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Article

Tomato plants colonization by endophytic entomopathogenic fungi for controlling the whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae)

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Abstract: Colonization of tomato plants by fungal endophyte isolates, *Beauveria bassiana* and *Cladosporium cladosporioides* by two inoculation methods (seeds and seedlings) was evaluated. Seed inoculation was the most efficient method resulted in highest recovery of both inoculated fungal strains from roots, stems and leaves tissues. Also, the effects of inoculated endophytes on survival of *B. tabaci* adults and nymphs were studied. In addition, Repellence effect and oviposition deterrence of inoculated endophytes were assessed. *C. cladosporioides* proved capable of colonizing tomato plants protecting it from *B. tabaci* attack by suppressing both adults and nymphs in addition to repel adults and deter oviposition.

Key words: Endophytic, *Beauveria bassiana*, *Cladosporium cladosporioides*, *Bemisia tabaci*, Repellence.

1. Introduction

Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) is an extremely dangerous pest threats several economic crops like tomato. This tiny insect damages crops through feeding and producing honeydew, the substrate of sooty molds. In addition, it transmits more than 350 plant viruses during feeding **(Polston *et al*., 2014)**. Despite several studies concerned with controlling this pest, it still problematic due to its polyphagous and multi-voltine nature. Entomopathogenic fungi were crucial candidates in integrated pest management program of *B. tabaci*. They were used either by direct inundative or inoculative application of fungal spores **(Liu *et al*., 2017)**. Endophytic fungi are apoplastic organisms live naturally inside the host plant tissues or artificially inoculated without causing any harm or disease symptoms to the host **(Wilson, 1995)**. Quite the contrary, it was found that the endophytic fungi play an important role in enhancing plant growth, providing protection of the plants from pathogens and insect attacks **(Saikkonen *et al*., 2004; Silva *et al*., 2020 and Garrido-Jurado *et al*., 2004)**. Inoculation techniques affect the ability of the endophytes to colonize different plant tissues and the period of the endophyte survive **(Muvea *et al*., 2014)**. Therefore, the present study aimed to evaluate the endophytic ability of native strains of *Beauveria bassiana* and *Cladosporium cladosporioides* in tomato plants by different inclusion methods. Also, their negative impacts on *B. tabaci* adults and nymphs, were studied. In addition, repellence effect and oviposition deterrence were assessed.

2. Materials and methods

2.1. Source and rearing of *B. tabaci*

B. tabaci culture was obtained from laboratorial stock colony maintained on tomato seedlings planted in small pots kept inside transparent plastic cages at 27±2 ºC, 70±5 RH and 14hr photoperiod at Plant Protection Research Institute, Mansoura Branch, Egypt.

2.2. Fungal inoculum source

The two native fungal isolates, *B. bassiana* and *C. cladosporioides* used in this study were previously recorded naturally infecting *B. tabaci* on tomato plants **(Ibrahim *et al*., 2024)**. They were morphologically identified according to **Humber`s key (1997)**.

Conidia were harvested from two-week old cultures grown on Sabouraud dextrose (SADA) media [40 g/l dextrose, 10 g/l peptone, and 20 g/l agar] supplemented with 20 mg/L Amoxicilline at 25± 2 ºC and 70±5% RH by scraping the sporulating cultures surface with a sterile scalpel. The collected conidia were suspended in 10 ml sterile distilled water containing 0.05 % Tween -80 with constant stirring for five minutes. The stock conidial concentrations were estimated using Neubauer haemocytometer. Through serial dilution, the conidial concentration of 1×10^8 conidia/ mL was prepared for seeds and seedlings inoculation.

For conidia viability assessment, 0.1 ml of each isolate conidial homogenate was inoculated onto the surface of 9-cm Petri dishes containing SDA, then a sterile microscope cover slip (2 × 2 cm) was placed on the surface of the agar in each plate and they were incubated at 25± 2 ºC and 70±5% RH. After 24hrs, the conidial germination percentage was determined by counting the germinated conidia out of 100 in one randomly selected field. Conidia germination was considered when germ tubes exceeded half of conidium diameter **(Muvea *et al*., 2014)**. Five replicates counts were used for each isolate.

