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Research Article

Impact of Some Entomopathogenic Fungi on the Growth of *Zea mays* L. and *Helianthus annuus* L.

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ABSTRACT

Entomopathogenic fungi, a promising alternative to conventional insecticides, have been widely used as biocontrol agents for the regulation of pest populations. Furthermore, recent studies have shown that entomopathogenic fungi also have growth-promoting properties in plants. The purpose of this investigation was to assess the effects of indigenous entomopathogenic fungi strains, *Beauveria bassiana* Pa4, *Cordyceps fumosorosea* KTU-42, and *Metarhizium flavoviride* As-18, on the germination and early development period of sunflower and maize plants. In this study, it was determined that EPF application did not affect the seed germination percentage in maize, but increased the germination percentage of sunflower seeds. It was determined that *M. flavoviride* application significantly increased root-stem weight, root length, stem diameter and biomass in sunflower samples compared to other EPF applications and control. In maize, it was determined that EPF applications had a positive effect only on root length. The most effective fungus to increase growth on maize plants was *C. fumosorosea* KTU-42. On the other hand, the same result was obtained with *M. flavoviride* As-18 on sunflower plants. The present study reported that these fungi promote plant development and should be considered an important factor in plant production besides pest management.

Anahtar Kelimeler: *Entomopathogen fungus, Germination, Plant growth, Maize, Sunflower*

Bazı Entomopatojenik Fungusların *Zea mays* L. ve *Helianthus annuus* L.'nin Büyümesi Üzerindeki Etkisi

ÖZET

Geleneksel insektisitlere umut verici bir alternatif olan entomopatojenik funguslar, bitki zararlı popülasyonlarının azaltılması için biyokontrol ajanları olarak yaygın olarak kullanılmaktadır. Ayrıca, son çalışmalar, entomopatojenik fungusların bitkilerde büyümeyi teşvik edici özelliklere de sahip olduğunu göstermiştir. Bu araştırmanın amacı, yerli entomopatojenik fungus suşları olan *Beauveria bassiana* Pa4, *Cordyceps fumosorosea* KTU-42 ve *Metarhizium flavoviride* As-18'in ayçiçeği ve mısırın çimlenme ve erken gelişme periyodu üzerindeki etkilerini değerlendirmektir. Bu çalışmada, EPF uygulamasının mısırda tohum çimlenme yüzdesini etkilemediği ancak ayçiçeği tohumlarının çimlenme yüzdesini artırdığı tespit edilmiştir. Ayçiçeği örneklerinde *M. flavoviride* uygulamasının diğer EPF uygulamalarına ve kontrole göre kök-gövde ağırlığını, kök uzunluğunu, gövde çapını ve biyokütleyi önemli ölçüde arttırdığı belirlendi. Mısırda ise EPF uygulamalarının sadece kök uzunluğuna olumlu etki yaptığı belirlendi. Mısır bitkilerinde büyümeyi arttırmada en etkili mantar *C. fumosorosea* KTU-42 oldu.

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Ayçiçeği bitkilerinde ise *M. flavoviride* As-18 ile aynı sonuç elde edilmiştir. Bu çalışma, bu mantarların bitki gelişimini desteklediğini ve zararlı yönetiminin yanı sıra bitki üretiminde önemli bir faktör olarak görülmesi gerektiğini bildirmiştir.

