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# Impact of Entomopathogenic Fungi on the Growth, Development, and Health of Cabbage Growing under Water Stress

Surendra K. Dara<sup>1\*</sup>, Sumanth S. R. Dara<sup>2</sup>, Suchitra S. Dara<sup>2</sup>

<sup>1</sup>University of California Cooperative Extension, San Luis Obispo, Solutions, Bakersfield, California, USA <sup>2</sup>Global Agricultural Solutions, Bakersfield, California, USA Email: \*skdara@ucdavis.edu

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### **Abstract**

Entomopathogenic fungi such as *Beauveria bassiana, Isaria fumosorosea*, and *Metarhizium brunneum* are commonly used in the form of biopesticides to manage various arthropod pests. These fungi also form endophytic and mycorrhiza-like relationships with plants. This study aimed at evaluating the impact of three entomopathogenic fungi on the growth, development, and health of cabbage plants grown under artificial lighting with limited water. *Beauveria bassiana* had a positive influence on the survival, growth, health, length, and dry weight of cabbage. Other fungi also had a positive impact on some of the measured parameters. Other beneficial bacteria and mycorrhizae were also compared with the entomopathogenic fungi. This is the first report evaluating the non-entomopathogenic role of three entomopathogenic fungi on cabbage plants.

# **Keywords**

Beauveria bassiana, Isaria fumosorosea, Metarhizium brunneum, Cabbage

# 1. Introduction

Entomopathogenic fungi *Beauveria bassiana* (Bals.-Criv.) Vuill. (Hypocreales: Cordycipitaceae), *Isaria fumosorosea* Wize (Hypocreales: Cordycipitaceae), and *Metarhizium brunneum* Petch (Hypocreales: Clavicipitaceae) are ubiquitous soilborne organisms that are pathogenic to various arthropod pests [1]-[8]. These fungi are commercially available for their primary use as biopesticides to control arthropod pests in various cropping systems. Recent studies show that entomopathogenic fungi form an endophytic relationship with banana [9], cotton [10], oilseed rape [11], sorghum [12], strawberry [13] and negatively impact

herbivores. Other reports indicated that *B. bassiana* and *Metarhizium anisopliae* (Metch.) Sorokin had activity against plant pathogenic fungi and a virus [14] [15] [16]. Additionally, *Metarhizium robertsii* J.F. Bisch., Rehner & Humber promoted root development [17] and nitrogen absorption [18] in switch grass and haricot beans and *M. anisopliae* mitigated salt stress in soybean [19] through endophytic interaction. These studies indicate a bigger role that entomopathogenic fungi could play in crop production in addition to their traditional role in crop protection. While some of the earlier studies used non-commercial isolates, species, or pure cultures of fungi, the current study was conducted using commercial formulations of *B. bassiana*, *I. fumosorosea*, and *M. brunneum* and comparing them with beneficial bacteria- and mycorrhizae-based products. The impact these materials have on the growth and health of cabbage under artificial lighting and reduced water conditions were evaluated.

