# Study Effect of Biochare, Earthworms and Mycorrhizal Fungi on Cauliflower Plant Growth and Some Soil Characters

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Abstract: The study aimed to determine the effect of different concentrations of biochare and there interaction with the mycorrhizal fungus Funneliformis mosseae and Earthworms Eisenia Fetida on growth of Cauliflower plant Brassica oleracea var. botrytis L. plant cultivated in plastic pots. The results of the study showed that there were changes in the properties of the studied soil, including organic matter, electrical conductivity, and the soil content of available phosphorus and potassium. However, the soil texture did not undergo any change, in addition to a decrease in the soil acidity compared to the control treatment. As for the effect of the presence of earthworms and biochare on mycorrhizal fungi, the results showed that the percentage of infection of cauliflower roots with mycorrhizal fungi increased, ranging from 60% to 100% in the interaction treatments compared to the mycorrhizal fungi only treatment, which was 20%. As for the severity of root infection with mycorrhizal fungi, it decreased in the interaction treatments of mycorrhizal fungi with earthworms and biochare , where it ranged from 23.33% to 42%, with the exception of the interaction treatment of mycorrhizal fungi with 2 gm of biochare, which was 56.66%, while the mycorrhizal fungi treatment was 50%. The results also showed that all treatments increased vegetative growth characteristics of cauliflower, including plant height, stem diameter, number of leaves, leaf area, and fresh and dry vegetative weight, as well as root growth characteristics, including root length and fresh and dry weight of the root system, compared to the control treatment without fertilizer. The results of this study contribute to environmental sustainability and soil conservation by reducing the use of chemicals as fertilizers.

## 1 INTRODUCTION

Cauliflower plant Brassica oleracea var. botrytis L., is an important winter vegetable crop it belongs to the Brassicaceae (cruciferae) family, which includes other crops such as cabbage and broccoli, The name cauliflower comes from the Latin word "cauliss," meaning stem, and "floris," meaning flower [1]. Cauliflower is an important food source rich in sulfur, which occurs in the form of compounds known as glucosinolates (GLS), GLS is one of the plant's defensive metabolic compounds and is responsible for its taste and flavor [2]. Eating vegetables rich in glucosinolates reduces the risk of cancer [3]. Several studies have indicated that eating cauliflower contributes to the prevention of metabolic disorders, asthma, and Alzheimer's disease, reduces the incidence of type 2 diabetes [4], and also reduces the risk of heart disease [5].

The extensive use of fertilizers over the past decades has significantly increased global food production capacity. However, in recent years, numerous studies have highlighted the inefficiency and unevenness of fertilizer use across countries, leading to environmental problems, soil nutrient imbalances, and suboptimal food production [6]. One alternative to chemical fertilization is the use of biofertilizers such as fungi, especially arbuscular mycorrhizal fungi represent a sustainable solution for enhancing plant growth because they form symbiotic relationships with many plants, They also contribute to plant growth by increasing the availability of nutrients in the soil and making plants more tolerant to abiotic stresses such as drought, salinity, heavy metals in the soil, and temperature changes and increase the plant's ability to withstand biotic stresses such as inducing plant resistance to pathogens and competing with pathogens in the rhizosphere [7], [8].

Biochare is one of the sustainable development goals because it contributes to the removal of carbon dioxide from the atmosphere by stabilizing atmospheric carbon. Therefore, it plays a role in reducing greenhouse gas emissions [9]. Biochare production also plays an important role in reducing waste. Many farmers have begun producing biochare from agricultural waste, which studies have shown contributes to improving soil properties, increasing organic matter, and increasing nutrient availability. It also enhances the growth of the soil's biological community, thus improving plant growth [10], [11], [12]. Earthworms are among the largest organisms that inhabit agricultural soils. They are among the largest animal groups in the soil and play a fundamental role as bioindicators for intensive agriculture [13]. They are of great importance in improving the physical, chemical, and biological properties of soil, as their presence accelerates the decomposition of soil pollutants, reduces heavy metal activity, and enhances crop growth [14], [15]. Due to the paucity of studies on the cauliflower crop Brassica oleracea var. Botrytis and the fertilization factors mentioned above. An experiment was carried out in plastic pots under greenhouse conditions with the aim of studying the effect of biofertilization with the mycorrhizal fungus Funneliformis mosseae and adding biochare and earthworms Eisenia fetida and the interaction between them in Some physical and chemical soil characteristics before and after planting, Mycorrhizal characteristics of cauliflower, and Vegetative and root growth characteristics of cauliflower plant.

