Pak. J. Agri. Sci., Vol. 58(2) 743-750; 2021
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI: 10.21162/PAKJAS/21.52
http://www.pakjas.com.pk

EFFICACY OF *Metarhizium anisopliae* AND *Beauveria bassiana* AGAINST *Tuta absoluta* (LEPIDOPTERA: GELECHIIDAE) EGGS UNDER LABORATORY CONDITIONS

Nagdy F. Abdel-Baky[1,3], Saleh S. Alhewairini[1,*], Mahmoud M. Al-Azzazy[1,4], Muhammad Z. Qureshi[2], Mohammed A. Al-Deghairi[1] and Jamal Hajjar[5]

[1]Department of Plant Production and Protection, College of Agriculture and Veterinary Medicine, Qassim University, Buraydah, Saudi Arabia. [2]Department of Biochemistry, Deanship of educational services, Qassim University, Buraydah, Saudi Arabia. [3]Economic Entomology Dept., Fac. Agric., Mansoura University, Egypt. [4]Agricultural Zoology and Nematology Department, Faculty of Agriculture, Al Azhar University, Cairo, Egypt. [5]College of agriculture and food sciences, King Faisal University, Alhassa, Saudi Arabia
[*]Corresponding author's e-mail: hoierieny@qu.edu.sa

Tuta absoluta Meyrick (pinworm), is a seriously crucial leaf mining, stalk borers and fruit damaging pest of solanaceae plants. Among solanaceous plants, tomato is considered to be the most preferred host to *T. absoluta*. Infestations caused by pinworm lead to reduction in tomato yield and its worldwide export. The current study has been conducted to evaluate effectiveness of two entomopathogenic fungi; *Beauveria bassiana and Metarhizium anisopliae* against eggs of *T. absoluta* under laboratory conditions. The potential of the two fungi was also assessed as an instrument of integrated pest management system through biological control. Eggs of *T. absoluta* were treated with four different conidial concentrations of each entomopathogenic fungus. These fungi were evaluated for their toxicity at four different conidial concentrations ($4x10^5$, $6x10^5$, $8x10^5$ and $1.0x10^6$ conidia/ mL). Twenty-one days post treatment, spore concentration $1.0x10^6$ conidia/mL, resulted in the highest death rate: 50 and 37.6% with eggs when treated by *B. bassania* and *M. anisopliae*, respectively. The lowest mortality 33.6 and 25.6% of eggs was observed when *T. absoluta* eggs were treated with $4x10^5$ conidia/ mL of *B. bassania* and *M. anisopliae*, respectively. Mortality values resulted from the experiments indicated that *B. bassania* showed superiority over *M. anisopliae* regarding its effect on controlling of *T. absoluta* eggs. In addition, $4x10^5$ conidia/ mL showed the minimum mortality effect among the two fungi used in the bioassay. Mortality and spore production on the eggs were significantly higher with the higher spore concentrations ($8x10^5$ conidia/ mL) of *M. anisopliae* and *B. bassiana*. Our findings suggest that the moderate ($8x10^5$ conidia/ mL) and the higher ($10x10^5$ conidia/ mL) spore concentration/ml of *B. bassania* and *M. anisopliae* are potent entomopathogenic and have the potential to be developed as effective and potent bio-control agents hostile to *T. absoluta* eggs in IPM programs.
Keywords: Biological control, *Tuta absoluta* Eggs, *Beauveria bassania*, *Metarhizium anisopliae*.

