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LABORATORY EVALUATION OF THE EFFECT OF *BEAUVERIA BASSIANA* ON THE VITAL ACTIVITY OF *GRAPHOLITHA MOLESTA* (*LEPIDOPTERA: TORTRICIDAE)*

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Improving yield and quality of the economic crops is a central task in agriculture. One of the great potentials of agriculture is to protect crops from pests. Chemical pesticides have widely used in agricultural production, but excessive amount of them has resulted in many environmental and pest-resistance problems. With the increasing demand for organic products, biopesticides have been an alternative to conventional chemical ones. Moreover, biopesticides are more and more favored by the pesticide market because of their safety for environment, and their unique mechanism of action which is not easy to produce resistance. Entomopathogenic fungi have a wide host range, and they are environment friendly and can markedly improve pest control efficacasy. Entomopathogenic fungi can also as biological control agent against the oriental fruit moth, Grapholita molesta (Busck) (Lepidoptera: Tortricidae), a significant pest of stone and pome fruits. Beauveria bassiana is one of the most widely studied entomopathogenic fungus and it is used as biopesticide. B. bassiana, is a broad spectrum entomopathogenic fungus with strong pathogenicity. It is easy cultured and its pathogenic is enough to keep pest populations below an economic threshold level. It has a special status in field of biological control. In order to better understand its mechanism and control effect on oriental fruit moth, in this study we analyzed the lethal effect under two different infection modes of B. bassiana and the affection of three concentrations of spore suspensions. The fourth instar larvae of oriental fruit moth were treated by impregnation method and the feeding method at different spore suspension of 1×10^5 conidia/mL, 1×10^6 conidia/mL, 1×10^7 conidia/mL, the mortality rate and body weight were calculated. The results showed that the maximum corrected mortality rate of cuticular infection and digestive tract infection on oriental fruit moth infected by B. bassiana was 65.7% and 22.7%, respectively. Compared with the control group, the body weight was decreased in different degrees. Under laboratory conditions, the concentration of B. bassiana of 1×10^7 conidia/mL is the economical and effective concentration for the control of the oriental fruit moth.

Key words: *plant pests, oriental fruit moth, plant protection, biological control, Beauveria bassiana.*

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Introduction. Entomopathogenic fungi are one of the most studied, produced and applied biological agents for biological control (St Leger & Screen, 2001). *Beauveria bassiana* is a broad-spectrum entomopathogenic fungus with strong pathogenicity, and it is widely used for agricultural and forestry pest control (Feng, 1998). When the temperature and humidity is suitable, conidia of *B. bassiana* can germinated and entered the host body cavity through the insect's body wall (Liu & Guo, 2019). Mycelium grows in the haemocoel, produces toxic metabolites that lead to host death and eventually invades all organs of the host, and re-produces spores through the body wall (Gupta et al., 1994). *B. bassiana* can infects its host by two ways: the first one – through contact with the insect from the body wall**,** spiracle, the invasion of internode membrane, stoma and wound, which is the main way of its infection (Hu & Fang, 1996). The other way is by feeding and breathing of insects: *B. bassiana* invades the host through internal channels such as digestive tract and respiratory tract. For the same insect, the development period is different, the infection mode is different as well (Quesada et al., 2013). For example, when *Myllocerinus aurolineatus Voss* is infected by *Beauveria bassiana*, the larvae directly infected the body wall, valve, and intermembrane after spore germination, while the adults are infected by swallowing a large number of spores, which entered the body and germinated (Wu et al., 2012). The larvae of *Culex spp* were mainly infected by *B. bassiana* through respiratory tract, while the adults were mainly infected through body wall (Vogels et al., 2014). Therefore, in order to improve the biological control effect of *B. bassiana* for the target insect, it is of great significance to study the infection mode.

