Intercropping with Oyster Mushroom (*Pleurotus columbinus*) Enhances Main Crop Yield and Quality

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Abstract. This research trial was conducted in the open field to assess the integrated cultivation of oyster mushroom *Pleurotus columbinus* and field-grown vegetable crops. Oyster mushroom intercropped within cabbage (*Brassica oleracea* var *capitata*) and cauliflower (*Brassica oleracea* var *botrytis*) rows at the beginning of even head or curd formation. Data revealed superior intercropped cabbage and cauliflower growth parameters and higher yields compared with the respective sole cultivation. *Pleurotus columbinus* fruiting bodies weight, cab diameter, and total yield were significantly higher for mushroom intercropped with cauliflower than with cabbage. However, no differences or inconsistent differences were observed in fruiting bodies number, cab thickness, stem length, and stem diameter. Analysis of some mushroom fruiting bodies chemical composition showed that mushroom/cabbage intercropping surpassed mushroom/cauliflower intercropping in total phenols. However, no significant difference was observed between cabbages and cauliflowers concerning crude protein, total antioxidant activity, and total flavonoids. It is concluded that oyster mushroom/cabbage or cauliflower intercropping can provide additional marketable protein-rich food as well as enhance cabbage and cauliflower crop productivity based on sustainable practice.

1. Introduction

Edible Mushrooms including oyster mushroom *Pleurotus* spp. are not only a high-valued nutritional food source but also have potential environmental positive impacts [1]. Mushroom mycelium can produce a group of complex extracellular enzymes [2] capable to decompose cellulosic wastes. Therefore, one of their extraordinary benefits in the ecosystem is the decomposition of organic wastes. The mycelium distribution underneath the soil can be beneficial if it is cultivated in the open field along with the other plant crops. Oyster mushroom is the second most widely cultivated mushroom in the world [3]. Its cultivation in Egypt is to some extent a recent activity and mushroom farms still produce less than the local market demand. The produced mushroom in Egypt is mostly *Agaricus*. However, *Pleurotus* cultivation needs less complicated procedures and relatively fast-growing [4, 5] thus integrate oyster mushrooms with short life cycle winter vegetable crops can increase soil profitable production. Usually, mushrooms grow in controlled growth rooms and their cultivation in the open fields is uncommon due to their need for especial micro-environmental conditions of high humidity and lack of direct sunlight. Very limited research has been conducted to investigate the intercropping of mushrooms with other vegetable crops. Restricted land availability and low soil fertility are major limitations to field crop yield optimization. Great efforts
are required to establish unconventional strategies of food production and achieve sustainability. Mushrooms have incredible potential for producing a great socio-economic impact on human welfare. Here, we aimed to study a novel cultivation approach that integrates oyster mushrooms with cabbage and cauliflower. It may help to increase field-crops production efficiency.

2. Materials and Methods
This research trial was carried out in The Mushroom House and the open field of the Experimental Farm of Horticulture division, Agriculture Research Station, Faculty of Agriculture, Sohag University during two consecutive winter seasons (2016/2017 and 2017/2018). Four treatments were assessed in a randomized complete block experiment with 4 replicates. These treatments were as follow: 1) sole cauliflower cultivation, 2) sole cabbage cultivation, 3) cauliflower intercropped with oyster mushroom, and 4) cabbage intercropped with oyster mushroom.

2.1. Plant Materials
Spawn of oyster mushroom (*Pleurotus columbinus*) was obtained from Agricultural Research Center, Horticulture Research Institute, Giza. Thirty-day-old cabbage (*Brassica oleraceae* var. *capitata*) and cauliflower (*Brassica oleraceae* var. *botrytis*) transplants named OS Cross and Rosetta, respectively, were obtained from a local nursery in Sohag, Egypt. Rice straw was used as an organic substrate for oyster mushroom cultivation.

2.2. Laboratory Groundwork
2.2.1. Substrate Preparation and Spawning. Rice straw substrate was chopped and soaked overnight in tap water to be cleaned and moistened. The moistened substrate was pasteurized in hot water at 80°C for 2 h [6]. Subsequently, the pasteurized substrate was left until moisture content reached about 70% then packed every 0.5 kg of wet substrate into clear polyethylene plastic bag. The spawning rate was 5% on the wet substrate weight basis. Incubation of the inoculated straw was at room temperature until complete mycelium running (21 days).

2.3. Field Groundwork
2.3.1. Main Crop Cultivation. Cabbage and cauliflower transplanting was on 29th October in both years. The transplants were spaced 50 cm apart on 70 cm wide and 3 m long rows. Each treatment was 4 rows per replicate. Agricultural practices for the field-grown cabbage and cauliflower crops such as tillage, fertilizing, irrigation, and plant protection products spraying were performed as necessary according to the recommended practices for the production of these crops.

2.3.2. Intercropping. Mushroom bags were transferred to the open field and placed within-rows between cauliflower and cabbage plants at the beginning of even curd of cauliflower or head formation of cabbage (on 10th December). Straw substrate high moisture was maintained by daily hand watering. Mushroom fruiting bodies first flush was (7-10) days after placing between the plants within the rows of cauliflower and cabbage. Harvesting of mushroom fruiting bodies continued until the end of the season.

