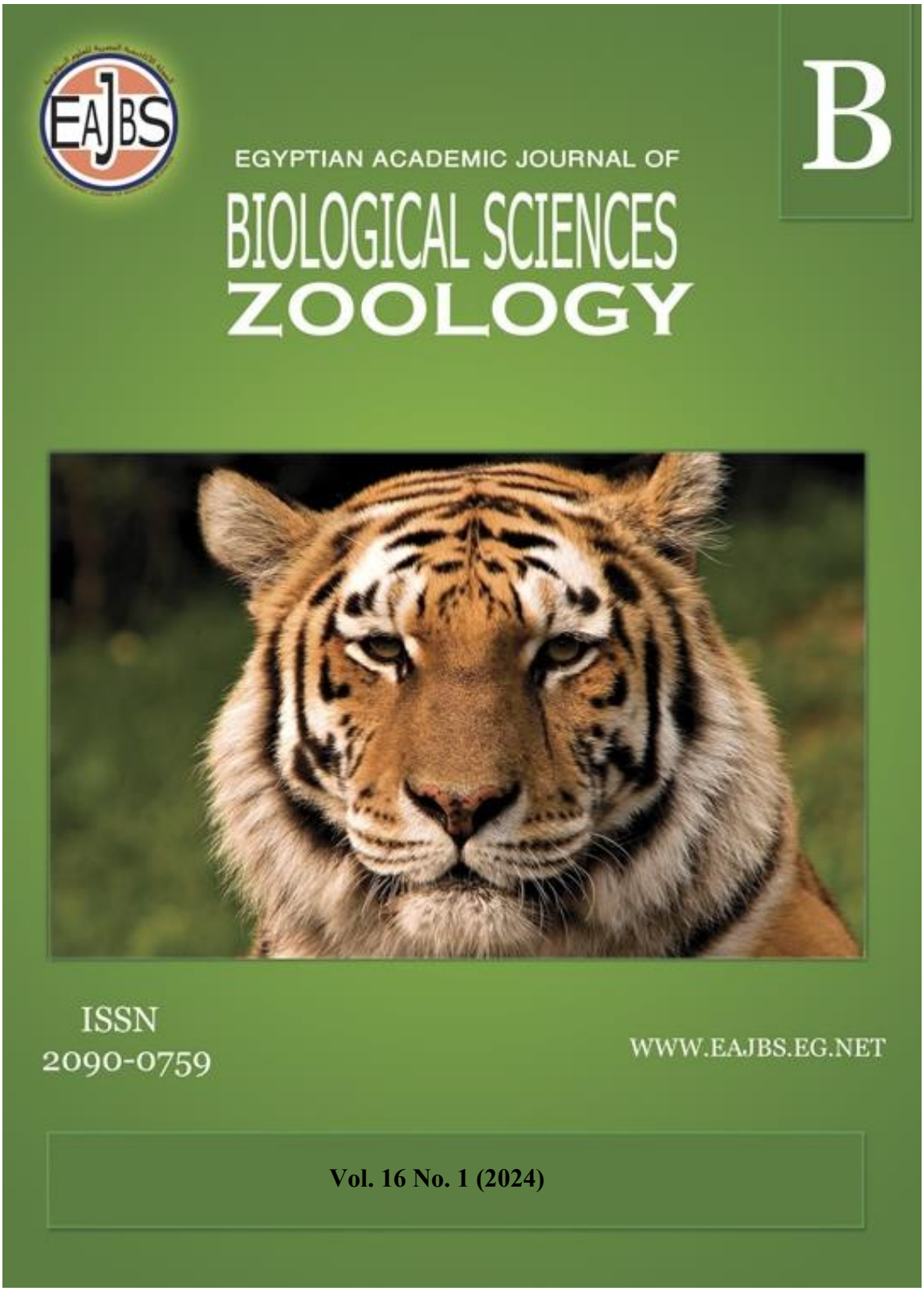
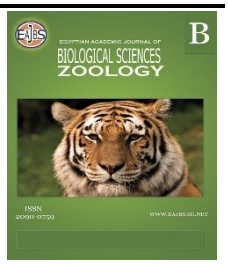


Citation: *Egypt. Acad. J. Biolog. Sci. (B. Zoology) Vol. 16(1) pp:129-143 (2024)*
DOI: 10.21608/EAJBSZ.2024.350554

Egypt. Acad. J. Biolog. Sci., 16(1):129-143(2024)

Egyptian Academic Journal of Biological Sciences
B. Zoology
ISSN: 2090 – 0759
http://eajbsz.journals.ekb.eg/



Unveiling the Biopesticidal impact of Entomopathogenic fungi, *Metarhizium anisopliae* AUMC 3262Against *Nezara viridula* (Linnaeus) (Hemiptera: Pentatomidae)

Rana H.M. Hussien; Mogeda M. Abdel Hafez ; Heba A. Ismail and Rehab M. El-Gendy*
Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, 12618, Egypt.
*** E-mail** : rehabgendy4@gmail.com ; hebaismail1379@gmail.com

ARTICLE INFO
Article History
Received:13/3/2024
Accepted:17/4/2024
Available:21/4/2024

Keywords:
Entomopathogenic fungi;
Metarhizium anisopliae; Nezara viridula;
Biological control

ABSTRACT

Nezara viridula (Linnaeus) (Hemiptera: Pentatomidae), commonly known as the southern green stink bug, poses a significant threat to agricultural productivity due to its destructive feeding nature which leads to substantial reductions in crop quality and yield. The entomopathogenic fungal genus Metarhizium (Hypocreales: Clavicipitaceae) has been known as a promising biological control agent with a wide range of arthropod hosts. Among them, *Metarhizium anisopliae* has shown potential as an alternative management strategy for controlling pests within crop ecosystems. This study aimed to assess the efficacy of different doses of *M. anisopliae* AUMC 3262 air-dried conidia (0.5, 2.5, and 5 g) against *N. viridula* adults and evaluate physiological and structural alternations under laboratory conditions. Results indicated that *M. anisopliae* AUMC 3262 dry conidia induced significant mortality in *N. viridula* in a time- and dose-dependent manner. Mortality rates reached 86.65%., 99.98% and 100 % of *N. viridula* after 5 and 6 days of exposure to 0.5, 2.5 and 5 g of dry conidia, respectively. Also, a significant reduction in the total soluble proteins was observed. Scanning Electron Microscope analysis confirmed deformation in the body structure of *N. viridula,* highlighting fungal penetration and proliferation within the insect's body. Overall, *M. anisopliae* AUMC 3262 is a virulent strain for *N. viridula,* which might qualify this strain to be used as a strong candidate for further investigation as a control agent for *N. viridula.*

INTRODUCTION

The green stink bug, *Nezara viridula* L. (Hemiptera: Pentatomidae), is a serious polyphagous insect pest known for causing severe damage to various crops, including economic crops, such as fruits, nuts, vegetables, and grains (Ademokoya *et al*., 2022). *N. viridula* infestations result in a yield reduction of up to 80% (Corrêa-Ferreira and De Azevedo, 2002). The extent of their damage varies depending on factors such as crop phenology, plant species, and developmental stage (Gore *et al.*, 2006; Mollah *et al.*, 2017).

These stink bugs feed on various plant parts, including stems, leaf veins, growing shoots, immature fruits, seeds, and flowers (Meglič *et al.*, 2001). Piercing-sucking feeding behavior allows the insects to inject toxic saliva and digestive enzymes into the plant,

producing discoloration, reduction of leaflets and pods, and delay in crop maturity. Additionally, the punctures they create may serve as entry points for microorganisms that affect fruit quality (Panizzi *et al.*, 2000). For instance, *N. viridula* can transmit *Pantoea agglomerans* an opportunistic bacterium, into green cotton bolls, leading to plant diseases (Gino Medrano *et al.*, 2009). Eventually, feeding damage significantly reduces the yield and the quality of infested crops (Soria *et al*., 2017).