The oriental fruit moth, *Grapholita molesta* (Busck) (*Lepidoptera: Tortricidae*), is a serious pest of fruit trees and fruits in most temperate regions of the world (Myers et al., 2006; Wang et al., 2017). The oriental fruit moth is a host-switching pest species. The stone fruit peach is considered as its primary host, whereas the pome fruits pear and apple are supposed as its secondary hosts (Rice et al*.,* 1972; Rothschild & Vickers, 1991).

Its larvae often bore into shoots or fruits and make damage, seriously affect the quality and yield of the fruit which always cause great economic losses (Kanga et al., 2003). *B. bassiana* as a better biological agent has been reported has potential for suppression of larvae pest (Ran et al., 2016; Sarker et al., 2020). However, there were few reports about the infection mode of oriental fruit moth by *B. bassiana*. In this study, we analyse different concentrations and infection modes of *B. bassiana* against larvae of oriental fruit moth under laboratory condition. These results will help a lay the foundation for further studying of relations between *B. bassiana* and oriental fruit moth, and better improving the control effect of *B. bassiana* for oriental fruit moth.

Materials and methods. ***Insect cultures.*** In the laboratory condition, the insects were reared on artificial diet for more than 30 generations before the experiment beginning. The adults were reared in beaker (2L in volume) with one fresh Fuji (it is a variety of apple, experiments show that different apple varieties have great influence on larval feeding, and this kind of apple is not easy to rot, and better feeding) apple and a waxed paper inside for egg laying and fed with 10% honey solution. Neonate larvae were reared in the apples, and late instar larvae (third to fifth instar) were picked out from the rotten apples (Wang et al., 2017; Zhang et al., 2021). The late instar larvae were reared on artificial diet until pupation, following the methods of Du et al (2009). All of them were reared under a photoperiod of 15: 9 L: D, 85% relative humidity and 26.5 ^{o}C till the appropriate assay instar.

Fungal Pathogen and Preparation of Conidial Suspension. B. bassiana BNCC 111705 was from BeNa Culture Collection, and cultured on potato dextrose (PDA) plates at 28^{o}C, 95% humidity under complete darkness. Conidia (spores) used for the infection were harvested from 5–7 days old cultures by scraping the surface of the mycelia with 40 ml ddH_2O, filtered with sterile gauze, and then washed with ddH_2O for third times. The spore concentrations were adjusted to 1×10^5 conidia/mL, 1×10^6 conidia/mL, 1×10^7 conidia/mL. The viability of conidia was determined before the bioassay, and greater than 95% conidia germination was observed in all tests.

Larva Bioassays infected by impregnation and feeding methods. Fourth instar larvae with consistent growth were selected and used for two different modes of infection.

Immersion method: infected insects were soaked in the spore suspension of 1×10^5 conidia/mL, 1×10^6 conidia/mL, 1×10^7 conidia/mL, for 10 s, while the insects of control group were treated with ddH_2O for the same duration.

Feeding method: the same size of artificial diet was smeared with the suspensions of conidia with different concentrations of *B. bassiana*, and place on absorbent paper for a few times to dry excess water surface. The treated group of insects were feed with the artificial diet coated with *B. bassiana.* The control groups were feeding with asepsis artificial diet.

A total of 120 insects for each treatment with three biological replicates, and each biological replicate with 30 larvae respectively. Insects were further dried with sterile filter paper, and reared separately in a dactylethrae with artificial diet and kept in Artificial Climate Chamber, at 26.5 ^{o}C, 95% RH, under a photoperiod of 15 L: 9 D.

Mortality was observed every 24 h for larvae and, until all insects in the control had died. Every larva in control group and treatment group was numbered, and the body weight of it was recorded on the first day of the fifth instar and pupal stages in each group.

Statistical Analysis. Statistical analyses were all performed using SPSS 18.0 Statistics software and graphs were constructed using Graph Pad Prism. 7.00. software. One-way analysis of variance (ANOVA) and Tukey's test were used, $P < 0.05$ was considered significant (Zar, 2010). Corrected mortality (%) = (treatment mortality – control mortality) /(1 – control mortality) ×100%.

