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Effects of *Beauveria bassiana* (Bals.-Criv.) Vuill. on the growth of *Vigna unguiculata* (Linnaeus) Walpers under laboratory and field conditions, and the resistance of the plant to insect attack

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Abstract. *Beauveria bassiana* (Bals.-Criv.) Vuill. is an established biocontrol agent in laboratory, but studies have shown negative effects of temperature and Ultraviolet radiation on its potential in vitro. Nevertheless, there is a lack of studies about its endophytic colonization in vivo, especially in field conditions in cowpea [*Vigna unguiculata* (L.) Walp.]. In this sense, this study aimed to evaluate the effects of *B. bassiana* applied to cowpea seed on the growth of the plants under laboratory and field conditions, and on their resistance to insect attack. Cowpea seeds were treated with suspension of 5 x 10^8 conidia/mL of *B. bassiana*. The plants were cultivated in two independent conditions (laboratory and field). The resistance to attack of the pest in field conditions was assessed by monitoring weekly the plants three times a week during 12 weeks, from the initial vegetative to the reproductive stage. According to the results, in laboratory conditions, there were significant differences among the inoculated and control plants for the variables of height of the plants (31.17 cm for treatment and 23.00 cm for control plants) and dry mass of the root (2.38 g for treatment and 0.97 g for control plants). In field conditions, the observed pest insects were miner fly [*Liriomyza* sp.] (Diptera: Agromyzidae), *Cerotoma arcuata* Olivier, 1791 (Coleoptera: Chrysomelidae), aphid (*Aphis craccivora* Koch, 1854) (Hemiptera: Aphididae) and the polyphagous hemipteran [*Crinocerus sanctus* (Fabricius, 1775)] (Hemiptera: Coreidae). The treated plants were less affected than control plants by these pests, especially at the beginning of the vegetative phase.

Keywords: biological control; cowpea; endophyte; growth promoter; UV radiation.

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Beauveria bassiana (Bals.-Criv.) Vuill. is known as a good biological control agent. As endophyte, it can colonize plants by living in the internal plant tissues and protecting the plants against pests and pathogens. Besides this, as well as the majority of endophytes, it also promotes the growth and development of plants (Jaber & Enkerli 2016; Tall & Meyling 2018).

Previous studies have found that *B. bassiana* colonized successfully common beans (Canassa *et al.* 2019), maize (Orole & Adejumo 2009), sorghum (Tefera & Vidal 2009), sugar beet, tomato (Ownley *et al.* 2004), and cowpea (Maketon *et al.* 2013). It protects plants against pathogens and insects (Jaber & Ownley 2018). In a non-insect arthropod, the biological parameters of the spider mite (*Tetranychus urticae* Koch, 1836) (Trombidiformes: Tetranychidae) were affected when it consumed common bean plants colonized by *B. bassiana* and *Metarhizium robertsii* J.F.Bisch., S.A.Rehner & Humber strains (Canassa *et al.* 2019).

Although *B. bassiana* have stood out as a good biocontrol agent in laboratory conditions, its application in field conditions is often limited. This entomopathogenic fungus is less effective when it is exposed to direct sunlight and UV radiation (Inglis *et al.* 1993; Fernandes *et al.* 2007). Researchers showed that high temperatures retarded conidial germination process of *B. bassiana* and, the optimal temperature for conidial germination is 25 °C with an upper limit at 30 °C (Devi *et al.* 2005; Mwamburi *et al.* 2015). The effects of temperature, and UV radiation on *B. bassiana* isolates *in vitro* have been well studied, nevertheless, there is a lack of study evaluating the endophytic colonization *in vivo*, especially in field conditions. This study aimed to evaluate the effects of *B. bassiana* applied to cowpea seed on the growth of the plants under laboratory and field conditions as well on the resistance of the plant to insect attack in the field conditions.