Keywords: *Entomopatojen fungus, Çimlenme, Bitki büyümesi, Mısır, Ayçiçeği*

I. INTRODUCTION

Some synthetic chemicals called pesticides are used to prevent losses in crop yield and quality in plants and to eliminate the effects of biotic-abiotic stresses. The use of these chemicals causes soil pollution and has long-term negative consequences for the environment [1], [2], [3]. In recent years, the use of entomopathogenic fungi (EPF) as an alternative to pesticides, especially in the biocontrol of insects, which is among the biotic stress factors, has come to the fore and attracted a lot of attention due to its agronomic importance [4], [5], [6]. Plants maintain a symbiotic life with endophytic fungi that live inside their tissues but cause no harm. Although the plant-fungi relationship is a symbiotic life, these fungi do not need plants to live and can survive without plants [7, 8]. Entomopathogenic fungi directly or indirectly promote plant growth. Transforming atmospheric nitrogen into a form that the plant can use, improving water transport, increasing the uptake of potassium and phosphorus, which are necessary for growth and development, and promoting the synthesis of phytohormones are among the direct effects it provides to the plant. It is known that they are effective in the synthesis of indole-3-acetic acid, which is in the auxin group of plant growth regulators, which is especially important in cell elongation, cell division, and differentiation. Indirect effects are antibiotics, siderophores, metabolites with low molecular weight such as hydrogen cyanide, synthesis of enzymes, inhibition of ethylene production, suppressing the negative effects of phytopathogens and thus reducing the stress effect [9], [5]. More than 700 fungal species belonging to 90 genera, including entomopathogenic fungi in the rhizosphere, are defined as insect pathogens. Research on these fungi; focused on the taxonomy, phylogeny, mode of action, and use of fungi as a biocontrol agent. *Beauveria bassiana* (Bals.-Criv.) Vuill., *Metarhizium anisopliae* (Metsch.) Sorokin, *Isaria* spp. and *Lecanicillium* spp. are some of the entomopathogenic fungi that can colonize the plant and are used as biopesticides against insect pests [8]. Different species of *Beauveria* produce oxalic and citric acids, as well as formic, lactic, orotic acids. These organic acids change the pH of the medium. Iron is required for fungal cell growth. *B. bassiana* produces siderophores that play an important role against cellular stress caused by iron deficiency [7]. *Metarhizium anisopliae* reduces the effects of salt stress on plants. The presence of *B. bassiana* and *Isaria fumosorosea* in cabbage plants grown under artificial light and in the absence of water reduces the effect of these stress factors [10].

Maize is an important industrial plant used as a raw material in the production of vegetable-origin protein, starch, glucose, and oil. Maize, which is a monocot plant, is very selective in terms of soil requirements. The plant has hairy roots, like warm, nutrient-rich, well-aerated soils with a pH of 6-7 [11], [7]. Sunflower is an important industrial plant cultivated in more than 70 countries with suitable climatic conditions [12], [13]. It is used in industry to make paint and soap and its pulp is used as feed, especially in cattle breeding, with its rich nutritional content. The optimum temperature for plant growth and flowering is between 21-24°C. It develops optimally in soils that hold moisture well [14].

The aim of this study is to reveal the effects of three different entomopathogenic fungi, *Beauveria bassiana*, *Cordyceps fumosorosea* (formerly known *Isaria fumosorosea*), and *Metarhizium flavoviride*, on germination and early development period in sunflower and maize by examining their morphological characteristics.

II. MATERIALS AND METHODS

A. PREPARATION OF FUNGAL SUSPENSIONS

Entomopathogenic fungi were provided from the entomopathogen culture collection at Karadeniz Technical University. Fungal strains, *B. bassiana* Pa4, *C. fumosorosea* KTU-42 and *M. flavoviride* As-18, were spread on Sabouraud dextrose agar (SDA) supplemented with 1% yeast extract and incubated at 28 ± 2°C, RH > 60% for two weeks in continuous darkness. Then, a sterile liquid solution of 0.1% Tween80 (10 ml) was added to the petri dishes, and conidia were harvested from the medium surface using a sterile scalpel. Conidial suspensions were filtered through a double layer of sterile cheesecloth and stirred for 5 min to homogenize the preparations. The conidial concentration was determined using a Neubauer hemocytometer and adjusted to 1×10^{8} conidia ml^{-1}. The viability of conidia was observed microscopically (100×) after incubation for 24 h at 26°C on SDA plates. Conidium was considered to have germinated if the germ tube was longer than the diameter of the conidium. Cultures that had more than 90 % conidial viability were used in experiments.

