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Original Article

Determination of certain growth parameters of cotton (*Gossypium barbadense* L.) due to Mealy Bug, *Phenacoccus solenopsis* (Tinsley) infection and endophytic, *Beauveria bassiana* treatments

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ABSTRACT

This investigation were carried to test effect of mealy bug infestation and two inoculation methods of the entomopathogenic fungal endophyte, *Beauveria bassiana* to cotton plant, *Gossypium barbadense* L. (var. Giza 90) on some chemical component of cotton plant. The change in growth photosynthetic pigment content, total protein content, total antioxidant capacity total flavonoid content and the activities of some antioxidant were determined. Results showed that, *B. bassiana* treatments could induce the length and weight of fresh stem. Pest infestation and/or *B. bassiana* treatments, an increase in the amounts of flavonoids was noticed as compared to the control while *B. bassiana* application caused decrease in the antioxidant activity of leaf extracts detected by DPPH assay. Cotton plants showed accumulation in total proteins as a defense mechanism against *Phenacoccus solenopsis* (Tinsley) treatments.

KEYWORDS
Endophytes,
Cotton,
***Phenacoccus solenopsis*,**
***Beauveria bassiana*,**
Growth parameters.

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INTRODUCTION

Egyptian cotton occupies a unique and important role in the world market because it is very high quality, long staple fiber. Cotton is threatened by infestation with many insect pests among which are mealy bugs. Cotton Mealy bug, *Phenacoccus solenopsis* (Pseudococcidae; Hemiptera) is an exotic pest of several crops especially cotton (**Noureen *et al*. 2016**).

Large populations of mealy bugs cause general weakening, defoliation, and death of susceptible plants, mealy bug not only causes direct damage to the host plant by sucking plant sap, but also excretes large quantities of honeydew which encourages the black sooty mold growth and hinders photosynthesis (**Arif *et al*. 2012**). Furthermore, the insect is suspected as vector of plant diseases (**Culik & Gullan, 2005**).

Endophytes are micro-organisms colonize and live part of their life cycle within the tissue of a plant as endosymbionts, without causing any disease symptoms (**Hardoim *et al*. 2015; Puri *et al*. 2016**). As well as, preventing colonization of the host by foreign parasitic organisms (**Martinuz *et al*. 2012**). Fungal endophytes are utilized as an indirect defense against herbivores (**Kim *et al*., 2007, 2008; Quesada-Moraga *et al*. 2009**).

There are various reports of possible artificial inoculation of plants with fungal entomopathogens to establish as plant endophytes artificially (**Gurulingappa *et al*. 2010**; **Brownbridge *et al*. 2012; Parsa *et al*. 2013; Qayyum *et al*. 2015; Greenfield *et al*. 2016**). *Beauveria bassiana* is a soil saprophyte, entomopathogen and plant endophyte (**Roy *et al*. 2006; Ownley *et al*. 2008**) Traditionally *B. bassiana* is known as insect pathogens and isolated as naturally occurring endophyte from asymptomatic plant tissues (**Bills & Polishook 1991; Cherry *et al*. 1999; Vega *et al*. 2008; Orole & Adejumo 2009**). It is being used as a biological insecticide to control a number of pests. As an endophyte, *B. bassiana* may play a role in protecting plants from herbivory and disease. **Ownley *et al*. (2008b)** reported that endophytic colonization of tomato and cotton seedlings through seed soaking in *B. bassiana* conidia protected the seedlings against biotic factors.

The present study was carried out to test effect of mealy bug infestation and two adding methods of the entomopathogenic fungal endophyte, *B. bassiana* to cotton plant, *Gossypium barbadense* L. (var. Giza 90) on some chemical component of cotton plant.

MATERIALS AND METHODS

Plant: Seeds of cotton were obtained from Cotton Research Institute, Agriculture Research Center. Seeds were planted in plastic pots containing of 2 kg of soil mixture (sand/ peat moss) (1:1) essential nutrient were added.

Fungus: *B. bassiana* was maintained on PDA medium, (**Yong Jia *et al*. 2013**) the fungus can grow in liquid or solid medium as follow in this experiment. Fungal conidia were harvested from 3-week-old PDA cultures.

Insects: *Phenacoccus solenopsis* (Tinsley) were collected from cotton field in Shandaweel research Station, Sohag Governorate. samples were transported and cultured in a laboratory which was maintained on potato tubers with vegetable shoots in a plastic cage (50 cm × 50 cm × 50 cm) in a climate controlled insectary (18 ± 2°C, 70 ± 5% relative humidity; L16:D8 photoperiod).