MATERIAL AND METHODS

The experiments were conducted at the Universidade Federal de Tocantins, Gurupi - TO, Brazil (-11.7447716,-49.0527617, 278 m) in the period from February to June of 2021. The fungus was obtained from the WP PL63 strain of *B. bassiana* by the commercial formulation Boveril® WP PL63, being each g of the product has 1 x 10^8 conidia. A suspension of the fungus was prepared by diluting 5 g of the product in 100 mL of autoclaved distilled water suspended in 1 mL of 0.1% (v/v) Tween 80 and vortexed for 10 secs to homogenization.

Before the inoculation, the seeds of cowpea were first surface-sterilized by immersing for two minutes in 0.5% sodium hypochlorite (NaClO), two minutes in 70% ethanol (EtOH), and rinsing three times in sterile distilled water. The inoculation was carried out by immersing the seeds in the fungus suspension described above for 60 min at 25 °C and the seeds were left on filter paper in Petri dishes for 5 min to dry (Canassa *et al.* 2019). The control was carried out by immersing the seed in autoclaved distilled water + 0.05% Tween 80. Then, three seeds were planted in each soil-filled pot. One week after germination, the least vigorous seedling in each pot was discarded. The plants were kept in laboratory conditions with artificial light, 180 µmol m^{-2} s^{-1}, 75% relative humidity, at 27 °C and 12 h of photoperiod.

The plants were cultivated in the conditions described above; they were watered with sterilized water when necessary. For the essays in field conditions, three days after the germination, the seedlings were transferred to the field and were watered twice a day.

The parameters height of the plants (HP), dry mass of the root (DMR), dry mass of the shoot (DMS), and the total dry mass of the plants (TDMP) were measured in plants sampled at the beginning of the reproduction stage to assess the effects of the fungal inoculation on plant growth. For the bioassays in field conditions the biometrics parameters evaluated were height of the plants and total dry mass of the plants and the number of pods. Plant height was measured from the base of the plant to the apex of the aerial part. For the dry mass, plants were carefully uprooted and washed in running water, then packed in paper bags and dried in a forced-air circulation oven at 50 °C for 72 h. An analytical balance (Shimadzu® model AUW220D) was used to record the dry mass.

A sample of 12 plants (six for treatment and six for control) was carried out in the laboratory conditions essays. In the field conditions essays, we sampled 20 plants (10 for treatment and ten for control).

The resistance to attack of the pest in field conditions was assessed by monitoring weekly the plants three times a week for 12 weeks, from the initial vegetative to the reproductive stage of the culture. The monitoring was carried out early in the morning and in the final of the day. The occurrence of the pest and its injuries in the plants were registered. Samples of insects were collected to be identified in laboratory conditions according to the taxonomic keys. Entomological glue was used as a trap to catch the more difficult species such as miner fly. A manual vacuum was used to catch beetles and bud. The observed injuries were compared with data available in the literature and associated with the insect observed in the plants.

The experimental design was completely randomized. The Sisvar 5.6 ® software was employed to carry out the statistical analyses (Ferreira 2011). The biometric data height of the plants, dry mass of the root, dry mass of the shoot, and the total dry mass of the plants were submitted to analysis of variance, and the original data were transformed into the square root of Y + 0.5 - SQRT (Y + 0.5) for total dry mass of the plants and, into square root of Y + 0.5 for dry mass of the root and dry mass of the shoot. A pairwise comparison was performed using Tukey's post hoc test with a 0.05 significance level when necessary.

RESULTS

Under laboratory conditions, there were significant differences among the inoculated and control plants for the variables of height of the plants and dry mass of the root. Inoculated plants exhibited higher height of the plants (ANOVA. $F_{1,11}$= 6.65, $P < 0.005$) and dry mass of the root (ANOVA, $F_{1,11}$= 5.08, $P < 0.005$) than control plants. *Beauveria bassiana* was effective in promoting the height of the plants. However, no significant difference was observed for the total dry mass of the plants, although the treatment plants showed a greater value than the control ones (Table 1).