# 2 MATERIALS AND METHODS

In order to evaluate the effect of treatment with mycorrhizal fungus Funneliformis mosseae, adding biochare, earthworms Eisenia fetida and their interaction on the growth of cauliflower (Brassica Oleracea var. botrytis), an experiment was carried out in plastic pots during the autumn of the 2024-2025 agricultural season in the greenhouse of the government nursery of the Directorate of Agriculture in Baqubah - Diyala Governorate. The area of the greenhouse was 50 m2 (length 10 m and width 5 m), and the land was leveled and weeds were removed to prepare it for the implementation of the experiment. The experiment was carried out according to the following steps:

## 2.1 Preparing Cauliflower Seedlings

Cauliflower seeds, Hybrid variety, were obtained from the local market in Baqubah, Diyala Governorate, For the purpose of growing cauliflower seedlings, plastic dishes were used, filled with peat moss sterilized by an autoclave at a temperature of 121°C and a pressure of 15 pounds. Inch2 for one hour for two consecutive days.The seeds were planted in them after being superficially sterilized with a 1% sodium hypochlorite solution, at a rate of one seed per hole. Planting took place on 9/10/2024 until they were transferred to plastic pots on 10/10/2024, with the seedlings being 30 days old.

## 2.2 Mycorrhizal Fungus Inoculum

Funneliformis mosseae mycorrhizal fungus was obtained from the Agricultural Research Department in Zafaraniya, affiliated with the Ministry of Science and Technology. The fungal inoculation consists of soil containing fungal spores and pieces of white corn roots infected with the mycorrhizal fungus. The mycorrhizal fungus was added to the soil at a rate.

## 2.3 Earthworms Inoculum

Earthworms Eisenia Fetida, they were obtained from the Khalis cow station of the National Company, northwest of Baqubah - Diyala Governorate, which was an organic soil containing immature worms, noting that every 100 grams of soil contains 100 worms of small and medium sizes. Earthworms were added at a rate of 20 worm to each plastic pot. Figure 1 shows the earthworms added to the soil.

Figure 1: Organic soil containing earthworms Eisenia fetida indicated in yellow.

## 2.4 Biochare

The biochare was obtained from the Mycology Laboratory at the College of Education for Pure Sciences, University of Diyala, which was prepared by one of the former graduate students from the woody branches of the eucalyptus plant. Biochare was added at three concentrations 0, 1, and 2 gm.

## 2.5 Experiment Implementation

The experiment was conducted in a greenhouse at the government nursery affiliated with the Diyala Agriculture Directorate in Baqubah, Diyala Governorate. Plastic pots were filled with a mixture of soil and peat moss, 15 kg per pot, after placing a filter paper at the bottom of the pot. For the addition of earthworms, a piece of plastic clip was placed at the bottom of the pot to prevent earthworms from escaping from the pot holes. Cauliflower seedlings were then planted in plastic pots on October 10, 2024, when they reached 3 or 4 leaves, at a rate of 2 plants per pot. These were subsequently thinned to one plant per pot. The experiment included 12 treatments with three replicates, bringing the number of treatments and their replicates to 36 experimental units as shown in Table 1 shows the treatments included in the experiment. The experiment was completed on 25 December, 2024, and several measurements were taken, including.

