

## Efficacy of Three Entomopathogenic Fungi on Tomato leaf miner, *Tuta absoluta* in Tomato crop in Egypt.

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The tomato leaf miner *Tuta absoluta* (Meyrick) has invaded tomato (*Solanum lycopersicum* L.) crop in Egypt and is representing today a major threat to the production of this crop. In This study, Efficacy of three concentrations (105; 106 and 107) of *Beauveria bassiana*, *Metarhizium anisopliae* and *Verticillium lecanii* were prepared and tested on *T. absoluta* larvae (Neonate "newly hatched", 2nd & 3rd instar) to study the effect of these Entomopathogenic fungi on larval mortality. In addition, eggs of *T. absoluta* were exposed to tested fungi to evaluate their effect on hatchability under laboratory conditions. Results showed that; the estimated LC50 of values of *B. bassiana*, *M. anisopliae* and *V. lecanii* were (0.28 x 105 & 0.11 x 105), (0.45 x 105 & 0.46 x 105) and (0.32 x 105 & 0.27 x 105 conidia/ml) for neonate, 2nd instar & 3rd instar *T. absoluta* larvae, respectively. The effect of pathogen application was dependent on the instar phase at which the larvae were fed on leaves treated with the pathogen. Concerning the most effective concentration of each Entomopathogenic fungi, the higher the concentration (107), the higher the mortality. The greatest percentage of mortality occurred in the newly hatched, 2nd instar followed by the third instar larvae when fed on leaves treated with tested fungi. After the exposure of the eggs to the three agents, the pathogenic effect was evident by the fourth day of evaluation after exposure in the three concentrations. The black appearance of eggs of *T. absoluta* took place after the exposure to the three concentrations of the three pathogenic fungi starting on the fifth day after exposure, and thereby no hatched larvae were detected in the three concentrations of the three pathogenic fungi compared to the control where the hatchability reached 86.7%, 85.3% and 84.0% during the evaluation time for *B. bassiana*, *M. anisopliae* and *V. lecanii*, respectively.

**Keywords** Entomopathogenic fungi, tomato leaf miner, *Tuta absoluta*.

### INTRODUCTION

The tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), first described in Peru in 1917, is now found throughout South America, where it is considered to be one of the most devastating pests for tomato crops (Barrientos, et al., 1998, Estay, 2000 and EPPO, 2006). In Spain, this pest was first detected at the end of 2006 in the north of Castellon (Eastern Spain) (Urbaneja, et al., 2008). During 2007, *T. absoluta* was detected in several locations throughout the Spanish Mediterranean Basin, the most important tomato growing region in the country. Since then, its presence has also been confirmed in Algeria, Canary Islands, France, Italy,

Morocco, and Tunisia in 2008, and in Albania, Bulgaria, Cyprus, Germany, Malta, Portugal, Switzerland, The Netherlands, and the United Kingdom in 2009 (Desneux, et al., 2010 and EPPO, 2010).

The tomato leaf miner *T. absoluta* (Meyrick) is one of the most devastating pests of tomato in South America (Barrientos, 1998). This pest was initially reported in eastern Spain in late 2006 (Urbaneja, 2008) and has subsequently spread throughout the Mediterranean Basin and Europe (Potting, 2009). Since the time of its initial detection, the pest has caused serious damages to tomato in invaded areas

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(Germain, 2009) and it is currently considered a key agricultural threat to European and North African tomato production. If no control measures are taken, then the pest can cause up to 80-100% yield losses by attacking leaves, flowers, stems and especially fruits (Lopez, 1991). The current management of *T. absoluta* in the Mediterranean Basin is mainly based on treatments with chemical insecticides. Nevertheless, few active ingredients are effective against *T. absoluta* and selectively beneficial to pollinators at the same time. (*T. absoluta* is considered a key pest in many areas where it is present, including Latin America (EPPO, 2005 and Anonymous, 2010).

A key pest is one that occurs regularly and will cause economic losses if left uncontrolled). Some consider *T. absoluta* to be the major limiting factor in tomato production in South America (Ferrara, 2002). It is known as the most devastating tomato pest in Brazil, at times causing 100% loss of production (Filho, et al., 2000). Tomatoes may lose their commercial value when severely attacked. *T. absoluta* can potentially become a pest of tomatoes in both field and greenhouses (EPPO, 2005& 2008). Its major host is *Solanum lycopersicum* (tomato) (Ismail and Abdel-Raheem, 2010), other hosts also exist such as *Capsicum* spp. (pepper), (Vargas, 1970, NAPPO, 2008, Korycinska and Moran, 2009 and Potting, 2009).