The primary management strategies to reduce *N. viridula* and other stink bugs rely heavily on using chemical insecticides which are environmentally unfriendly agronomic practices (Giacometti *et al.*, 2020), leading to the development of insect resistance. However, the availability of chemical insecticide treatments worldwide, controlling piercing-sucking insects like *N. viridula* remains challenging due to their highly mobile nature (Snodgrass *et al.*, 2006; Takeuchi and Endo, 2012; Portilla, 2014).

Therefore, a pressing need for the development of safe alternatives to eradicate or reduce chemical pesticide utilization has become an urgent necessity. Unlike viruses, bacteria, and protozoa, which infect their hosts through the digestive tract, Entomopathogenic fungi EPF conidia germinate and penetrate insect host cuticle then rapidly proliferate and colonize the insect's hemocoel before emerging and sporulating on its cadavers (St. Leger and Wang, 2010). *M. anisopliae*, is one of the most important EPF that have been mass-produced and prepared for use in various pest management programs. Numerous studies have demonstrated the broad host range of Metarhizium species against various stink bug populations (Martins *et al.*, 2004; Xavier and Ávila, 2006). For instance, three strains of *M. brunneum* (ARSEF 4556, ARSEF 3297, native strain) were tested for their effectiveness against adult and nymph of brown stinkbug (*Euschistus heros*) and green-belly stinkbug (*Diechops furcatus*). The findings showed that nymphs in their early instars were more vulnerable to fungal strains than adults and late instars. Additionally, it emphasized the potential for focusing on adults to decrease oviposition and on eggs to stop nymphs from emerging and spreading (Resquin-Romero *et al.*, 2020). Given the evidence of *M. anisopliae's* high pathogenicity in insects and its capacity for laboratory multiplication (Rohde *et al.*, 2006), selecting isolates of this fungus for controlling *N. viridula* may provide valuable insights into pest management. Therefore, the objective of this study was to evaluate the pathogenicity of *M. anisopliae AUMC 3262* isolate on *N. viridula* adults and assess their physiological and structural impacts under laboratory conditions. The findings from this study may offer a promising avenue for developing practical solutions to combat this pest, thereby mitigating associated risks and making a meaningful contribution toward addressing this critical issue. Implementing these findings in Integrated Pest Management (IPM) programs could significantly enhance pest control strategies and promote sustainable agricultural practices.

MATERIALS AND METHODS

Insect Maintenance:

Adult individuals of *N. viridula* L. were collected from sesame, *Sesamum indicum L.* fields in El-Sharkia province (latitude 30.608754 - longitude 31.490958)Egypt. The obtained adult groups were put in gauze cages (28X28X25 cm). *N. viridula* adults were fed every day by placing fresh Purslane plant (*Portulaca oleracea* L.). The cages were housed at 27 ±2 ºC, RH 65±5 % and 16:8H (L:D) photoperiod. The bugs were adapted to the laboratory condition for at least three days before starting the assays.

Fungal Isolates and Inoculum Preparation:

The origin and molecular identity of *M. anisopliae* AUMC 3262 were characterized by Ezzat *et al.* (2019) and Kortsinoglou *et al.* (2020). Before use in this study, *M. anisopliae* AUMC 3262 was first passed through the insect host, *G. mellonella,* to restore virulence,

before being re-isolated on Sabouraud dextrose agar supplemented with 0.2% w/w yeast extract (SDAY) (Inglis *et al*. 2012). Briefly, Aerial conidia of *M. anisopliae* AUMC 3262 were mass-produced on autoclaved broken rice following the protocol outlined by (Jaronski and Jackson, 2012). Erlenmeyer flasks (250 ml) containing 50 ml of Sabouraud dextrose broth Yeast (SDBY) used for biomass production by liquid fermentation. Three autoclaved flasks (replicates) of SDBY medium were inoculated with 0.8 cm discs of *M. anisolpiae* AUMC 3262 collected from 7 days old culture plates and placed for 5 days in an orbital shaker incubator at 26 ± 0.5°C and 150 rpm. Three replicates of 1 L flasks containing 200 g of autoclaved rice grains were then inoculated with 20 ml of each liquid media, manually homogenized, and incubated for a further 15-20 days at 26 ± 1°C, then dried at 35±2°C until moisture content was 5%. The harvested dry conidia were stored in air-tight plastic containers in the dark at 4 °C for further use. Before each assay, the viability of the spores was assessed according to the method outlined by Inglis *et al*. (2012).

Effect of *M. anisopliae* AUMC 3262 Dry Conidia on *N. vridula:*

To evaluate the efficacy of *M. anisopliae* AUMC 3262 dry conidia against *N. viridula*, sets of transparent plastic boxes (25X25X15 cm) with 9 cm Petri dishes placed in the box centre, were prepared. Three boxes were prepared for each fungal treatment of doses 0.5, 2.5, and 5 g. Subsequently, okra pods (*Abelmoschus esculentus*) were placed on the central Petri dish and coated with different doses of fungal dry conidia as a food source, then covered the boxes with perforated lids to allow airflow. Control boxes consisted of a central Petri dish with okra pods introduced without any treatment in the center. Batches of five *N. viridula* adults were released on each box. After 24h of exposure to okra pods treated with different doses of dry conidia, the treated and untreated Petri dishes were replaced by new dishes with untreated food daily. The experiment was conducted at 27 ±2 ºC, RH 65±5 % and 16:8H (L:D) photoperiod. Mortality rates were assessed every 24 h for 7 days. The number of deceased *N. viridula* insects in each treatment and control was counted, and the percentage of mortality was calculated accordingly.

Total Soluble Protein Content:

The total soluble protein content was determined calorimetrically following the procedure outlined by Gornall *et al*. (1949). A violet-purple color developed upon the addition of Biuret reagent and the protein intensity was measured at a wavelength of 546 nm.

Scanning Electron Microscopy (SEM):

Scanning electron microscopy (SEM) analysis was performed at the Faculty of Agriculture, Mansoura University, Egypt, aiming to study the attachment and germination of the *M. anisolpiae* AUMC 3262 dry conidia on *N. viridula* cuticle in the thorax and abdomen leg. The insect cadavers were chosen from the 5 g treatment and control group. The cadavers were surface sterilized with 1% sodium hypochlorite for 2 min then rinsed twice with sterilized water before being incubated in a sterilized Petri dish lined with moistened filter paper to encourage fungal sporulation for further investigation. Conidia attached to the cuticle of *N. viridula* and germination was observed with SEM. Because of the large size of the insects, the cadavers were cut into small pieces using sterilized razor blades (dorsal, ventral, abdomen, and legs). Infected pieces were fixed in 2.5 % buffered glutaraldehyde in 0.1 M phosphate-buffered saline (PBS), pH 7.4 at 4 °C for 24 h, and then washed three times in PBS (10 min each). The samples were then fixed in 1 % osmic acid for 30 min and rewashed three times in PBS (10 min each). Dehydration was carried out in an ascending series of ethyl alcohol concentrations 30, 50, 70, 90 % and absolute alcohol infiltrated with acetone, each concentration for 30 min. For observation, the samples were then dried and mounted on aluminum stubs coated with gold in an SPI-ModuleTM Vac/Sputter 7. Photographs of the samples were then taken using a scanning electron microscope (JEOL JSM 6510 LV) equipped with an imaging system. (-7600F).

Statistical Analysis:

The effects of different amounts of fungal dry conidia and time exposure on *N. viridula's* mortality were analyzed using logistic regression under a generalized linear models (GLM) framework. Multiple comparisons between treatment pairs (Hothorn *et al.*, 2008). The data obtained from the biochemical studies were presented as means and statistically analyzed using one-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD) test. Significance was established at $p \leq 0.05$ using computer statistical software (CoStat, 2005).