Table 1: Experimental treatments.

| Treatment code | Treatment details |
|---|---|
| Con | Control |
| 1BCH | 1 gm of Biochare |
| 2BCH | 2 gm of Biochare |
| MF | Mycorrhizal fungus |
| MF+1BCH | Mycorrhizal fungus+1 gm of Biochare |
| MF+2BCH | Mycorrhizal fungus +2 gm of Biochare |
| EW | Earthworms |
| EW+1BCH | Earthworms+1 gm of Biochare |
| EW+2BCH | Earthworms + 2gm of Biochare |
| MF+EW | Mycorrhizal fungus+ Earthworms |
| MF+EW+ 1BCH | Mycorrhizal fungus + Earthworms+1gm of Biochare |
| MF+EW+ 2BCH | Mycorrhizal fungus+Earthworms +2gm of Biochare |

### 2.5.1 Physical and Chemical Properties of Soil

Two months after planting, soil samples were taken from each treatment from a depth of 20 cm. They were air-dried and placed in plastic bags, three samples for each treatment. The samples were sent to the soil analysis laboratory of the Soil and Water Division of the Agriculture Directorate in Baqubah, Diyala Governorate, to determine the physical and chemical properties of the soil after planting.

### 2.5.2 Mycorrhizal Characteristics

Mycorrhizal traits, including the percentage and severity of mycorrhizal fungal infection, were measured in the Mycorrhizal Laboratory at the Agricultural Research Department in Zafaraniya, affiliated with the Ministry of Science and Technology , The method of Phillips and Hayman [16] was followed to calculate the percentage of mycorrhizal fungal infection of roots. and the infection rate was calculated according to the equation mentioned in [17] . The severity of mycorrhizal infection was calculated according to the equation mentioned in [18].

### 2.5.3 Plant Growth Characteristics

Shoot growth parameters includes Plant height (cm), stem diameters, fresh and dry weight for shoot part, leaves number , leaf area [19]. The characteristics of root system growth included root length (cm) , The fresh and dry weight of root group.

## 2.6 Statistical Analysis

A factorial experiment was conducted using a Randomized Complete Block Design (R.C.B.D.). The results were statistically analyzed using SPSS. The differences between the means were compared using Duncan's multiple range test at a probability level of 0.05 also the percentage increase between treatments compared to the control treatment was calculated.

# 3 RESULTS

## 3.1 Physical and Chemical Properties of the Soil after Planting

The results in Table 2 showed that there was a change in the concentration of organic matter in the soil, and the highest concentration was in the treatment of interaction between mycorrhizal fungi, earthworms and 1 gm of biochare, which was 3.5% compared to the control treatment, which was 2.4%. The table also shows that the soil pH decreased in all treatments and ranged between 7.3 to 7.8, while the soil pH in the control treatment was 8.1. The results in the table also show a change in the degree of conductivity in the soil in all treatments, which ranged between 0.7 to 1.3 $dsm^{-1}$ compared to the control treatment, which was 1.0 $dsm^{-1}$. As we find

Table 2: Physical and chemical properties of soil after and before planting.

| Treatments | pH | Organic Matter (%) | Available phosphorus (mg.kg$^{-1}$) | Available potassium (mg.kg$^{-1}$) |
|---|---|---|---|---|
| Control | 8.1 | 26 | 35.1 | 2.4 |
| MF | 7.6 | 27 | 29.9 | 2.8 |
| EW | 7.4 | 32 | 40.3 | 2.9 |
| MF+EW | 7.3 | 30 | 39.7 | 2.6 |
| 1BCH | 7.8 | 32 | 22.8 | 3.2 |
| 2BCH | 7.8 | 33 | 14.1 | 1.6 |
| MF+1BCH | 7.6 | 29 | 45.6 | 1.6 |
| MF+2BCH | 7.5 | 26 | 35.1 | 3.0 |
| EW+1BCH | 7.5 | 27 | 22.4 | 3.1 |
| EW+2BCH | 7.3 | 34 | 38.2 | 2.6 |
| MF+EW+1BCH | 7.5 | 38 | 40.6 | 3.5 |
| MF+EW+2BCH | 7.3 | 29 | 48.6 | 2.4 |

Table 3: Effect of Earthworms and Biochare on percentage and severity of Cauliflower roots infection with the mycorrhizal fungus.

| Treatments | Percentage of infection, % | Severity of infection, % |
|---|---|---|
| Control | 0 | 0 |
| MF | 20 | 50 |
| MF+EW | 100 | 33.33 |
| MF+1BCH | 60 | 42.5 |
| MF+2BCH | 71.4 | 56.66 |
| MF+EW+1BCH | 100 | 23.33 |
| MF+EW+2BCH | 100 | 25 |

from the results shown in Table 1, there was a change in the soil content of available phosphorus, and the interaction treatment between mycorrhizal fungi and earthworms and the addition of 2 grams of biochare achieved 48.6 mg.kg$^{-1}$ compared to the control treatment, which was 35.1 mg.kg$^{-1}$. As for the available potassium in the soil, the results show that the interaction treatment between mycorrhizal fungi and earthworms and the addition of 1 gram of biochare achieved the highest content of available potassium in the soil, which was 38 mg.kg$^{-1}$ compared to the control treatment, which was 26 mg.kg$^{-1}$.