INTRODUCTION

Tuta absoluta Meyrick (Lepidoptera: Gelechiidae) is identified by various names in the same and different regions of the world; like tomato borer (TB), tomato leaf miner (TLM), tomato pinworm and the tomato pinworm found in South America. The pinworm (tomato leaf-miner) is a serious and persistent agricultural pest reported to occur all around the world (CABI, 2016; Abdel-Baky and Al-Soqeer, 2017; Abdel-Baky *et al.,* 2019). It is an oligophagous notorious insect pest that invades a huge number of solanaceae plants worldwide (Anastasios *et al*., 2014; Amizadeh, *et al*., 2015; Chidege *et al.,* 2016; Materu *et al*., 2016). *T. absoluta* is a cosmopolitan species that affects a variety of wild and cultivated solanaceous species including *Solanum habrochaites* S. Knapp and D, pepper (*Capsicum anuum* L.), pepino (*Solanum muricatum* Aiton), black nightshade (*Solanum nigrum* L.), tree tobacco (*Nicotiana glauca* Graham), devil's apple (*Datura stramonium* L.), tomato (*Solanum lycopersicum* L.), aubergine (*Solanum melongena* L.). M. Spooner, *Solanum lyratum* Thunb., *Solanum elaeagnifolium* Cav., *Solanum puberulum* Phil. and *Datura ferox* L. (EPPO, 2005; F.E.R.A. 2009; CABI, 2016). Some researchers consider that outbreaks of *T. absoluta* are vague due to the lack of certain ecological and biological information (Illakwahhi and Srivastava, 2017).

The use of chemical insecticides has become complicated due to development of insecticide resistance in *T. absoluta* after repeated exposure (Abdel-Baky *et. al.,* 2019; Fanigliulo *et al.,* 2012). In certain countries, insecticides are not applied according to the need but they are used regularly on a calendar date basis. In most cases, early-season chemical insecticidal remedial therapies by broad-spectrum insecticides, especially organophosphates are considered responsible for the outbreak of *T. absoluta* and other insect pests.

Tomato crop in Saudi Arabia and Middle East is under constant threat due to rapidly increasing infestations of *T. absoluta*. It is therefore, development of a sustainable management system (SMS) is crucial to cope with it so that the role of tomato is made sustainable in diversification of the economy, alleviation of poverty, and improvement of its nutritional quality (Tropea-Garsia *et al.,* 2012; Abdel-Baky and Al-Soqeer, 2017; Abdel-Baky *et al.,* 2019; Mansour *et al.,* 2019). Natural control, also called biological control is one of the safe management methods of pest's treatment as it is considered safe for non-target species and the environment. Among biological control methods, entomopathogenic fungi have been found potentially very strong for their effective use against *T. absoluta* (Alikhani *et al.,* 2019). This potential of entomopathogenic fungi was also observed against a variety of other economically important pests (Tadele and Emana, 2017).

Recently, entomologists are convinced to adopt more environmentally benign practices. In the pest control programs using insecticides, insecticides management is extremely crucial to: (i) maintain ecological balance, (ii) curtail injurious effects on non-target organisms, and (iii) ensure human health and safety (Deedat, 1994; Balzan and Moonen, 2012)

In the context of biological control system, the use of microbial agents would be an ideal option to avoid issues like environmental hazards and pesticide resistance. The need to discover environmentally safe insecticides as well as bio-pesticides to fight *T. absoluta* has triggered enthusiastic interest in alternatives to insecticides such as utilization of entomopathogenic fungi and bacteria, which are currently evaluated for insecticidal efficacy (Sabino *et al.,* 2019). Currently, *Metarhizium anisopliae* (Metschnikoff) Sorokin and *Beauvaria bassiana* (Balsamo-Crivelli) are considered two of the most notable entomopathogenic fungi used widely against insect contagions and being the most popular and commonly found species developed as bio-pesticides (Butt *et al*., 2001; Zimmerman, 2007a,b). *B. bassiana* and *M. anisopliae* are the most common fungal parasite of Arthropods. Recently, selected strains of *B. bassiana* and *M. anisopliae* have been granted allowed to be used commercially against whiteflies, aphids, thrips, and numerous other insect pests (Shah and Pell, 2003). These fungi, which are commonly found in natural populations of arthropods in particular with insects (Abdel Rahman *et al*., 2010), were observed very toxic against larvae when applied to plant foliage (Abdel Rahman *et al*., 2010; Zaki and Abdel-Raheem, 2010).

