula*

Results presented in Table (1) indicated significant decreased the Calcium (Ca), Magnesium (Mg) and Phosphorus (Ph) contents in the adult of *N. viridula* after fungal infection.

Ca content recorded 62.8±2.17 ug/g.b.wt in the adult of *N. viridula* after 24h. of fungal infection. That it was 27.7% significantly decreased in Ca content comparing with the untreated *N. viridula* adult which recorded 86.87±3.16ug/g.b.wt. The content of calcium then, fluctuated after 48h and 72h (73.83±2.83 and 61.97 ±4.21ug/g.b.wt), respectively. (Table.1).

Table 1. Effect of *B. bassiana* treatment on some minerals profile of *N. viridula* adults

Time (hours)	Minerals (ug/g.b.wt)		
	Calcium	Magnesium	Inorganic Phosphorus
Control	86.87±3.16 ^a	146.33±12.7 ^a	703.3±24.8 ^a
24	62.8±2.17 ^b	102.67±20.9 ^c	480.3±10.5 ^c
48	73.83±2.83 ^a	112±19 ^b	507.7±9.07 ^b
72	61.97±4.21 ^b	127.67±7.64 ^b	533.7±14.8 ^b

Values are means of 3 replicas ± S.E.

Means followed by the same letter within the same column are not significantly different at 0.05 probability level.

The most significant decrease in Mg content was 102.67±20 ug/g.b.wt after 24h of treatment, which stand for 29.8% regression in Mg content comparing with control (146.33±12.7 ug/g.b.wt.). Also, treatment of *N. viridula* adult with *B. bassiana* for 48h. and 72h., significantly reduced Mg content comparing with control (112±19 and 127.67±7.64 ug/g.b.wt, respectively) (Table. 1).

Also, Ph take the same trend as magnesium did. Treatment of *N. viridula* adult with *B. bassiana* for 24h resulted in the most significant decreases in Ph content, that it reached 480.3± 10.5 ug/g.b.wt which represent 31.7% regression in Ph content comparing with control (703.3±24.8 ug/g.b.wt.). After 48h and 72h of fungal infection Ph content was significantly lower than control (507.7±9.07 and 533.7 ± 14.8 ug/g.b.wt, respectively) (Table,1).

DISCUSSION

Beauveria bassiana is recognized as an essential source of mycopesticides and entomopathogenic control agents for a variety of pests all over the world (Asi *et al.*, 2013; Han *et al.*, 2014). The efficacy of entomopathogenic fungus in curatively controlling white grubs can be improved if those are integrated with other pathogens such as *Bacillus thuringiensis var. kurstaki* and *Heterorhabditis bacteriophorabur* (Laznik *et al.*, 2012).

Many investigations on the histological changes which occurred as a result of entomopathogenic fungal infection on different insect species using light and electron microscopy were discussed (Ragavendran *et al.*, 2017; Benzina *et al.*, 2018 ; Intodia *et al.*, 2019).

In the present investigation the mid gut was chosen part to carry out study the histological study, as it was noted that gastric cells were significantly damaged after infection with the fungus compared to other parts of the gastrointestinal tract.

In the present study, the crypts of the midgut of infected adult were damaged. The columnar epithelial

cells slipped into the gut lumen, and became enlarged and granular especially 48h post treatment. The epithelial cells were completely dissolved after 72h. Infection through the digestive system has been observed histologically for *B. bassiana* in adult of *N. viridula* (Fig. 2). After 72h of fungal treatment, the majority of the midgut epithelium had been destroyed and the cells lacking nuclei were also observed. This may be the reason of the death of infected insects. On contrast, a slight effect was observed in the adult's mid gut 24h post infection. These results corroborate with Quesada-Moraga *et al.* (2006) who attributed the death of *S. littoralis* larvae after fungal infection by *B. bassiana* may due to the fungal toxic protein resulting in a gradual bleeding of the midgut epithelium cells into the gut lumen. Also, the epithelium layer lysed. Similarly, the present results agree with those found by Jun-Nan *et al.* (2020) who noticed the invasion of the host by *B. bassiana* has triggered the host's defense mechanism as well as the immune response by induction of immune proteins and enzymes. But, the propagation of *B. bassiana* fungi throughout the host's body disrupted the host's normal physiological functions. This research results show similarities with those observed by Fan *et al.* (2007), they suggested that host's mortality is often caused by the action of fungal toxin, the physical obstruction of blood circulation and the destruction of the host internal organs caused by the fungal invasion.

Metabolism of carbohydrates-which maintain the structures and function of insect tissues- is controlled mainly by amylase, trehalase and invertase enzymes, (Wigglesworth, 1972). These enzymes are perplexed when challenged with unusual circumstances such as fungal and bacterial attack (Mead, 2000). The activity of α -Amylases showed a significant increase after 24h during; the time needed for spores' penetration through the cuticle, that was in consistent with the findings of Abdou *et al.* (2017) who declared that after administration of *B. bassiana*, activities of α -Amylase in *A. craccivora* adults slightly not significantly increased with respect to control.