Results. *Morphology of larvae infected with Beauveria bassiana.* Studies have shown that the insects of treated groups infected with different concentration of *B. bassiana* suspension, were characterized by decreasing body size with the increase of the concentration of *Beauveria bassiana* solution after 4-th day infected. The control group larvae pupated at 8-th day, while some of the treatment group larvae not pupated and their body covered with white mycelium and conidia formed on top of the mycelial mass.

Larval Mortality infected by B. bassiana.

Infected through body wall. Larvae of oriental fruit moth fastly died when the spore concentration was 1 × 10^7 conidia/mL, and the corrected mortality for this was 65.7%. The corrected mortality infected with 1 × 10^5 conidia/mL suspension of *B. bassiana* was 44.9%, which reached the maximum death rate on 7 days. And the lethal effect of these two groups (1 × 10^6 conidia/mL and 1 × 10^7 conidia/mL) were significantly higher than that of 1 × 10^5 conidia/mL *B. bassiana* concentration 11.7%).

Compared with the control group, the body weight of larvae treated with 1×10^5 conidia/mL decreased to 24% and 21% at the fifth and pupa ages, in the group treated with 1×10^6 conidia/mL, body weight decreased to 36% and 31% at the fifth and pupa ages. In the group treated with 1×10^7 conidia/mL body weight decreased to 75% and 74% at the fifth and pupa ages which was significantly lower than that of the control group and the lower concentrations (Figures 1, 2).

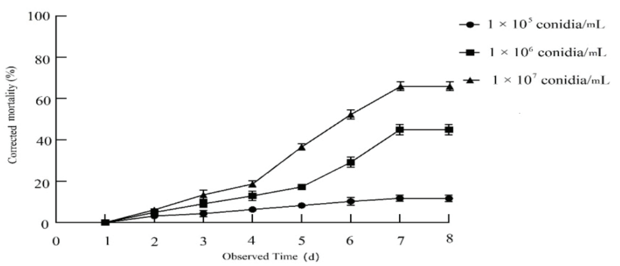

Fig. 1. The corrected mortality of cuticular infection of oriental fruit moth infected by different concentration of *B. bassiana*
(The bars represent the mean ± SD (n = 3)

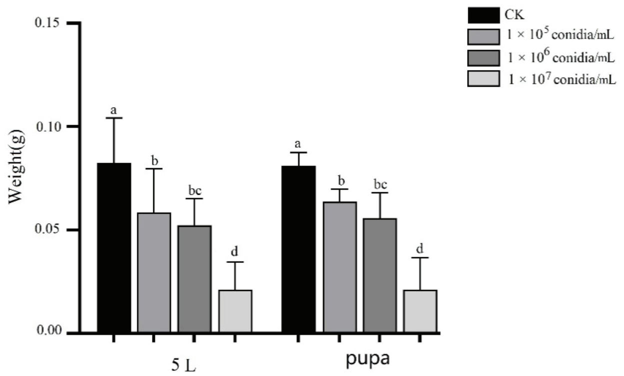

Fig. 2. Weight of oriental fruit moth infected by *B. bassiana* (cuticular infection)
(Data represent mean ± SD, the same age, different concentrations according to ANOVA and Turkey test method significance difference analysis, means followed by the same letter are not significantly different (α=0.05)

Infected by feeding. The results showed that the corrected mortality of *B. bassiana* was up to 22.7% when the concentration of conidia suspension was 1×10^7 conidia/mL. The corrected mortality of larvae was less than 20% when the concentration was reduced (7.6% and 16.7%). When the concentration of conidia suspension was 1×10^7 conidia/mL, it shows that the larval body weight significantly decreased. Meanwhile, comparing to other groups (1×10^5 conidia/mL and 1×10^6 conidia/mL), the difference is statistically significant. And there was no significant difference in body weight between different instars and control group, when the concentration of conidia suspension was 1×10^5 conidia/mL and 1×10^6 conidia/mL (Figures 3,4).