Recently the developments of bio-insecticides have been directed towards specific insects. No bio-insecticide has, however, been heretofore identified as having ovicidal activity against *T. absoluta* eggs (Vega *et al.,* 2008). It is therefore the objectives of this study were to determine toxic effects of two entomopathogenic fungi namely; *B. bassiana* and *M. anisopliae* against eggs of *T. absoluta*. Moreover, the current study examines strategies for the use of certain significant fungal entomopathogens as a future microbial instrument to reduce the insecticidal resistance management program in sustainable pest management systems.

MATERIALS AND METHODS

Rearing technique of T. absoluta: Culture of *T. absolulta* was developed from freshly field collected larvae at Qassim region. Larvae were reared under laboratory condition (25±2 ^{o}C, and 60±5 % RH) until emergence of adult stage and naturally start mating and new egg were obtained (Krechemer and Foerster, 2015). Egg-masses of *T. absoluta* were collected and placed in cylindrical glass jars (1 lb.) with small pieces of tomato leaf (*Solanum lycopersicum* L.) and covered with muslin cloth that was gripped with a rubber strip. Once eggs were hatched, the freshly hatched larvae were moved into larger rearing jars (4 lb.) using a fine hair camel brush. In order to absorb any surplus moisture, a filter paper was provided at the bottom of the jar. Larvae were nurtured on fresh tomato leaves until pupation. As getting close to the end of the 4th instar larvae, moist sawdust was placed at the bottom of the rearing jars to provide pupation sites. The formed pupae were collected in due course, and placed in clean jars until their adult growth. The newly born moths were sexed and kept in pairs in clean jars (1 lb.). Each rearing jar was administered with 10% honey solution and drenched in cotton fiber that was tied with wire for moth feeding. To avoid any growth of microorganisms and fermentation, honey solution was refreshed on daily basis. Fresh green leaves of tomatoes were introduced into clean jars (4 lb.) as oviposition sites of mated adults. The freshly laid egg-masses were collected on everyday basis and moved into the rearing jars (5 egg-masses/jars). All rearing jars were kept under laboratory conditions of 25±2 °C and RH 60±5 %.

***Pathogen (fungi) source*:** Entomopathogenic fungi, *B. bassiana* and *M. anisopliae* were obtained from Plant Production and Protection Department, Faculty of Agriculture and Veterinary Medicine, Qassim University, KSA. These fungi were isolated from soils and decayed insects.

The spores of *B. bassiana* and *M. anisopliae* were routinely grown on potato dextrose agar (PDA) media and incubated at 25±2°C till the fungi growth developed dense sporulation within 14 to 15 days of incubation (Krechemer and Foerster, 2015). The spores of each fungus were removed with a spatula and kept in sterile water containing 0.05% of Tween 80. Haemocytometer was used to prepare the required spore concentration. Hundred microlitre of each spore concentration ($4x10^5$, $6x10^5$, $8x10^5$ and $1.0x10^6$ conidia/ mL) was applied topically on the eggs of *T. absoluta* using a micro pipette.

***Spray treatment*:** The bioassay method was conducted according to Goettel and Johnson (1992). Spray application

on Eggs of *T. absoluta was administered by the direct method* that involved a direct application of conidia (water-based suspension). Each egg group administered with 1 ml of fungus suspension with partial doses; $4x10^5$, $6x10^5$, $8x10^5$ and $10x10^5$ conidia/ mL / replicate, respectively. 1 ml of distilled water was used to treat the control. Five sets of one-day old *T. absoluta* eggs were prepared. Egg count was fixed to 50 eggs in each group. Group on was used as a control and it was treated with distilled water. Group 2, 3, 4 and 5 were treated fungal concentrations $4x10^5$, $6x10^5$, $8x10^5$ and $10x10^5$ conidia/ mL, respectively. Each trial was run in five replicates.