Five seeds of cotton were planted in each pot and then irrigated every three days until germination. The best plant was selected for each pot and the rest of the plants were eliminated. Soon after 21 days of germination, seedling pots were arranged in a randomized complete block design (RCBD) with two factors, A: fungus adding methods; spray, watering and without adding (10 pots for each) and B: Each fungal treatment and zero treatment were divided into two parts (5 pots for each), one receiving infection with mealy bugs and the other without infection. 200 ml of the fungus solution were prepared for each treatment (Four flasks, each containing fifty mm liquid media -previously inoculated with the fungus- were mixed by a mixer) to watering one third of the planting pots, while the solution was used to spray the other third and the fungus was not added to the last third (zero treatment). These treatments were applied every three days for five times and then the addition of fungus was stopped, after that all plants were washed by water, then 5 adults of mealy bugs were used to make an artificial infection to half of pots according to experiment design and infestation development was recorded for 15 days.

Growth parameters

Germination and growth measurements including fresh weight (FW), dry weight (DW) and stem, root length were recorded.

Plant biochemical analysis

1. **Estimation of photosynthetic pigments**: The photosynthetic pigments (Chlorophyll a, Chlorophyll b and Carotenoids) were determined using spectrophotometric method recommended by (**Lichtenthaler 1987**).
2. **Estimation of total protein contents**: Total protein contents of leaves were determined at 700 nm, according to **Loweryetal (1951)** calibration curve was constructed using albumin and the data were expressed as mg/g DW.

3. **Determination of total flavonoid contents**: Aluminum chloride colorimetric method was used for estimation of total flavonoids (**Hegazy *et al*. 2012; Bhaigyabati *et al*. 2014**).
4. **Determination of total antioxidant, DPPH free radical scavenging assay**: The free radical-scavenging activity of leaf extract in methanol was measured using the method described by **Shimada *et al*. (1992)**. The percentage of DPPH scavenging activity was calculated according to **Azeez *et al*. (2012).**

$$\textbf{DPPH scavenging activity} = \frac{absorbance\ of\ control - absorbance\ of\ test}{absorbance\ of\ control} \times 100$$

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using F-test, followed by calculating the least significant difference (LSD) at 5 % level of probability, using Mstat-C computer-program, to exhibit the differences among all treatments.

RESULTS AND DISCUSSION

Effect of entophytic *B. bassiana* and mealy bug infestation on growth parameters

The inoculation of fungi and insect infestation as variable factors were significantly influencing some vegetative and morphological characteristics of the cotton plant as shown in Table (1) Data illustrated that the inoculation with *B. bassiana* in general, led to a significantly increase on average stem length with 41.55 and 42 cm for spray and watering treatments respectively compared with non-treated by 29.55 cm (were, LSD= 5.87), data also showed that, spraying treatments were insignificantly lower than watering treatments, however mealy bug infestation has no significant effect. Fresh stem weight not affected by fungus inoculation in general, but un-infested and non-inoculated treatment was decreased significantly gained 4.68 gm (were, LSD = 3.02). In the same table, fresh root length was increased significantly in watering treatment (35.8 cm) and insignificantly with spraying treatment (28.4) compared with control treatment (25.85 cm), however, no significantly differences between inoculation methods (LSD = 9.67), in the same time un-infested mealy bug treatments were gained the highest root length by 40.8 and 35.8 cm for watering and spraying treatments (were, insignificantly among them) and with un-inoculated and watering for mealy bug-infested treatments by 31.8 and 30.8 cm respectively, and significantly differed with fungus spraying insect infested and uninoculated uninfested treatment by 21 and 19.9 cm respectively (were, LSD= 13.68). Fresh root weight also was decreased significantly when inoculation B. bassiana by watering (2.26 g) compared with spraying and control by 1.38 g and 1.52 g respectively (were, LSD = 0.65), interaction between fungus watering and un-infested treatment record the highest fresh root weight by 3.04 g, while the lowest weight was recorded in un-treated sit by 0.66 g (were, LSD = 0.91). With regard to the dry weight of the stems and roots, the un-inoculated fungus treatment was achieved the highest dry weight of 5.38 and 0.82 g followed insignificantly by watering treatments by 3.74 and 0.74 g and insignificantly differed with control treatment by 1.66 and 0.48 g respectively.

