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Productivity and physiology of kale inoculated with entomopathogenic fungi of Amazon region to control caterpillars

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Abstract

The use of biological control agents such as entomopathogenic fungi is an alternative for the control of kale (*Brassica oleracea* L.) defoliating caterpillars. The objective was to investigate the efficacy of entomopathogenic fungi of Amazon region in the control of defoliating caterpillars in kale and their impacts on the physiological and agronomic responses of the crop. Field and greenhouse experiments were conducted in randomized block design and completely randomized design, respectively. The treatments consisted in the application of isolates of entomopathogenic fungi: *Beauveria bassiana*, *Isaria* sp., *Metarhizium anisopliae* and *Trichoderma asperellum*. The control treatment consisted of the application of an chemical insecticide based on deltamethrin.Variables referring to the development, yield and physiology of the plants were evaluated. Field results revealed that plants treated with the fungi *B. bassiana, M. anisopliae* and *T. asperellum* showed levels of severity, number of leaves and commercial yield that did not differ from the standard treatment; however, they showed a lower population density of the defoliating caterpillar complex. The application of the treatments with *M. anisopliae* and chemical insecticide showed better photosynthetic performance. In greenhouse, the fungus *T. asperellum* provided greater plant height and robustness index in relation to the treatment with chemical insecticide. The entomopathogenic fungi of Amazon region can be contributed to the integrated management of leaf defoliating caterpillars in kale. These microorganisms have similar efficiency with chemical insecticides, being ecologically and economically viable to mitigate the negative impacts caused by the systematic use of chemicals.
Keywords: Biological control, *Brassica oleracea*, *Metarhizium anisopliae*, *Spodoptera spp.*

Introduction

Kale (*Brassica oleracea* L. var. Acephala) is a leafy vegetable widely consumed and cultivated throughout the world (Chiu et al., 2018). A limiting factor for culture cultivation is the attacks of insect pests such as defoliating caterpillars, which are pests of worldwide occurrence and can cause serious damage to vegetables (Ibrahima et al., 2018).

Defoliating caterpillars can cause direct damage to the growth, physiological development and productivity of leafy plant. Leaf removal in these species is detrimental to productivity since it is the marketable part. From the photosynthetic point of view, photosynthesis is directly altered by the removal of leaf tissue and damage to vascular tissues, directly affecting the flow of substrates necessary for photosynthesis (Meza-Canales et al., 2016).

Regarding the main caterpillars in kale plants, the following stand out *Spodoptera eridania* (Montezano et al., 2014), *Spodoptera cosmioides* (Freitas et al., 2019), *Plutella xylostella* (Ferreira et al., 2021), and *Chrysodeixis includens* (Specht et al., 2015). (Bortoli et al., 2013) comparing different cultivars and hybrids of cruciferous in relation to resistance to *Plutella xylostella* attack reported that kale is the most susceptible among the cultivars evaluated.

The control of these pests is considered difficult because the caterpillars have the ability to develop resistance to synthetic insecticides, in addition to the negative effects of insecticides on their natural enemies (Magalhaes et al., 2014). The most commonly used method to control defoliating caterpillars is the application of non-selective insecticides, however, the emergence of resistant populations is already described (Arruda et al., 2020).

Besides the growing number of resistance reports, the indiscriminate use of synthetic and non-selective

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insecticides in vegetables is a fact that can compromise food safety (Ngosong et al., 2020). According to (Nogueira et al., 2015), the use of alternative methods is necessary as a strategy to control pests, aiming to reduce the quantify of insecticides application and the levels of residues in food.

Biological control with predators, parasitoids and entomopathogens can be crucial for the control of caterpillars (Farias et al., 2020). An important tool in this type of control is the use of entomopathogenic fungi because they are able to provide protection to plants against insects and nematodes (Vidal & Jaber, 2015). According to (Barros et al., 2020), the use of entomopathogenic fungi should be studied due to their pathogenicity against different insects, being a potential bioinsecticide capable of controlling pest insects such as defoliating caterpillars. Thus, the use of entomopathogenic fungi contributes to increase plant resistance against pest attack, as well as increased nutrient uptake by plants.

Therefore, this study aims to investigate the efficacy of entomopathogenic fungi of Amazon region in the control of caterpillars in kale plants and their impacts on the physiological and agronomic responses of the crop.

Material and Methods

Field experiment

A field experiment was conducted from January to March 2020 in a commercial vegetable growing are located in the Marituba city, Pará state, Brazil. (**Figure 1**). The experimental area is located in theAmazon biome, with climate type "Ami" according to the international classification of Köppen (Alvares et al., 2013), with rainfall distribution defined in two annual seasons, rainy from December to May and less rainy from June to November. During the conduction of the study the mean wind speed was 0.7 m s^{-1}, mean temperature 26 °C, mean relative humidity 85% and annual precipitation 1,457.4 mm.