## 3.2 Mycorrhizal Characteristics

The results of Table 3 indicate that there are differences between the treatments for the infection rate and infection severity trait, as the treatment of mycorrhizae and metabolite worms, the treatment of mycorrhizae and metabolite worms and biochare at a concentration of 1%, and the treatment of mycorrhizae and metabolite worms and biochare at a concentration of 2% were superior, giving the highest value of 100% in the infection rate compared to the treatment of adding mycorrhizae, which was 20%. Table 2 also indicates the superiority of the treatment of interaction between mycorrhizae and biochare at a concentration of 2%, as it achieved the highest value of 56.66% in infection severity compared to the treatment of adding mycorrhizae, which was 50%.

## 3.3 Shoot Growth Parameters of Cauliflower Plant

The results in Table 4 and Table 5 indicate an increase in the height of the cauliflower plant if the interaction treatment between mycorrhizal fungus and biochare at a concentration of 1% was superior, as it achieved the highest increase of 47 with an increase rate of 65.90% compared to the control treatment, which was 28.33. The table shows an increase in the fresh weight of the urban group without the flower, as the biochare treatment at a concentration of 2% was superior, as it achieved the highest increase of 33 - 523 with an increase rate of 223.72% compared to the control treatment, which was 161.66. The table also shows an increase in the dry weight of the vegetative group without the flower, as the interaction treatment between mycorrhizal fungus and biochare at a concentration of 1% was superior, as it recorded the highest increase of 43.33 with an increase rate of 282.43%

compared to the control treatment, which achieved 11.33. The table indicates an increase in the diameter of the stem when the interaction treatment between mycorrhizal diameter and biochare at a concentration of 1% was superior. 1% compared to the control treatment, which was 4.00%1, where it achieved the highest increase, which was 5.66, with an increase rate of 41.5. Table 5 shows an increase in the number of cauliflower leaves if the treatment with mycorrhizal fungi was superior, as it achieved the highest increase, which was 15.66, with an increase rate of 42.36% compared to the control treatment, which was 11. The table indicates an increase in the leaf area of the cauliflower plant when treated with metabolite worms and biochare at a concentration of 2%, as it recorded the highest increase, which was 259.06, with an increase rate of 193.98% compared to the control treatment, which recorded 88.12. The table shows an increase in the leaf area of the cauliflower plant if the interaction treatment between metabolite worms and biocarbon at a concentration of 2% was superior, as it achieved the highest increase, which was 3737.4, with an increase rate of 283.42% compared to the control treatment, which was 974.73. The table shows an increase in the content of Chlorophyll in cauliflower leaves, as the interaction treatment between worms and biochare at a concentration of 1% and the interaction treatment between worms and biochare at a concentration of 2% were superior, as they recorded 50.7, an increase of 31.92% compared to the control treatment, which was 38.43 .

Table 4: Effect of inoculation with Earthworms and mycorrhizal fungus and their interaction with biochare on Root growth for Cauliflower plant.

| Treatments | Plant height, (cm) | Stem diameter, (mm) | Leaves number |
|---|---|---|---|
| Control | 28.33 d | 4.00 c | 11.00 e |
| MF | 44.00 ab | 4.66 bc | 15.66 a |
| EW | 32.00 cd | 4.00 c | 13.00 cd |
| MF+EW | 35.66 cd | 3.83 c | 13.33 c |
| 1BCH | 35.66 cd | 4.66 bc | 15.33 ab |
| 2BCH | 44.33 ab | 4.66 bc | 15.33 ab |
| MF+1BCH | 47.00 a | 5.66 a | 15.33 ab |
| MF+2BCH | 45.00 ab | 5.00 ab | 14.33 abc |
| EW+1BCH | 35.00 cd | 4.16 bc | 14.00 bc |
| EW+2BCH | 38.66 bc | 4.33 bc | 14.33 abc |
| MF+EW+1BCH | 33.33 cd | 3.83 c | 12.00 de |
| MF+EW+2BCH | 36.33 cd | 4.00 c | 12.00 de |

Note: Similar letters indicate that there are no significant differences between the means , at a probability level of 0.05. Note that the probability value p-values ≤ 0.00.

