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ORIGINAL RESEARCH ARTICLE

Susceptibility of fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to natural products and entomopathogenic fungi

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Abstract

Synthetic insecticides have a direct adverse effect on the natural enemies and long-term residual effects causing serious environmental pollution as well. Public awareness of a clean environment increased the attention to developing alternative eco-friendly approaches. The objectives of this study are the detection of the effect of *Beauveria bassiana*, *Metarhizium anisopliae*, and natural products of plant-extract origin on the fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) under laboratory conditions. The drench-bioassay results showed that mortality of larvae by *B. bassiana* KACC40224 increased from 10 to 80% as the dose was increased from 10×10^5 to 10×10^9 conidia ml^{-1}. However, mortality by *M. anisopliae* KACC40029 reached maximally 60% at the dose of 10×10^9 conidia ml^{-1}. All natural-extract products tested against the insect pest were effective, except lavender oil, which caused mortality to vary between 10 and 100%. Rosemary oil was found to be the most effective essential oil, showing 10% to 100% mortality indices at a concentration of 0.1 and 0.2% (v/v), respectively. *S. frugiperda* eggs tend to be more susceptible to entomopathogenic fungi rather than the larvae. The essential oils exhibited significant insecticidal properties against the larvae of *S. frugiperda*. This study could help in the development of potential biopesticides for the environment-friendly management of the fall armyworm *S. frugiperda* pest and emphasize the advantages of entomopathogenic fungi application.

Keyword Biopesticides · Natural products · Fall armyworm · Biocontrol

Introduction

The fall armyworm *Spodoptera frugiperda* (J.E. Smith) has recently become an invasive pest in Africa, the Far East, and North America (Jamil et al. 2021). It's a voracious pest that attacks sorghum, corn, sugarcane, forage grasses, turf grasses, cotton, rice, and peanuts (Naharki et al. 2020; Sarkowi and Mokhtar 2021; Anjorin et al. 2022). The polyphagous pest *S. frugiperda* was first detected in the corn-growing area Cagayan Valley, Philippines (Navasero et al. 2019). The recent introduction of this insect into other countries causes economic losses in yield crops. It is a good flier and disperses long mileage annually during the summer months (Montezano et al. 2018). In 2016 it appeared for the first time in Central and West Africa, currently, it threatens Africa and Europe. A high reproductive rate and fast development of the insect were demonstrated when fed with young corn leaves. Corn is the most important main cereal crop grown by small farmer (Naharki et al. 2020; Grote et al. 2021). Approximately 90% of corn production is used as food in many countries, particularly in Africa (Onu et al. 2018). Generally, the genus *Spodoptera* (family: Noctuidae) is responsible for great economic damage in important plant crops such as maize, cotton, and rice. Montezano et al. (2018) reported thirty-one insects of the genus *Spodoptera* (Noctuidae) are responsible for great economic loss in important plant crops. For example, *S. littoralis* is one of the most damaging pests of several fruits and vegetables worldwide (Wakil et al. 2020). Despite various control strategies, this pest is still able to cause significant destruction. The cotton leafworm *S. littoralis* is considered a key pest of cotton plants (*Gossypium hirsutum* L.), as well as corn (*Zea mays* L.) and tobacco (*Nicotiana tabacum* L.), in the Middle East and