B. GROWING OF PLANTS

The effects of fungi on seed germination and the early development of *Z. mays* and *H. annuus* were evaluated. Sunflower seeds (SY Suzuka) were obtained from Ziya Organic Agricultural Enterprises and maize seeds (ADA523) were obtained from Sakarya Maize Research Institute. The pots (15×25 cm) containing soil (N (%), 3.07; Ca, 617.4 ppm; K, 1163.0 ppm; Mg, 651.0 ppm; and P, 7.396 ppm; Cu, 4.736 ppm; Fe, 50.3 ppm; Mn, 12.73 ppm; Zn, 24.13 ppm; and B, 2.48 ppm in 100 g soil) were inoculated with 100 ml of 1×10^{8} conidia/ml fungal suspensions and incubated in a climate chamber at 25°C, 60% RH, and an L16:D8 light cycle [15]. A sterile liquid solution of 0.1% Tween80 was used in the control group. Three days after fungi inoculation, randomly selected ten seeds sterilized by 10% sodium hypochlorite (NaOCl) were planted in each pot, irrigated with sterile distilled water, and grown in the climate chamber for two months. Experiments were repeated three times.

C. PLANT GROWTH MEASUREMENTS

Two months after seed planting, root and stem length were measured with a ruler. Stem diameters were measured with a digital caliper. After weighing the root and stem fresh weights with precision scales, the samples were left to dry overnight at 105°C. The weight of the dried root and stem samples was measured with a precision balance. Then root and stem biomass were calculated according to Sulus and Leblebici (2020) with minor adjustments [16].

D. STATISTICAL ANALYSIS AND EVALUATION OF RESULTS

The results obtained by comparing three different fungi applications with the control group were evaluated statistically by applying one-way ANOVA and Duncan test, one of the multiple comparison tests, in the Graphpad program.

III. RESULTS

All maize seeds germinated in fungi-treated and control group (Table 1). The treatment of conidial suspensions (*B. bassiana* Pa4, *C. fumosorosea* KTU-42 and *M. flavoviride* As-18) to maize plants increased root and stem lengths as compared to control treatment with 0.1% Tween80 two months after application ($p<0.01$). Also, the best root and stem growth was seen in *C. fumosorosea* treated plants. Two months after inoculation the following average root and stem lengths ± SD were found, respectively: *B. bassiana* = 70.57 cm ± 0.55; 65.4 cm ± 0.36; *M. flavoviride* = 65.9 cm ± 0.53; 66.4 cm

± 0.3; *C. fumosorosea* = 75.75 cm ± 0.46; 70.57 cm ± 0.21; and control = 62.33 cm ± 0.76; 62.83 cm ± 0.25 (Figure 1a and 1b).

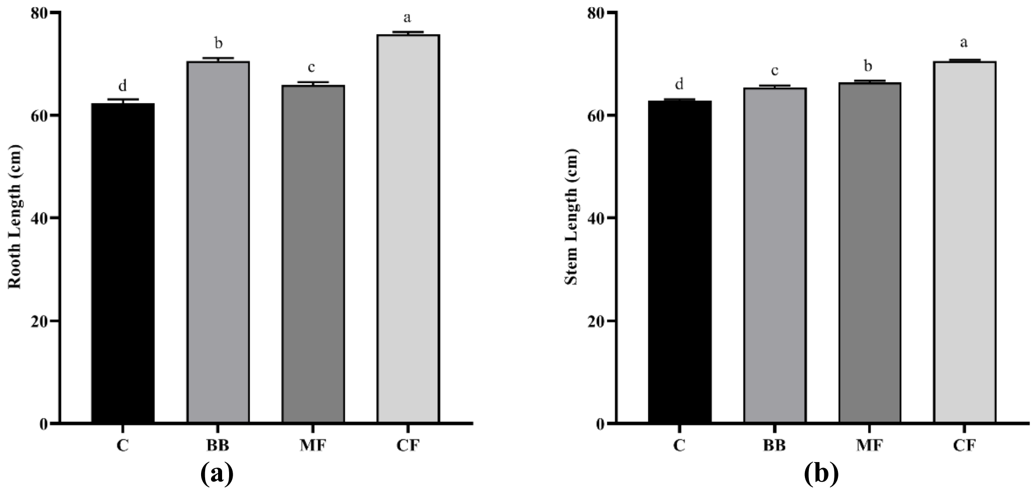


Figure 1. (a) *Root length of maize samples* ***(b)*** *Stem length of maize samples (Means (n = 9) ± SD are shown, p<0.01, C: Control, BB: Beauveria bassiana, CF: Cordyceps fumosorosea, MF: Metarhizium flavoviride).*