The tomato leaf miner *T. absoluta* Meyrick has invaded tomato (*Solanum lycopersicum* L.) crop in Egypt and is representing today a major threat to the production of this crop. Therefore, integration with other control methods (cultural, biological and biotechnological methods) becomes imperative, as the continued use of chemical insecticides could harm non-target organisms (Landgren, et al., 2009, Abdel-Raheem, 2005& 2013 Urbaneja, et al., 2007, , Abdel-Raheem, et al., 2009, Abdel-Raheem and Zakia A.Ragab, 2010, Abdel-Raheem, et al., 2011a&b, Zaki and Abdel-Raheem, 2010, Ismail and Abdel-Raheem, 2010, Sabbour, et al., 2012, Sabbour and Abdel-Raheem, 2013,2014, Lacey, 1997 and Marta Rodríguez, et al., 2006).

The aim of this study was to estimate the susceptibility of *T. absoluta* larvae to *B. bassiana*, *M. anisopliae*, and *V. lecanii* and the effect of *B. bassiana*, *M. anisopliae* and *V. lecanii* on egg hatchability.

## MATERIALS AND METHODS

### Tomato Plants

Tomato seeds were sown in the nursery in 100 cell foam trays and kept for 45 days until transplanted to the laboratory under conditions (25 °C, 70±2% R.H.). Seedlings of 45 days old were transplanted in 30 cm diameter plastic pots containing a sterilized soil-peat moss mixture, one seedling per pot. Pots were held in rearing cages (60 cm<sup>2</sup> high, 50 cm<sup>2</sup> wide and 50 cm<sup>2</sup> long).

### Tuta Absoluta Colony

A laboratory colony of *T. absoluta* was established with pupae from field strain. This colony was maintained in the laboratory. Pupae were dislodged from leaves and were housed in a wooden and nylon cage. Adults were fed on 10% honey solution (Taphla leaves were used as a carrier for honey droplets as a food source for adults) and provided with tomato terminal buds and leaves for oviposition overnight so that *T. absoluta* pupation could take place either on leaves or on the soil. When pupation was completed, the cocoons were

carefully collected to be used for starting the experiment. *T. absoluta* adults were reared on tomato plants (45 days old). Tomato plants were placed in Pots and held in rearing cages (60 cm<sup>2</sup> high, 50 cm<sup>2</sup> wide and 50 cm<sup>2</sup> long) provided weekly by seedlings for feeding and egg laying. When required for our assays, newly emerged adults were collected using an aspirator (Fargalla and Shalaby, 2013 and Hussein Salama, et al., 2014).

### Fungi Cultures

Three concentrations of *B. bassiana*, *M. anisopliae* and *V. lecanii* were (105; 106; and 107 conidia/ml). Two Entomopathogenic fungi were used in this study; *B. bassiana* and *M. anisopliae* were isolated from *Cassida vittata* and *Scrobipalpa ocellatella* insects from Kafr Elshikh Governorate in Egypt (Abdel-Raheem, 2005). The third fungus was isolated from the soil from El-Behira Governorate in Egypt, and grown on peptone media (10g Peptone, 40g Dextrose, 2g Yeast extract, 15g Agar and 500 ml. Chloramphenicol and completed to one liter with distilled water). The media was autoclaved at 120 °C for 20 minutes, and poured in Petri-dishes (10 cm diameter x 1.5 cm) then inoculated with the entomopathogenic fungi and kept at 25 ±2°C and 85 ±5 R.H. The fungal isolates were re-cultured every 14-30 days and kept at 4 °C.

To obtain a huge number of conidia, both *B. bassiana* and *M. anisopliae* isolates were propagated on wetted rice. Two Kilogram wetted rice was washed in boiled water for 10 min. and put in thermal bags. These bags were autoclaved at 120°C for 20 min., then infected by isolates and incubated at 25 ±2°C for 15 days. The Conidia were harvested by distilled water and filtered through cheese cloth to reduce mycelium clumps and Tween 80% was added (Lacey, 1997).