RESULTS

Pathogenicity Assay:

Exposure of *N. viridula* adults to okra pods treated with different doses of *M. anisopliae* AUMC 3262 dry conidia (0.5, 2.5 and 5g) for 24 h revealed that mortality was dose-time dependent (Fig.1). On the first day post-treatment, no mortality was observed across all treatments. The group treated with 0.5 g exhibited increased mortality levels on the 6th day, reaching a maximum average mortality of 86.65%. Whereas, the 2.5 g treatment resulted in significantly higher mortality rates, reaching 99.98% on the 6th day. Remarkably, the group treated with 5 g displayed cumulative mortality reaching 100% after 5 days. Conversely, the control group exhibited a mortality rate of 33.31% after 6 days. The lower dose of 0.5 g exhibited accumulative mortality of 86.65%, while 2.5 and 5 g displayed 99.98 and 100 respectively, after 6 days compared to control which exhibited 33.3%.

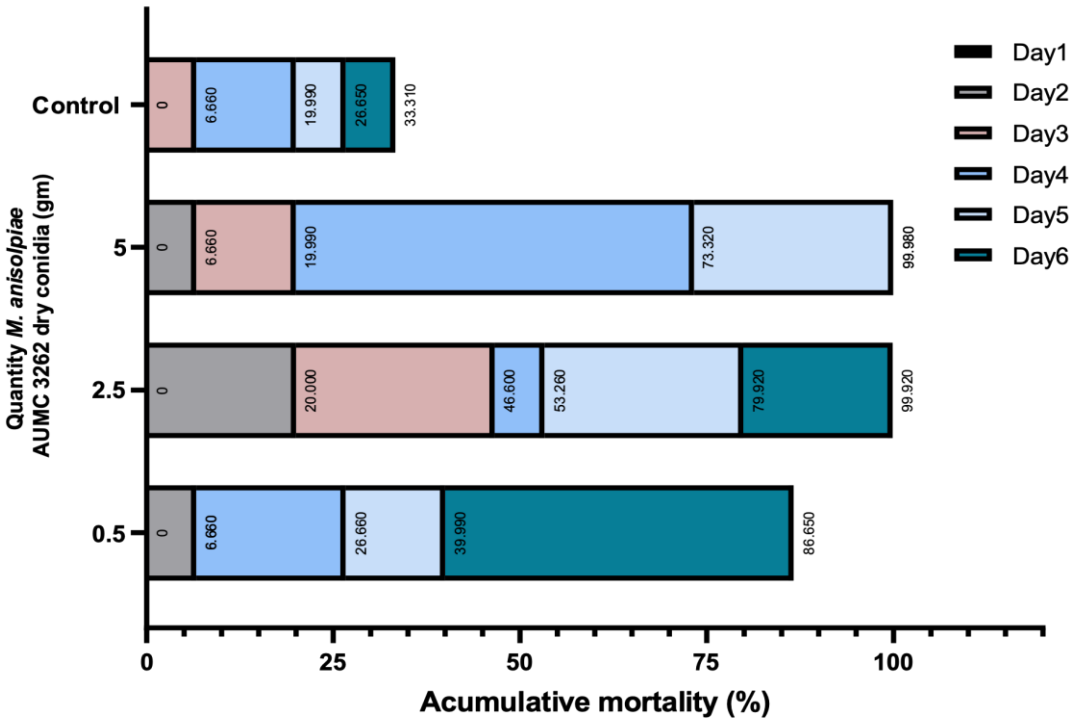


Fig.1: Accumulative mortality % of *N. viridula* after direct exposure to different doses of 0.5, 2.5, and 5 g of *M. anisolpiae* AUMC 3262 dry conidia at 25±2 ºC and 16:8H (L:D).

Total Soluble Protein (TSP):

The data presented in Figure 2, indicate that the TSP (mg/g b. wt.) levels of *N. viridula* adults treated with different doses of *M. anisolpiae* AUMC 3262 dry conidia were significantly reduced with time compared to the control group. Specifically, the groups

administered higher amounts of dry conidia 2.5 and 5 g exhibited a slight increase in protein levels with values of 4.81 ±0.38 and 5.56 ±0.4 after 1 h, respectively, compared to the control group with values 4.017±0.09 (mg/g b. wt.). Whereas, after 24 hr, 2.5 and 5 g exhibited a decrease in protein levels with values of 2.82 ±0.48 and 5.59 ±0.8, respectively, compared to the control group with a value of 9.52 ±0.34(mg/g b. wt.).

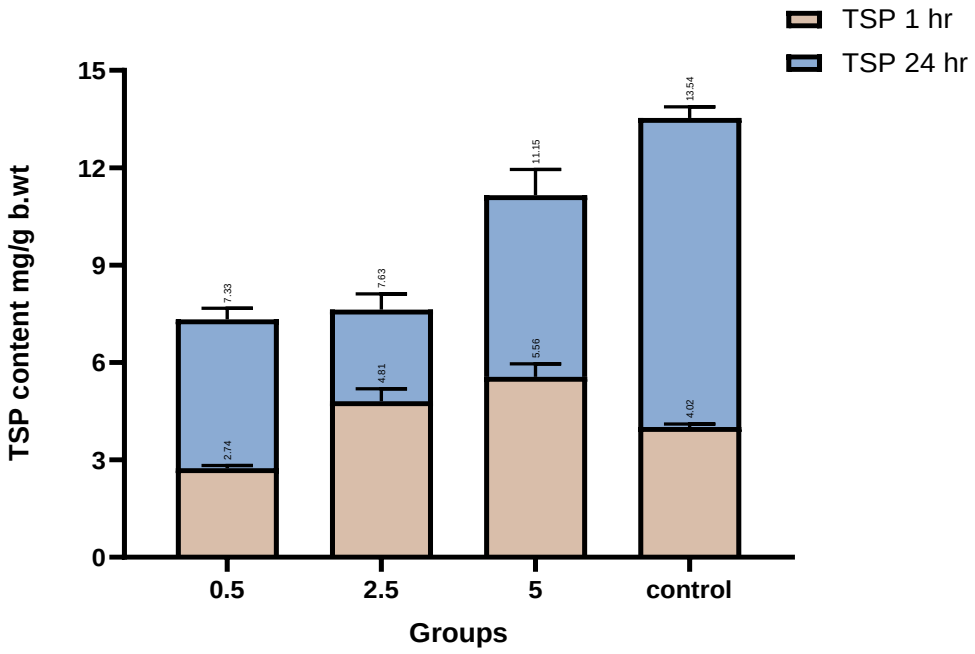


Fig. 2: Effect of *M. anisolpiae* AUMC 3262 dry conidia on total soluble protein content in *N. viridula* after 1 and 24 h.

Scanning Electron Microscopic Observation:

It was clear that the dry conidia of *M. anisolpiae* AUMC 3262 adhered to almost any part of the *N. viridula* cuticle in the thorax, abdomen and leg. Complete colonization of the stink bugs by the fungal isolate was visually apparent at 10 days post-inoculation (Fig. 3). Characteristic green, powdery masses of fruiting bodies were observed on the surface of the bug cadavers, Individuals killed by fungi had a dry appearance and external conidiogenesis that was evident before the end of the 10-day observation period. No cases of fungal infection were observed on insects collected from the control.

The impact of *M. anisolpiae* AUMC 3262 dry conidia on the external morphology of crucial anatomical structures, namely the cuticle, leg and abdomen within adult *N. viridula* were systematically investigated in this study by using SEM. The whole body was covered by *M. anisopliae* dry conidia causing cuticle degradation (Fig.4E). In the present study, SEM observation showed the appearance of the external thoracic surface of the *N. viridula* in the control group (Fig. 4A). In adults exposed to *M. anisopliae* AUMC 3262, the dry conidia were observed attaching to the surface of the adult body also germination of the conidia and growing of few hyphae were observed (Fig. 4B). No fungal growth was observed in untreated control external abdominal surface (Fig.4C) whereas; little conidiogenesis occurred on external abdominal surface due to adult activity (Fig.4D) fungal isolate showed fungal hyphae growth on the exoskeleton of treated adult: conidia adhesion and different stages of germination adhered to the surface of the cuticle, fungal hyphae grew heavily and together formed a dense network of mycelium covered the insect body after ten days of inoculation (Fig. 4E) in high magnification view, conidia and many spores appeared scattered in between the mycelia (Fig. 4F). The mycelial extrusion of *M. anisopliae* AUMC

3262 was more in the intersegmental areas resulting in a process of cuticle degradation along the whole body of the insect.