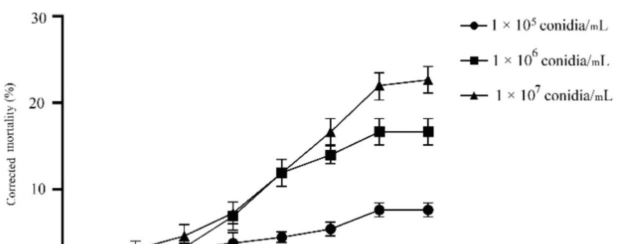

Fig. 3. The corrected mortality of digestive tract infection of oriental fruit moth infected by different concentration of *B. bassiana. (*The bars represent the mean ± SD (n = 3).

Discussion. The entomopathogenic fungus of *B. bassiana* is well known as a potential alternative to chemical pesticides for control of insect pests and is commercially available for such purpose in numerous countries worldwide (Glare et al., 2008; Sevim et al., 2010; Glare & Inwood, 2014; Saranraj & Jayaprakash, 2017). Compared with bacterial and viral insecticides, fungal insecticides have stronger spreading ability and wider control spectrum due

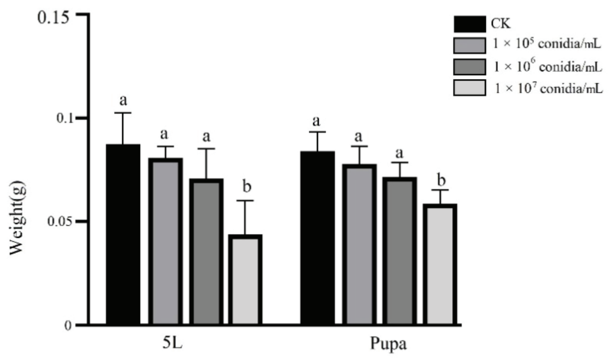

Fig. 4. Weight of digestive tract infection of oriental fruit moth infected by *B. bassiana*
(Data represent mean ± SD, the same age, different concentrations according to ANOVA and Turkey test method significance difference analysis, means followed by the same letter are not significantly different (α=0.05)

to its unique way of infecting insect body walls. In this study, when the concentration is 1×10^7 conidia/mL, the mortality of cuticular infection of oriental fruit moth infected by *B. bassiana* is significantly higher than that of digestive tract infection. In previous researches are reported that for other larvae of *Lepidopteran* the similar phenomenon is observed. In *Plutella xylostella*, the correct mortality of cuticular infection with *B. bassiana* Bb02 (1×10^7 conidia/ mL) was 73.79%, while that of digestive tract infection was 33.79% (Lei et al., 2010). However, sometimes, for the same insect, due to the different concentration and host strain type, the results are significantly different. In *Plutella xylostella*, infected with *B. bassiana* GDS at concentration of 1×10^8 conidia/mL the corrected mortality of digestive tract infection was 80.5%, (Yan *et al.,* 2013). The corrected mortality was 92.4% when infected through cuticular with *B. bassiana* MZ041016 at concentration of 2.3×10^8 conidia/mL (Yuan *et al.,* 2007). *B. bassiana* HFW-05 was successfully infected in *Helicoverpa armigera* through the digestive tract (feeding method), and the corrected mortality rate was 75.8% after 6th days infected. The body weight and food intake of the insects infected with *B. beauversiana* HFW-05 through the cuticle surface were similar to those of the control group (the corrected mortality rate of 6th days was only 17.3%, and the pathogenic effect could not be achieved through the cuticle surface (Cao et al*.,* 2011). However, Yu et al (2020) reports that in *Helicoverpa armigera*, after 10th day infected by *B. bassiana* at concentration of 1.5×10^8 conidia/mL, the corrected mortality of cuticular infection was 63%, and that of digestive tract infection was only 38% (Yu et al., 2020). It is generally believed that the host relationship between *B. bassiana* and its hosts is established mainly through the germination of conidia on the insect body surface, producing bud tubes and forming appressorium to penetrate the host body wall, and then invading the host body for reproduction (Cao et al*.,* 2013; Holder & Keyhani, 2015). However, the hyphomycetes of *B. bassiana* can also entry through the digestive tract, conidia enter the digestive tract with food, germinate in the digestive tract, grow into mycelia, and then invade and expand into the body cavity through the intestinal wall cells (Ferron, 1978; Huang et al., 2002; Lei et al., 2010). Thus, it is of great significance to better understand its infection mode and to achieve better control effect. Until now, only a few entomopathogenic fungus have been studied to determine their effects on *G. molesta*. And, there was no reports on the infection mode of *G. molesta* with *B. bassiana*.