The stem diameter of maize plants increased significantly in *C. fumosorosea* treated plants compared to the control group ($p<0.01$). However, there was no difference in stem diameters among the other fungal treatment ($p>0.05$). Stem diameters were measured as *B. bassiana* = 6.86 cm ± 0.06; *M. flavoviride* = 6.72 cm ± 0.03; *C. fumosorosea* = 7.66 cm ± 0.11; and control = 6.7 cm ± 0.18 (Figure 2).

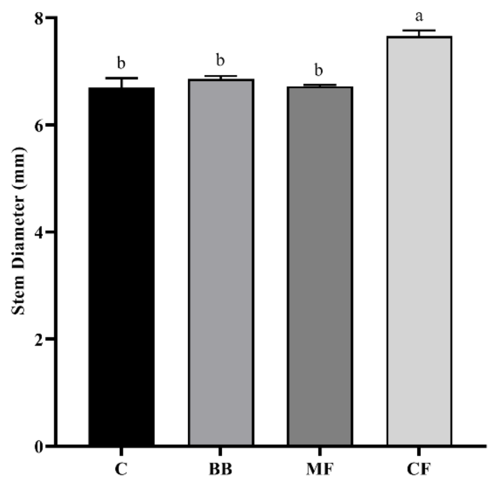
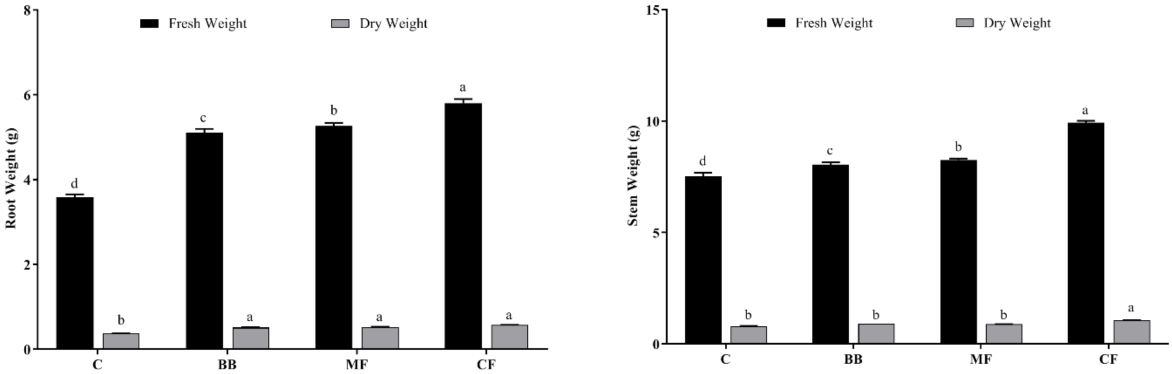


Figure 2. *Stem diameter of maize samples (Means (n = 9) ± SD are shown, p<0.01, BB: Beauveria bassiana, CF: Cordyceps fumosorosea, MF: Metarhizium flavoviride).*

There was no significant difference between the leaf number of the fungi-treated plants and the control group ($p>0.01$). The average leaf number ± SD were calculated, respectively: *B. bassiana* = 4 ± 0.0; *M. flavoviride* = 3.83 ± 0.29; *C. fumosorosea* = 4.33 ± 0.58; and control = 4.17 ± 0.29. The root fresh and dry weight of *C. fumosorosea* treated plants was higher than control and the other fungi treated plants (Table 1). The fresh and dry weight of roots ± SD were calculated, respectively: *B. bassiana* = 5.11 g ± 0.08; 0.51 g ± 0.01; *M. flavoviride* = 5.27 g ± 0.07; 0.53 g ± 0.005; *C. fumosorosea* = 5.8 g ± 0.1; 0.57 g ± 0.004; and control = 3.59 g ± 0.07; 0.37 g ± 0.007 (Figure 3a). Similarly, the stem fresh and dry weight was higher with *C. fumosorosea* treated plants. The fresh and dry weight of stems ± SD were calculated, respectively: *B. bassiana* = 8.04 g ± 0.12; 0.92 g ± 0.002; *M. flavoviride* = 8.26 g ± 0.06; 0.89 g ± 0.004; *C. fumosorosea* = 9.92 g ± 0.09; 1.06 g ± 0.008; and control = 7.53 g ± 0.16; 0.80 g ± 0.007 ($p<0.01$, $p<0.05$ respectively) (Figure 3b).