2.3. Inoculation of tomato seed and seedling by fungal endophytes

Tomato seeds (*Solanum lycopersicum* L. cv. 023 F1) (Sakata Seeds Corporation, Japan) were surface-sterilized by successive washing in 70% ethanol for 2 min followed by 1.5% sodium hypochlorite for three min then rinsed thrice in sterile distilled water **(Clair *et al*., 1997)**. For confirming surface sterilization efficiency, 0.1 mL of the last rinse water was inoculated onto SDA supplemented with 20 mg/L Amoxicilline and incubated at 25ºC for 14 days. Sterilization reliability was confirmed by zero fungal growth on the culture media **(Schulz *et al*., 1998** and **Parsa *et al*., 2013)**. The surface sterilized seeds were let dry for 20 min on sterile filter paper in a laminar cabinet, then, they were divided into two parts, one was designated for the seed while the other for the seedling inoculation.

For conducting seed inoculation process, 10 g of surface-sterilized seeds were divided into three equal parts, two of them were separately immersed in 10 ml of each tested fungal conidial suspension at concentration of 1×10^8 conidia/ ml for 12 hrs. The third portion (control) was immersed in sterile distilled water containing 0.05% Tween- 80. All seeds were air dried for 20 min in a laminar flow cabinet, then sown at 1 cm depth in plastic pots (8 cm diameter × 10 cm height) containing ~50g of sterile peat moss planting substrate. They were maintained at room temperature (~25±2ºC, 60± 5% RH and 12hrs photoperiod) and watered when needed without any fertilizer use. Immediately after germination, pots were transferred to glasshouse (3 m length × 3 m width × 2.5 m height) at 25 ± 2 °C, 60± 5% RH and 12hrs photoperiod for four weeks.

For seedlings inoculation, the surface-sterilized seeds were sown as outlined previously but without immersing in the conidial suspensions. They were maintained for two weeks in a glasshouse at room temperature (~25± 2ºC and 60± 5% RH). Then, the two weeks old seedlings (height 8 -10 cm) were carefully uprooted and the residual attached soil was removed then rinsed with sterile water. Roots of four well-developed seedlings were dipped in an endophyte conidial suspension of 1×10^8 conidia/ ml for 12 hours, whereas, another four seedlings (control) were dipped only in sterile distilled water containing 0.05 % Tween -80. Both control and inoculated seedlings were transplanted in pots containing sterile soil and maintained under similar conditions of seed inoculation as stated earlier.

2.4. Colonization assessment

For verifying fungal isolates colonization in tomato, four weeks old seedlings were gently uprooted from their pots and rinsed with sterile water. Five randomly selected sections of leaves, stems and roots were sampled from each plant and these sampled parts were surface-sterilized according to **(Clair *et al*., 1997)**, then aseptically cut inside a laminar cabinet into 1 × 1 cm pieces that placed 4 cm apart on SDA plates supplemented with a 0.05% streptomycin sulphate salt as antibiotic **(Akutse *et al*., 2013** and **Muvea *et al*., 2014)**. Plates were incubated at 25 ± 2 °C for 10 days for endophytes presence determination. Positive colonization of the plant parts was scored according to Koch's postulates **(Petrini and Fisher, 1986)** by counting number of pieces of different plant parts that showed inoculated fungal growth. Slides were prepared from the mother plates to confirm that the endophytes were those initially inoculated. Each treatment was performed with four replicates. The fungal endophyte colonization (%) of host plant parts was determined as following:

$$\text{Colonization frequency} = \left(\frac{\text{Number of Plant Pieces Colonized}}{\text{Total Number of Plant pieces}}\right) \text{x}\, 100$$

2.5. Impact of endophytically-colonized tomato plants on whitefly, *B. tabaci*

2.5.1. Repellence effect and oviposition deterrence (Choice test)

The repellence effect of the endophytic entomopathogenic fungal isolates *B. bassiana* and *C. cladosporioides* was tested. A tomato seedling (4 weeks old) inoculated as seeds with one of the inoculated fungal isolate was placed with another untreated one (control) in a net cage (60cm x 60cm x 60cm) inside the glass house under stable conditions (~25±2ºC and 60± 5% RH and 12hrs photoperiod). The distance between both the inoculated and untreated seedling was 30cm distance. At the center of the cage, release of 100 adult whiteflies were done and let spread. The numbers of settled whiteflies and deposited eggs on both inoculated and non-inoculated seedlings were counted at 24hrs after release. The experiment has been repeated three times (3 replicates).