### Preparation of the Concentrations

Conidia of fungal isolates were harvested by rinsing with sterilized water, 0.5% Tween 80 from 14 days old culture rice media. The suspensions were filtered through cheese cloth to reduce mycelium clumping. Conidia were counted in the suspension by using a haemocytometer (Hirschmann 0.1 mm x 0.0025 mm<sup>2</sup>). The suspension was put in plastic bottles (2 liter). To restore the virulence of the isolates, it was passed through their natural host, wax moth larvae *Galleria mellonella*. Three concentrations were prepared, (C1) 105, (C2) 106 and (C3) 107 conidia/ml in all isolates.

### Treatment Procedures

Three concentrations of each agent and three replicates for each were tested on *T. absoluta* larvae (Neonate "newly hatched", 2nd & 3rd instar) to study the effect of these materials on larval mortality. *B. bassiana*, *M. anisopliae* and *V. lecanii* were prepared with concentrations of (105; 106; 107). Tomato seedling pots of approximately 45 days old were placed in rearing cages and were exposed to ten *T. absoluta* couples in rearing cages for 24 hrs. Then *T. absoluta* adults were removed and the plants were checked daily until egg hatching. Potted plants were removed after exposure period and transferred in other cages until eggs start to hatching. Nine randomly selected leaves for each concentration were cut and dipped into the suspensions (three leaves per replicate), transferred onto white, clean paper for water evaporation then treated leaves were put in Petri dishes with filter papers and supplied with moisture as needed, then treated leaves infested with neonate larvae obtained from the laboratory colony (15

larvae/replicate). The treated disks were only used once at the beginning of the bioassay. Subsequently, the larvae were fed with untreated leaves when needed. A similar method of experiment was performed to estimate the effect of the three entomopathogenic materials on larvae from the second instar and third instar. In addition, eggs of *T. absoluta* were exposed to *B. bassiana* and *M. anisopliae* to evaluate their effect on hatchability. In these cases, the experiments were conducted in the same way. In order to obtain larvae of the 2nd & 3rd instar used in these experiments, larvae were reared to the desired instar on tomato plants.

The leaves were collected from the tomato plants, arranged in Petri dishes and infested with larvae obtained from the laboratory colony. Larvae were allowed to feed on untreated leaves until they reached the second and third instar. Discs were transferred to Petri dishes and larvae in the appropriate instar were placed in the dishes. The bioassay lasted for 7 days and the median lethal concentration (LC50) values were obtained by the software computer program. The larval mortality was evaluated daily for 10 days and at the end of the experiment. The mortality was corrected using Abbott's formula (Abbott, 1925)

$$\text{Corrected Mortality \%} = 100 \times 1 - \frac{\text{Insect population in treated after treatment}}{\text{Insect population in control after treatment}}$$

## RESULTS AND DISCUSSION

Efficacy of three concentrations of *B. bassiana*, *M. anisopliae* and *V. lecanii* were prepared with concentrations of (105; 106; 107) and tested on *T. absoluta* larvae (Neonate "newly hatched", 2nd & 3rd instar) to study the effect of these materials on larval mortality. In addition, eggs of *T. absoluta* were exposed to *B. bassiana*, *M. anisopliae* and *V. lecanii* to evaluate their effect on hatchability under laboratory conditions. The estimated LC50 of *V. lecanii* were  $3.25 \times 105$  spores/ml,  $5.47 \times 105$  &  $3.28 \times 105$  for neonate, 2nd instar & 3rd instar *T. absoluta* larvae, respectively. While the LC50 values of *B. bassiana* & *M. anisopliae* were  $(0.28 \times 105$  &  $0.11 \times 105)$ ,  $(0.45 \times 105$  &  $0.46 \times 105)$  and  $(0.32 \times 105$  &  $0.27 \times 105$  conidia/ml) for neonate, 2nd instar & 3rd instar *T. absoluta* larvae, respectively.