Conclusions. In this study, *G. molesta* infected with *B. bassiana* through cuticular infection had higher corrected mortality and better weight inhibition than that of digestive tract infection. These results provide reliable theoretical support for the development and production of fungal preparations for the control of *G. molesta*. Meanwhile, *B. bassiana* at concentration of 1×10^7 conidia/mL may have potential to be used as control measure against *G. molesta* in fruit orchards, but the field experiment needs further verification. Moreover, *B. bassiana* is also affected by many other environmental factors, and it need to be further researched ways of improving its control effect on *G. molesta* in the future.

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Лабораторна оцінка впливу Beauveria bassiana на життєдіяльність Grapholita molesta (Lepidoptera: Tortricidae)

Підвищення врожайності та якості господарських культур є центральним завданням у сучасному сільському господарстві. Одним з найбільших потенціалів сільського господарства є захист сільськогосподарських культур від шкідників. Хімічні пестициди широко використовуються в сільськогосподарському виробництві, але їх надмірне застосування призвело до багатьох екологічних проблем і підвищення резистентності шкідників. Зі зростанням попиту на органічні продукти біопестициди стали альтернативою звичайним хімічним препаратам. Більш того, біопестициди користуються все більшим попитом на ринку пестицидів через їх безпеку та захист навколишнього середовища, а також через їх унікальний механізм дії, до якого нелегко виробити стійкість шкідникам. Ентомопатогенні гриби мають широкий спектр господарів і нешкідливі для навколишнього середовища. Вони можуть помітно підвищити ефективність боротьби зі шкідниками, а також бути важливим біологічним засобом боротьби зі східною плодожеркою Grapholita molesta (Busck) (Лускокрилі: Tortricidae), що є небезпечним шкідником кісточкових і зерняткових культур. Beauveria bassiana – один з найбільш широко вивчених ентомопатогенних грибів, який широко використовується як біопестицид. Цей ентомопатогенний гриб має широкий спектр дії, сильну патогенність, легко культивується. Рівня патогенності гриба достатньо, щоб підтримувати популяції шкідників нижче економічного порогу шкодочинності. Він має особливий статус у галузі біологічного контролю. Щоб краще зрозуміти його механізм і контрольовану дію на східну плодожерку, в цьому дослідженні нами проаналізовано летальний ефект при двох різних способах зараження B. bassiana і ураженні трьома концентраціями суспензій спор. Личинок східної плодожерки четвертого віку обробляли методом просочення і методом годування суспензією спор різної концентрації ($1×10^5$ конідій/мл, $1×10^6$ конідій/мл та $1×10^7$ конідій/мл), фіксували рівень смертності та масу тіла. Результати показали, що максимальний скоригований рівень смертності від інфекції кутикули та інфекції травного тракту в східної плодожерки, зараженої B. bassiana, становив 65,7% та 22,7% відповідно. У порівнянні з контрольною групою маса тіла була знижена. У лабораторних умовах концентрація B. bassiana $1×10^7$ конідій/мл є економічно ефективною для боротьби зі східною плодожеркою.

Ключові слова: *шкідники рослин, східна плодожерка, захист рослин, біологічний контроль, Beauveria bassiana.*