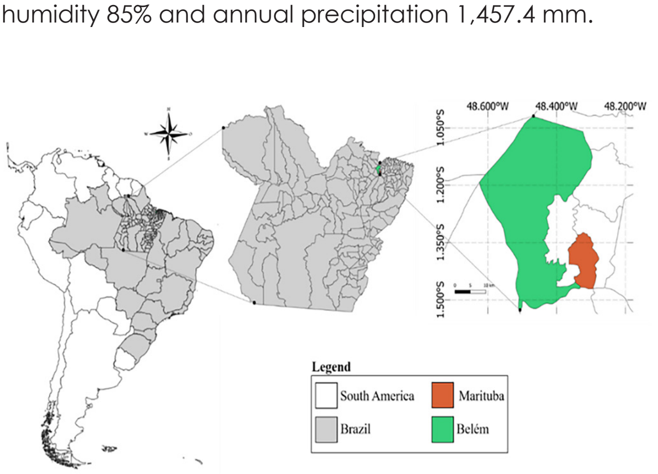

Figure 1. Geographic location of field and greenhouse experiments.

The experimental design was in randomized blocks, with five treatments and six replications. The treatments consisted in the application of isolates of entomopathogenic fungi, being *Beauveria bassiana*, *Isaria sp*, *Metarhizium anisopliae* and *Trichoderma asperrellum*, plus insecticide based on deltamethrin as standard treatment (routinely used in commercial areas).

Characterization and preparation of entomopathogenic fungi

The entomopathogenic fungi *Beauveria, Isaria, Metarhizium* and *Trichoderma* used in this study belong to the microorganism collection of the Plant Protection Laboratory of the Federal Rural University of the Amazon. Fungal isolates were multiplied in Petri dishes containing PDA (potato, dextrose and agar) culture medium and incubated in a growth chamber for four days at 28° C. After the formation of colonies and obtaining monosporic isolates, aqueous suspensions of each isolate were prepared. These aqueous suspensions were prepared in 10 ml tubes containing sterile water and 0.1% Tween 80 spreader sticker, were then agitated in vortex shaker for one minute. The suspension obtained was quantified in a Neubauer chamber to standardize the concentration of the fungus at 1 x 10^8 spores ml^{-1} for inoculation into autoclaved rice. Then, 500 g of rice was placed in 5 L plastic box (previously sterilized with 1% hypochlorite for five minutes and washed in distilled water) and inoculation was performed by spraying the spore suspension on the rice, after 96 h the colonization of fungi occurred. Subsequently, the inoculated rice was stored in a freezer at -18° C until the moment of use.

Installation and conduction of experiment

The seeds of the kale hybrid HI CROP (TAKII®) were sown in plastic trays, filled with substrate composed of soil and chicken litter at a ratio of 2kg of soil to 1kg of chicken litter and 1 kg of limestone in each plastic tray. At six days after germination the seedlings were transplanted to 0.5 dm^3 pots containing the same substrate with which they were sown, at 25 days after sowing they were transplanted to the field. Above the plants a tunnel-type covering was built with galvanized steel roofing in the form of an arch, with semi-open sides, covered with agricultural film of the LDPE (Low Density Polyethylene) type of 100 micrometers. The area was divided in six blocks (4.5 m long and 1.0 m wide) and the planting was performed in rows. The spacing used was 0.5 x 0.5 m between plants and rows. The experimental plot consisted of three plants.

Soil preparation was done manually, with weeding to remove spontaneous vegetation. The cover fertilization was performed with organic compost, at a dose of 150 g plant^{-1} of chicken litter every 21 days, being the first performed at five days after transplanting. The area was irrigated daily (manually) to soil saturation. Manual weeding was carried out throughout the experimental period to remove weeds.

The occurrence of the caterpillars occurred naturally, due to the large occurrence of this type of pest in peri-urban commercial vegetable areas. It was verified the presence of the caterpillars *Spodoptera spp.*, *Chrysodeixis includens* and *Plutella xylostella*.

Application of treatments

Fungal suspensions were prepared by adding 10 g of rice colonized with fungus in one liter of water, proportion to obtain the concentration of 1.0 x 10^8 spores ml^{-1}. After mixing and homogenization, the suspensions were manually filtered using cotton fiber filter. To prepare the spray solution of the chemical insecticide, 0.3 ml of the concentrated product was added in 1 liter of water (the dose recommended by the manufacturer of the product).

To control the caterpillar complex, applications were made in the morning at 30, 37, 44 and 51 days after transplanting (DAT) directly on the aerial part of the plant and on the soil, using a manual compression sprayer.