2.5.2. Effect of the endophytic fungi on *B. tabaci* adults and nymphs

For exploring the impact of the endophytic fungi on adult *B. tabaci*, three tomato seedlings (4 weeks old) inoculated as seeds with *B. bassiana* and another three inoculated as seeds with C. *cladosporioides*, in addition to three non- inoculated seedlings (control) were individually (one plant/ cage) placed in net cage (30 cm × 30 cm × 60 cm high) under glass house. Afterward, 50 adult *B. tabaci* were released in each cage and adults were allowed to feed and oviposit for 48 hr, then the seedlings with eggs laid were replaced with another of the same kind to provide the adults with nutrition. Adult mortality % was recorded daily till the 5th day after release. Seedlings with deposited eggs were kept in another cages under the glass house to track the nymphs that will hatch from the eggs. As soon as the nymphs emerge, 100 nymphs/ seedling were counted and marked using a water proof pen under dissecting microscope to represent a replicate. Mortality % of *B. tabaci* nymphs were recorded after 7 days of their hatch.

2.6. Statistical analysis

The Repellence index **(Baldin and Lara 2001; Schilick-Souza *et al*., 2011 and Baldin *et al*., 2013)** was estimated according to the equation: RI=2T / (T+C), where T is the number of insects settled on the inoculated seedling and C is the number of insects on the non-inoculated seedling. When RI values <1, it indicated repellence of *B. tabaci* by inoculated seedling compared with the control; RI>1, that indicated *B. tabaci* attractiveness to the inoculated seedling compared with the control.

Estimation of oviposition deterrence index (ODI.) **(Hang *et al.* 1982)** was performed according to the equation: ODI= [(T-C)]/ (T+C)] x 100, where T is the number of eggs counted on the inoculated seedling, and C is the deposited eggs number in control. ODI values ranged between +100 (very attractive) and -100 (complete deterrence). Values of RIs and ODIs were subjected to One-Way ANOVA analysis using **CoStat Software (2004).**

Mortality percent of adults and nymphs of *B. tabaci* was determined according **Abbott (1925).**

3. Results and Discussion

3.1. Colonization of tomato seed and seedling by fungal endophytes

Both fungal isolates yielded over 90% of germinated conidia in the viability test. They were able to colonize tomato plants via seed or seedling inoculation with varying degrees depending on the fungal isolate and the inoculation method (Figs. 1, 2). Seed inoculation resulted in highest recovery of both inoculated fungal strains from roots, stems and leaves comparing with seedlings inoculation. Also, *C. cladosporioides* showed the highest colonization of all plant parts through both colonization methods. It successfully colonized 100, 95 ±8.66% and 85 ± 8.66% of roots, stem and leaves of seed inoculated-tomato plant, respectively. In addition, it resulted in 100, 80 ± 14.14 and 75 ± 8.66% of root, stem and leaves of seedling inoculated tomato plant, respectively.

On the other hand, *B. bassiana* showed satisfactory colonization degree of root in both inoculation method, but low and very low colonization of stem and leaves in seed and seedling inoculated tomato plant. The current data agreed with previous studies proved the great success of seed inoculation method for colonizing many plant tissues with entomopathogenic fungi **(Kabaluk and Ericsson 2007; Ownley *et al.* 2008; Powell *et al.* 2009; Akello and Sikora 2012; Akutse *et al.* 2013; Lopez *et al.* 2014 and Russo *et al.* 2015)**.