The fungal dry conidia attached to and penetrated some vulnerable sites in the insect's body, such as leg joints. Most of the observed conidia were on the adult leg joints. Colonization covers the insect leg, as depicted in (Fig. 5 B) these areas were easily invaded, with many conidia adhering and producing hyphae (Figs. 5 C-E) a higher magnified view for extruding conidia in the legs of *N. viridula varicella* (Fig. 5 F).

Microscopic examination of untreated *N. viridula* adult showed a normal structure of the cross-section of the abdomen (Fig 6 A) with no fungal growth observed inside the body cavity. On the other side, the treated *N. viridula* at low magnification (25 X) showed a mycelium network covering the whole-body cavity of the adult on the cross-section of the abdomen, with a deformation in the abdomen structure (Fig.6 B). At higher magnification (2500 X) the internal body surface was covered completely by adhering hyphae and conidia. Details of hyphal penetration of insect internal structure cuticles as well as proliferation (Fig.6 D) in comparable with normal ones was characterized by the presence of special orientation at 2500X (Fig 6 C). Conidiophore formation and conidiogenesis inside the body cavity with degradation of the visceral contents were noticed in (Figs. 6E-G).

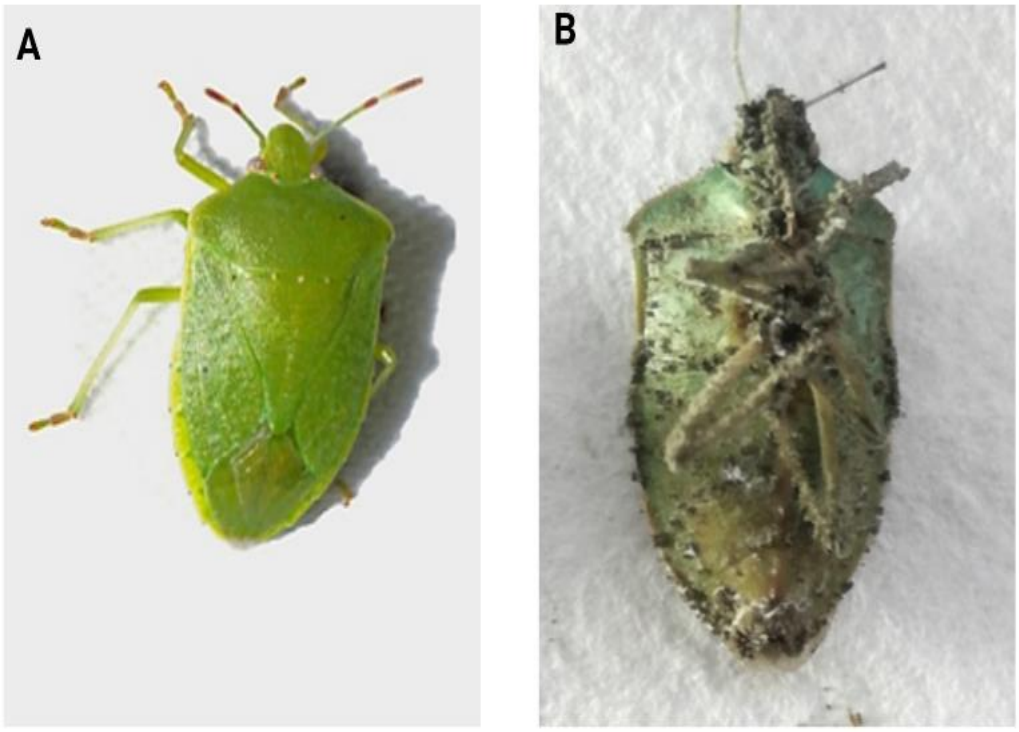


Fig. 3: (A)Normal *N. viridula*, (B) Conidiogenesis of *M. anisolpiae* AUMC 3262 on the body surface of adult *N. viridula* 10 days after insect death.

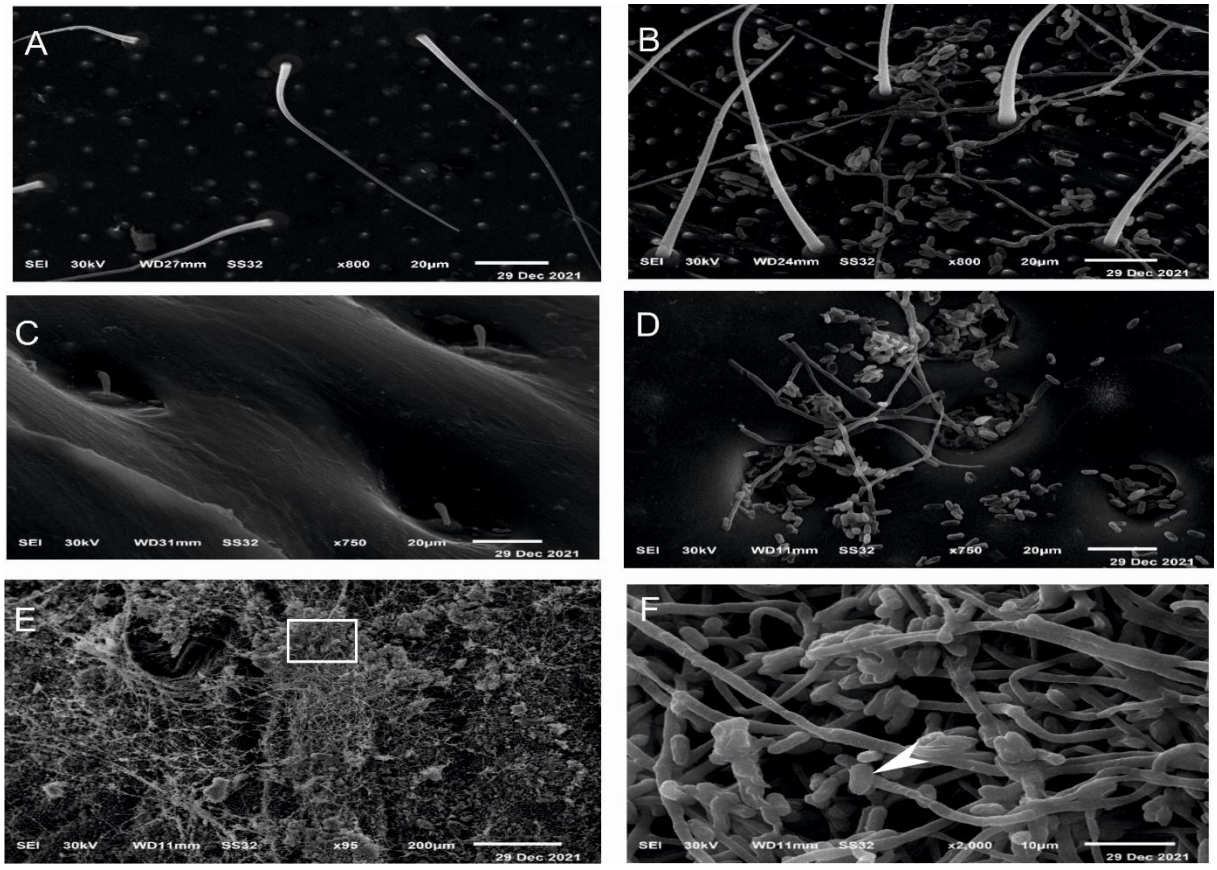


Fig. 4: SEM photomicrograph for thorax and abdominal region of *Nezara viridula* adult (normal and treated with *Metarhizium anisopliae* AUMC 3262 after 10 days.
(A): Dorsal view of the thoracic region of normal adult (B): dorsal view of infected Adult treated with *M. anisopliae* AUMC 3262 after 10 days showing scattering with conidia covering the thoracic surface. (C): ventral view of normal abdominal region (D): ventral view of infected adult in the abdominal region treated with *M. anisopliae* AUMC 3262 after 10 days showing hypha with little conidiogensis covering the surface (E): Adult Exoskeleton with spread heavily growth fungal hypha (F): high magnification of condensed fungal growth on adult exoskeleton.