Lactate dehydrogenase catalyzes the inter conversion of pyruvate and lactate with concomitant inter-conversion of NADH and NAD⁺ in glycolysis cycle and when there is a lack of oxygen or tissue degradation, LDH levels rise, and it is used as a medicinal diagnostic tool (Kaplan and Pesce, 1996). Some research attributed the elevation of LDH activity after challenging with *B. bassiana* to the production of toxic secondary metabolites by the entomopathogenic fungi to disable immune system of insects (Mirhaghparast *et al.*, 2013). In addition, increasing the LDH activity meets to the energy requirements for removing parasite from haemolymph by elevation of

glycolysis rate leading to conversion of pyruvate to lactate.

On the contrary, in the present study, activity of lactate dehydrogenase was significantly decreased after 24h of treatment as compared with control. The obtained result agreed with Chaurasia *et al.* (2016) who reported that the decrease of LDH activity in the haemolymph of treated cockroaches *Periplaneta americana* (Linnaeus) with entomopathogenic fungi *Isaria fumosoroseus*, may be caused by the insect's low nutritional ability, which will affect all subsequent vital activities at the same time. So, an entomopathogenic fungi may occur via overcoming on immune responses and discrepancies of intermediary metabolism. These finding show significant role of these agents against agricultural pests and might be used to improve their quality and efficiency in future.

Calcium is a second critical transmitter in all cells and is essential for proper functioning of the nervous system, muscles and eggshell structure (Dow, 2017). The fluctuation of Ca content level in this study may be due to the toxic secondary metabolites produced from *B. bassiana* invading. Wei *et al.* (2017) reported that *B. bassiana* produced oosporein toxin which affect the host immunity defenses, increased the virulence and harm the muscular system.

Magnesium is an essential mineral that helps to keep polyphosphates like ATP stable and polyphosphates included in DNA and RNA synthesis. Magnesium is also involved in the development of over 300 enzymes (Dow, 2017).

Visanuvimol and Bertram (2011) reported that Nitrogen and phosphorus are required by organisms to build proteins, RNA, DNA, and ATP. Phosphorus available during growth can have a major impact on lifelong history characteristics. The phosphor-rich ribosomal RNA rRNA is essential for cell growth because it makes up 50-60% of the ribosome, the cell's growth machinery and an average cell needs several million ribosomes to help protein synthesis, which could be affected by any disturbance in phosphorus content (Elser *et al.* 2000).

In line with these results, the mineral content in the infected adult results suggest that *B. bassiana* weakens the host's immune system, inhibiting the activities of various protective and detoxifying enzymes and finally, causing mortality of the host. These results may due to the fungal toxin and its destruction of the host internal organs and the fungal effect on the host fat cells.

CONCLUSIONS

In the present study, fungal infection strongly affected the midgut epithelial tissue. It caused damage in the different parts of the midgut after 48 and 72h. Also, disturbance in the activity of some enzymes like α -amylase and Lactate dehydrogenase (LDH) is observed under the fungal treatment which is associated with the detoxifying and immune systems. So, it is believed that *B. bassiana* could play a vital role as an encouraging pest-control agent against *N. viridula* through integrated pest Management (IPM) programs.

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الملخص العربي

تقييم تأثير المعاملة بفطر *Beauveria bassiana* الممرض للحشرات على بعض التغيرات النسيجية والفسيلولوجية لحشرة البقعة الخضراء

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في الحشرات المعاملة. حيث سجلت أعلى درجة لنشاط الإنزيم بعد ٢٤ ساعة من بدأ المعاملة ثم إنخفضت تدريجياً بعد ٤٨ و ٧٢ ساعة. على العكس من ذلك ، إنخفض نشاط إنزيم لاكتيت ديهيدروجينيز بشكل كبير في الحشرات المعاملة بعد ٢٤ ساعة من بدأ المعاملة. فيما يتعلق بالمعادن ، إنخفض محتوى جميع المعادن المختبرة بشكل ملحوظ في الحشرات المعاملة بغض النظر عن الوقت بعد المعاملة الفطرية. وقد أشارت هذه الدراسة إلى أنه يمكن اعتبار الفطريات الممرضة للحشرات *B. bassiana* نموذجاً واعدًا للمكافحة البيولوجية لحشرة البقعة الخضراء *N. viridula*

الكلمات المفتاحية: الفطريات الممرضة للحشرات، بوفاريا باسيانا، البقعة الخضراء، دراسات نسيجية، النشاط الإنزيمي.

أجريت الدراسة الحالية لتحديد تأثير المعاملة بالفطريات الممرضة للحشرات *Beauveria bassiana* على بعض التغيرات النسيجية في القناة الهضمية الوسطى للطور البالغ لحشرة البقعة الخضراء *Nezara viridula* بعد ٢٤، ٤٨ و ٧٢ ساعة من المعاملة. كذلك تأثير المعاملة علي بعض الخصائص الفسيولوجية كنشاط إنزيمي الفا أميليز ولاكتيت ديهيدروجينيز و محتوى بعض المعادن كالكالسيوم والفسفور غير العضوي والماغنسيوم. أظهرت الدراسات النسيجية وجود العديد من التشوهات في المعدة الوسطى للطور البالغ لحشرة البقعة الخضراء، كما تسببت العدوى الفطرية في حدوث أضرار في مواقع مختلفة من المعدة الوسطى بعد ٤٨ و ٧٢ ساعة من المعاملة. زاد نشاط إنزيم الفا أميليز بشكل ملحوظ