According to LC50 values, *B. bassiana* and *M. anisopliae* were most effective on larval phase of *T. absoluta* than *V. lecanii* (Table 1). Daily mortality (%) of larval phase of *T. absoluta* fed in the newly hatched, second instar and third instar larva with leaves treated with *V. lecanii*. The results revealed that when neonate larvae fed on *V. lecanii* the pathogen effect was evident by the third day of evaluation after exposure in the three concentrations (105; 106; 107 spores/ml) with recorded mortality (13.3, 13.3, 33.3%) respectively. Thereafter, the values of the corrected mortalities of neonate larvae increased gradually from the 4th day after exposure until the last day (10th) with mortality 88.8% for the first concentration 105 spores/ml. For the second concentration (106 spores/ml), the mortality values were increased and reached its maximum in the 8th day of exposure (100% reduction). The mortality values reached its maximum during the 7th day and in the 5th day for the second and third concentration (106 & 107 spores/ml) and recorded 100% reduction for the two concentrations. Thus, it was evident that the higher effective concentration of *V. lecanii* on neonate larvae of *T. absoluta* was 107 spores/ml followed by 106 spores/ml while the other concentrations (105 spores/ml) showed a moderate effect. For the second instar larva of *T.*

*absoluta* the pathogenic effect was evident by the fifth day for (105 spores/ml), by the 4th day for (106 spores/ml) and by the 3rd day for (107 spores/ml) of evaluation after exposure with corresponding mortalities (53.3, 40.0, and 6.6 %) for the three concentrations, respectively. Then, the values of the corrected mortalities of the second instar larvae increased gradually until the 9th day after exposure to record 88.8% mortality for the first concentration (105 spores/ml).

The mortality values reached its maximum in the 9th day, 8th day and in the 7th day for the three concentrations and recorded 100% reduction. Daily mortality (%) of larval phase of *Tuta absoluta* fed in the newly hatched "neonate", second instar and third instar larva with leaves treated with *Beauveria bassiana* was recorded. The results stated that when neonate larvae fed on *Beauveria bassiana*, the pathogenic effect was evident by the 3rd day of evaluation after exposure in the first concentration (105 conidia/ml) with recorded mortality (20.0%). While the pathogenic effect was evident by the 2nd day of evaluation after exposure in the 2nd & 3rd concentrations (106 & 107) with recorded mortalities (6.7%) for the three concentrations.

Thereafter, the values of the corrected mortalities of neonate larvae increased gradually until the 7th day with maximum mortality 100.0% for 105 & 106 conidia/ml. While for the 2nd and 3rd concentration (106 & 107 conidia/ml) the mortality values were rapidly increased and reached its maximum in the 5th and 4th day of exposure (100% reduction). Thus, it was evident that the highest effective concentration of *B. bassiana* on neonate larvae of *T. absoluta* was 107 conidia/ml where this concentration gave 100% reduction rapidly in the 4th day of the evaluation, followed by 106, 107 and 105 conidia/ml, respectively. For the second instar larva, the results confirmed that when larvae fed on *B. bassiana* the pathogenic effect was evident by the 3rd day of evaluation after exposure in the concentration (105 & 106 conidia/ml) with recorded mortality (6.7 & 13.3%), respectively.

While the pathogenic effect was evident by the 2nd day of evaluation after exposure in the 2nd & 3rd concentrations (106 & 107 conidia/ml) with recorded mortalities (6.7%) for the two concentrations. After that, the values of the corrected mortalities of the second instar larvae increased gradually until the 7th day and 8th day with maximum mortality 100.0% for 105 & 106 conidia/ml, respectively. While for the 3rd concentration (107 conidia/ml), the mortality values were rapidly increased and reached its maximum in the 5th day of exposure (100% reduction).

Thus, it was clear that the higher effective concentration of *B. bassiana* on the second instar larvae of *T. absoluta* resulted from the two concentrations 107 and 106 conidia/ml evenly, followed by 107 and 106 conidia/ml, respectively. When the third instar larvae fed on *B. bassiana*, the pathogenic effect was evident by the 3rd day of evaluation after exposure in the first concentration (105 conidia/ml) with recorded mortality (20.0%). While the pathogenic effect was evident by the 2nd day of evaluation after exposure in the 1st, 2nd & 3rd concentrations (105, 106 & 107) with recorded mortalities (6.7%) for the three concentrations. Afterward, the values of the corrected mortalities of the third instar larva increased gradually until the 7th day and 6th day where it reached its maximum mortality 100.0% for 105 & 106 conidia/ml, respectively. Whereas, for the 3rd concentration (107 conidia/ml), the mortality values were rapidly increased and reached its maximum in the 5th day of exposure (100% reduction).