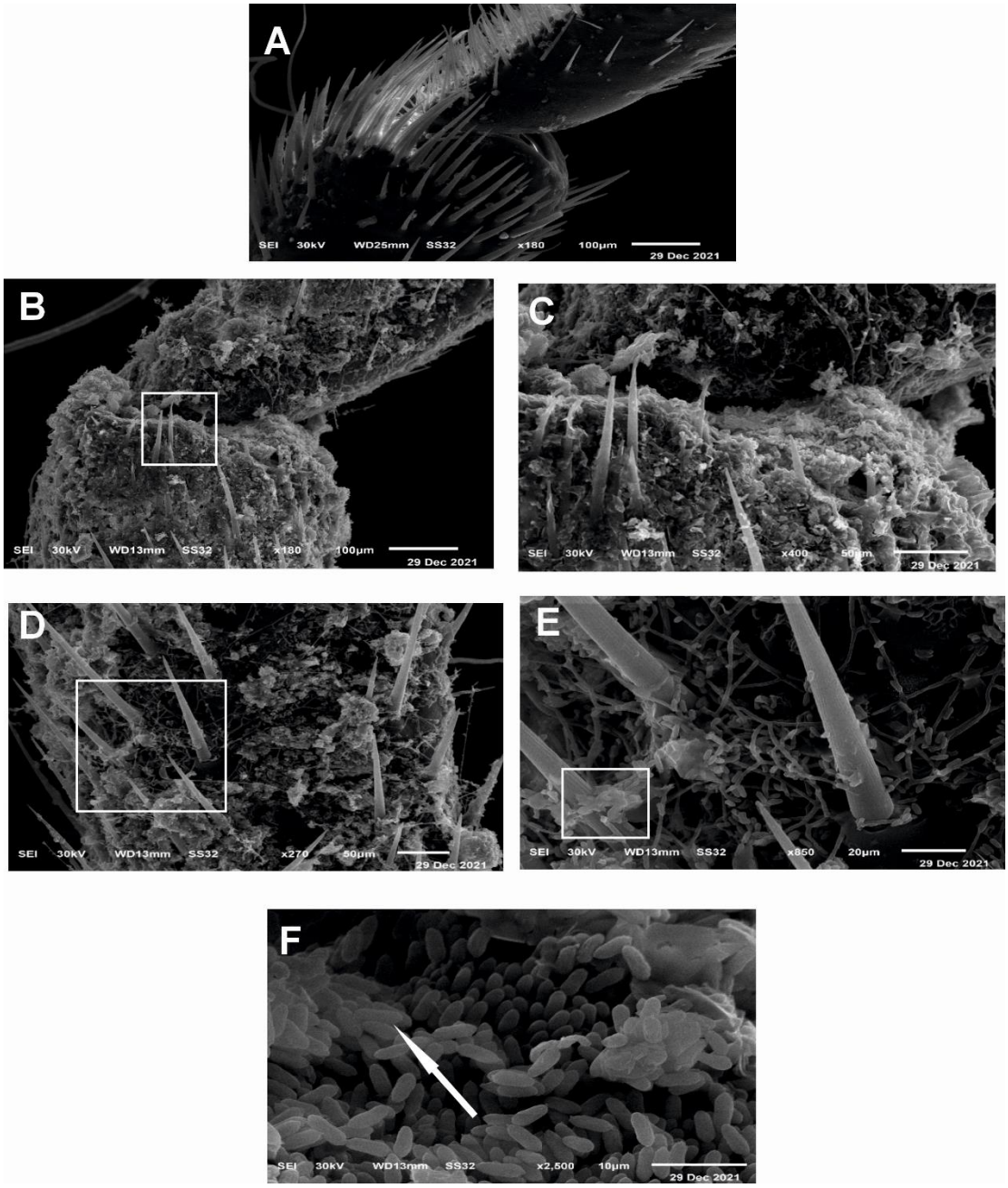


Fig. 5: SEM photomicrograph Leg joints of *Nezara viridula* adult (normal and treated with *Metarhizium anisopliae* AUMC 3262 after 10 days.
(A): Normal Leg of adult of *N. viridula* (B): treated Leg showing collapsed conidia with *M. anisopliae* AUMC 3262 after 10 days (C,D,E,F): Different magnification of *M. anisopliae* conidia covering leg joint.

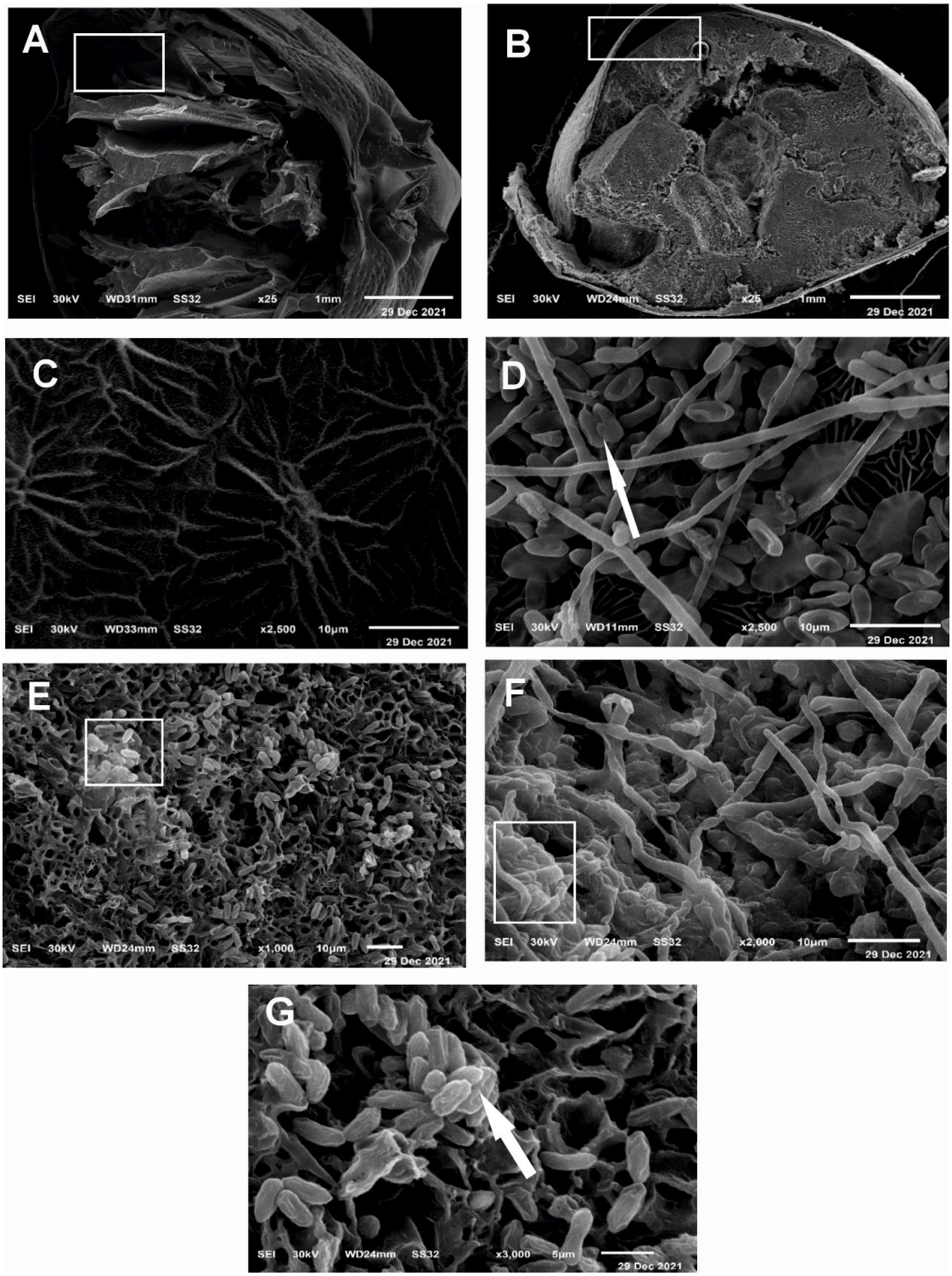


Fig. 6: SEM photomicrograph of cross-section for internal body surface in descent abdominal region of *Nezara viridula* adult (normal and treated with *Metarhizium anisopliae* AUMC 3262 after10 days.