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Asian countries. Larvae are voracious, causing important economic losses in both open fields and greenhouses on a broad range of industrial and vegetable crops (Naharki et al. 2020). Because of the severe damage to various plant crops, controlling this pest is a mandatory part of the integrated pest management programs where it exists. The cutworm *S. litura* (Lepidoptera: Noctuidae) is known to attack over one hundred twenty plant species among fruits, vegetables, and ornamentals worldwide (Wakil et al. 2020; Xia et al. 2020). The overwintering conditions of warm temperature and high humidity favor the reproduction and survival of fall armyworm, moreover, protect them from natural enemies. Therefore, although fall armyworm has several natural enemies few can succeed enough to prevent crop injury (Naharki et al. 2020). Synthetic insecticides have been used to control insect pests during the 1940s. Because of the adverse effects of these insecticides on the environment, their use is being controlled. Globally, there is a growing desire to reduce the use of synthetic chemical pesticides on agricultural lands, in respect of their toxicity to human health and the environment (Xia et al. 2020; Kalyabina et al. 2021). Likewise, the development of alternative techniques for crop pest management is encouraged. These techniques include the use of pathogens of insect pests and bioactive compounds of plant extracts (Flonc et al. 2021; Anjorin et al. 2022). Many efforts have been exerted to control this pest species using several chemical pesticides e.g. organophosphate and carbamates to which this insect has developed resistance. Therefore, finding new options is a critical demand to control this insect pest. The chemical diversity of plants is a potentially powerful source for controlling pests of this genus. There is an increasing interest in the use of entomopathogens for the biological control of insect pests as alternatives to chemical insecticides (Wakil et al. 2020). Microbial insecticides neither have toxic residues in the environment nor induce resistance in their insect hosts (Flonc et al. 2021). The public awareness of the unpolluted environment increased the attention on developing biological control agents (FAO 2017; Wakil et al. 2020). At present, chemical insecticides are still the most frequently used method for controlling insect-pest outbreaks (Barratt et al. 2018). However, many pesticides harm the natural enemies and cause serious environmental pollution due to long-term residual effects (Bottrell and Schoenly 2012). Besides the severe harmful effect on human health, toxic insecticides also damage the Earth. The spray application can spread through contaminated groundwater or wind may affect fish and wildlife, and subject delicate ecosystems to danger. The real environmental risk of these chemicals can be conceived by knowing that some chemicals banned nearly forty years ago are still detected in food products today (Kalyabina et al. 2021). Thus, to offer promising alternatives to detrimental synthetic chemical pesticides, it is necessary to explore eco-friendly pest management strategies utilizing bio-control agents that could achieve similar efficacy against this pest. The use of native entomopathogens such as viruses, bacteria, and fungi for the biological inhibition of *Spodoptera* species has become a goal for pest control (Vega 2018). In addition, natural compounds can play an important role in the management of insect pests through their insecticidal effects (Stankovic et al. 2020). The present study evaluated the virulence of *B. bassiana* KACC40224 and *M. anisopliae* KACC40029 strains against the voracious *S. frugiperda* and the effects of some natural products on the insect-pest larvae as well. The use of entomopathogenic fungi is one of the promising strategies with great potential to control insect pests and minimize the adverse effects of synthetic insecticides. This study aimed to detect the effect of *Beauveria bassiana*, *Metarhizium anisopliae*, and natural products of plant-extract origin on the fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) under laboratory conditions.

Materials and methods

Fungi and insects

The two fungi used in this study, i.e., *Beauveria bassiana* KACC40224 and *Metarhizium anisopliae* KACC40029 were identified and confirmed as entomopathogenic strains by the Korean Agricultural Cultural Collection (KACC), Jeonju, Korea. The larvae and eggs of the noctuid polyphagous pest fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) were provided by the National Institute of Agricultural Science and Technology, Chung-Cheong Bukdo, Korea.

Spore suspensions' preparation

Beauveria bassiana KACC40224 and *Metarhizium anisopliae* KACC40029 were grown on Difco™ Sabouraud Dextrose Agar and incubated at 25 ^{0}C under static conditions. After ten days of incubation, their conidia were harvested from the surface culture using a surgical scalpel. The plate was flooded with sterile distilled water (SDW) and the colony scraped under aseptic conditions. The conidia were transferred to Tween 80 solution (0.05%) and mixed well using a magnetic stirrer for 20 min (Inglis et al. 2012). The conidial suspension was passed through clinical gauze to separate the trapped hyphae. The number of conidia in the stock spore suspension was counted and using the cell counting chamber (Neubauer-improved, Marienfeld, Germany) a serial dilution (1×10^6 to 1×10^{10}) was prepared and the LC_{50} was detected.