In the essay carried out in field conditions, there was no significant difference between the treatments. The fungus was ineffective in promoting the growth of the cowpea bean in such conditions despite the greater values of the treatment plants for the variables total dry mass of the plants, number of pods, and height of the plants (Table 2).

Table 1. Effect of *B. bassiana* on the plant in laboratory conditions.

	Dry mass of the root (g)	Dry mass of the shoot (g)	Total dry mass of the plants (g)	Height of the plants (cm)
Treatment	2.38b^{b}	4.41^{a}	6.78^{a}	31.17^{b}
Control	0.97^{a}	2.68^{a}	3.66^{a}	23.00^{a}
CV	40.42	37.52	28.36	20.25

Averages followed by the same letter in the column do not differ significantly from each other by the Tukey test (p = 0.05), data were transformed into the square root of Y + 0.5 - SQRT (Y + 0.5) for TDM and, into square root of Y + 0.5 for dry mass of the root and dry mass of the shoot. CV: Coefficient of variation.

Table 2. Effect of *B. bassiana* on the plant in field conditions.

	Total dry mass of the plants (g)	Number of pods	Height of the plants (cm)
Treatment	5.94	1.90	33.20
Control	4.77	2.00	31.10
CV	25.5	17.97	10.48

CV: Coefficient of variation.

In the experiments conducted in field conditions, the insects observed were miner fly [*Liriomyza* sp.] (Diptera: Agromyzidae), *Cerotoma arcuata* Olivier, 1791 (Coleoptera: Chrysomelidae), aphid (*Aphis craccivora* Koch, 1854) (Hemiptera: Aphididae) and, the polyphagous hemipteran [*Crinocerus sanctus* (Fabricius, 1775)] (Hemiptera: Coreidae). The treatment plants showed lower incidence insect pests when compared to the control plants. However, this pattern is decreasing all over time.

In monitoring conducted three times a week, injuries by the leaf miner fly were observed in the plant's leaves. Miner was detected in the second week in the control plants; it increased with time and was more significant in all the weeks in the control plants. (Figure 1A). However, the difference between the control and the treatment decreases with time.

In monitoring carried out three times a week, we noticed the occurrence of *C. arcuata,* and its injuries in the leaves were recorded. The occurrence of *C. arcuata* started in the second week in the treatment plants (Figure 1B). Its incidence increases until the 7th week, and, from the 8th, a decrease was observed. Its incidence increases until the 7th week and, from 8th a decrease was observed. The incidence was higher in control than the treated plants for all the weeks. However, the difference between the control and the treatment was more obvious in the first weeks.

In Figure 1C, we can observe that aphid occurrence started in the third week in the control plants and the fourth week in the treatment plants. Its incidence increases until the 8th week and, from 9th a decrease was observed. The incidence was superior in the control plants until the 10th.

The polyphagous hemipteran *C. sanctus* was noticed in the reproduction stage of the experiment. The number of pods with *C. sanctus* injuries was 16 for the control and 14 for the treatment.

DISCUSSION

As showed in the results, *B. bassiana* inoculated via seed treatment effectively promoted the plant's height and the