(A): Normal internal body cavity in abdominal region (B): internal body cavity of infected adult showing mycelium network of *M. anisopliae* AUMC 3262 after 10 days (C): Normal internal surface in abdominal region (D): internal body surface of infected adult was covered completely by adhering hyphae and conidia in abdominal region treated with *M. anisopliae* AUMC 3262 (E, F, G): Conidiophore formation and conidiogenesis inside the body cavity with degradation of the visceral contents at 3 high magnification.

DISCUSSION

Entomopathogenic fungi (EPF), are known for their wide host range and effectiveness in combating insect pests. EPF utilization as a biocontrol agent has increased dramatically as a safe, cost-effective alternative to chemical pesticides (Khan *et al.*, 2012). With diverse applications, they offer a versatile solution for addressing a broad spectrum of insect pests (Khan *et al.*, 2012; Castro *et al.*, 2016). The study provided evidence that 24-h exposure to dry conidia of *M. anisolpiae* AUMC 3262 enhanced conical attachment and facilitated *N. viridula* adult infection. Our findings revealed a significant increase in mortality, with rates reaching 86.5%, 99.98% and 100% after 5 and 6 days of exposure to 0.5, 2.5 and 5 g of dry conidia, respectively. Similarly (Dromph and Vestergaard, 2002) recorded that continuous exposure to high concentrations of conidia may increase the likelihood of infection and increase mortality. Also flying ability of *N. viridula* might play an important role in the dispersal of these fungi. Although *N. viridula* adults have been reported to be fungi static to EPF and caused low natural infection rates in this insect population (Sosa-Gómez *et al*., 1998), the observation in this study indicates that the mortality is dose-dependent, and suggests that the adhesive power of the dry conidia to the cuticle was influenced by the fungal strain and genetic variability among fungal isolates (da Silva *et al*., 2015; Raafat *et al*., 2015). Furthermore, *M. anisolpiae* AUMC 3262 is characterized by high viability and rapid germination which is an attribute of virulent strains (Hussien *et al*., 2021). Moreover, *M. anisolpiae* AUMC 3262 conidia possess a high level of spore-bound Pr1 which may collaborate with other enzymes and degrade fungi static compounds at the host surface and concomitantly release nutrients that stimulate germination (Hussien *et al*., 2021). Ultimately, the susceptibility of *N. viridula* to *M. anisopliae* infection was dependent on the composition of hydrocarbons and aldehydes found in cuticle extracts and scent glands (da Silva *et al*., 2015; Bot *et al*., 2002).

The release of antifungal compounds might be spatially and temporally regulated. In general, the physiological levels of such anti-fungal agents vary with the life stage and therefore may dictate stage-specific susceptibility of insect phytopathogens. Furthermore, Some EPF produces secondary metabolites to stop the action of antifungal compounds. These metabolites can be produced in different amounts by different isolates of the same species. For instance, *B. bassiana* fungus produces destruxins that modify the activity of P-glycoprotein, a mediator of fungal detoxification in insects. (Rohlfs and Churchill, 2011).

Proteins serve as essential building blocks within all living cells, encompassing various substances such as enzymes, hormones, and antibodies which are crucial for an organism's proper functioning (Fagan *et al.,* 2002). Our data demonstrate a reduction in TSP content in groups treated with 2.5 and 5 g of dry conidia after 24 h compared to the untreated group. This decline could be attributed to using proteins in enzymatic detoxification processes (El-Gendy and El-Shafiey, 2018) aimed at counteracting the insecticidal effects of *M. anisopliae* AUMC 3262 dry conidia. These results are supported by the findings of Nada (2015) who revealed that *M. anisopliae* reduces TSP, carbohydrates, and lipids in *N. viridula* after treatment with *B. bassiana EPF.* In the same context, varying quantitative of EPF, including *M. anisopliae*, *B. bassiana*, and *Paecilomyces lilicanu* causes decreases in TSP in the 3rd instar larvae of *Culex pipiens* (Hamama *et al.,* 2022). Moreover, *M. anisopliae* causes a decline in protein content in desert locust *Schistocerca gregaria* two days post-infection (Ouedraogo *et al.,* 2002; Seyoum *et al.,* 2002). This decrease in TSP leads to the retardation of many physiological processes in insects, which require protein for promoting ovulation and egg development contributing to the mortality of treated insects (Elbanna *et al.,* 2012).

The utilization of SEM has significantly enhanced our understanding of EPF's mode of action with their hosts. Our SEM analysis revealed that *N. viridula* possesses multiple penetration sites which enable fungal dry conidia to rapidly colonize the host (Altre &

Vandenberg, 2001). Fungal conidia appeared with characteristic attachment and germination at vulnerable sites such as leg joints, some parts of the hind wing, and mouthparts which allow faster infection with fungal dry conidia. Furthermore, *M. anisopliae* dry conidia are characterized by hydrophobic proprieties which allow them to adhere to the waxy epicuticle of the host by the combination of passive hydrophobic forces, electrostatic forces, and protein interactions between the conidia and the epicuticle.

Our research observation revealed widespread cuticle degradation throughout the insect's body after exposure to *M. anisopliae* AUMC 3262 dry conidia, which might be explained by the mechanical pressure exerted by the germinated conidia combined with the production of a mixture of cuticle-degrading enzymes (proteases, lipases and chitinases) and eventually penetrating insect cuticle (St. Leger and Wang, 2010; Butt et al., 2016). Similarly, Moino Jr *et al.*, 2002 observed haloes appearing on the insect cuticle at the sites of germination and penetration points for *M. anisopliae.* The occurrence of these haloes was due to the production and excretion of exoenzymes by the entomopathogen during the infective process (Dutta *et al.*, 2018).

This study revealed significant deformations in the structure of the insect abdomen cross-section, characterized by hyphal proliferation and limited colonization into the integument of infected *N. viridula*. Limited conidiogenesis occurred on the abdomen of the *N. viridula,* this may be attributed to its feeding habits and behavior, which harbor a significant number of microorganisms in its digestive system. These microorganisms may compete with the fungal pathogen, thereby impeding the optimal growth of the entomopathogen (Moino Jr *et al.*, 2002).

Furthermore, our findings indicate that the fungus persists, after the death of the host; the hyphae extrude the host cuticle forming a denser network and spores on the cadaver of the infected host and transmitting to the outer environment to infect a new host. This underscores the tenacity of *M. anisopliae* AUMC 3262 efficacy and its potential use as an effective biocontrol agent in *N. viridula* integrated management strategies.