***Mortality evaluation*:** Number of dead eggs was noticed and recorded daily, and the observations were continuously carried out to distinguish between dead eggs as a result of the fungal infection, as well as, the egg hatchability was counted for the eggs which escaped from the fungal infection. Replicates and treatments were scored for mortality for three days after exposure, and the surviving eggs were also monitored until pupation (one larva/ Petri-dish).

Any morphological irregularities in inoculated eggs were detected. Microscopic analyses and the growth of bio-pesticides on eggs were verified. As per the method described by Lacey *et al*., (2007), dead eggs were taken away and surface was sterilized. The dead eggs were soaked in 75% ethanol solution for a few seconds and then rinsed thoroughly in sterile distilled water. Abnormal eggs were treated by placing them into 0.5% sodium hypochlorite for two minutes that was followed by two more rinses with sterile distilled water and then they were left to dry for 48h. For the examination purpose if fungus infection was lethal for abnormal eggs or not, the eggs were incubated in a Petri-dish with wet cotton under sterile conditions inside the clean desiccators at room temperature.

***Data analysis*:** All the experiments were replayed three to four times in five replicates. The data was recorded, tabulated and subjected to statistical analysis. Treatment means, standard deviations (SDs), and significant differences were analyzed using Costat software (1993) test program. One-way analysis of variance (ANOVA) was applied to adjudge the importance of its main effects. The crucial variations between treatments were guaged using Tukey's multiple range tests ($P \leq 0.01, 0.05$). Abbott (1925) was resorted to for calculating percent mortality:

$$\text{Corrected \%} = 1 - \frac{\text{N in T after Treatment}}{\text{N in Co after Treatment}} \times 100$$

Where: N= Number of eggs, T= Treated, Co= Control

RESULTS

***Efficacy of Beuveria bassaina against T. absoluta eggs*:** The chronic pathogenicity (mortality within 21 days) associated with four spore concentrations of *B. bassania* applied against *T. absoluta* eggs were assessed *in vivo*. The laboratory studies revealed that the four spore concentrations of the fungus *B. bassania* were toxic to *T. absoluta* eggs (Table 1). Although, there were no initial deaths (mortality %= 0) among the four spore concentrations, however, their activities showed a moderate effect against *T. absoluta* eggs (Table 1, Fig. 1).

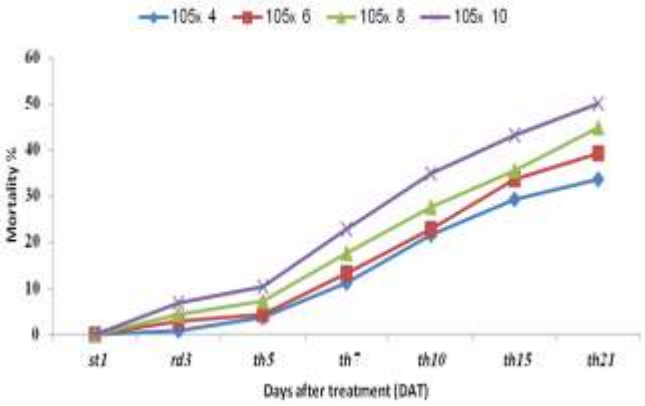

Figure 1. Daily mortality percentage of *T. absoluta* eggs treated by four spore concentrations of *B. bassiana* under laboratory conditions *in vitro*.

Twenty-one days of treatment, the use of a low fungal concentration ($4x10^5$ conidia/ mL), resulted in lower mortality rates compared to other concentrations after (16.8±1.14 eggs of 50 eggs; which formed 33.6% of the total) (F value = 123.8 with 6/28 df; and P≥0.0001). With $6x10^5$

Table 1. Mortality of *T. absoluta* eggs after treatment by four spore concentrations of *B. bassiana* under laboratory conditions.