Table 5: Effect of inoculation with Earthworms and mycorrhizal fungus and their interaction with biochare on Root growth for Cauliflower plant.

| Treatments | Leaf area ($cm^2$) | Shoot fresh weight (gm) | Shoot dry weight (gm) |
|---|---|---|---|
| Control | 88.12 g | 133.33 e | 11.33 b |
| MF | 166.62 cdef | 431.67 a | 36.33 a |
| EW | 175.37 cde | 190.00 cd | 17.67 b |
| MF+EW | 214.49 abc | 170.67 de | 17.00 b |
| 1BCH | 119.24 f | 256.67 b | 18.67 b |
| 2BCH | 194.75bcde | 443.33 a | 38.00 a |
| MF+1BCH | 230.00 ab | 425.00 a | 43.33 a |
| MF+2BCH | 226.68 ab | 418.33 a | 42.33 a |
| EW+1BCH | 162.50 def | 157.33 de | 16.67 b |
| EW+2BCH | 259.06 a | 237.00 bc | 16.67 b |
| MF+EW+1BCH | 157.37 ef | 139.33 de | 14.00 b |
| MF+EW+2BCH | 209.37abcd | 178.00 de | 17.00 b |

Note: Similar letters indicate that there are no significant differences between the means , at a probability level of 0.05. Note that the probability value p-values ≤ 0.00.

Table 6: Effect of inoculation with Earthworms and mycorrhizal fungus and their interaction with biochare on Root growth for Cauliflower plant.

| Treatments | Root length (cm) | Root fresh weight (gm) | Root dry weight (gm) |
|---|---|---|---|
| Control | 32.00 $^{f}$ | 50.00 $^{f}$ | 6.33 $^{e}$ |
| MF | 41.00 $^{bcde}$ | 116.66 $^{b}$ | 15.00 $^{ab}$ |
| EW | 33.66 $^{ef}$ | 98.33 $^{bc}$ | 10.66 $^{bcd}$ |
| MF+EW | 36.00 $^{def}$ | 45.33 $^{def}$ | 11.66 $^{abc}$ |
| 1BCH | 45.33 $^{bc}$ | 73.33 $^{cd}$ | 11.33 $^{abc}$ |
| 2BCH | 43.33 $^{bcd}$ | 115.00 $^{b}$ | 15.66 $^{a}$ |
| MF+1BCH | 48.00 $^{b}$ | 106.66 $^{b}$ | 12.66 $^{ab}$ |
| MF+2BCH | 57.66 $^{a}$ | 158.33 $^{a}$ | 13.66 $^{ab}$ |
| EW+1BCH | 32.00 $^{ef}$ | 53.00 $^{def}$ | 14.33 $^{ab}$ |
| EW+2BCH | 33.66 $^{ef}$ | 59.00 $^{de}$ | 12.33 $^{abc}$ |
| MF+EW+1BCH | 40.00 $^{cde}$ | 26.66 $^{f}$ | 7.00 $^{de}$ |
| MF+EW+2BCH | 33.66 $^{ef}$ | 35.00 $^{ef}$ | 8.33 $^{cde}$ |

Note: Similar letters indicate that there are no significant differences between the means , at a probability level of 0.05. Note that the probability value p-values ≤ 0.00

### 3.4 Root Growth Parameters of Cauliflower Plant

Table 6 shows an increase in root length in cauliflower, as the treatment that combined mycorrhizal fungi with 2% biochare achieved the highest increase of 57.66, representing an increase of 80.18% compared to the control treatment, which recorded 32.00. The table also indicates an increase in fresh root weight, as the treatment with mycorrhizal fungi outperformed, recording the highest increase of 116.66, representing an increase of 133.32% compared to the control treatment, which recorded 50.