(a) **(b)**

Figure 3. (a) *Root fresh and dry weight of maize samples* ***(b)*** *Stem fresh and dry weight of maize samples (Means (n = 9) ± SD are shown, p<0.01, BB: Beauveria bassiana, CF: Cordyceps fumosorosea, MF: Metarhizium flavoviride).*

The root and stem biomass of fungi treated plants was higher than the control. Also, *C. fumosorosea* had the highest root and stem biomass. The root and stem biomass ± SD were calculated, respectively: *B. bassiana* = 45.32 g ± 0.90; 81.06 g ± 0.13; *M. flavoviride* = 46.85 g ± 0.43; 78.29 g ± 0.38; *C. fumosorosea* = 50.71 g ± 0.33; 93.83 g ± 0.66; and control = 32.87 g ± 0.58; 71.03 g ± 0.61 (p<0.01) (Figure 4).

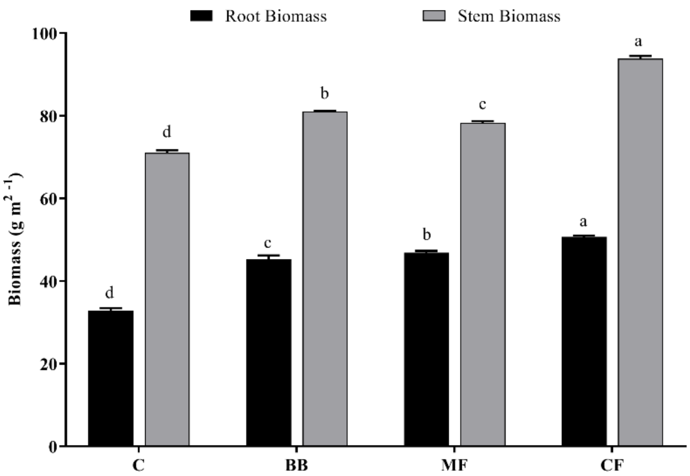


Figure 4. *Root and stem biomass of maize samples (Means (n = 9) ± SD are shown, p<0.01, BB: Beauveria bassiana, CF: Cordyceps fumosorosea, MF: Metarhizium flavoviride).*

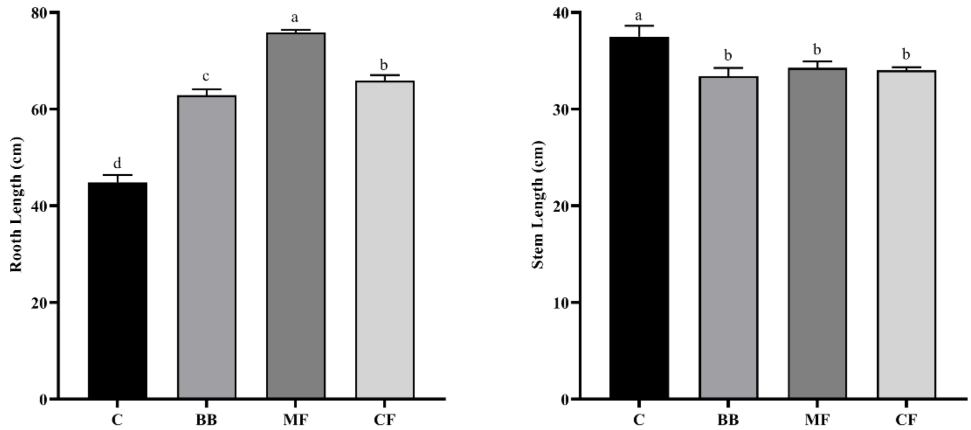
The germination of fungi-treated sunflower seeds was higher than in the control group. The germination rate was highest (89% ± 2.3) in *C. fumosorosea* treated seeds (Table 1).