spore Concentrations/ ml	N	Initial Kill (0 Time)	Mortality average ±SE/replicate (Days after treatment)						Residual Activity % (RA)	Total Activity % (TA)	Statistical analysis	
			3	5	7	10	15	21			F Value	P > F
4 x 10^5	50	0f	0.8±0.37ef	1.8±0.37e	5.6±0.51d	10.8±0.66c	14.6±0.68b	16.8±1.14a	16.68	8.35	123.80 with 6/28 DF	<0.0001
6 x 10^5	50	0f	1.4±0.40e	2.2±0.37e	6.6±0.25d	11.4±0.25c	16.8±0.58b	19.6±0.25a	19.33	9.67	520.93 with 6/28 DF	<0.0001
8 x 10^5	50	0g	2.2±0.58f	3.6±0.51e	8.8±0.2d	13.8±0.37c	17.8±0.37b	22.4±0.25a	22.87	11.43	514.30 with 6/28 DF	<0.0001
10 x 10^5	50	0g	3.4±0.68f	5.2±0.68e	11.4±0.51d	17.4±0.51c	21.6±0.25b	25.0±0.63a	28.00	14.00	363.68 with 6/28 DF	<0.0001

*Averages followed by same letter with in a row are not significantly different from each other at 5% (Duncan)

Table 2. Mortality of *T. absoluta* eggs after treatment by four spore concentrations of *M. anisopliae* under laboratory conditions.

spore Concentrations/ ml	N	Initial Kill (0 Time)	Mortality average ±SE/replicate (Days after treatment)						Residual Activity % (RA)	Total Activity % (TA)	Statistical analysis	
			3	5	7	10	15	21			F Value	P > F
4 x 10^5	50	0 e	0.2±0.02e	0.6±0.25e	2.0±0.32d	4.8±0.38c	8.6±0.51b	12.8±0.80a	9.7	4.83	136.52 with 6/28 DF	<0.0001
6 x 10^5	50	0 f	0.2±0.02f	1.0±0.00e	2.8±0.20d	6.2±0.38c	10.2±0.20b	14.2±0.38a	11.43	5.77	530.77 with 6/28 DF	<0.0001
8 x 10^5	50	0 f	0.8±0.20f	2.0±0.45e	4.0±0.45d	8.0±0.32c	12.0±0.32b	15.8±0.37a	14.2	7.1	327.61 with 6/28 DF	<0.0001
10 x 10^5	50	0 g	1.6±0.25f	3.2±0.37e	6.4±0.25d	10.4±0.25c	14.2±0.37b	18.8±0.37a	18.2	9.1	566.07 with 6/28 DF	<0.0001

*Averages followed by same letter with in a row are not significantly different from each other at 5% (Duncan)

spores/ml, the accumulative mortality reached 19.6±0.25 eggs of the total used (50), which formed 39.2% (F value = 520.93 with 6/28 df; and P≥0.0001). Additionally, use of 8x10^5 spores/ml caused accumulative mortality equal 22.4±0.25 eggs of the total (50 eggs), which formed 44.8% (F value = 514.30 with 6/28 df; and P≥0.0001). On the other hand, the higher fungal concentration (10x10^5 conidia/ mL) induced 50% mortality within *T. absoluta* eggs (25.0±0.63 eggs, N=50; F value = 363.68 with 6/28 df; and P≥0.0001) (Table 1).

Also, it could be concluded from these data that, the mortality rates were extremely low in the beginning of the treatment and then increased gradually, causing the highest mortality rates among the treated eggs, after 15-21 days of treatment (Fig. 1).

This study revealed that the pathogenic efficiency of *B. bassania* varies significantly in relation to the initial mortality, post-treatment time, residual activity, total activity and spore concentrations.

Efficacy of M. anisopliae against T. absoluta eggs: *In vivo*, mortality rates within 21 days associated with four spore concentrations of *M. anisopliae* applied against *T. absoluta* eggs, were evaluated. The application of *M. anisopliae* against *T. absoluta* eggs with all four spore concentrations used within the laboratory resulted on 25.6 to 36.4% mortality of eggs compared to the control, which achieved significant mortalities of *T. absoluta*. Data showed that *M. anisopliae* were pathogenic to *T. absoluta* eggs (Table 2) with the four spore concentrations applied. In spite of, the initial mortality through fungus were zero (mortality %= 0) among the four spore concentrations, their activities ranged from lower mortality percentage to a moderate effect against *T. absoluta* eggs (Table 2, Fig. 3).