Greenhouse

An experiment was conducted under greenhouse conditions at the Federal Rural University of the Amazon, located in the municipality of Belém, in March 2021, with the aim of evaluating the use of entomopathogenic fungi to control caterpillars in kale plants. The institution is located 20 km from the commercial area where the field experiment was conducted, sharing the same biome and similar climatic characteristics, however under controlled environment conditions.

The experimental design was entirely randomized, with six replications. Treatments, concentration and preparation of entomopathogenic fungi were similar to the field experiment.

Kale seeds were grown in polyethylene trays filled with compost from tree pruning waste. At 10 days after germination, seedlings were transplanted to larger containers (0.5 dm^3 plastic pots) with composting. 25 days after sowing, the seedlings were transplanted into plastic pots (4 dm^3) filled with soil from the field experiment.

Fertilization of the plants occurred at 3-week intervals with the application of 100 g of organic compost based on poultry litter. During the experimental period, four fertilizations and three harvests of material were made to determine the evaluated variables. Irrigation occurred daily to maintain the soil saturation

Agronomic characteristics evaluated

The following agronomic traits were evaluated: plant height (cm), plant diameter of the lap (mm), robustness index, severity, commercial leaves, non-commercial leaves, total leaves, commercial yield (kg ha^{-1}; g planta^{-1}) and total yield (kg ha^{-1}; g plant^{-1}).

Plant height was measured with the aid of a graduated ruler, from ground level to the apical bud of the highest leaf, and the plant diameter of the lap was checked with a digital pachymeter. The robustness index was calculated by the ratio between plant height and plant diameter of the lap. Severity was estimated by dividing the number of holes produced by the caterpillar complex by the number of leaves harvested from kale plants.

The total leaves were evaluated by direct counting of the kale leaves. After harvesting, the commercial leaves were separated from the non-commercial leaves, being considered non-commercial the leaves that presented six or more holes. Then, the collected leaves were packed in Kraft paper bags and taken to the laboratory for determination of yield on analytical balance. Total and commercial yields were calculated by the crop production in the sampled area (0.25 m^2 for field and plant^{-1} in greenhouse) and made the ratio for one hectare in field experiment (kg ha^{-1}) and kept the value per plant in greenhouse experiment (g planta^{-1}). A total of three harvests of leafy kale were carried out, which occurred at 36, 51 and 66 DAT.

Population density was determined by the ratio of the number of caterpillars present on the plants and the total number of plants observed. To control the caterpillar complex, applications were made during 4 consecutive weeks, at 30, 37, 44 and 51 DAT directly in the aerial part of the plant and on the soil, with a manual compression sprayer, with a capacity of 1.5 L. The application of the treatments was carried out early in the morning (07:00 h).

Leaf gas exchange

The parameters of gas exchange were performed on fully expanded leaves located in the upper middle third of the plant, 66 days after transplanting the kale seedlings. A portable open flow gas exchange system was used (LI-6400XT, LI-COR, Lincoln, NE) with constant light source of 900 μmol photons m^{-2} s^{-1} and external CO_2 concentration of 400 μmol mol^{-1}, between 11:00 and 13:00 hours. Net assimilation of CO_2 (*A*), stomatal conductance e to water vapor (gs), intercellular CO_2 concentration (Ci) and transpiration rate (E) were estimated. The instantaneous carboxylation efficiency (A/Ci) was calculated by the ratio between A and Ci and the instantaneous water use efficiency was evaluated by dividing A/E.

Chlorophyll content

The quantification of chlorophyll content of kale leaves was evaluated using SPAD-502 portable chlorophyll meter. Measurements were taken prior to the evaluation of gas exchange, at three points of the leaf area, on the adaxial side of the same leaves.

Statistical analysis

Initially, the data collected was evaluated using the Shapiro-wilk test for normality and homoscedasticity (Levene). Subsequently subjected to analysis of variance by F test and, when significant, the means were compared by the student-Newman-Keuls (SNK) test at 5% significance using the Sisvar software version 5.6 (Ferreira, 2011). Averages are presented alongside the standard error in the graphs.