**Table (1):** Efficacy of three entomopathogenic Fungi against *Tuta absoluta*

| Entomopathogenic fungi | LC <sub>50</sub>       |                               |                               |
|------------------------|------------------------|-------------------------------|-------------------------------|
|                        | Neonate                | 2 <sup>nd</sup> instar larvae | 3 <sup>rd</sup> instar larvae |
| <i>B. bassiana</i>     | 0.28 x 10 <sup>5</sup> | 0.45 x 10 <sup>5</sup>        | 0.32 x 10 <sup>5</sup>        |
| <i>M. anisopliae</i>   | 0.11 x 10 <sup>5</sup> | 0.46 x 10 <sup>5</sup>        | 0.27 x 10 <sup>5</sup>        |
| <i>V. lecanii</i>      | 3.25 x 10 <sup>5</sup> | 5.47 x 10 <sup>5</sup>        | 3.28 x 10 <sup>5</sup>        |

**Table (2):** % Hatchability of *Tuta absoluta* eggs treated with *B. bassiana*.

| Concentration   | 1 <sup>st</sup> | 2 <sup>nd</sup> | 3 <sup>rd</sup> | 4 <sup>th</sup> | 5 <sup>th</sup> | 6 <sup>th</sup> | 7 <sup>th</sup> day | 8 <sup>th</sup> | 9 <sup>th</sup> | 10 <sup>th</sup> |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|---------------------|-----------------|-----------------|------------------|
|                 | day             | day             | day             | day             | day             | day             |                     | day             | day             | day              |
| % Hatchability  |                 |                 |                 |                 |                 |                 |                     |                 |                 |                  |
| 10 <sup>5</sup> | 0.0             | 0.0             | 0.0             | 0.0             | 45.2            | 46.2            | 46.3                | 46.3            | 46.3            | 46.3             |
| 10 <sup>6</sup> | 0.0             | 0.0             | 0.0             | 0.0             | 43.0            | 43.0            | 43.0                | 43.0            | 43.0            | 43.0             |
| 10 <sup>7</sup> | 0.0             | 0.0             | 0.0             | 0.0             | 0.0             | 33.0            | 36.9                | 38.7            | 38.7            | 38.7             |
| Control         | 0.0             | 0.0             | 0.0             | 0.0             | 86.7            | 86.7            | 86.7                | 86.7            | 86.7            | 86.7             |

**Table (3):** % Hatchability of *Tuta absoluta* eggs treated with *M. anisopliae*.

| Concentration   | 1 <sup>st</sup> | 2 <sup>nd</sup> | 3 <sup>rd</sup> | 4 <sup>th</sup> | 5 <sup>th</sup> | 6 <sup>th</sup> | 7 <sup>th</sup> day | 8 <sup>th</sup> | 9 <sup>th</sup> | 10 <sup>th</sup> |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|---------------------|-----------------|-----------------|------------------|
|                 | day             | day             | day             | day             | day             | day             |                     | day             | day             | day              |
| % Hatchability  |                 |                 |                 |                 |                 |                 |                     |                 |                 |                  |
| 10 <sup>5</sup> | 0.0             | 0.0             | 0.0             | 0.0             | 43.0            | 45.3            | 45.3                | 45.3            | 45.3            | 45.3             |
| 10 <sup>6</sup> | 0.0             | 0.0             | 0.0             | 0.0             | 35.0            | 37.0            | 37.0                | 37.0            | 37.0            | 37.0             |
| 10 <sup>7</sup> | 0.0             | 0.0             | 0.0             | 0.0             | 33.0            | 35.3            | 36.9                | 36.9            | 36.9            | 36.9             |
| Control         | 0.0             | 0.0             | 0.0             | 0.0             | 85.0            | 85.3            | 85.3                | 85.3            | 85.3            | 85.3             |

**Table (4):** % Hatchability of *Tuta absoluta* eggs treated with *V. lecanii*.

| Concentration   | 1 <sup>st</sup> | 2 <sup>nd</sup> | 3 <sup>rd</sup> | 4 <sup>th</sup> | 5 <sup>th</sup> | 6 <sup>th</sup> | 7 <sup>th</sup> day | 8 <sup>th</sup> | 9 <sup>th</sup> | 10 <sup>th</sup> |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|---------------------|-----------------|-----------------|------------------|
|                 | day             | day             | day             | day             | day             | day             |                     | day             | day             | day              |
| % Hatchability  |                 |                 |                 |                 |                 |                 |                     |                 |                 |                  |
| 10 <sup>5</sup> | 0.0             | 0.0             | 0.0             | 0.0             | 46.0            | 46.9            | 46.9                | 46.9            | 46.9            | 46.9             |
| 10 <sup>6</sup> | 0.0             | 0.0             | 0.0             | 0.0             | 40.4            | 40.7            | 40.8                | 40.8            | 40.8            | 40.8             |
| 10 <sup>7</sup> | 0.0             | 0.0             | 0.0             | 0.0             | 36.0            | 36.4            | 38.8                | 38.8            | 38.8            | 38.8             |
| Control         | 0.0             | 0.0             | 0.0             | 0.0             | 83.0            | 84.0            | 84.0                | 84.0            | 84.0            | 84.0             |