#### 2. Materials and Methods

Approximately 3-week old cabbage (variety Supreme Vantage) transplants, obtained from a commercial nursery (Plantel Nurseries Inc, Santa Maria, CA), were planted in Miracle-Gro Moisture Control Potting Mix (The Scotts Company, LLC, Marysville, OH) in 650 ml plastic containers. This potting medium contained 0.21% nitrogen (0.12% ammonical and 0.09% nitrate nitrogen), 0.07% available phosphate (P<sub>2</sub>O<sub>5</sub>), 0.14% soluble potash (K<sub>2</sub>O), and 0.10% iron (Fe). Each treatment had 10 plants and each plant was treated by pouring 100 ml of respective treatment solution, prepared according to the field application rates, around the base of the plant. Water alone was added to control plants. Treatment solutions were made by adding the following materials to make up a final volume of 100 ml: 1) 1 ml of BotaniGard ES (LAM International Corp, Butte, MT)-active ingredient B. bassiana strain GHA, 2) 1 ml of Met52 (Novozymes Biologicals, Inc, Salem, VA)-active ingredient M. brunneum ( = M. anisopliae) strain F52, 3) NoFly WP (Natural Industries, Inc, Spring, TX)-active ingredient Isaria fumosorosea (=Paecilomyces fumosoroseus) strain FE 9901, 4) 2.3 ml of SumaGrow (SumaGrow USA, Westminster, CA)-active ingredients 12% humic acid and 1% of beneficial microbes that included Azorhizobium caulinodans, Bacillus subtilis, Pseudomonas fluorescens, Rhizobium phaseoli, and Trichoderma virens, 5) and 1 ml of CropSignal (Advanced Soil Technologies, Bakersfield, CA)-active ingredient is a proprietary blend of minerals and micronutrients, 6) 0.03 ml of Mykos Liquid (Reforestation Technologies International, Gilroy, CA)-active ingredient Rhizophagus irregularis, and 7) H2H or Harvestto-Harvest (California Safe Soil, LLC, West Sacramento, CA) with 1% water soluble nitrogen, 1% available phosphate, and an undefined blend of beneficial microbes. One plant from each treatment was placed in a tray and each tray represented a replication. Trays were arranged on a table and one 75 w Philips Agro-Lite BR30 (Philips North America Corporation, Andover, MA) was placed in each corner of the table. Plants were grown in a dark room at a mean temperature of 22°C under 14:10 light: dark cycle and 50 ml of water was added to each container on 42, 50, 64, and 81 days after planting. With artificial lighting and a

limited amount of water, less than favorable field or greenhouse conditions were created to subject plants to stress and evaluate the impact of entomopathogenic fungi compared to other commercial products that are meant to promote plant growth, health, and yield (Table 1).

Various parameters were measured during the study. Plant stand was counted 40, 70, and 90 days after planting (DAP). Plant health was rated 40 and 70 DAP using a 0 - 5 rating where 0 = dead, 1 = weak, 2 = moderate-low, 3 = moderate-high, 4 = good, and 5 = very good in terms of general health. Length of the roots and shoots was measured 90 DAP. Plants from each treatment were placed in a paper bag and dried for 8 days at 36.5°C. After taking the dry weights, plants were sent to the University of California Davis analytical laboratory to measure nitrogen, phosphorous, potassium, and iron content. Samples were analyzed following standard analytical procedures using the combustion method (<a href="http://anlab.ucdavis.edu/using-the-lab/analysis/plant/522">http://anlab.ucdavis.edu/using-the-lab/analysis/plant/522</a>) for total nitrogen and the nitric acid digestion method

(<a href="http://anlab.ucdavis.edu/using-the-lab/analysis/plant/590">http://anlab.ucdavis.edu/using-the-lab/analysis/plant/590</a>) for the remaining nutrients. Since the plant material was insufficient due to small size of the plants, analysis was not conducted for individual plants, but for all plants within each treatment. Data were subjected to analysis of variance and significant means were separated using LSD test.

#### 3. Results

Plant growth was stunted during the study period indicating less than favorable growth conditions as intended for this study. There were significant differences ( $P \le 0.001$ ) among various treatments based on different measured parameters (**Figure 1**).

#### 3.1. Plant Stand

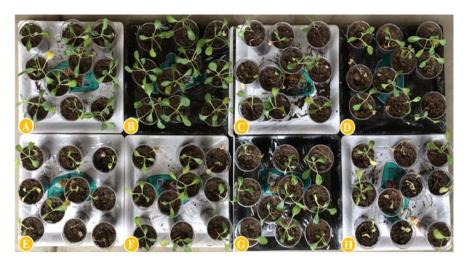
Beauveria bassiana was the only treatment where all the plants survived for 90

**Table 1.** Average dry weight of the plant, nitrogen (N), phosphorous (P), potassium (K), and iron (Fe) content, and the ratio of plant weight and respective nutrients.