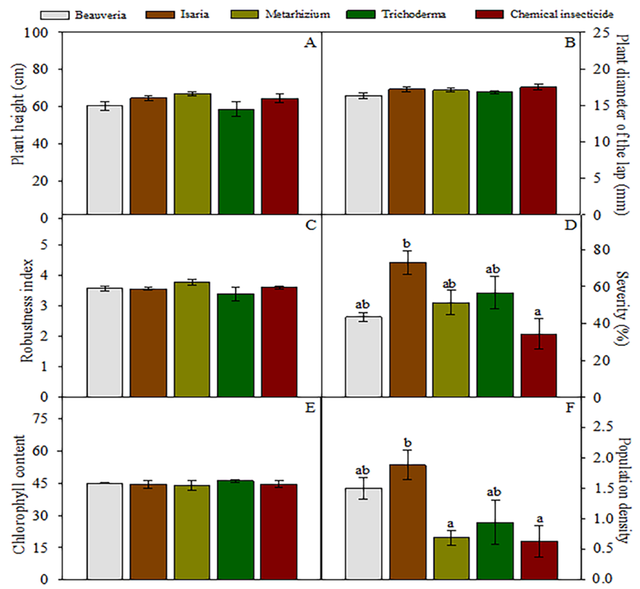

Figure 2. Plant height (A), plant diameter of the lap (B), robustness index (C), severity (D), chlorophyll content (E) and population density (F) of caterpillars in commercial area of kale after application of entomopathogenic fungi in field. Bars followed by different letters present significant difference at 5% probability by the SNK test.

Results and Discussion

Field experiment

There was difference significant for severity and population density ($p \leq 0.05$). Plant height, plant diameter of the lap, robustness index and chlorophyll content showed no differences ($p \geq 0.05$) between treatments with entomopathogenic fungi and chemical treatment (**Figure 2**A, B, C and E).

The height plants remained at 63.0 cm, regardless of the treatment applied. Similarly, the plant diameter of the lap and robustness index remained at 17.0 mm and 3.6, respectively. The chlorophyll index showed an average value of 44.8.

Regarding the severity of the natural attack of the complex of defoliating caterpillars, there was less efficiency of the treatment with the *Isaria* fungus in relation to the chemical treatment, but similar to the other fungi evaluated (Figure 2D). Plants that received the *Isaria* fungus showed a severity of 73%, and this value was 29%, 21%, 16% and 39% higher compared to treatments with *B. bassiana*, *M. anisopliae*, *T. asperellum* and the chemical insecticide.

For population density, lower populations of defoliating caterpillars were observed on plants that received treatments with the fungus *M. anisopliae* and chemical insecticide (Figure 2F). The highest population density was observed in the treatment with *Isaria sp.* presenting 198% higher in relation to the chemical insecticide.

For the production variables of kale plants as a function of the application of treatments, there was significant difference only for the variable's commercial leaves and commercial yield (**Figure 3**A and B). The commercial leaves were higher in the treatments with chemical insecticide (5.5 leaves) and the fungus *B. bassiana* (4.5 leaves) and *M. anisopliae* (6.5 leaves), which were higher than the treatment with *T. asperellum* (1.5 leaves) (Figure 3A). Non-commercial leaves showed no difference between the treatments applied (Figure 3B). The kale plants presented an average of 12 leaves that had no potential for commercialization.

For total leaves, plants that received the application of the treatment with *M. anisopliae* (21.7 leaves) produced a greater number of leaves compared to the treatment with fungus *T. asperellum* (16.7 leaves) and similar significantly ($p > 0.05$) to the other treatments (Figure 3C).

As for the commercial yield of leaves, only the treatment with *Isaria sp.* showed inferior results to the treatment with chemical insecticide (Figure 3D). Treatments with *B. bassiana*, *M. anisopliae* and *T. asperellum* showed no difference ($p > 0.05$) from treatment with chemical insecticide. The total yield averaged 6,800 kg ha^{-1} (Figure 3E).

For photosynthetic rate (A), the highest means were found in the treatments with *M. anisopliae* and chemical insecticide (**Figure 4**A). However, plants treated with *T. asperellum*, *Isaria sp.* and *B. bassiana* exposed reductions in 10%, 8% and 3%, respectively, for *gs* and 8%, 2% and 3%, respectively for *E*, when compared to the chemical insecticide.

For *A/Ci* and *A/E* ratio, treatments with entomopathogens induced reductions of 17% and 15% (*Isaria sp.*), 12% and 15% (*B. bassiana*), 4% and 9% (*M. anisopliae*) and 15% and 9% (*T. asperellum*) respectively (Figure 4E and F).