The literature suggests that genotype specific interactions between plants and entophytes may either enhance, reduce, or have no effect on plant figure (**Rodriguez *et al*. 2009**) According to **Surendra *et al*. (2017)** used *B. bassiana* to promote plant growth and had a positive influence on the survival, growth, health, length, and dry weight of cabbage.

Table (1) fresh and dry parameter affected by mealy bug infestation and *B. bassiana* inoculation

		Control	Spraying	Watering	Pest infestation	F&LSD
Fresh stem length	**Infested**	30.2	39	40.5	**36.57**	**0.97 & 4.79**
	Un-infested	28.9	44.1	43.5	**38.83**	
Fungus inoculation		**29.55**	**41.55**	**42**	**F= 0.67 & 8.3**	
F&LSD		**12.61 & 5.87**				
Fresh stem weight	**Infested**	11.72	11.72	7.72	**10.39**	**2.89 & 3.02**
	Un-infested	4.68	8.42	10.68	**7.93**	
Fungus inoculation		8.2	10.07	9.2	**4.06 & 5.23**	
F & LSD		0.56&3.7				
Fresh root length	**Infested**	31.8	21	30.8	**27.87**	**1.29 & 7.9**
	Un-infested	19.9	35.8	40.8	**32.17**	
Fungus inoculation		25.85	28.4	35.8	**4.71 & 13.68**	
F & LSD		2.49 & 9.67				
Fresh root weight	**Infested**	2.38	1.24	1.48	**1.63**	**0.18 & 0.53**
	Un-infested	0.66	1.52	3.04	**1.74**	
Fungus inoculation		1.52	1.38	2.26	**12.53 & 0.91**	
F & LSD		5.17 & 0.65				
Dry stem weight	**Infested**	5.38	3.96	3.74	**4.36**	**1.77 & 1.98**
	Un-infested	1.66	3.68	3.96	**3.1**	
Fungus inoculation		3.52	3.82	3.5	**1.71 &3.42**	
F & LSD		0.05 & 2.42				
Dry root weight	**Infested**	0.82	0.7	0.74	**0.75**	**0.75 & 0.28**
	Un-infested	0.48	0.9	1.22	**0.87**	
Fungus inoculation		0.65	0.8	0.98	**3.34 &0.47**	
F & LSD		2.13 & 0.33				

Effect of endophytic *B. bassiana* inoculation and mealy bug infestation on Chlorophyll a & b and Carotenoids

Data in Table (2) illustrate the changes in pigments due to fungus inoculation and pest infestation, where chlorophyll a in spraying and watering treatments was slightly decreased than control; Chlorophyll b was decreased than control in two treatments. Carotenoids in watering treatment were slightly decreased than control but in spray slightly increase. Also, pest infestation didn’t have significant changes in pigment values. Statistically, there were no significant differences were observed in pigment values as results for using fungus or insect.

The level of photosynthetic pigment like chlorophyll and carotenoids contents of plant in response to the insect attack is mainly determined by the species of host plant and scale of insect abundance. However it can also get influenced with respect to the environmental factors (**Mary *et al*. 2006**). The changes in the chlorophyll content in the stress leaves due to less synthesis might be part of adaptive response of plant (**Golawska *et al*. 2010**).

Effect of endophytic *B. bassiana* inoculation and mealy bug infestation on protein concentration

Data in Table (3) indicated that, fungus inoculation caused accumulation of total proteins in cotton plants, where spraying and watering treatments were 30.36% and 26.16% respectively compared with non-treated plant (22.73%). Statistically, there were significant differences between fungus inoculation and control treatment and within inoculation methods where, LSD= 1.13.

Table (2) Chlorophyll a & b and Carotenoids affected by mealy bug infestation and *B. bassiana* inoculation

		Control	Spraying	Watering	Pest infestation	F&LSD
Chlorophyll a	**Infested**	1.853	1.541	1.616	1.67	0.08 & 0.31
	Un-infested	1.872	1.688	1.759	1.773	
Fungus inoculation		1.863	1.614	1.668	0.08 & 0.54	
F & LSD		0.96 &0.38				
Chlorophyll b	**Infested**	0.801	0.700	0.709	0.737	0.001 & 0.17
	Un-infested	0.658	0.801	0.741	0.733	
Fungus inoculation		0.703	0.751	0.725	0.78 & 0.78	
F & LSD		0.04 & 0.21				
Carotenoids	**Infested**	0.293	0.286	0.333	0.304	2.018 & 0.05
	Un-infested	0.337	0.350	0.327	0.338	
Fungus inoculation		0.315	0.318	0.330	0.76 & 0.08	
F & LSD		0.14 & 0.06				