Low fungal concentration (4x10^5 spores/ml) caused lower mortality rates compared to other concentrations after 21 days of treatment (12.8±0.80 eggs of 50 eggs; which formed 25.6% of the total) (F = 136.52 with 6/28 df; and P≥0.0001). As increasing the fungal concentrations to 6x10^5 spores/ml, the accumulative mortality was increased to 14.2±0.38 eggs of the total eggs used (50 eggs), which represents 28.4% (F value = 530.77 with 6/28 df; and P≥0.0001). Use of 8x10^5 conidia/ mL induced slight increases in accumulative mortality which reached 15.8±0.32 eggs of 50 eggs, accounting for 31.6% (F value = 327.61 with 6/28 df; and P ≥ 0.0001). The higher fungal concentration (10x10^5 conidia/ mL) induced 37.6% mortality (18.8±0.37 eggs, N=50; F value = 566.07 with 6/28 df; and P ≥ 0.0001) (Table 2).

In general, it can be concluded that *M. anisopliae* induced a lowest mortally among *T. absoluta* eggs in the beginning of the study, and then increased gradually with the prolonging the exposure period, causing varied mortality rates, during 15-21 days post-treatment (Fig. 3).

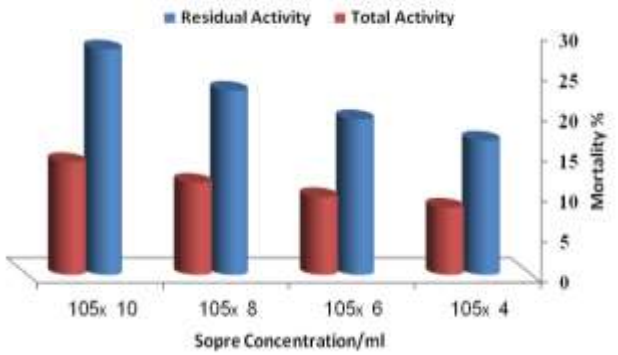

Figure 2. Pathogenicity of *B. bassiana* against *T. absoluta* eggs treated by four spore concentrations under laboratory conditions *in vitro*.

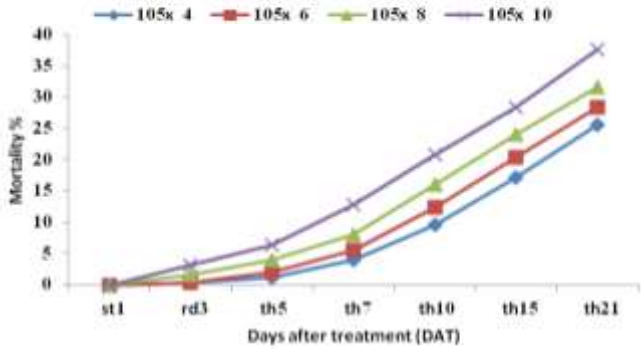

Figure 3. Daily mortality percentage of *T. absoluta* eggs treated by four spore concentrations of *M. anisopliae* under laboratory conditions *in vitro*.

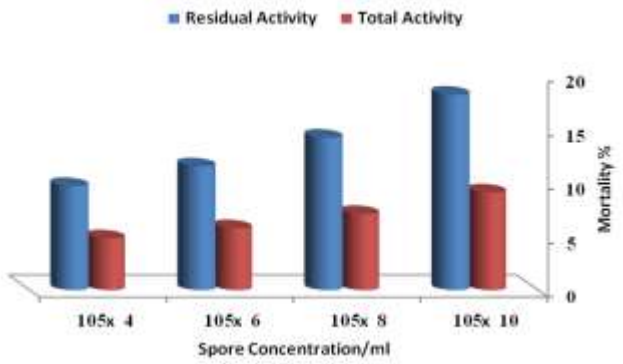

Figure 4. Pathogenicity of *M. anisopliae* against *T. absoluta* eggs treated by four spore concentrations under laboratory conditions *in vitro*.