CONCLUSION

In light of the challenges posed by insecticide resistance in managing the green stink bug, *N. viridula*, our study underscores the potential of *M. anisopliae* AUMC 3262 dry conidia as a promising eco-friendly alternative. Through our experiments, we have demonstrated its significant impact on adult *N. viridula*, with notable mortality rates and TSP reduction observed, complemented by structural abnormalities identified through SCM. These findings provide valuable insights into the mode of action of *M. anisopliae* AUMC 3262 dry conidia. The implications of our research extend to sustainable pest control, offering a novel approach to safeguarding crops and maintaining yield quality. Future efforts may concentrate on product development and application strategies, thereby advancing sustainable and effective agricultural practices on a global scale.

Declarations:

Ethical Approval: This study does not involve any research activities that require ethical approval.

Conflicts of Interest: The authors declare that they have no competing interests.

Informed consent: All the authors of this manuscript accepted that the article is submitted for publication in the Egyptian Academic Journal of Biological Sciences, B. Zoology, and this article has not been published or accepted for publication in another journal, and it is not under consideration at another journal.

Authors Contributions: All authors have contributed equally and significantly to the manuscript and gave final approval for publication. All authors have read and agreed to the published version of the manuscript.

Funding: No funding was available for this research.

Availability of Data and Materials: All relevant data have been presented within the manuscript text, tables and figures. Further information can be provided by the corresponding author upon reasonable request.

Acknowledgements: The authors would like to express their gratitude to Prof. Hend M. Sabry for her invaluable assistance in analyzing and illustrating the SEM photos.

REFERENCES

Altre, J. A., & Vandenberg, J. D. (2001). Penetration of cuticle and proliferation in hemolymph by Paecilomyces fumosoroseus isolates that differ in virulence against lepidopteran larvae. *Journal of Invertebrate Pathology, 78(2), 81-86.*

.Ademokoya, B.; Athey, K. and Ruberson, J. (2022). Natural Enemies and Biological Control of Stink Bugs (Hemiptera: Heteroptera) in North America, *Insects*, 13(10): p. 932.

Castro, T.; Mayerhofer, J., Enkerli, J., Eilenberg, J., Meyling, N. V., de Andrade Moral, R., Demétrio, C. G. B. and Delalibera Jr, I. (2016). Persistence of Brazilian isolates of the entomopathogenic fungi Metarhizium anisopliae and M. robertsii in strawberry crop soil after soil drench application. *Agriculture, Ecosystems & Environment*, 233, 361-369.

Corrêa-Ferreira, B.S. and De Azevedo, J. (2002). Soybean seed damage by different species of stink bug. *Agricultural and Forest Entomology*. 4(2): 145–150.

Costat Statistical Software (2005): Microcomputer program analysis version, 6.311. Co Hort Software,Monterey, California, USA.

da Silva, R.A.; Quintela, E.D., Mascarin, G.M., Pedrini, N., Lião, L.M. and Ferri, P.H. (2015). Unveiling chemical defense in the rice stalk stink bug against the entomopathogenic fungus *Metarhizium anisopliae*. *Journal of Invertebrate Pathology*,127: 93–100.

Dutta, P.; Pegu, J., Kaushik, H., Kaman, P. and Das, A. (2018). Scanning Electron Microscopy (SEM) study showed the mode action of *Metarhizium anisopliae* on *Odontotermes obesus*. *International Journal of Current Microbiology and Applied Sciences, 7*: 901-906.

Dromph, K.M. and Vestergaard, S. (2002). Pathogenicity and attractiveness of entomopathogenic hyphomycete fungi to collembolans. *Applied Soil Ecology*, 21:197–210.

El-Gendy, R.M. and El-Shafiey, S.N. (2018). Eco-Friendly Control Strategies of Green Stink Bug, *Nezara viridula* L. (Hemiptera: Pentatomidae): Repellency and Toxicity Effects of *Callistemon citrinus*, Bottle Brush Essential Oil. *Journal of Plant Protection and Pathology*. 9(12): 807–813.

Elbanna, S. M.; Elhadidy, N. M., Semida, F. M. and Abdel Rasool, T. (2012). Physiological and biochemical effect of entomopathogenic fungus *Metarhizium anisopliae* on the 5th instar of *Schistocerca gregaria* (Orthoptera: Acrididae). *Jornal. Research. Environmint. Science. Toxicology*, 1(1):7–18.

Ezzat, S.M.; El-Sheikh, A.A. and Hussien, R.H.M. (2019). Mass production of *Metarhizium anisopliae* AUMC 3262 strain isolated from Egyptian habitat and its virulence against *Spodoptera littoralis* larvae (Boisd.). *Annals of Agri-Bio Research*, 24: 277–282.

Fagan, W. F.; Siemann, E., Mitter, C., Denno, R. F., Huberty, A. F., Woods, H. A., and Elser, J. J. (2002). Nitrogen in insects: implications for trophic complexity and species diversification. *The American Naturalist*. 160(6);784–802.

Giacometti, R.; Jacobi, V., Kronberg, F., Panagos, C., Edison, A. S., and Zavala, J. A. (2020). Digestive activity and organic compounds of *Nezara viridula* watery saliva

induce defensive soybean seed responses. *Scientific Reports.*10(1):15468.

Gino Medrano, E.; Esquivel, J. F., Nichols, R. L., and Bell, A. A. (2009). Temporal analysis of cotton boll symptoms resulting from southern green stink bug feeding and transmission of a bacterial pathogen. *Journal of Economic Entomology*. 102(1): 36–42.

Gore, J.; Abel, C. A., Adamczyk, J. J., and Snodgrass, G. (2006). Influence of soybean planting date and maturity group on stink bug (Heteroptera: Pentatomidae) populations. *Environmental Entomology*.35(2): 531–536.

Gornall, A.G.; Bardawill, C.J. and David, M.M. (1949). Determination of serum proteins by means of the biuret reaction. *Jornal of biological Chemistry*, 177(2): 751–766.

Hamama, H. M.; Zyaan, O. H., Ali, O. A. A., Saleh, D. I., Elakkad, H. A., El-Saadony, M. T., and Farag, S. M. (2022). Virulence of entomopathogenic fungi against *Culex pipiens*: Impact on biomolecules availability and life table parameters. *Saudi Journal of Biological Sciences*, 29(1): 385–393.

Hothorn, T.; Bretz, F. and Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal: Journal of Mathematical Methods in Biosciences*, 50(3): 346–363.

Bot, A.N.M.; Ortius-Lechner, D., Finster, K., Maile, R. and Boomsma, J.J. (2002). Variable sensitivity of fungi and bacteria to compounds produced by the metapleural glands of leaf-cutting ants. *Insectes Sociaux*, 49: 363–370.

Butt, T.M.; Coates, C.J., Dubovskiy, I.M. and Ratcliffe, N.A. (2016). Entomopathogenic fungi: new insights into host–pathogen interactions. *Advances in Genetics*, 94: 307–364.

Hussien, R.H.M.; Ezzat, S.M., El Sheikh, A.A., Taylor, J.W.D. and Butt, T.M. (2021). Comparative study of fungal stability between Metarhizium strains after successive subculture. *Egyptian Journal of Biological Pest Control*, 31: 1–6.

Inglis, G.D.; Enkerli, J. and Goettel, M.S. (2012). Laboratory techniques used for entomopathogenic fungi: Hypocreales. *Manual of techniques in invertebrate pathology*, 2: 189–253.

Jaronski, S.T. and Jackson, M.A. (2012). Mass production of entomopathogenic Hypocreales. *Manual of techniques in invertebrate pathology*. 2nd Ed. Academic press, London, U.K. 255:284.

Khan, S.; Guo, L., Maimaiti, Y., Mijit, M., and Qiu, D. (2012). Entomopathogenic fungi as microbial biocontrol agent. *Molecular Plant Breeding* 3(7). 63-79.

Kortsinoglou, A. M.; Saud, Z., Eastwood, D. C., Butt, T. M., and Kouvelis, V. N. (2020). The mitochondrial genome contribution to the phylogeny and identification of Metarhizium species and strains. *Fungal biology*. 124(10): 845–853.