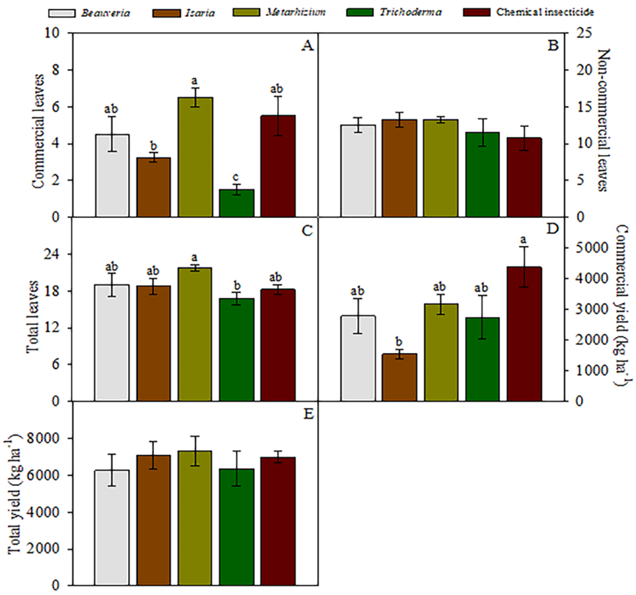

Figure 3. Commercial leaves (A), non-commercial leaves (B), total leaves (C), commercial yield (D) and total yield (E) of kale after application of entomopathogenic fungi for the control of caterpillars in field. Bars followed by different letters present a significant difference at 5% probability by the SNK test.

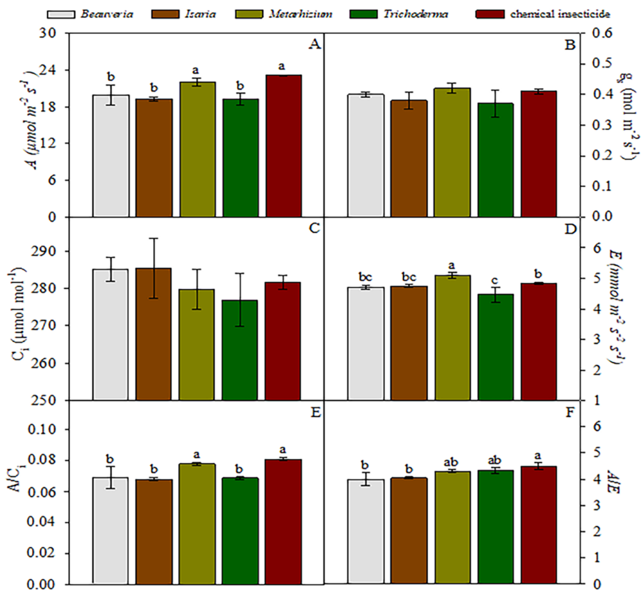

Figure 4. Leaf gas exchange of kale after application of entomopathogenic fungi for the control of caterpillars in field. A: Net assimilation rate (A), B: stomatal conductance (gs), C: intercellular concentration of CO_2 (Ci), D: transpiration (E), F: instantaneous efficiency of carboxylation (A/Ci), E: instantaneous efficiency of water use (A/E). Bars followed by different letters present a significant difference at 5% probability by the SNK test.

Greenhouse

In greenhouse, there was difference ($p<0.05$) for agronomic variables plant height, robustness index and population density among the studied treatments. The plant diameter of the lap and chlorophyll index variables had average values of 9.5 mm and 44.3, respectively (**Figure 5**).

Inoculated plants with *Isaria sp.* and *T. asperellum* showed greater plant height compared to treatments with *M. anisopliae* and chemical insecticide (Figure 5A). For robustness index, there was highlight for plants inoculated with *T. asperellum*, being 10% higher than the treatment with insecticide (Figure 5C). Severity maintained an average value of 30% (Figure 5D).

For population density, inoculated plants with *B. bassiana* showed higher density than the other treatments, being 110, 145, 133 and 358% higher than treatments with *Isaria sp.*, *M. anisopliae*, *T. asperellum* and insecticide, respectively (Figure 5F).

The Productive variables commercial leaves, non-commercial leaves and commercial yield showed significant difference according to the application of entomopathogenic fungi in kale plants grown in the greenhouse (**Figure 6**).

The commercial leaves with insecticide were 39% more than the leaves of plants inoculated with *Isaria sp.* (Figure 6A). In contrast, plants inoculated with *Isaria sp.* showed the greatest number of non-commercial leaves, being 90% greater than the treatment with insecticide (Figure 6B). Other fungi showed similar values to the insecticide.

The highest commercial yield was seen in plants with chemical insecticide (91.5 g plant^{-1}), but significantly similar to treatments with *B. bassiana* (71.5 g plant^{-1}), *M. anisopliae* (75.3 g plant^{-1}) and *T. asperellum* (78.3 g plant^{-1}). Treatment with chemical insecticide showed commercial yield 37% higher than plants inoculated with *Isaria sp.* (Figure 6D).

Variables corresponding to the leaf gas exchange did not show significant difference for kale plants as a function of the applied treatments (**Figure 7**).

The net assimilation rate, stomatal conductance, intercellular CO_2 concentration, evapotranspiration, instantaneous carboxylation efficiency and water use efficiency presented average values of 13.87 μmol m^{-2} s^{-1} 0.154 mol m^{-2} s^{-1}, 248.62 μmol mol^{-1}, 3.15 mmol m^{-2} s^{-2} s^{-1}, 0.047 and 2.77, respectively.