Insect fungi-exposure bioassay

The immersion technique was followed during this investigation; ten larvae of the third instar per replicate were soaked in the conidial suspensions of each *B. bassiana* KACC40224 or *M. anisopliae* KACC40029. After drenching in the conidial suspension, each replicate was placed in a sterile Petri dish padded with wet filter paper. A solution of 0.05% Tween 80 was served as a control. The Petri dishes were incubated at 25 °C; 15 cm of fresh corn leaves was catered daily to each (Butt and Goettel 2000). The larvae were examined daily for two weeks, and the dead larvae were transferred to another moist chamber. The investigation was conducted in triplicate and average values were used. Similarly, the upper layer of eggs mass was removed using a sterile camel-hair brush No. 1 and using binocular dissecting (Model VT-II, Olympus, Japan), and the successive layer eggs was counted. Three replicates each containing fifty eggs was treated as mentioned above, placed in moist Petri dishes, and incubated at 25 ºC. The mortality % was checked daily for ten days. Dead eggs were counted and transferred to another moist chamber of Petri dishes. About 150 eggs in three replicates were used for each conidial concentration as well as the control (sterile 0.05% Tween 80 Soln). Mortality was calculated according to the Eq. (1):

$$Morality\ (\%) = \frac{Number\ of\ infected\ (dead)}{Total\ number\ of\ insects} \times 100 \qquad (1)$$

Insect natural product-exposure bioassay

The test was conducted using 100% pure essential oils purchased from the producer (Essential Oil Co. Ltd., Sydney, Australia). The natural products Deltamethrin (flowers extract of *Chrysanthemum indicum* L. family: Asteraceae), kefama1 (pyrethrin, 3%; matrine, 10%; *Syzygium aromaticum*, 20%; root extract of *Pulsatilla koreana*, 20%; parafine oil, 40%; polysorbate, 7%), and kefama2 (rotenone, 5%; matrine, 15%; *Phytolacca decandra*, 10%; *Helianthus tuberosus*, 40%; neem oil, 30%) were provided from the National Institute of Agricultural Science and Technology, Chung-Cheong Bukdo, Korea. Different dilutions (1, 0.2, 0.1% v/v) were prepared from each of the obtained natural products. The insect-dip bioassay technique was used against the *S. frugiperda* larvae as described above.

Scanning electron microscopic observation

Beauveria bassiana KACC40224 and *Metarhizium anisopliae* KACC40029 strains were inoculated in malt extract agar (MEA) medium and incubated at 25 °C. Ten days later, fungi were fixed using glutaraldehyde (2.5%) for 24 h at 4 °C followed by rinsing in the cacodylate buffer (0.1 M) for 15 min at 4 °C. The secondary fixation of fungi was done using osmium tetraoxide (1%) at 4 °C for 1 h. The samples were washed again in cacodylate buffer (0.1 M) at 4 °C to remove the unfixed osmium tetraoxide. After fixation, the fungi stubs were dried in a glass desiccator containing silica gel (RH 0%) for 72 h. Ultimately, the gold–palladium coating was performed in a Balzers BA 360 M high-vacuum evaporator for 120 S. Samples were then mounted on SEM holders and viewed using a JEOL JSM-T200 (Co. Ltd., Japan) scanning electron microscope.

Statistical analysis

The obtained data were statistically analyzed using SAS software (SAS 2011) for all results using Tukey's test (version 11.0) to compare the averages ($P > 0.05$).