St. Leger, R.J. and Wang, C. (2010). Genetic engineering of fungal biocontrol agents to achieve greater efficacy against insect pests, *Applied Microbiology and Biotechnology*, 85: 901–907.

Martins, J. F. D. S.; Botton, M., Carbonari, J. J., and Quintela, E. D. (2004). Eficiência de *Metarhizium anisopliae* no controle do percevejo-do-colmo *Tibraca limbativentris* (Heteroptera: Pentatomidae) em lavoura de arroz irrigado. *Ciência Rural*, 34:1681–1688.

Meglič, V.; Virant-Doberlet, M., Šuštar-Vozlič, J., Sušnik, S., Čokl, A., Miklas, N. and Renou, M. (2001). Diversity of the southern green stink bug *Nezara viridula* (L.) (Heteroptera: Pentatomidae). *Journal of Central European Agriculture*. 2(3–4): 241–250.

Moino Jr, A.; Alves, S. B., Lopes, R. B., Neves, P. M. O. J., Pereira, R. M. and Vieira, S. A. (2002). External development of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in the subterranean termite *Heterotermes*

tenui., *Scientia agricola*, 59: 267–273.

Mollah, M. M. I.; Rahman, M. M., Soyema, K., Mukta, M., and Akon, M. R. (2017). Toxicity of botanical and chemical insecticides on stink bug complex (Heteroptera: Pentatomidae) in lablab bean (Lablab purpureus Lin.) field, *Journal of Entomology and Zoology Studies*; 5 (2). 537–541.

Nada, M.S. (2015) 'Response of green stinkbug *Nezara viridula* (Linnaeus), to the activity of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae. Journal of Plant Protection and Pathology*, 6(12): 1633–1644.

Ouedraogo, R. M., Kamp, A., Goettel, M. S., Brodeur, J. and Bidochka, M. J. (2002). Attenuation of fungal infection in thermos regulating *Locusta migratoria* is accompanied by changes in hemolymph proteins. *Journal of Invertebrate Pathology*, 81(1):19–24.

Panizzi, A. R.; McPherson, J. E., James, D. G., Javahery, M. and McPherson, R. M. (2000). Stink bugs (Pentatomidae), *Heteroptera of economic importance*, 828.

Portilla, M. (2014). Biological control as an alternative measure for tarnished plant bug control in Mississippi., *MidSouth Entomology*, 7: 70–78.

Raafat, I.; Meshrif, W.S., El Husseiny, E.M., Seif, A.I.and El-Hariry, M. (2015). *Nezara viridula* (Hemiptera: Pentatomidae) cuticle as a barrier for *Beauveria bassiana* and Paecilomyces sp. infection. *African Entomo*logy, 23: 75–87.

Rohlfs, M. and Churchill, A.C.L. (2011). Fungal secondary metabolites as modulators of interactions with insects and other arthropods. *Fungal Genet. Biol*. 48: 23–34.

Soria, M.F.; Degrande, P.E., Panizzi, A.R. and Toews, M.D. (2017). Economic injury level of the neotropical brown stink bug *Euschistus heros* (F.) on cotton plants. *Neotropical Entomology*, 46: 324–335.

Resquín-Romero, G., Cabral-Antúnez, C., Sarubbi-Orue, H., Garrido-Jurado, I., Valverde-García, P., Schade, M., & Butt, T. M. (2020). Virulence of Metarhizium brunneum (Ascomycota: Hypocreales) strains against stinkbugs Euschistus heros and Dichelops furcatus (Hemiptera: Pentatomidae). *Journal of Economic Entomology*, *113*(5), 2540-2545.

Rohde, C.; Alves, L.F.A., Neves, P.M.O.J, Alves, S.B., Silva, E.R.L. and Almeida, J.E.M. (2006). Selection of isolates of *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metsch.) Sorok. against the mealworm *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae). *Neotropical Entomology*, 35(2): 231-240.

Seyoum, E.; Bateman, R.P. and Charnley, A.K. (2002). The effect of *Metarhizium anisopliae* var acridum on haemolymph energy reserves and flight capability in the desert locust, *Schistocerca gregaria. Journal of Applied Entomology*, 126(2-3): 119–124.

Snodgrass, G. L.; Scott, W. P., Abel, C. A., Robbins, J. T., Gore, J. and Hardee, D. D. (2006). Suppression of tarnished plant bugs (Heteroptera: Miridae) in cotton by control of early season wild host plants with herbicides. *Environmental entomology*, 35(5):1417–1422.

Sosa-Gómez, D.R. and Moscardi, F. (1998). Laboratory and Field Studies on the Infection of Stink Bugs, *Nezara viridula*, *Piezodorus guildinii* and *Euschistus heros* (Hemiptera: Pentatomidae) with *Metarhizium anisopliae* and *Beauveria bassianain* Brazil', *Journal of Invertebrate Pathology*, 71(2), pp. 115–120.

Takeuchi, H. and Endo, N. (2012). Insecticide susceptibility of *Nezara viridula* (Heteroptera: Pentatomidae) and three other stink bug species composing a soybean pest complex in Japan. *Journal of economic entomology*, 105(3):10 24–1033.

Xavier, L.M.S. and Ávila, C.J. (2006). Patogenicidade de isolados de *Metarhizium anisopliae* (Metsch.) Sorokin e de *Beauveria bassiana* (Bals.) Vuillemin a

Scaptocoris carvalhoi Becker (Hemiptera, Cydnidae). *Revista Brasileira de Entomologia*, 50: 540–546.

ARABIC SUMMARY

الكشف عن تأثير المبيد الحيوي الفطري الممرض للحشرات *Metarhizium anisopliae* AUMC 3262على البقه الخضراء *Nezara viridula* (Linnaeus) (Hemiptera: Pentatomidae)

رنا حسين محمد حسين ، مجده محمد عبد الحافظ ، هبه عبد الله إسماعيل ، ريحاب محمود الجندي
معهد بحوث وقاية النباتات- مركز البحوث الزراعية- الدقي- جيزه-12618- مصر

تشكل البقه *الخضراء Nezara viridula* (Linnaeus) (Hemiptera: Pentatomidae) تهديدًا كبيرًا للإنتاجية الزراعية بسبب طبيعتها الغذائية المدمرة التي تؤدي إلى انخفاض كبير في جودة المحاصيل وإنتاجيتها. يُعرف الفطر الممرض للحشرات *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae) بأنه أحد العوامل الواعده في المكافحة البيولوجية لمفصليات الأرجل. أظهر *Metarhizium anisopliae* إمكانية استخدامه كأحد عوامل استراتيجية إدارة بديلة لمكافحة للآفات داخل النظم البيئية للمحاصيل. تهدف هذه الدراسة إلى تقييم فعالية جرعات مختلفة من الكونيديا المجففة بالهواء 0.5،2.5، 5 جرام *M. anisopliae* AUMC 3262 ضد الأفراد البالغه من *N. viridula*. أيضًا تقييم التحورات الفسيولوجية والهيكلية تحت ظروف المعمل. أشارت النتائج إلى أن *M. anisopliae* AUMC 3262 conidia الجافة تسبب في حدوث نسبة موت كبيرة في *N. viridula* بطريقة تعتمد على الوقت والجرعة. وصلت معدلات الموت إلى 86.65% و99.98% و100% من *N. viridula* بعد 5 و6 أيام من التعرض لـ 0.5 و2.5 و5 جرام من الكونيديا الجافة على التوالي. كما لوحظ انخفاض كبير في إجمالي البروتينات القابلة للذوبان. أكد تحليل المجهر الإلكتروني الماسح وجود تشوهات في جسم حشرة البقه الخضراء *N. viridula* ، مما يسلط الضوء على اختراق الفطريات وتكاثرها داخل جسم الحشرة. بشكل عام، يعتبر *M. anisopliae* AUMC 3262 سلاله فتاكه للبقه الخضراء ، مما قد يؤهل هذه السلالة لاستخدامها كمبيد قوي في مكافحة البقه الخضراء.