Inoculated plants with *B. bassiana*, *M. anisopliae* and *T. asperellum* showed similar severity levels to the chemical insecticide (Figure 2D). Results observed in this

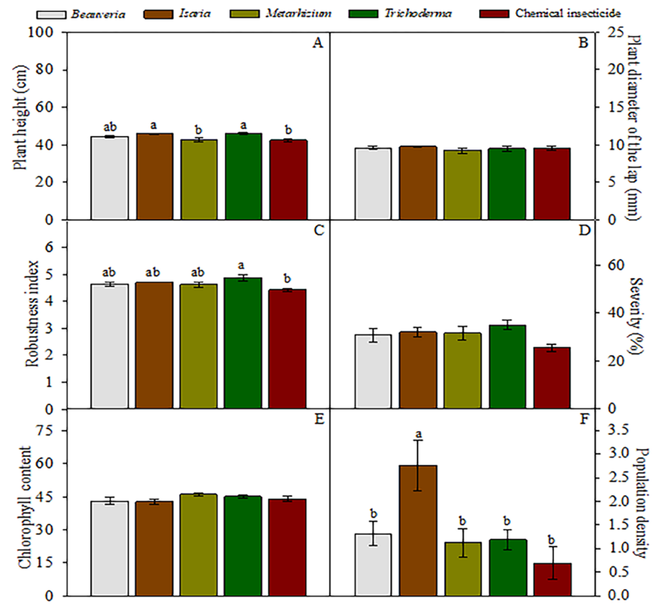

Figure 5. Plant height (A), plant diameter of the lap(B), robustness index (C), severity (D), chlorophyll content (E) and population density (F) of caterpillars in kale after application of entomopathogenic fungi in greenhouse. Bars followed by different letters present significant difference at 5% probability by the SNK test.

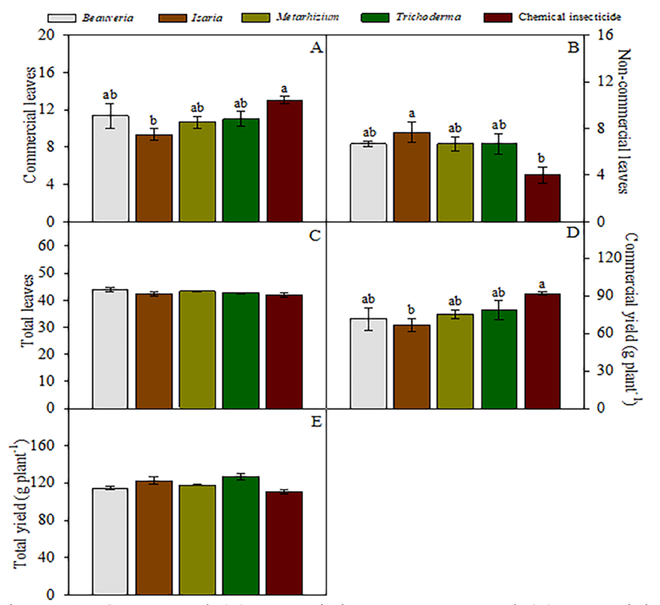

Figure 6. Commercial leaves (A), non-commercial leaves (B), total leaves (C), commercial yield (D) and total yield (E) of kale plants after application of entomopathogenic fungi for the control of caterpillars in greenhouse. Bars followed by different letters present a significant difference at 5% probability by the SNK test.

work are similar to those observed by (Nurhidayati et al., 2020), who studied the effects of *Plutella xylostella* attack on the yield of *Brassica oleracea* and reported that the intensity of attack by this pest significantly affects the commercial yield of the plant. Similarly, although different genus, (Al-kherb, 2014), found that *Beauveria* and *Metarhizium* were efficient in biological control of caterpillars of the genus *Spodoptera*.