Results

Entomopathogenic fungi pathogenicity

In this study, *B. bassiana* KACC40224 and *M. anisopliae* KACC40029 strains were evaluated, as suspension drenches, against the *S. frugiperda* eggs and the third instar larvae as shown in Table 1 and S1. Treatment efficacy was detected by assessing the mortality during development from the third instar through to pupae. Both strains were pathogenic to the *S. frugiperda* larvae but overall mortality varied between 13.4 and 80.0% (3.0% for control). In the case of eggs, mortality was more severe and varied between 41.0 and 91.0% (5.0–6.0% for control). The LC_{50}s of *S. frugiperda* larvae were 10×10^7 and 1.5×10^9 conidia ml^{-1} for *B. bassiana* KACC40224 and *M. anisopliae* KACC40029 strains, respectively. The LC_{50}s of *S. frugiperda* eggs were 10×10^6 for both entomopathogenic fungal strains. Our results showed that the mortality of larvae was increased by *B. bassiana* KACC40224 from 10 to 80%. However, mortality by *M. anisopliae* KACC40029 increased from 13 to 60% as the dose was increased from 10×10^5 conidia ml^{-1} to 10×10^9 conidia ml^{-1}. Eggs mortality after exposure to *B. bassiana* increased from 41 to 91%. However, mortality by *M. anisopliae* KACC40029 increased from 43 to 82% as the dose was increased from 10×10^5 conidia ml^{-1} to 10×10^9 conidia ml^{-1}. Also, the results indicated that *B. bassiana* KACC40224 strain was more virulent than *M. anisopliae* KACC40029 strain (Fig. 1). The *S. frugiperda* eggs were markedly more susceptible to the two entomopathogenic fungi (Fig. 2). The larvae of *S. frugiperda* were more resistant to infection by the entomopathogenic fungi than the eggs.

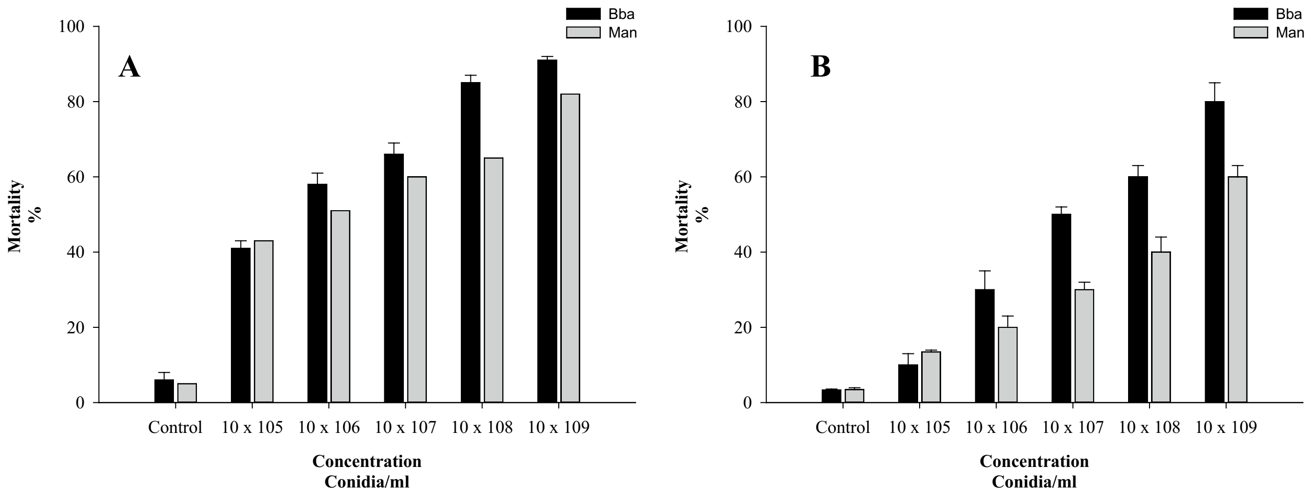

Fig. 1 Mortality caused by Beauveria bassiana KACC40224 and Metarhizium anisopliae KACC40029 application against Spodoptera frugiperda (**A**) eggs and; (**B**) their larvae of the third instar. There is a proportional relationship between the applied conidial concentration of the entomopathogenic fungi and the mortality of the insect-pest

Scanning electron microscopy (SEM)