## 4 DISCUSSION

The changes in soil properties two months after planting cauliflower, treatment with mycorrhizal fungi, earthworms, two concentrations of biochare, and their interaction confirm the direct impact of these factors on soil properties. These changes are expected to increase with the duration of their presence in the soil, particularly in the percentage of organic matter, which increased in the earthworm treatment as a result of the earthworms' biological activity in the soil [20]. Biochare also contributes to the incorporation of organic carbon into the soil [21], improving soil structure, promoting the formation of soil aggregates [22], and providing an ideal environment for soil microorganisms [23]. Mycorrhizal fungi (AM) play an important role in the formation and stabilization of soil organic matter (SOM) [24]. Soil additives also contribute to increasing the availability of phosphorus and potassium in the soil, as mycorrhizal fungi contribute to reducing nutrient loss in the soil and increasing its availability [25]. The results also show that soil texture did not change. This is attributed to the short duration of the experiment, as changing soil texture takes a long time.

The results also showed that the percentage of root infection by mycorrhizal fungi was positively affected by the presence of earthworms and biochare. Earthworms and arbuscular mycorrhizal fungi (AMF) belong to the soil community and are beneficial soil organisms at different trophic levels. Both improve soil fertility and structural development, which enhances plant growth and nutrient uptake. Earthworm activities redistribute mycorrhizal spores and exert diverse effects on mycorrhizal colonization. Co-inoculation with both earthworms and AMF significantly enhances plant growth response by increasing soil enzyme activities and altering nutrient availability [26], also the application of biochar generally increased both arbuscular mycorrhizal fungi (AMF) root colonization and relative amount of neutral lipid fatty acid [27].

The current study also confirmed a significant increase in the vegetative and root growth characteristics in all treatments, compared to the control treatment without adding. the inoculation with AMF enhances plant growth, increases yield and improves quality, especially under stressful conditions, especially elements and nutrients [28]. It enhances the plant's ability to tolerate drought, salinity, temperature changes and heavy metal toxicity, thus contributing to reducing damage caused by climate change [29]. It increases water absorption through fungal hyphae outside the root or by changing the root structure, enhances water and nutrient use efficiency, regulates hormone balance (auxin, abscisic acid and cytokinin), regulates stomata function, and increases the rate of photosynthesis [30].

Earthworms contribute to improving plant growth by enhancing soil fertility and increasing the mixing of organic matter in the soil, which facilitates the access of plant roots to it, in addition to improving soil aeration and decomposition of organic matter, thus increasing the availability of nutrients in the soil, in addition to enhancing the growth of microscopic soil organisms [31].

Biochare has demonstrated numerous positive environmental effects, such as carbon sequestration, reduced greenhouse gas emissions, and soil improvement. Biochare application has also shown tremendous benefits when applied to agricultural systems, including improved plant growth, either under optimal conditions or under biotic or abiotic stress. Several mechanisms have been described, such as enhancing soil microbial diversity and thus increasing soil nutrient cycling functions, improving soil physical and chemical properties, stimulating microbial colonization, or increasing soil phosphorus, potassium, and nitrogen content, to exert these positive effects on plant growth [32], [33].

# 5 CONCLUSIONS

The results of the current study confirm the importance of protecting natural resources in achieving sustainable development goals by avoiding the use of chemical fertilizers in agriculture. The results showed the positive effect of adding biocarbon, earthworms, and endophytic mycorrhizal fungi, either individually or in combination with each other. These treatments achieved a significant increase in the vegetative and root growth characteristics of cauliflower, which is considered one of the important economic plants around the world. The results obtained from the GC-MS analysis identified several phytochemical constituents with known biological activities, including 2,3-butanediol, oxirane derivatives, and 6-oxa-bicyclo [3.1.0] hexan-3-one. These compounds are known to exert antimicrobial, antioxidant, and anti-inflammatory effects, which may act through mechanisms such as membrane disruption, protein synthesis inhibition, or interference with bacterial metabolic pathways. The results also showed that the above treatments had an impact on some physical and chemical soil properties, which are likely to show greater variation with increasing time. Therefore, the study recommends adopting environmentally friendly methods to improve plant growth.

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