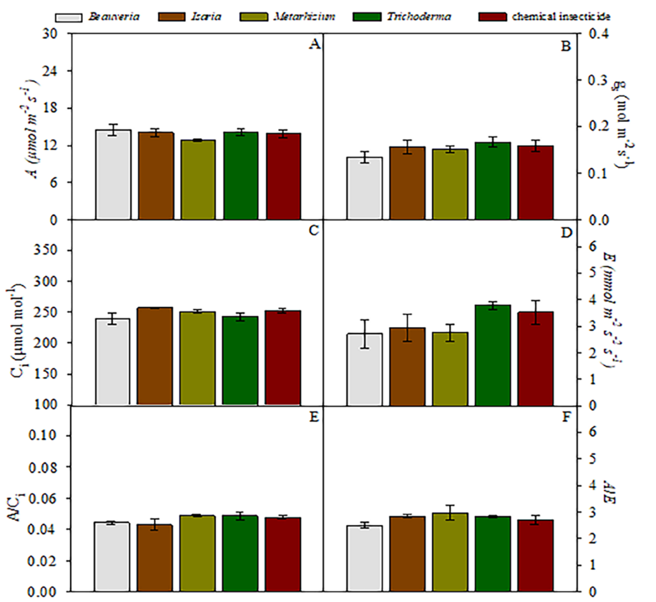

Figure 7. Leaf gas exchange of kale after application of entomopathogenic fungi for the control of caterpillars in a greenhouse. A: Net assimilation rate (A), B: stomatal conductance (gs), C: intercellular concentration of CO_2 (Ci), D: transpiration (E), F: instantaneous efficiency of carboxylation (A/Ci), E: instantaneous efficiency of water use (A/E). Bars followed by different letters present a significant difference at 5% probability by the SNK test.

Inoculated plants with *B. bassiana*, *M. anisopliae* and *T. asperellum* showed lower rates of caterpillar population density (Figure 2F and 5F), showing similar action to the chemical insecticide, which has contact action. Highlights include that entomopathogenic fungi require more time to cause the mortality of caterpillars, however, they remain longer in the environment (soil and plant), which may have caused systematic mortalities, protecting the plant for longer, since the plants received three consecutive applications of the fungus suspensions. Study of (Fred et al., 2012) proved the effectiveness of the *Metarhizium* on *Spodoptera exigua* larvae, with mortality of 87.5% in laboratory and 81.3% in a greenhouse. Treatment with *B. Bassiana* also proved to be an efficient alternative to control caterpillars. (Batcho et al., 2018) reported that *B. Bassiana* has ample spectrum and effective biomonitoring agent for control of *P. xylostella*.

Commercial leaves were influenced according to caterpillar population density (Figure 2F and 5F), that is, the lower the number of caterpillars, the less damage to the leaves and the quantity greater the commercial leaves, especially for inoculated plants with *M. anisopliae* (Figure 2A). This fungus is one of the most promising for use in the biological control of pests, and there are several strains against a diversity of insect-pests (Steinwender et al., 2015). Unlike chemicals that can cause harm to soil, plants and humans, entomopathogenic fungi such as

Metarhizium are considered clean technology and have been reported to be naturally occurring in areas with *B. oleracea* (Steinwender et al., 2015).

According to the results obtained, there was less damage in plants inoculated with entomopathogenic fungi, for number of commercial leaves, therefore, enabling the replacement or reduction of chemical insecticide applications. For this, it is recommended the use of fungi *B. bassiana* and *M. anisopliae* in the field and *B. bassiana*, *M. anisopliae* and *T. asperellum* in greenhouse (Figure 3A and 6A). The action of these entomopathogenic microorganisms occurs through asexual spores (conidia) that penetrate by direct contact through the tegument of the host insect, and subsequently, begins the infection leading the host to death (Hesketh et al., 2010). They can also be ingested by eating through the leaves, a fact that can favor a more rapid infection. Therefore, *Beauveria* and *Metarhizium* play important roles in regulating pest insect populations (Zimmermann, 2007; 2008; Tuncer et al., 2019).

In the present study, *B. bassiana* and *M. anisopliae* have proven to be an efficient alternative for the control of caterpillars including the moth. According to (Agboyi et al., 2020) are the two fungi among the most marketed components of biopesticide products in the world. Evaluating use of *B. bassiana* in the control of *P. xylostella* on Brassicas in West Africa, (Agboyi et al., 2020), observed that the chemical insecticide used (deltamethrin) was ineffective against the larvae of the pest. They also highlighted that the populations of this pest are increasingly resistant to deltamethrin, and the use of *B. bassiana* is a promising tool in the biocontrol of pests resistant to chemical insecticides.

In greenhouse, inoculated plants with *T. Asperellum* presented a higher height and robustness index, in relation to chemical insecticide (Figure 5A and C). Possibly, these results are related to the ability of this microorganism, traditionally, to promote greater plant growth by the release and or stimulation in the synthesis of growth phytohormones (Garnica-Vergara et al., 2016). Additionally, the environment seems to have influenced the height of the plants, since there was no difference between the treatments in the field, the average height was 60 cm. In the greenhouse the plants grew less and reached an average of 40 cm, possibly due to soil limitation.

For total leaves in field conditions, treatment with *M. anisopliae*, provided 14%, 16%, 29% and 19% greater number of leaves compared to treatments with *B. bassiana*, *Isaria sp.*, *T. asperellum* and chemical insecticide, respectively. Variables such as number of leaves and fresh mass are of great importance in evaluating the productivity of vegetables, because these two variables are important in the commercialization of leafy vegetables, because they are responsible for the weight and volume of the aerial part (Silva et al., 2016).