2.4. Measurements
2.4.1. Soil Characteristics Alterations. Some main soil characteristics were determined before planting and after harvesting mushroom cultivation including PH, EC, organic matter (%), organic carbon (%), available N (mg/kg), available P (mg/kg), and available K (mg/kg). Five random core samples from each plot for each treatment were collected from the top 30 cm soil layer and homogenized. Soil organic
carbon (SOC) was determined as described by [7], available N by the Kjeldahl method [8], available P using Olsen's method [9], and available K using The Flame Photometric method.

### 2.4.2. Mushroom, Cauliflower and Cabbage Agronomic Parameters

Average head or curd weight (g), curd or head diameter (cm), leaf number, and total yield (kg/feddan) for both cabbage and cauliflower crops were determined. Mushroom fruiting bodies grown between the two vegetable crops were evaluated for their fruiting bodies yield (g/kg substrate) and (kg/fed.), fruiting bodies number/kg substrate and fruiting bodies number/fed., average fruiting bodies weight (g), cap diameter (cm), cap thickness (cm), stem length (cm) and stem diameter (cm) in both seasons.

### 2.4.3. Mushroom Nutritional Composition

#### 2.4.3.1. Crude Protein Determination

Total protein content present in mushroom fruiting bodies was analyzed on a fresh basis according to the method described in [10].

#### 2.4.3.2. Total Antioxidant Activity (TAA)

Radical-scavenging activity of Pleurotus columbinus was determined by the DPPH method previously described by [11]. Total antioxidant activity was calculated using the following equation: Total antioxidant activity = (A - B) / A x 100 where, A-Absorbance of the Blank, B- Absorbance of the sample.

#### 2.4.3.3. Total Phenolic Content (TPC)

TPC in the mushroom extracts was measured using Folin-Ciocalteu’s phenol reagent according to the method of [12]. The total phenol content is expressed as mg of gallic acid equivalent (GAE)/100g of sample on a fresh weight basis using the following equation: Y=0.047X-0.0028; Where: X =Absorbance, Y=Concentration, R² = 0.9891.

#### 2.4.3.4. Total Flavonoids Content (TFC)

TFC was determined as described by [13]. Results were expressed as micrograms of (+)-catechin equivalents per 100g fresh mushrooms using the following equation: Y=175.82X-3.4048 Where, X =Absorbance, Y=Concentration, R²=0.9957.

### 2.5. Statistical Process

Three different mean comparisons were piloted using the Student's “t” test analysis at 0.05 level of probability. These mean comparisons were 1) Cabbage sole crop vs. cabbage with mushroom intercrop, 2) Cauliflower sole crop vs. cauliflower with mushroom intercrop and 3) cabbage intercropped with mushroom vs. cauliflower intercropped with mushroom. Mean values were obtained from a sample of sixteen plants; i.e., four plant/plot/replicate.

### 3. Results

#### 3.1 Soil Analysis

As shown in Table (1), some alterations occurred in soil components after mushroom cultivation. There were noticeable changes in EC, organic matter, organic carbon, and available P and K. However, pH remained almost unchanged. Observational detection confirmed extensive belowground fungal hyphae in the soil underneath the straw substrate (Figure 1 A and B).
Table 1. Some main soil characteristics determined before mushroom cultivation as an intercrop with cabbage and cauliflower and after mushroom harvesting.

| Status           | pH  | EC  | Organic matter (%) | Organic carbon (%) | Available N (mg/kg) | Available P (mg/kg) | Available K (mg/kg) |
|------------------|-----|-----|--------------------|--------------------|---------------------|---------------------|--------------------|
| Before cultivation | 7.79| 743 | 2.19               | 1.27               | 50                  | 34.41               | 609.88             |
| After cultivation | 7.78| 801 | 2.43               | 1.41               | 50                  | 43.71               | 525.04             |

Figure 1. (A and B) Mushroom hyphal roots penetrating the soil, (C) Mushroom grown between cauliflower plants, (D) Mushroom grown between cabbage plants.

3.2. Cabbage and Cauliflower Agronomic Parameters
Statistical student’s t-test analysis showed significant values for all parameters tested except cabbage leaf number in the two years of the study (Table 2). Inter-planting oyster mushroom (*Pleurotus columbinus*) with cabbage and cauliflower rows significantly increased cauliflower leaf number as well as head weight (g), head diameter (cm), and total yield (Mt/fed) of both cabbage and cauliflower crops.

Table 2. Means of cabbage and cauliflower head weight (g), head diameter (cm), outer leaves number, and total yield (Mt/fed.) as affected by oyster mushroom (*Pleurotus columbinus*) intercropping intra-rows between cabbage and cauliflower plants.
As shown in Table 2, head weight (g) of cabbages intercropped with *Pleurotus columbinus* was significantly higher than those produced in the sole cultivation. This increase was estimated by (15.83, 20.37%) in the 1st and 2nd years, respectively. Head diameter (cm) of cabbages grown with *Pleurotus columbinus* bags placed within their rows surpassed the non-intercropped ones by (5.41%, 18.56%) in the 1st and 2nd years, respectively. *Pleurotus columbinus* intercropping, subsequently, showed a magnificent significant increase in the cabbage total yield in contrast to non-intercropped ones. This increase was evaluated as (15.40%, 20.21%) in the 1st and 2nd years, respectively.