Also, the current results were compatible with those of **(Agbessenou *et al*., 2020)** reported the high recovery of the fungus was detected from root of seed inoculated tomato plant but low colonization rate found in aerial parts especially, leaves. It may be due to the unsuitable environment of tomato leaf which contain some substances inimical to the fungal growth **(Posada *et al*., 2007 and Martin, 1964).** The high recovery of *B. bassiana* from the roots may be attributed to rhizosphere microbiota origin of this fungus which attracts the fungus to the root exudates and rhizodeposits **(Philippot *et al*., 2013).**

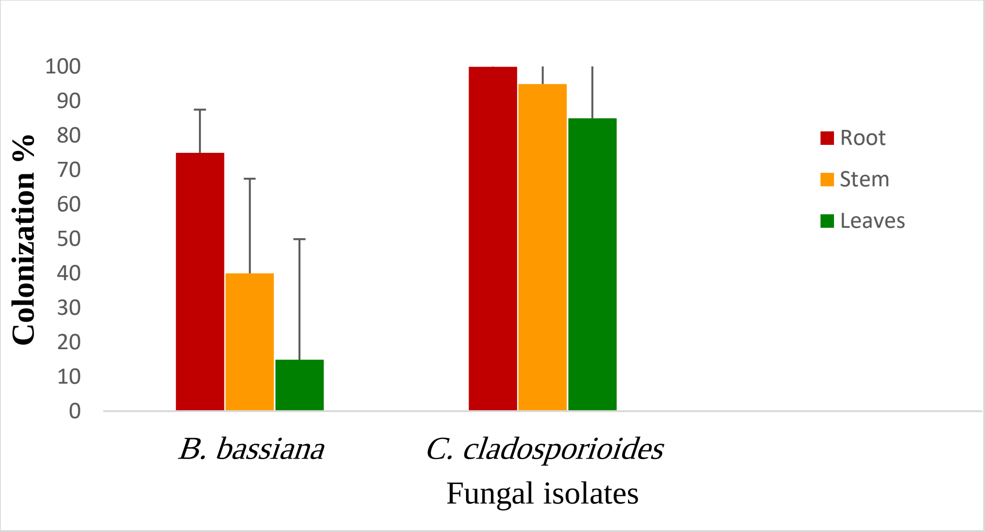


Fig. (1). Colonization % of tomato plant parts (root, stem and leaves) by different fungal endophytes through seeds inoculation. Bar chart represents Mean± SE at p< 0.05