Table 1. *Seed germination rate (%) and leaf numbers of maize and sunflower samples.*

	Zea mays L.		*Helianthus annuus* L.	
	Germination rate (%)	Leaf number	Germination rate (%)	Leaf number
Control	100±0a	4.17±0.29a	72.22±9.62a	6±0a
BB	100±0a	4.00±0.00a	83.33±28.87a	6±0a
MF	100±0a	3.83±0.29a	83.33±0.00a	6±0a
CF	100±0a	4.33±0.58a	88.89±19.25a	6±0a

(Means (n = 9) ± SD, BB: *Beauveria bassiana*, CF: *Cordyceps fumosorosea*, MF: *Metarhizium flavoviride*. Different case letters in a coloumn represent statistically significant differences amongst the means according to the Duncan multiple comparison test, p<0.01)

The treatment of conidial suspensions to sunflower plants increased root lengths as compared to control treatment with 0.1% Tween80 two months after application (p< 0.01). Also, the best root and stem growth was seen in *M. flavoviride* treated plants. However, the stem lengths of fungi-treated plants were decreased compared to the control treatment. Two months after inoculation the following average root and stem lengths ± SD were found, respectively: *B. bassiana* = 62.87 cm ± 1.27; 33.4 cm ± 0.87; *M. flavoviride* = 75.57 cm ± 1.01; 34.27 cm ± 0.68; *C. fumosorosea* = 65.9 cm ± 1.15; 34.03 cm ± 0.31; and control = 44.87 cm ± 1.51; 37.5 cm ± 1.11 (Figure 5a and 5b). There was no significant difference between the leaf number of the fungi-treated plants and the control group (p>0.01). The stem diameter of sunflower plants increased significantly in *M. flavoviride* treated plants compared to the control and other fungi-treated groups (p<0.01) (Table 1). However, there was no difference in stem diameters among the other fungal treatment (p>0.01). Stem diameters were measured as *B. bassiana* = 4.59 cm ± 0.09; *M. flavoviride* = 4.95 cm ± 0.11; *C. fumosorosea* = 4.44 cm ± 0.09; and control = 4.58 cm ± 0.04 (Figure 6).



(a) **(b)**

Figure 5. (a) *Root length of sunflower samples* ***(b)*** *Stem length of sunflower samples (Means (n = 9) ± SD are shown, p<0.01, BB: Beauveria bassiana, CF: Cordyceps fumosorosea, MF: Metarhizium flavoviride).*

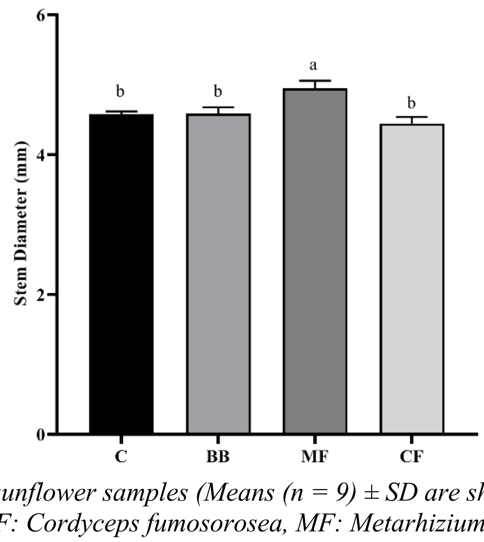
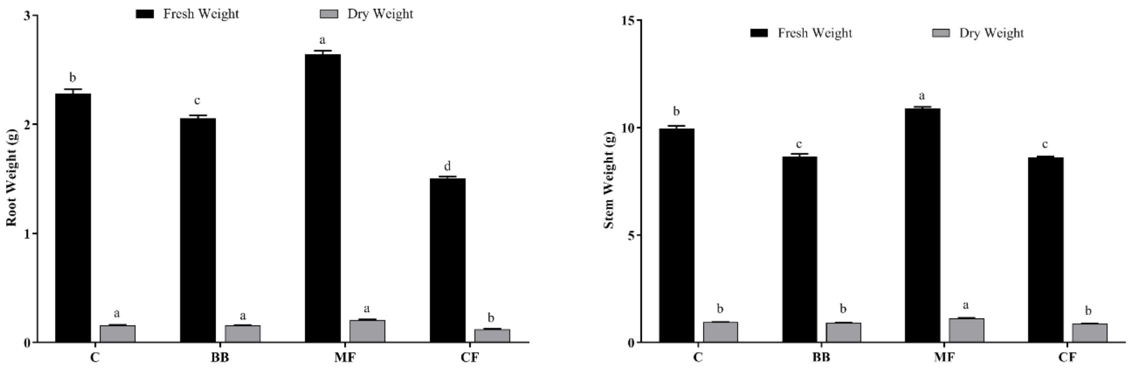