Thus, it was evident that the highest effective concentration of *B. bassiana* on the second instar larvae of *T. absoluta* was 106 & 107 conidia/ml where these concentrations gave 100% reduction rapidly in the 5th day of the evaluation, followed by 106 and 105 conidia/ml, respectively. Daily mortality (%) of larval phase of *T. absoluta* fed in the newly hatched "neonate", second instar and third instar larva with leaves treated with *M. anisopliae*. When neonate larvae fed on *M. anisopliae* (the

pathogen effect was evident by the 3rd day of evaluation after exposure in the first concentration (105 conidia/ml) with recorded mortality (20.0%). While the pathogenic effect was evident by the 2nd day of evaluation after exposure in the three concentrations (105, 106 & 107) with recorded mortalities (6.7%) for the three concentrations.

Subsequently, the values of the corrected mortalities of neonate larvae increased gradually until the 7th day with

maximum mortality 100.0% for 105 & 106 conidia/ml. While for the 3rd concentration (107 conidia/ml) the mortality values were rapidly increased and reached its maximum in the 5th day of exposure (100% reduction) for the two concentrations. Hence, it was evident that the highest effective concentration of *M. anisopliae* on neonate larvae of *T. absoluta* was 106 & 107 conidia/ml evenly, where these two concentrations gave 100% reduction rapidly in the 5th day of the evaluation, followed by 105 conidia/ml, respectively. The results confirmed that, when the second instar larvae fed on *M. anisopliae*, the pathogenic effect was evident by the 3rd day of evaluation after exposure in the concentration (105 & 106 conidia/ml) with recorded mortality (6.7 & 13.3%), respectively.

While the pathogenic effect was evident by the 2nd day of evaluation after exposure in the 2nd & 3rd concentrations (106 & 107 conidia/ml) with recorded mortalities (6.7%) for the two concentrations. Thus, it was clear that the highest effective concentration of *M. anisopliae* on the second instar larvae of *T. absoluta* resulted from the concentration 107 conidia/ml followed by the concentration 106 conidia/ml, and 105 conidia/ml. While the third instar larvae when fed on *M. anisopliae*, the pathogenic effect was evident by the 3rd day of evaluation after exposure in the first concentration (105 conidia/ml) with recorded mortality (20.0%). While the pathogenic effect was evident by the 2nd day of evaluation after exposure in the 1st, 2nd & 3rd concentrations (105, 106 & 107) with recorded mortalities (6.7%) for the three concentrations.

Afterward, the values of the corrected mortalities of the third instar larvae increased gradually until the 7th day and 6th day where it reached its maximum mortality 100.0% for the three concentrations, (105, 106, & 107 conidia/ml). Eggs of *T. absoluta* were exposed to *B. bassiana* and *M. anisopliae* to evaluate their effect on hatchability with concentrations of (105, 106, & 107 conidia/ml) under laboratory conditions (Tables 2 & 3). Hatchability of *T. absoluta* eggs treated with *B. bassiana* and *M. anisopliae* demonstrated in (Tables 2 & 3) revealed that; the pathogenic effect was evident by the fourth day of evaluation after exposure in the three concentrations (105, 106, & 107 conidia/ml). The black appearance of eggs of *T. absoluta* took place after exposure to the three concentrations of the two pathogenic fungi starting from the fifth day after exposure, and thereby no hatched larvae appeared in the three concentrations of the two pathogenic fungi compared to the control where the hatchability reached 87.7% and 80.0% during the evaluation time for *B. bassiana* and *M. anisopliae*, respectively.