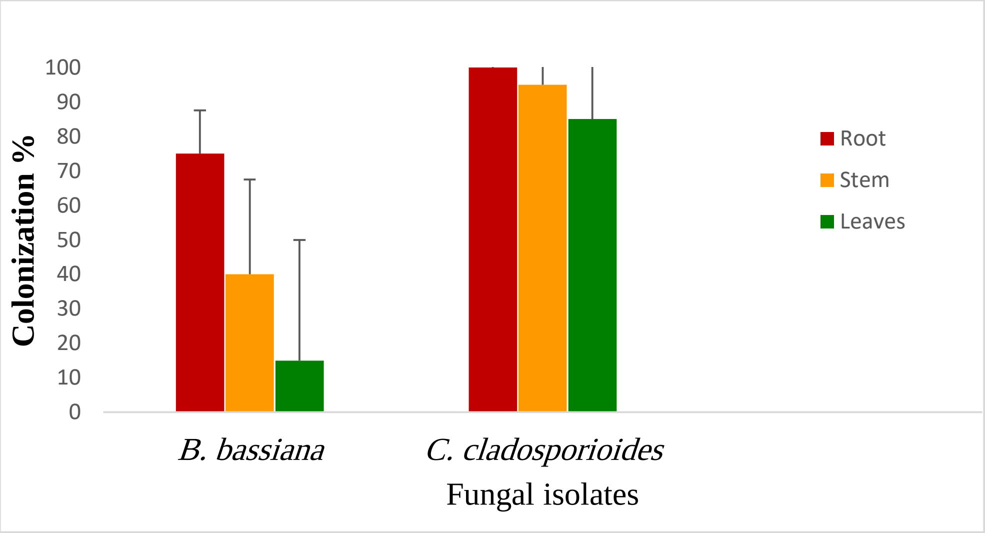
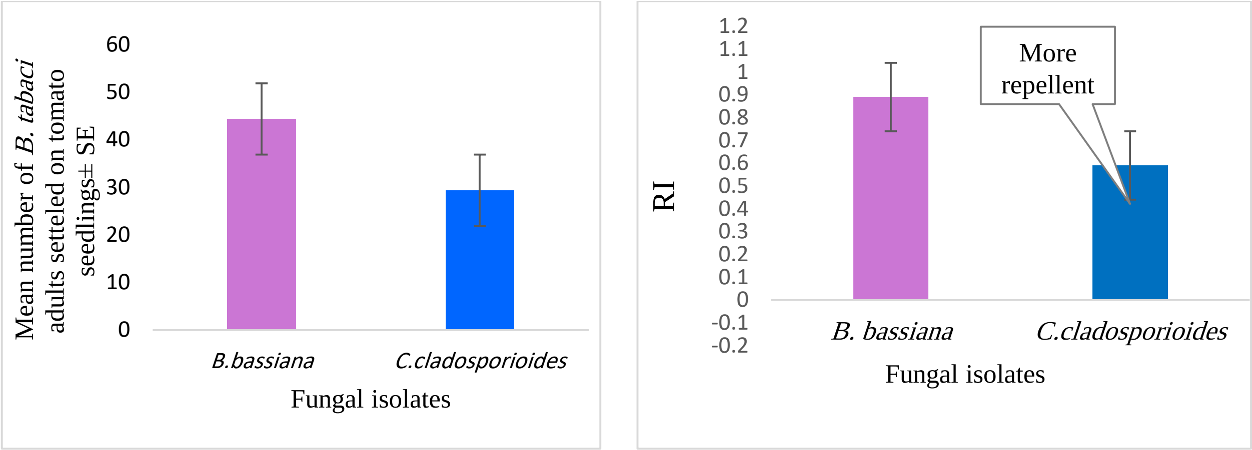


Fig. (2). Colonization % of tomato plant parts (root, stem and leaves) by the tested fungal endophytes through seedkings inoculation. Bar chart represents Mean± SE at p< 0.05

3.2. Impact of endophytically-colonized tomato plants on whitefly, *B. tabaci*

3.2.1. Repellence effect and oviposition deterrence (Choice test)

Repellence of endophytically colonized tomato plants against *B. tabaci* adults were studied via choice test to enable the insect to exercise full choice in host plant selection. It was noticed that both of *B. bassiana* and *C. cladosporioides* endophytically colonized tomato plants showed repellence effect towards *B. tabaci* adults. There were significant differences between the two fungi (df= 1, f: 46.023, p< 0.005**). Fig. 3 (A& B) showed the mean number of adult *B. tabaci* on the tested endophytic colonized tomato seedlings in addition to RI of both of them. *C. cladosporioides* colonized tomato plants recorded the lowest mean number of *B. tabaci* adults (29.33± 1.7) and RI: 0.887%, thereby placing it at the top as repellent of *B. tabaci* adults. *B. bassiana* colonized tomato plants showed lower repellent effect where they received more adult insects (mean 44.33 ± 2.625 adults) and RI: 0.587%.



(A) (B)

Fig. (3). (A)Mean number of *B. tabaci* adults settled on fungal endophytic colonized tomato seedlings ± SE, (B): Repellence Index of the endophytic colonized tomato plants

Also, the oviposition deterrence of the tested endophytic colonized tomato plants was tested against *B. tabaci* adults. The oviposition deterrence indices (ODIs) (Fig. 4) indicated that *C. cladosporioides* colonized tomato plants showed the high oviposition deterrence effects (-62.33%) followed by *B. bassiana* colonized tomato plants (-25.33%). There was high significant differences between the two fungal isolates (df= 1, f: 112.009, p<0.001***)