Treatment	Dry Weight (mg)	N (mg)	P (mg)	K (mg)	Fe (mg)	Weight/N	lWeight/P	Weight/k	(Weight/Fe
Miracle – Gro	202.22	1.139	0.130	1.610	0.0105	177.51	1558.44	125.61	19271.95
MG + BotaniGard	234.00	0.889	0.136	1.617	0.0097	263.16	1715.27	144.72	24213.08
MG + Met52	157.50	0.852	0.132	1.333	0.0074	184.76	1197.60	118.17	21276.60
MG + NoFly	92.22	0.509	0.074	0.748	0.0035	181.09	1246.54	123.29	26162.79
MG + SumaGrow	55.00	1.312	N/A*	N/A	N/A	41.93	N/A	N/A	N/A
MG + CropSignal	88.89	0.521	0.075	0.667	0.0042	170.45	1178.01	133.33	21028.04
MG + Mykos Liquid	90.00	0.532	0.072	0.778	0.0026	169.17	1253.48	115.68	35294.12
MG + H2H	70.00	0.658	0.137	1.071	0.0043	106.38	510.73	65.36	16129.03

<sup>\*</sup>Not available due to insufficient amount of dried plant material for analysis.





**Figure 1.** Condition of the surviving plants 90 days after planting (DAP) in different treatments [A. Miracle – Gro (MG), B. MG+*B. bassiana*, C. MG + *M. brunneum*, D. MG + *I. fumosorosea*, E. MG + SumaGrow, F. MG+CropSignal, G. MG + Mykos Liquid, and H. MG + H2H].

days of the observation period (**Figure 2(a)**). There was a 10% to 80% reduction in the plantstand in other treatments during this period. Highest plant death was seen in SumaGrow and H2H treatments (P = 0.001 at 40 DAP and <0.00001 at 70 and 90 DAP).

#### 3.2. Plant Health

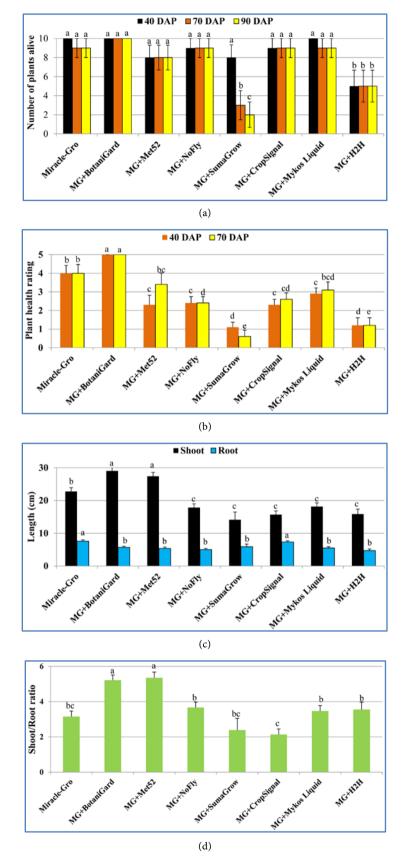
Plants treated with *B. bassiana* were significantly and uniformly healthier (*P* < 0.00001) than the rest of the treatments on both observation dates with a 'very good' rating (**Figure 2(b)**). Health of the plants growing in Miracle-Gro with no supplemental materials also had a 'good' rating and was better than the health of plants in most of the remaining treatments. Plants treated with SumaGrow and H2H had poor health with a 'weak' rating.

# 3.3. Shoot and Root Length

Length of the shoots was significantly higher (P < 0.00001) for plants treated with B. bassiana (29 cm) and M. brunneum (27.6 cm) compared to the rest of the treatments (**Figure 2(c)**). Plants treated with Miracle-Gro alone had a mean shoot length of 22.9 cm, but the remaining treatments had significantly shorter shoots that varied from 13 - 18 cm. Plants growing in Miracle-Gro alone and those supplemented with Crop Signal had significantly longer (P < 0.00001) roots.

# 3.4. Shoot-to-Root Ratio

Shoot-to-root ratio, which indicates the shoot growth in relation to the root growth, was significantly higher (P < 0.00001) for plants that were treated with *B. bassiana* and *M. brunneum* (**Figure 2(d)**) followed by those treated with *I. fumosorosea* and others.



**Figure 2.** Plant stand (a); plant health (b); shoot and root length (c); and shoot-to-root ratio (d) in different treatments at 40, 70, or 90 days after planting (DAP).