SEM study exposed that the *B. bassiana* colony developed slowly with white creamy powdery growth on MEA media. The colony of *M. anisopliae* grew rather slowly, at first appearing floccose but later becoming olive-green with plentiful conidiation and white shadow (S2). The scanning electron microscopy study illustrated that the conidiogenous cells of *B. bassiana* KACC40224 arise singly from the vegetative hyphae with swollen stalks. The conidiogenous cells are cylinder-shaped with sympodial elongation and a zig-zag shape. Generally, the conidiogenous apparatus forms dense clusters. Conidia are likely to be sub-globose with a slightly pointed base. *M. anisopliae* KACC40029 conidiophores and phialides are aggregated in dense clusters with a branched verticillate and parallel arrangement. The conidia are cylindrical and produced in chains (S3).

Insecticidal properties of natural products

The current investigation showed that essential oils were active against the *S. frugiperda* larvae; whereas rosemary and ginger oils displayed a high ability to kill third instar larvae. The highest effect was shown by rosemary oil which caused mortality of 100% and 10% at 0.2% and 0.1% v/v of the oil, respectively. In this study, rosemary oil showed high mortality indices (100%) against third instar

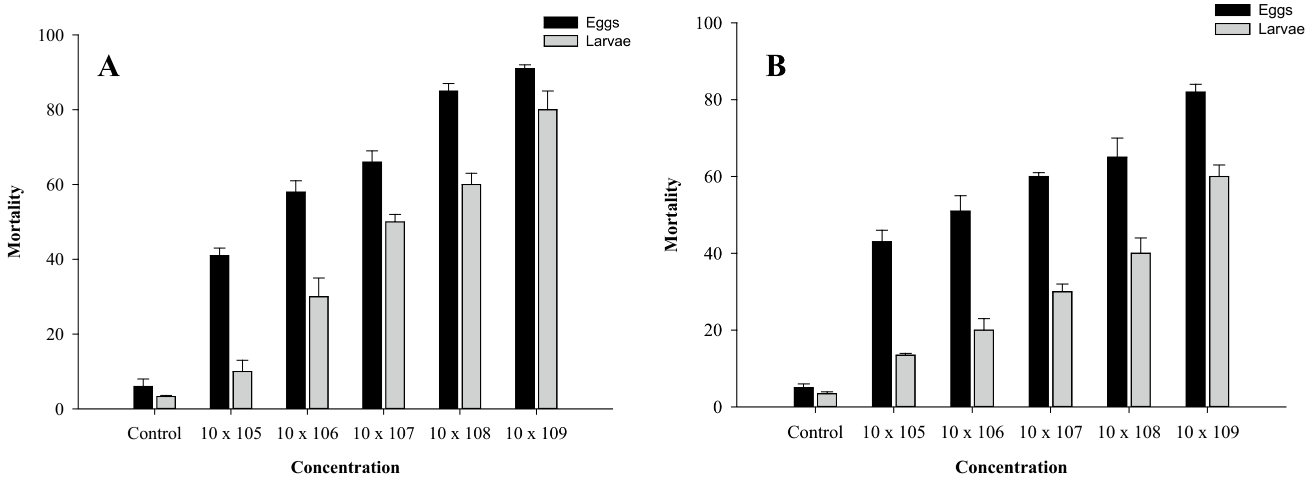

Fig. 2 Mortality caused by Beauveria bassiana KACC40224 (**A**); and Metarhizium anisopliae KACC40029 (**B**) application against Spodoptera frugiperda eggs and their larvae of the third instar using different concentrations (conidia/ml)

Table 1 Mortality caused by *Beauveria bassiana* KACC40224 and *Metarhizium anisopliae* KACC40029 application against the *Spodoptera frugiperda* larvae of the 3rd instar

B. bassiana KACC40224			*M. anisopliae* KACC40029		
	Eggs	Larvae		Eggs	Larvae
Control	6±2f	3.3±0.3f	Control	5.0±1e	3.4±0.51e
10 x 10^5	41±2e	10±3e	10 x 10^5	43±3d	13.4±0.51d
10 x 10^6	58±3d	30±5d	10 x 10^6	51±4c	20±3c
10 x 10^7	66±3c	50±2c	10 x 10^7	60±1b	30±2b
10 x 10^8	85±2b	60±3b	10 x 10^8	65±5b	40±4b
10 x 10^9	91±1a	80±5a	10 x 10^9	82±2a	60±3a