Figure 6. *Stem diameter of sunflower samples (Means (n = 9) ± SD are shown, p<0.01, BB: Beauveria bassiana, CF: Cordyceps fumosorosea, MF: Metarhizium flavoviride).*

The root fresh and dry weight of *M. flavoviride* treated plants was higher than control and the other fungi-treated plants. However, the root fresh and dry weight of *C. fumosorosea* and *B. bassiana* treated plants were lowest than control. The fresh and dry weight of roots ± SD were calculated, respectively: *B. bassiana* = 2.05 g ± 0.03; 0.16 g ± 0.002; *M. flavoviride* = 2.64 g ± 0.03; 0.21 g ± 0.006; *C. fumosorosea* = 1.51 g ± 0.02; 0.12 g ± 0.003; and control = 2.28 g ± 0.04; 0.16 g ± 0.003 (p<0.01, p<0.05 respectively) (Figure 7a). Similarly, the stem fresh and dry weight was higher with *M. flavoviride* treated plants. The fresh and dry weight of stems ± SD were calculated, respectively: *B. bassiana* = 8.66

g ± 0.11; 0.92 g ± 0.005; *M. flavoviride* = 10.89 g ± 0.07; 1.14 g ± 0.005; *C. fumosorosea* = 8.59 g ± 0.06; 0.88 g ± 0.006; and control = 9.95 g ± 0.13; 0.95 g ± 0.005 ($p<0.01$, $p<0.05$ respectively) (Figure 77b). The root and stem biomass of *M. flavoviride* treated plants was higher than the control. However, the root and stem biomass of *C. fumosorosea* and *B. bassiana* treated plants were lowest than the control. The root and stem biomass ± SD were calculated, respectively: *B. bassiana* = 13.91 g ± 0.18; 81.18 g ± 0.39; *M. flavoviride* = 18.13 g ± 0.53; 100.87 g ± 0.39; *C. fumosorosea* = 10.79 g ± 0.26; 77.81 g ± 0.54; and control = 14.12 g ± 0.22; 83.92 g ± 0.41 ($p<0.01$) (Figure 8).



(a) (b)

Figure 7. *(a) Root fresh and dry weight of sunflower samples (b) Stem fresh and dry weight of sunflower samples (Means (n = 9) ± SD are shown, p<0.01, BB: Beauveria bassiana, CF: Cordyceps fumosorosea, MF: Metarhizium flavoviride).*