### 3.5. Nutrient Content

Since all the surviving plants, 90 DAP, were combined for the dry weight or analyzed for the nutrient content as a single sample for each treatment, data were not sufficient for statistical analysis. Plants treated with *B. bassiana* had relatively higher biomass. When the plant weight, as a result of accumulated nutrients, was calculated by dividing the weight with respective nutrient content, *B. bassiana* appeared to have relatively higher output for nitrogen, phosphorus, and potassium based on numerical values. Such an effect for iron was seen in all treatments compared to Miracle-Gro alone. However, these values are only indicative as they were not subjected to statistical analysis.

#### 4. Discussion

Although both SumaGrow and H2H contained beneficial microbes, plants treated with these materials did not survive well and were not able to maintain good health. Mycorrhizal products are typically not used in cole crops as crucifers are thought to have poor or no mycorrhizal relationship. Both SumaGrow and Mykos Liquid, which contained mycorrhizae, served as positive controls in this study. In general, *B. bassiana* had a significant positive impact on the survival, health, and growth of the plants compared to the remaining treatments while *M. brunneum* had a similar positive effect on the shoot length and shoot-to-root ratio.

The positive impact of entomopathogenic fungi on plants is generally attributed to the pest control they provide. A recent review [20] discussed multiple roles of entomopathogenic fungi as endophytes and plant disease antagonists, but contemplated on rhizosphere colonization and possible plant growth promotion as there was no published evidence of the direct impact of entomopathogenic fungi on plant growth at the time of the review. However, a recent study showed the positive impact of *B. bassiana* on strawberry plant growth regardless of the pest damage [21]. In a different study, strawberry plants treated with *M. brunneum* withstood infestations by two spotted spider mite, *Tetranychus urticae* Koch better than untreated plants [22]. Although the mode of action was not clear, *M. brunneum* could have promoted the root growth in this study similar to *M. roberts ii* in switch grass and haricot beans [17]. Spider mites generally thrive on water stressed plants [23] and *M. brunneum* could have improved the water absorption and helped strawberry plants withstand spider mite pressure.

Insects cause a significant loss to plant nutrients through their feeding damage, but plants also seem to have developed a mechanism to recover those nutrients, especially nitrogen, from insects through entomopathogenic fungi that have endophytic interactions with plants. As a result, whether or not insects are present in the soil, plants have an advantage to form an endophytic or mycorrhiza-like relationship with soilborne entomopathogens. It also appears that *B. bassiana* and *Metarhizium* spp. transfer insect-derived nitrogen to plants as seen in a study with monocots and dicots [24]. The current study was conducted in

the absence of pest insects and both *B. bassiana* and *M. brunneum* promoted plant growth demonstrating their role as nutrient and moisture absorption channels for plants. Mycorrhizae are known to improve plant growth, yield, water or nutrient absorption in citrus [25], corn [26], pepper [27], olive [28], onion [29], and snapdragon [30] and their role is especially important for plants growing in poor soils or under drought or saline conditions. Mycorrhizae also helped the seedlings of a tropical tree, *Diconyina guianensis* to grow well under poor light conditions [31]. Crucifers are previously thought to be non-mycorrhizal [32], but later studies reported varying degrees of mycorrhizal relationships in crucifers sometimes dependent on glucosinolate content [33] [34] [35] [36]. However, mycorrhizae-based products are generally not used in cole crop production. As the current study indicated that *B. bassiana* and *M. brunneum* helped cabbage plants to survive well, grow better, and maintain good health under low water and light conditions, these fungi may play a role in cabbage and other cole crop production.

#### 5. Conclusion

This is the first report of the direct impact of entomopathogenic fungi on cabbage plant growing under stressful conditions. The positive effect of *B. bassiana*, and to some extent *M. brunneum*, is important to expand their role in practical agriculture beyond pest management. If they are used to promote plant growth, improve water and nutrient absorption, withstand saline or drought conditions, and increase yields in addition to their typical use as biopesticides, then they can play a critical role as holistic tools in sustainable agriculture. The higher cost of biopesticides, compared to chemical pesticides, is a limitation for their extensive use. If products based on entomopathogenic fungi have multiple roles in both crop production and protection, their cost effectiveness improves and opens up new avenues for their increased use.

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