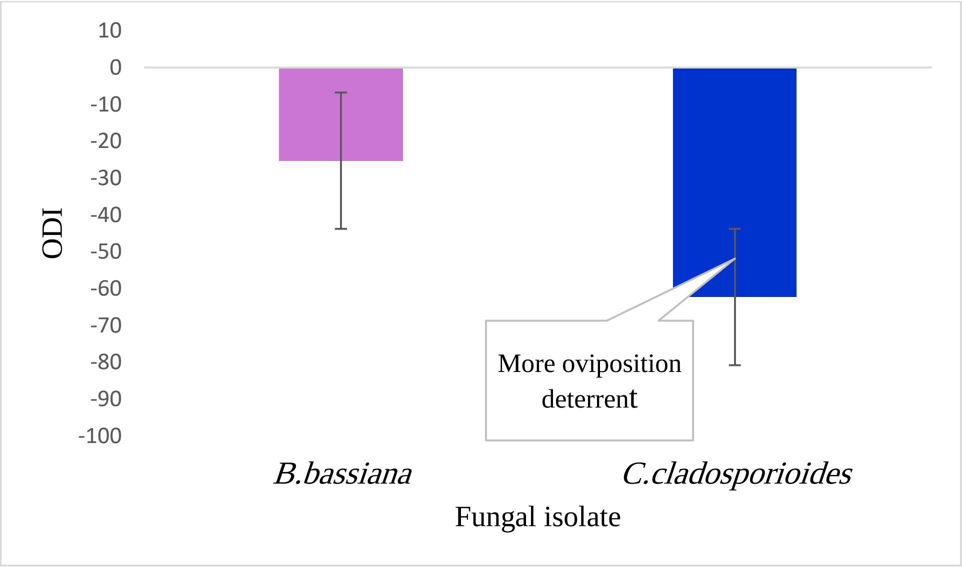


Fig. (4). Repellence Index of fungal endophytically colonized tomato plants

3.2.2. Effect of the endophytic fungi on *B. tabaci* adults and nymphs

Endophytic colonized tomato plants revealed a clear population reduction of both *B. tabaci* adults and nymphs. *B. bassiana* colonizing tomato plants caused moderate reduction in adult population (40.67± 3.4%), while, *C. cladosporioides* colonizing tomato plants revealed significant reduction in *B. tabaci* adults (60.67± 1.89%). There was significant differences between the endophytic fungi (df=1, f: 52.941, p< 0.005**)

Moreover, the negative impact of the endophytic colonized tomato plants extended to *B. tabaci* nymphal stages. Although nymphs were less affected compared to adults, its impact remains significant. The endophytic colonized fungus, *C. cladosporioides* revealed mortality in *B. tabaci* nymphs (58± 2.16 %) higher than that of *B. bassiana* (38.67± 1.7%). The high significant differences between the endophytic fungi was evidenced (df= 1, f: 98.941, p< 0.001***).

The current data is compatible with previous studies which reported negative effects of the endophytic fungus *B. bassiana* as endophyte in tomato plant on different developmental stages of *T. vaporariorum* **(Pourtaghi *et al*., 2020)**. Also, our data agreed with **(Barra-Bucarei *et al*., 2020)** who reported significant reduction in whitefly, *Trialeurodes vaporariorum* eggs and nymphs number infesting *B. bassiana*- inoculated tomato plants in the greenhouse.

Several previous studies demonstrated that endophytic entomopathogenic fungi negatively impacted the herbivores, especially phloem feeders (sap-sucking pests) **(Vidal and Jaber, 2015; Gange *et al*., 2019 and Ment *et al*., 2020)**. It was proved that the insecticidal activity of the endophytic fungi against endophytically-colonized plant feeder is attributed to feeding deterrence, antibiosis or toxic secretion **(Klieberand and Reineke, 2016; Greenfield *et al.* 2016; Allegrucci *et al*., 2017 and Russo *et al*., 2018)**.

Despite the trending of *C. cladosporioides* as entomopathogenic candidate in insect pest microbial control, researches on plant inoculations with *C. cladosporioides* to control *B. tabaci* and other insect pests is scarce. In the current study, it showed promising activity as endophytic fungi. It was investigated that *C. cladosporioides* produce a phytohormone, 3-indole acetic acid, which possess an insecticidal activity or turned into toxin upon ingestion **(Lajide *et al*., 1993)**. Also, it inhibits ecdysone hormone which plays a major role in moulting, leading to insect death **(Jeyasankar *et al*., 2013; Sivaraman *et al*., 2014; Chennaiyan *et al*., 2016 and Abo Elsoud *et al*., 2021).** It proved worthy of further study for understanding the mechanism of its activity as endophytic fungi.