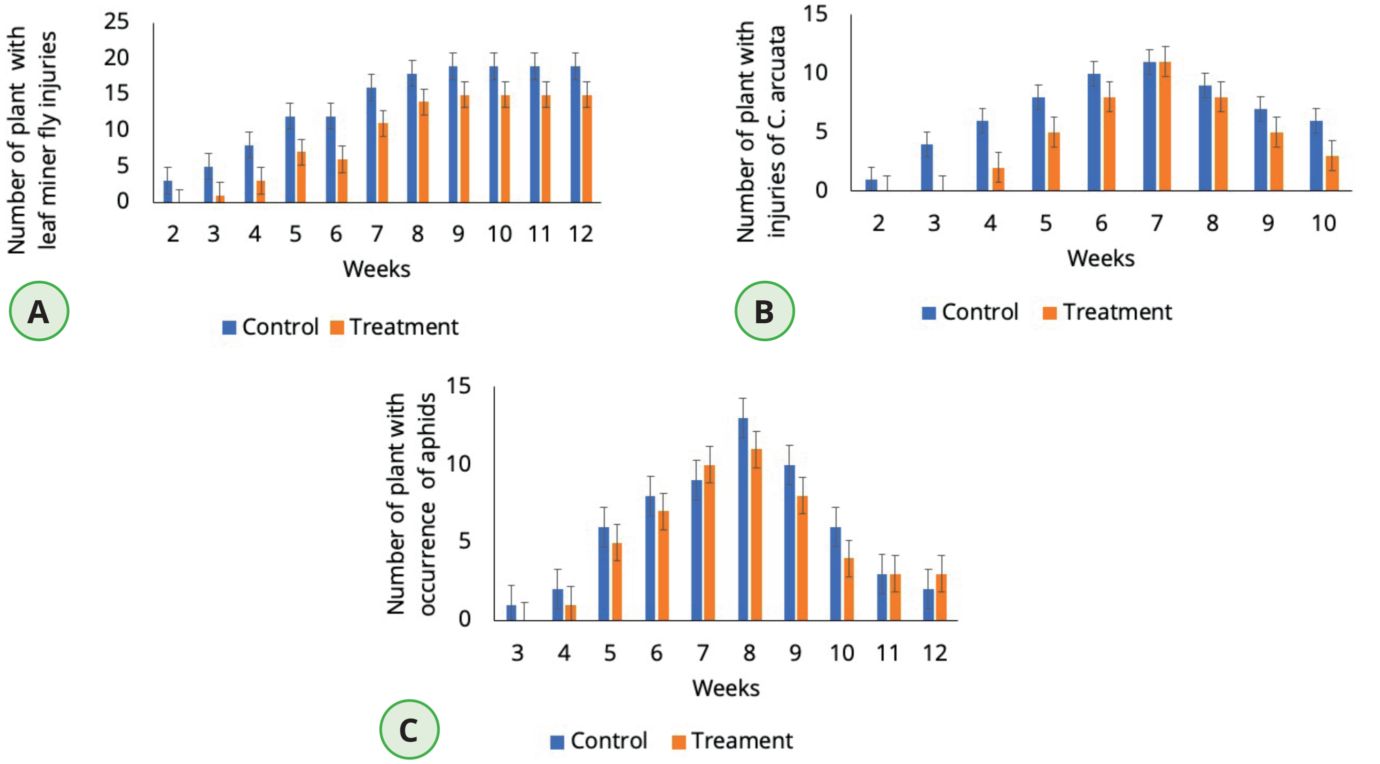

Figure 1: A. Number of plants injured by leaf miner fly; B. Plant with injuries of *C. arcuata*; C. Plant with occurrence of aphids.

root's mass when cultivated in laboratory conditions. Despite the lack of research carried out on the endophytic colonization on cowpea by *B. bassiana,* it is a confirmed endophyte in cowpea (Maketon *et al.* 2013). Common bean (*Phaseolus vulgaris* Wall.) seeds treated with *B. bassiana* enhanced the growth of the plant (Dash *et al.* 2018; Canassa *et al*. 2019). This fungus also effectively promoted other species such as cotton (Lopez & Sword 2015)

The growth promotion by microorganisms is linked to their potentiality to regulate plant hormones (auxin, gibberellins, cytokinins, and ethylene), produce growth regulator metabolites, and fixate nutrients from the soil (Berg 2009; Pierik *et al.* 2006). The seed treatment did not significantly influence the dry mass of the plants, that shows the necessity of the search for more effective methods of inoculation of this fungus in cowpea.