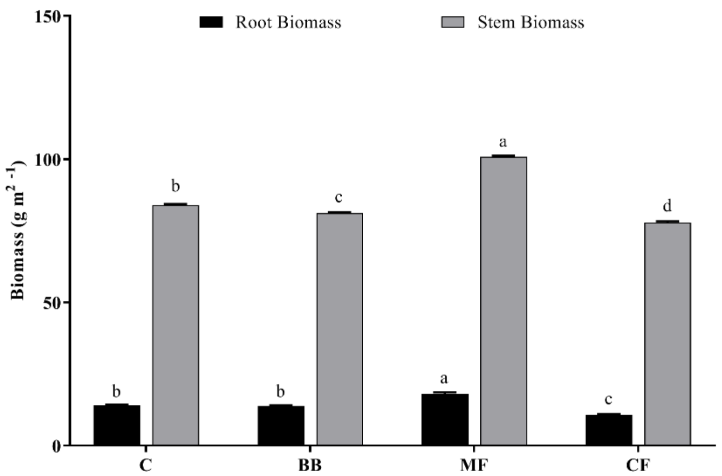


Figure 8. *Root and stem biomass of sunflower samples (Means (n = 9) ± SD are shown, p<0.01, BB: Beauveria bassiana, CF: Cordyceps fumosorosea, MF: Metarhizium flavoviride).*

IV. DISCUSSION

Numerous studies reported that entomopathogenic fungi not only protect the plants from insects' damage but also promote plant growth. In this study, the effects of three different EPF, *B. bassiana*, *M. flavoviride* and *I. fumorosorosea*, on the early development period of sunflower and maize were investigated. The impact of successful colonization of EPF isolates on plant growth parameters was generally positive. Several studies have reported the positive effects of EPF on the growth of different plants such as tomato, wheat, maize, cotton and bean [17], [18], [19].

In our study, maize seed germination percentage was not affected by EPF treatment which was similar to the results reported by Kuzhuppillymyal-Prabhakarankutty et al (2020), who showed that *B. bassiana* did not alter maize seed germination. Likewise, *M. anisopliae* strains did not increase maize seed germination [20], [21]. Also, it has been reported that EPF does not affect seed germination in many plants such as sweet pepper, peanut, eggplant [22], [23], [24]. In contrast, Russo et al. (2019) reported an increment in the germination of *B. bassiana*-treated maize seeds. Similarly, we observed that the germination percentage of sunflower seeds treated with EPF was higher compared to the control treatment [25].

Fungi-treated maize plants had higher root and stem biomass compared to control plants. These results were similar to those reported in previous studies, which indicated that *Beauveria* sp. and *Metarhizium* sp. isolates increase the biomass of maize [4], [25], cotton [19], faba bean [26], and tomato [7]. However, a few studies reported no significant difference in the growth of *B. bassiana* inoculated maize plant [27] and chives [28] over the control treatment. Limited studies about the effect of *C. fumosorosea* on plant growth revealed that the fungus did not improve growth parameters in sweet sorghum [29] and citrus [30]. In contrast, we observed a significant enhancement in root weight and length, stem weight and length, and stem diameter of maize plants treated with *C. fumosorosea*. In addition, the effect of this fungus on the growth parameters of maize plants was higher than *B. bassiana* and *M. anisopliae*. In contrast to this, the growth parameters of *M. flavoviride* treated sunflower plant was generally higher compared to other fungi treated plants and control treatment. Although the root weight and length, stem weight, stem diameter and biomass of *M. flavoviride* treated sunflower plants increased significantly when compared with control treatment, stem length was decreased. However, other fungi only increased the root length of sunflower plants but decreased the stem weight and length, root weight and biomass. Miranda-Fuentes et al. (2021) treated two different strains of *B. bassiana* in sunflower plants and observed that one of them did not affect the growth parameters while the other caused a decrease in the parameters [31]. Although it is known that *M. flavoviride* is able to colonize some plant roots and shows endophytic properties, its effects on plant growth have not been investigated until now. To the best of our knowledge, this is the first study about the effects of *M. flavoviride* on plant growth.

V. CONCLUSION

This study revealed that the plant-enhancing effects of EPF may vary according to fungal species, and even different strains of the same species exhibit different properties when compared with other studies. *Cordyceps fumosorosea* for maize and *Metarhizium flavoviride* for sunflower can be used to improve plant growth. Considering that fungi have lots of ecological roles, these fungi should also be tested on pathogenic microorganisms and insects that harm these plants. Thus, with the application of a single agent, an important step will be taken towards solving the problems encountered in plants.

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