Means followed by similar letters in a column are not significantly different at $P < 0.05$

larvae. In this study, cloves oil was the inferior insecticide, showing mortality indices between 10 and 90% at the concentration of 1.0 and 0.2% (v/v), respectively. Neem oil showed mortality indices of only 20% at 0.2% (v/v) concentration, but caused 100% mortality at 1.0% (v/v) concentration (Table 2). Except for lavender oil, all essential oils were effective and overall mortality varied between 10 and 100% (0% for control); the average survival time was 24 h. Essential oils were weakly to highly toxic to *S. frugiperda* larvae, rosemary and ginger oils were strong even at 0.2% (100% mortality). Neem and cloves oils were moderately effective, while lavender oil didn't display any mortality. A large number of herbal plants possess pesticide activity, because of their bioactive components. Therefore, some of them are new potential biocontrol agents (Hussein and Joo 2018). Deltamethrin was highly toxic to *S. frugiperda* larvae of the third instar (90% mortality) only at a concentration of 1.0% (v/v), and it showed a moderate toxic effect (70% mortality) at 0.1% (v/v) concentration. Kefama1 insecticides showed mortality indices of only 20% at the same concentrations. However, kefama2 insecticides showed mortality indices between 40 and 30% at the same concentrations (Table 2). Generally, the two kefama insecticides used in this study exhibited mortality indices of less than 50% in *S. frugiperda* larvae of the third instar. Deltamethrin showed mortality indices between 90 and 30% at the same concentrations (Table 2).

Discussion

The entomopathogenic Hyphomycetous fungi e.g. *Beauveria bassiana*, are naturally soil dwellers (Butt and Goettel 2000). Hence, they can be technologically developed as biocontrol agents against soil-inhabiting pests without threat to non-target insects (Bamisile et al. 2021). Entomopathogenic fungi *B. bassiana* and *Isaria fumosorosea* are efficient biological agents in the management of multiple arthropod pests (Robles-Acosta et al. 2019). In a previous study, *B. bassiana* Bb10331and *B. bassiana* Bb7725 exhibited high virulence against the fall webworm *Hyphantria cunea* (family: Erebidae) larvae (Bai et al. 2015; Hu et al. 2021). Garrido-Jurado et al. (2019) demonstrated that all tested *B. bassiana* and *M. brunneum* isolates against *S. littoralis* prepupae using the soil drenches technique were pathogenic and caused mortality ranging from 31.7 to 83.3%. Their LC_{50} was 1.7×10^7 and 1.8×10^7 conidia ml^{-1}, respectively (Garrido-Jurado et al. 2019). The use of entomopathogenic fungi could be a key component of the IPM strategies due to the direct effect on soil-dwelling life stages and the significant reduction in the reproductive potentiality of insect pests (Bamisile et al. 2021; Deka et al. 2021; Idrees et al. 2021). Ismail et al. (2020) reported that the development of an effective method to control the cotton leafworm *S. littoralis* was urgently needed since it became a serious pest to many important economic crops in Egypt. The fungal species *Isaria fumosorosea* showed an adverse effect on the pupal development of *S. litura* (Idress et al. 2022). Also, Hussein et al. (2013) showed that the entomopathogens *I. fumosorosea* and *B. bassiana* had a significant effect on reducing the food consumption, growth rate, and egg hatchability of *S. litura*. These findings suggested *B. bassiana* and *I. fumosorosea* as effective eco-friendly mycoinsecticides against *S. litura* (Garrido-Jurado et al. 2020). Although several microbial pathogens have demonstrated the ability to reduce the incidence of fall armyworm pests in crops, only fungal entomopathogens are most feasible, due to direct penetration of the insect cuticle on foliage. Indigenous strains of entomopathogenic bacteria tend not to be robust, but the genetically modified strains improve their performance (Koppenhöfer et al. 2020).