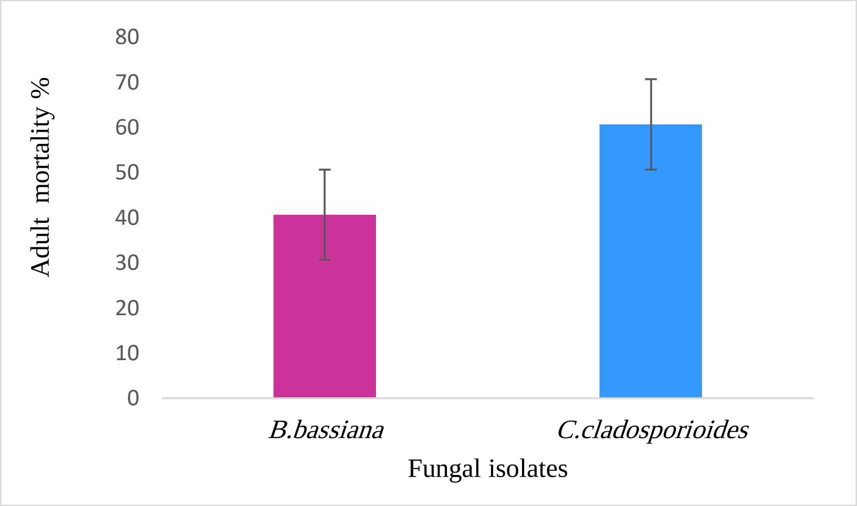


Fig. (5). Impact of fungal endophytically colonized tomato plants on Adults of *B. tabaci*

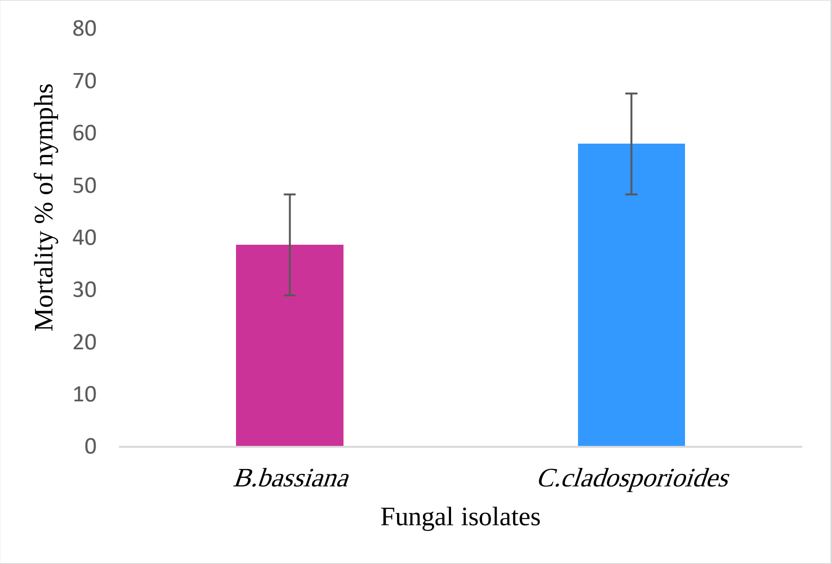


Fig. (6). Impact of fungal endophytically colonized tomato plants on nymphs of *B. tabaci*

4. Conclusion

Both of the entomopathogenic fungi, *B. bassiana* and *C. cladosporioides* effectively colonized various tomato plants parts through both of seeds and/or seedlings colonization, but inoculation through seeds was the almost efficient. The endophytic *C. cladosporioides* exerted significant repellence effect, oviposition deterrence and toxic effect against *B. tabaci* adults and nymphs higher than *B. bassiana*. It would be very crucial to investigate the underlying mechanism of its potency as endophytic fungi colonizing tomato or other plants for controlling the insect pests under the framework of Integrated Pest Management.

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