In this regard, 36 & 17 stated that; eggs of *T. absoluta* are deposited singly or (rarely) in batches. Immediately after egg deposition they are yellowish, becoming coppery-red and with two red eye-spots about 1 day before hatching. The incubation period is between 4 and 7 days at 27 °C. Also, 22 revealed that, According to statistical analysis of observed data, effect of *B. bassiana* was 41.67% and 66.67%, while effect of *M. anisopliae* was 91.67% and 100.00% on egg stage of *T. absoluta* at the end of the 7th and 9th days after application. In spite of that, on first larval stage of *T. absoluta* at the end of 9th day after application, effect of *B. bassiana* and *M. anisopliae* were 12.50% and 91.67% respectively.

Consequently, this laboratory experiment suggested that *M. anisopliae* has a potential effect on both egg and first larval stage of *T. absoluta* but *B. bassiana* is effective just on egg stage. The greatest percentage of mortality occurred in the newly hatched "neonate" for the three concentrations followed by the third instar larvae and the second instar larvae for the three concentrations which gave the lowest larval mortality.

These results agree with findings of 21 where stated that; the higher mortality of neonate larvae, than later instars can be explained by the feeding behaviour differences. Neonate larvae scratch the leaf for 20±45 min before penetrating the mesophyll and are therefore exposed to a higher dose of bacterial spores and toxins. For second instar larvae as well, the low mortality was probably related to the lack of leaf scratching as observed with neonates.

For the third instar larvae, high mortality was probably due to greater leaf consumption since this instar consumed the entire treated leaf disc, consequently ingesting a higher dose of the pathogen and its toxin. The accumulative larval mortality of larval phase of *T. absoluta* fed with leaves treated with *B. bassiana* the greatest percentage of pathogenic effect occurred for in the newly hatched "neonate" followed by the second and the third instar larva which gave similar larval mortality values. Similarly, when larval phase of *T. absoluta* were fed with leaves treated with *M. anisopliae*, the pathogenic effect was clearly high on the newly hatched "neonate" for the three concentrations followed by the second and the third instar larva which gave similar larval mortality values. In this regard (Hussein Salama, et al., 2014) stated that, the experiment used the immersion method conducted with four replicates in laboratory.

After application, leaves on which larvae and eggs were laid on blotting paper in order to dry and then they were placed in petri dishes. Dead and survival individuals were taken census at 3rd, 5th, 7th and 9th days. According to statistical analysis of observed data, effect of *B. bassiana* was 41.67% and 66.67% while effect of *M. anisopliae* was 91.67% and 100.00% on egg stage of *T. absoluta* at the end of 7th and 9th days after application. In spite of that, on first larval stage of *T. absoluta* at the end of 9th day after application, effect of *B. bassiana* and *M. anisopliae* were 12.50% and 91.67% respectively. Generally, it could be concluded that; the effect of pathogen application was dependent on the instar at which the larvae were fed on pathogen-treated leaves. Where in our experiment, the greatest percentage of mortality occurred on the neonate larvae for three treatments (*V. lecanii*, *B. bassiana* & *M. anisopliae*).

In addition, the pathogen *B. bassiana* gave the highest effect on larval phase of *T. absoluta* during the evaluation time and similar to the effect of *M. anisopliae* followed by the effect of *V. lecanii*. Concerning the most effective concentration of each agent, it was found that the higher the concentration (107), the higher the mortality. Thus our laboratory experiment suggested that *B. bassiana* and *M. anisopliae* has a potential effect on both egg and neonate "newly hatched larvae" of *T. absoluta* and *V. lecanii* is effective on neonate and third larval stage. Results showed that using fungus *Beauveria bassiana* was affected on all parameters of growth stages of *T. absoluta*. The use of this fungus led to a decrease of egg hatching of *T. absoluta* compared with control treatment; meanwhile, this fungus had a significant effect on mortality ratio of larval instars of studied insect and led to increase of this ratio compared with control treatment which had 0% of mortality ratio. Also, results showed increases in mortality ratio when using *B. bassiana* on adults. The effect of pathogen application was dependent on the instar phase at which the larvae were fed on pathogen-treated leaves also, the greatest percentage of mortality occurred on the neonate larvae for three agents. Concerning the most effective concentration of each agent, the higher the concentration (107), the higher the mortality. The greatest percentage of mortality occurred in the newly hatched "neonate" followed by the third instar larvae when fed with leaves treated with *B. bassiana*. Also, the greatest percentage

of pathogenic effect occurred in the newly hatched “neonate” followed by the second and the third instar larva which gave similar larval mortality values when larvae were fed with leaves treated with *M. anisopliae* and *V. lecanii*.

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