Table (3) Protein concentration affected by mealy bug infestation and *B. bassiana* inoculation

		Control	Spraying	Watering	Pest infestation	F&LSD
Protein (conc. %)	**Infested**	22.73	30.36	26.16	26.417	0.99
	Un-infested	26.52	27.36	32.78	28.887	
Fungus inoculation		24.63	28.86	29.47	0.74	
F & LSD		1.13				

Pest infestation also was recorded significant decrease in protein concentration 26.417% comparing with un-infest treatment (28.886%). The effect of interaction between fungus inoculations and pest infestation were recorded insignificant differences among them.

Protein synthesis is essential for normal cell differentiation and growth. A variety of environmental stressors have been reported to influence the synthesis of plant proteins (**William 1989**). Plants responded to pathogen attack by formation of new families of proteins called pathogenesis-related proteins or PR proteins (**van Loon 1985**). Applying of *B. bassiana* to cotton leaves caused prominent accumulation of total proteins. Formation of new proteins and protein accumulation is may be considered a way and an indicator of resistance towards fungus inoculation. Like the same way of formation of new protein and protein accumulation as a result to virus infestation (**Radwan *et al*. 2010**).

Effect of endophytic *B. bassiana* inoculation and mealy bug infestation on Flavonoids and Anti-Oxidants

Flavonoids and Anti-oxidants values were decreased from 52.98 to 38.6 and 38 µg QE/mg d w. (Insignificantly) and from 78.91 to 71.88 and 71.88 DPPH Scavenging ability % (Significantly), due to applying *B. bassiana* on leaves (spray) and on roots (watering) of un-infested cotton plants respectively (Table 4). Data in the same table showed insignificantly increase in flavonoids in watering treatment on infested plants, where anti-oxidants were decreased significantly comparing with inoculated plants and among inoculation methods in infested plants.

In general, pest infestation with mealy bug resulted in increased flavonoids from 43.19 to 48.6 µg QE/mg d w., but anti-oxidants were decreased from 74.22 to 73.14 DPPH Scavenging ability %. Also, fungus inoculations resulted in increased flavonoids insignificantly from 45.72 µg QE/mg d w. in non-treated plants to 59.63µg QE/mg d w. in watering plant and decreased in spraying plants to 40.72 µg QE/mg d w., where anti-oxidants affected significantly by fungus inoculation methods.

Flavonoids mainly act as antioxidants, to prevent the pest penetration into cells, and to trigger the host cell self-defense mechanisms (**Friedman 2007**). This supports a role of watering of *B. bassiana* in the induction of antioxidants and enhanced resistance.

Table (4) Flavonoids and Anti-Oxidants affected by mealy bug infestation and *B. bassiana* inoculation

		Control	Spraying	Watering	Pest infestation	F&LSD
Flavonoids	**Infested**	45.72	40.72	59.36	48.6	0.68 & 12.66
	Un-infested	52.98	38.6	38	43.19	
Fungus inoculation		49.348	39.66	48.681	2.002 & 21.93	
F & LSD		1.05 & 15.51				
Anti-Oxidants	**Infested**	79.11	66.21	74.11	73.15	5.82 & 17.5
	Un-infested	78.91	71.88	71.88	74.22	
Fungus inoculation		79.012	69.045	72.994	1.79 & 30.31	
F & LSD		1.38 & 21.44				

CONCLUSION

The inoculation with *B. bassiana* in general, led to a significantly increase on average stem length for spray and watering treatments compared with non-treated. Fresh root length was increased significantly in watering treatment and insignificantly with spraying treatment compared with control treatment. Also data revealed that, there were no significant differences observed in pigment values as results for using fungus or insect. While, fungus inoculation caused accumulation of total proteins in cotton plants compared with non-treated plant. Pest infestation also was recorded significant decrease in protein concentration comparing with un-infest treatment. Applying of *B. bassiana* to cotton leaves caused prominent accumulation of total proteins. In general, pest infestation resulted in increased flavonoids, but anti-oxidants were decreased. Also, fungus inoculations resulted in increased flavonoids and decreased in spraying. This supports a role of watering of *B. bassiana* in the induction of antioxidants and enhanced resistance.

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