For commercial yield (field and greenhouse), chemical insecticide was significantly similar to the fungi *M. anisopliae*, *B. bassiana* and *T. asperellum*. In this variable, we can observe the direct damage caused by defoliating caterpillars on crop production, corroborating with (Costa et al., 2014) who reported significant reduction in quality and commercial productivity in kale by insect attack.

The production of organic vegetables is a growing activity in the world, as a consequence of concerns about human health and preservation of the environment (Sediyama et al., 2014; Thavarajah et al., 2019). However, the relationship of lower productivity and nutritional value in organic crops compared to non-organic systems, make it difficult to adopt this system (Thavarajah et al., 2019). Results obtained in our study demonstrated that the inoculation of kale plants with entomopathogenic fungi promoted productivity similar to chemical insecticide, serving as an alternative in organic or traditional crops. In addition, most of these microorganisms are recognized as growth promoters in plants, enabling improvement of the nutritional quality of cultivation systems.

As for leaf gas exchange, in the field, kale treated with *M. anisopliae* and chemical insecticide showed higher values of liquid CO_2 assimilation (*A*) (Figure 4A). This fact evidences the ability of *M. anisopliae* to protect the leaves against the attack of caterpillars, resulting in less damage to photosynthesizing tissues and higher number of commercial leaves in this treatment. Photosynthesis is one of the physiological processes most affected by the occurrence of damage to leaf tissues (Visakorpi et al., 2018). The loss of leaves reduces the leaf area of the plant and consequently, the photosynthetic rate, decreasing resources available to plants. However, depending on the level of defoliation, plants can increase the photosynthetic efficiency of the remaining leaves in order to minimize the damage to their development (Wirf, 2006; Wang et al., 2016; Peschiutta et al., 2018). On other hand, interaction with entomopathogenic fungi can increase photosynthesis in plants in suboptimal situations, such as in conditions of biotic stress (Hossain et al., 2017).

The lowest mean for net assimilation CO_2 (*A*) and transpiration rate (*E*) found in plants treated with *T.*

asperellum, *Isaria sp.* and *B. bassiana* (Figure 4A and D) may be due to the increased closure of stomata from the lower stomatal conductance (*gs*) found in this study. Lower stomatal conductance results in lower influx of CO_2 into the chloroplasts, while decreasing water loss due to transpiration via the stomatal pore (Shimazaki et al., 2007; Taiz & Zeiger, 2019). In addition, the repercussions on *A* and *E* depend mainly on the intensity of the damage and the size of the leaves left on the plants after the attack of the caterpillars (Wenda-Piesik et al., 2017).

The reductions in instantaneous carboxylation efficiency (A/Ci) and instantaneous Water Use Efficiency (A/E) found in treatments with *T. asperellum*, *Isaria sp*. and *B. bassiana* (Figure 4E and F) are related to the lower levels of *A* and *gs* found in these treatments. Removal of leaf tissue caused by caterpillars may decrease carbon assimilation and stomatic conductance in kale (Meza-Canales et al., 2017). At the same time, these phenomena can cause stomatic dysregulation and low CO_2 levels can lead to decreased water use efficiency (Grimmer et al., 2012).

Even with the highest population density of caterpillars found in plants treated with *Isaria sp*. (Figure 5F), there was no significant difference between treatments for gas exchange under greenhouse conditions. Damage caused by the removal of tissue by caterpillars on plant photosynthesis may present varied results (Meza-Canales et al., 2017). This is due to variations in the pest and plant stage, level of damage caused, part of the attacked plant, among others (Tang et al., 2006). Corroborating this statement, even with significant differences in the population density of the caterpillars observed, the severity of these attacks was similar for all treatments (Figure 5D).

Conclusions

Entomopathogenic fungi isolated from the Amazon presented potential for protection of kale plants against the attack of defoliating caterpillars, in relation to the traditional use of chemical insecticides, especially for *Metarhizium anisopliae*. Plants inoculated with *B. bassiana*, *M. anisopliae* and *T. asperellu*m showed lower caterpillar population density rates. Commercial leaves were larger in the treatments with chemical insecticides compared to the treatments with fungi. However, the plants inoculated with *Isaria* sp. showed the highest number of non-commercial leaves than the insecticide treatment. Plants inoculated with *Isaria* sp. and *T. asperellum* had greater plant height. Plants inoculated with *B. bassiana*, *M. anisopliae* and *T. asperellum* showed similar levels of severity as the chemical insecticide. For the total number of leaves under field conditions, the treatment with *M. anisopliae* provided more leaves compared to the other treatments. Regarding the robustness index, the plants inoculated with *T. asperellum* were superior than the insecticide treatment. The highest commercial yield was observed in plants with chemical insecticide.

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