Moreover, our data brought out that the four spore concentrations used against *T. absoluta* eggs vary significantly with reference to the initial death-rate, post-treatment period, residual activity, total activity and the fungal concentration used (Table 3). It could be concluded that *T. absoluta* eggs were more susceptible to *B. bassania* and the death rates were varied based on the fungal concentrations which was low in the beginning and then increased gradually causing the highest mortality rates among the treated larvae, after 15-21 days of treatment (Fig. 5).

DISCUSSION

Agricultural scientists all over the sphere are facing frustration due to crop losses and limitations of pest control methods. It was estimated that a two-third production of all crops would be lost, depriving a big human population from their daily foods, if pesticides were not used (Deedat, 1994). Due to the heavy dependence on insecticides, a variety of ecological problems are emerging that include toxicity to human and the occurrence of insecticidal immunity in insect pests (Balzan and Moonen, 2012). In the present scenario use of certain entomopathogenic fungus is important as bio-control agent and/or as a natural controller of pest populations and has potential as myco-insecticide agent against varied insect pests in agro-ecosystem (Abdel-Baky *et al.*, 1998). Victims of these fungi are infected by them as they penetrate through the cuticle, have access to the hemolymph, produce toxins, and grow by making use of nutrients present in the haemocoel to avoid insect immune responses (Hajek and St. Leger, 1994). Consequently, these entomopathogenic fungi may be administered in conidia form or mycelia which sporulate after their administration (Abdel-Baky and Abdel-Salam, 2003).

Effect of two fungai; *B. bassiana* and *M. anisopliae* on eggs of *T. absoluta* after treatment by four spore concentrations, under laboratory conditions as given in tables 1 and 2 are in coordination with the findings of Studdert and Kaya (1990), who found that more soybean caterpillars, *S. exigua* were easily vulnerable to *B. bassiana* in the event of their exposure to drier soils compared with moist soils that contain residues of insecticides. This also explain that the success of *B. bassiana* and *M. anisopliae* (Tables 1, 2 ,3 and 4) depends on conidial viability and number of spores/ml (Bidochka and Hajek, 1998; Olivera and Neves, 2004), where the start of epizootics is conditioned to the capacity of these structures to grow on the host. Moreover, the results obtained in this study show that, although the initial mortality was zero, but the epizootics occurred within 15 days and reached their maximum levels after 3 weeks of treatment (Tables 1, 2, 3 and 4). *B. bassania* was more infective at any spore concentration than *M. anisopliae* and caused death among eggs up to a maximum average percent. Keeping in view the earlier reports, we suppose that the cumulative effects of following factors could be responsible for the pathogenic potential of *B. bassania* and *M. anisopliae*:

1. The existence of adhesions on the surface of conidia for attachment to the cuticle of insects (Wang and Leger, 2007, Anand *et al.*, 2009).
2. Toxic factors like chitinases, Pr1 and Pr2 proteases, etc. (Freimoser *et al.*, 2003; Anand *et al.*, 2009)
3. The existence of collagenous protective film that enables fungi to breach the innate immunity of insects when the fungus gets accessed by hemolymph (Wang and Leger, 2006; Anand *et al.*, 2009; Freed *et al*., 2012).