Table 2 Mortality caused by selected natural products preparations against the *Spodoptera frugiperda* larvae at the third instar

Conc.	Deltamethrin	Kefama1	Kefama2	Lavender oil	Neem oil	Rosemary oil	Cloves oil	Ginger oil
1%	90±10a	20±10cd	40±10b	0±0e	100±0a	100±0a	90±10a	100±0a
0.20%	70±10b	20±10cd	30±10c	0±0e	20±10cd	100±0a	10±10de	100±0a
0.10%	30±10c	0±0e	0±0e	0±0e	0±0e	10±10de	0±0e	0±0e
Control	0±0e	0±0e	0±0e	0±0e	0±0e	0±0e	0±0e	0±0e

Means followed by similar letters in a column are not significantly different at $P < 0.05$

Recently, essential oils and bioactive substances are considered organic, broad-spectrum, and low-risk pesticides (Hussein and Joo 2017). Essential oils are volatile liquid compounds obtained from plant extracts of flowers, fruits, seeds, leaves, peel, and roots (Hyldgaard et al. 2012). The ancient Egyptians utilized them in perfumery and mummification of their bodies (Dandlen et al. 2011; Lang and Buchbauer 2012). SEM is a convenient tool to observe the criteria of entomopathogenic fungi so that the fungal units can be determined (Luz et al. 1998; Sun et al. 2016). The SEM technique was used in the present study to investigate the detailed morphological features of *B. bassiana* and *M. anisopliae* after ten days of inoculation on MEA media, to compare the two fungal biocontrol agents. Rosemary essential oil was very effective against all the pathogenic strains of the ginseng plant, whereas it displayed a high ability to control *Sclerotinia* spp. (Hussein et al. 2020). Hussein and Joo (2018) reported that ginger essential oil represents a rich source of natural pesticides and may be useful as promising non-synthetic agent to control ginseng fungal pests. Wu et al. (2016) concluded that essential oils and particularly their bioactive molecules are considered insecticidal compounds. Cloves, ginger, lavender, neem, and rosemary are the most frequent herbal plants across the globe with medicinal significance. They have been recommended for multiple medical conditions and are claimed to have anti-inflammatory and digestive effects (Hussein and Joo 2018; Hussein et al. 2020). This study shows a significant role for mycoinsecticides in pest control management, presenting a potential alternative for the control of *S. frugiperda* using environment-friendly substances instead of chemical insecticides. Also, however, limited numbers of investigations are available on the interaction of natural products with insect pests under lab conditions. Such investigations where natural products are exploited would be helpful in the development of eco-friendly pest control programs.

Conclusions

The entomopathogens *B. bassiana* and *M. anisopliae* are aerobic pathogenic fungi that parasitize insect hosts. This study may hasten their application to control *S. frugiperda* larvae one of the key pests of corn and other economic crops (S4). Moreover, the chemical diversity of plants is a potentially powerful source for controlling pests of this genus. The insecticidal indices demonstrated in this study introduced evidence of the potentiality of natural products from plant origin to manage plant crop damage caused by fall armyworm *S. frugiperda* pest. This data could be used to propose and produce natural bioinsecticides that could reduce the potential risk to the environment. The study also suggests feasible examples of pesticide compounds extracted from herbal plants which are biodegradable products that could be safe for application soon.

Abbreviations KACC: Korean Agricultural Cultural Collection; SDW: Sterile distilled water; MEA: Malt extract agar; SEM: Scanning electron microscopy; LC: Lethal concentration

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Author contributions Jin Ho Joo: Supervision, Visualization, Writing—Original Draft, Project administration. Khalid Abdallah Hussein: Supervision, Conceptualization, Visualization, Writing—Review & Editing.

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Availability of data and materials All data and materials are available.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare that they have no competing interests.

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