As described in the results, *B. bassiana* was ineffective to promote the growth of the cowpea bean when the plant were expose in field conditions despite the greater mathematic values observed in the treatment plants. Considering the temperature of the study zone that could reach up to 40 °C, these results could be explained by the fact that *B. bassiana* does not like high temperatures. It was observed that the optimal temperature for conidial germination of *B. bassiana* is approximately 25 °C with an upper limit at 30 °C, and high temperatures retarded its conidial germination process (Devi *et al*. 2005; Mwamburi *et al.* 2015). Previous study found that *B. bassiana* grew 0.1cm, 9.0cm, 9.00 cm and 2.5cm at 4 °C, 16 °C, 29 °C and 40 °C temperature respectively, on 10th day of incubation (Pratibha & Pal 2021).

Besides this, in the field essay, the plants were exposed to sunlight to increase the plant tissues' temperature. Authors also found that *B. bassiana* is highly sensitive to UV radiation (Acheampong *et al*. 2020), that could consequently prejudice the performance of *B. bassiana* in the plants.

As observed in the results, occurrence of miner fly, *C. arcuata,* aphid and red bug of cowpea was higher in the control plants. *Beauveria bassiana* is a good control agent of aphid (*A. craccivora*). It could affect its mortality and reproduction (Abdou *et al*. 2017). Akutsze *et al.* (2013) found that *B. bassiana* as an endophyte could reduce the emergence or adult of leaf miner *Liriomyza huidobrensis* (Blanchard, 1926) (Diptera: Agromyzidae).

When some endophytes fungi colonize plants, they could develop repellence capacity to become more resistant to insect attack. *Beauveria bassiana* reduced the population of spider in common bean plant (Canassa *et al*. 2019). *Hypocrea lixii* Pat. conferred resistance to onion against *Thrips tabaci* Lindeman, 1889 (Thysanoptera: Thripidae) (Muvea *et al*. 2015).

It is worth mentioning that the resistance of non-preference was more evident in the first weeks of the experiments in the field conditions, and it was decreasing all over time. Considering that the inoculation was carried out in the laboratory conditions, and then the plants were transferred in the field after germination, that explains that the fungus was more effective in protecting the plants in the first weeks. Besides, if we considered the hypothesis that *B. bassiana* does not tolerate high temperature and UV radiation (Mwamburi *et al*. 2015; Acheampong *et al*. 2020), we could deduce that the capacity of *B. bassiana* to protect the plants is decreasing with the time as much as the pants were exposed to high temperature and UV radiation in field conditions.

Beuaveria bassiana is inactivated when exposed to direct sunlight and UV radiation (Inglis *et al.* 1993; Fernandes *et al*. 2007). To achieve good results with *B. bassiana* in field conditions, it is necessary to use some natural substances to protect its conidia (Kaiser *et al*. 2019). Many researchers have shown the effect of temperature, and UV radiation on *B. bassiana* isolates *in vitro*. Nevertheless, there is a lack of study explaining sunlight exposure effects on endophytic mechanisms in vivo.

Occurrence of polyphagous hemipteran (*C. sanctus*) was noticed in the reproduction stage of the experiment. Despite the incidence of this pest being higher in the treatment (16) compared to the control plant (14), the effects of *B. bassiana* were not expressive. This insect, in Brazil, is a critical pest known for its capacity to consume the pods during the reproduction stage and consequently affects seed filling and reduces the yield. Due to its injuries to the quality of the grain and yield, *C. sanctus* has been considered as a primary economic pest of cowpea crop in Brazil (Moreira *et al*. 2006; Sousa *et al*. 2019). Despite the incidence of this pest was higher in the treatment (16) when compared to control plant (14), the effects of *B. bassiana* was not expressive. We could explain this with the same argument above. Sunlight exposure negatively affects the conidia of *B. bassiana* consequently its capacity to protect the plant against pest attack principally in the reproductive stage.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to disclose.

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