These findings are similar to the findings of Yoon *et al.* (1999); Sabbour and Sahab (2005) and Freed *et al*. (2012) who reported that both of *B. bassiana* and *M. anisopliae* can be used as microbial control agent against *T. absoluta* eggs and larvae in greenhouses and in field conditions. As per the laboratory bioassay findings, we suggest that the four fungal concentrations can be used against the eggs of *T. absoluta* in a dose–dependent manner. It is clear from the data that the use of different concentrations of both entomopathogenic fungi resulted in different mortality depending on the pathogenic species, fungal concentrations used and the target stages of insect pests (Figs 1-4, and tables 1-2). This is in agreement with Balasubramaniyam and Sundaresan, (2009), who found that use of *B. bassiana* at varied concentrations of 10^3, 10^4, 10^5, 10^6, 10^7 and 10^8 mL^{-1} displayed insecticidal activity of 5-33% on *Hypothenemus hampei*. From these results, it could be concluded that *B. bassiana* and *M. anisopliae* contain virulent characteristics that make them suitable to use in effective bio-control programs against *T. absoluta*. However, more work is needed to be addressed to determine the efficiency and the characters of those fungi against other life stages of *T. absoluta* in both greenhouses and open field strains. This study also showed that egg stage was resistance to the entomopathogenic infection. These results are consistent with the study of Tanada and Kaya (1993) which mentioned that during the life cycle of an insect, the larvae

and adults are more susceptible to attack by entomopathogenic as compared with the eggs and pupae. The use of *B. bassiana* and *M. anisopliae* to control eggs of *T. absoluta* were not reported in the past, probably because the control was achieved by the integration of other tools such as egg parasitoids of the genus *Trichogramma* or bacteria larvicides such as *B. thuringiensis* (Theoduloz *et al*., 1997). Results proved that at all concentrations tested, spinosad and indoxacarb were compatible with the two *M. anisopliae* isolates. Chlorfenapyr displayed compatibility with URPE-6 and abamectin with URPE-19 at the average recommended concentration. The fungal antagonist of *B. bassiana* and *M. anisopliae* was previously mentioned as being pathogenic on *T. absoluta*. The interaction between *B. bassiana* and *M. anisopliae* and their probable hosts of insects are not less complex since their entomopathogenicity had evidently developed independently in many main taxa of fungi (Rodríguez *et al.,* 2006). This may elucidate the dissimilarity in degree of precision and total or partial dependence on the presence of a suitable host or its appropriate stages for their survival. In case of confronting with an appropriate host, a complex interconnection begins between the insect and the fungus, starting with invasion, then evasion, leading to augmentation and dispersion of conidia to new potential hosts. Such coinciding development may also be the reason that evasion from host defense by the mode of avoiding recognition as "non-self," a strategy employed by many entomopathogenic fungi, is based on variable characteristics amongst the various species. To sum up, this chronology of events, may either directly or indirectly be influenced by a formation of biotic and abiotic factors, including the insect's host plant.

***Conclusion*:** Results of this study reveal that local strains of *B. bassiana* and *M. anisopliae* are effective to stop hatching of *T. absoluta* eggs under laboratory conditions and hence limiting the emergence of mites. Efficacy of the mycospores increased with increase in exposure time and conidial concentrations/mL. The study indicates the possibility of developing a pest control management system by using *B. bassiana* and *M. anisopliae*, leading to: (i) a major decrease in pesticide usage; (ii) minimized exposure of non-target organisms to pesticides; (iii) escalation in activities of natural enemies; (iv) slashed amounts of pesticide residues in food; and (v) an environment that is healthier and conducive to live. These conclusions, based on the results, got under laboratory conditions, cannot easily be concluded to field efficacy. Therefore, field experiments on tomato crop are necessary to fully adjudge the potential of entomopathogenic fungi isolates against *T. absoluta* in green houses of the Qassim region, Kingdom of Saudi Arabia.

Acknowledgement: The authors gratefully acknowledge Qassim University, represented by the Deanship of Scientific " Research, on the financial support for this research under the number (10144-cavm-2020-1-3-I) during the academic year 1441 AH / 2020 AD".

Conflict of interest: The authors declare that they have no conflict of interest.

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[Received 7 Jan 2021; Accepted 24 March 2021; Published (online) 18 April 2021]