Obviously, in comparison with sole cauliflower cultivation, oyster mushroom intercropping with *Pleurotus columbinus* had the same trend in all studied parameters. Head weight, head diameter, and total yield of intercropped cauliflowers were significantly surpassed sole cauliflower by (20.40 %, 20.47%), (7.28 %, 20.63 %) and (20.44 % and 23.55 %), respectively, in the 1st and 2nd years.

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Results from the two years of the study showed a significant difference in fruiting bodies yield and average fruiting bodies weight of (*Pleurotus columbinus*) between mushrooms intercropped within cabbage and cauliflower rows. Data in (Table 3) revealed that mushroom intercropped with cauliflower achieved much larger fruiting bodies’ cab diameter and higher yield than mushroom intercropped with cabbage in both years. Fruiting bodies number (Table 3) and stem length (Table 4) were significant in one year only. Cab thickness and stem diameter did not differ in both years (Table 4) concerning the different crops.

**Table 3.** Means of oyster mushroom (*Pleurotus columbinus*) fruiting bodies number (kg/substrate), fruiting bodies number/feddan and average fruiting bodies weight (g) grown intra-rows between cabbage and cauliflower plants.

| Treatments                  | Fruiting bodies number/kg substrate | Fruiting bodies number/feddan |
|-----------------------------|-------------------------------------|-------------------------------|
|                             | (2016/2017)                         |                               |
| Mushroom with cabbage       | 32.00±1.36                          | 64000±3265.9                  |
| Mushroom with cauliflower   | 28.40±0.46                          | 56800±923.7                   |
| **Significance**            | 4.24*                               | 4.24*                         |
|                             | (2017/2018)                         |                               |
| Mushroom with cabbage       | 28.65±1.92                          | 57300±3841.8                  |
| Mushroom with cauliflower   | 28.90±0.96                          | 57800±1918.3                  |
| **Significance**            | 0.23 ns                             | 0.23 ns                       |

| Treatments                  | Fruiting bodies yield (g/kg substrate) | Fruiting bodies yield kg/feddan |
|-----------------------------|----------------------------------------|---------------------------------|
|                             | (2016/2017)                            |                                |
| Mushroom with cabbage       | 449.40±5.20                           | 898.80±48.14                   |
| Mushroom with cauliflower   | 553.40±13.30                          | 1106.60±87.1                   |
| **Significance**            | 14.56**                               | 14.58**                        |
|                             | (2017/2018)                            |                                |
| Mushroom with cabbage       | 403.80±11.46                          | 807.60±22.93                   |
| Mushroom with cauliflower   | 483.40±7.56                           | 946.70±49.79                   |
| **Significance**            | 11.58**                               | 5.07**                         |

| Treatments                  | Average fruiting bodies Weight (g)    |
|-----------------------------|---------------------------------------|
|                             | (2016/2017)                           |
| Mushroom with cabbage       | 14.07±0.69                            |
| Mushroom with cauliflower   | 19.49±0.55                            |
| **Significance**            | 12.14**                               |
|                             | (2017/2018)                           |
| Mushroom with cabbage       | 14.12±0.72                            |
| Mushroom with cauliflower   | 16.73±0.39                            |
| **Significance**            | 6.35**                                |

*Sample size (n) =16; ns, * and ** are insignificant, significant at 0.05 and 0.01, respectively, using student’s t-test.*
### Table 4. Means of oyster mushroom (*Pleurotus columbinus*) fruiting bodies cap diameter, cap thickness (cm), stem length (cm), and stem diameter (cm) grown intra-rows between cabbage and cauliflower plants.

| Treatments                  | Cap diameter (cm) | Cap thickness (cm) |
|-----------------------------|-------------------|--------------------|
| (2016/2017)                 |                   |                    |
| Mushroom with cabbage       | 6.01±0.05         | 0.81±0.04          |
| Mushroom with cauliflower   | 6.77±0.27         | 0.89±0.03          |
| Significance                | 1.93*             | 2.13 ns            |
| (2017/2018)                 |                   |                    |
| Mushroom with cabbage       | 5.54±0.32         | 0.84±0.05          |
| Mushroom with cauliflower   | 7.36±0.28         | 0.89±0.02          |
| Significance                | 6.6**             | 2.01 ns            |

| Treatments                  | Stem length (cm) | Stem diameter (cm) |
|-----------------------------|------------------|-------------------|
| (2016/2017)                 |                  |                   |
| Mushroom with cabbage       | 1.97±0.07        | 0.91±0.09         |
| Mushroom with cauliflower   | 2.07±0.17        | 1.09±0.15         |
| Significance                | 1.01 ns          | 2.01 ns           |
| (2017/2018)                 |                  |                   |
| Mushroom with cabbage       | 2.39±0.12        | 0.86±0.03         |
| Mushroom with cauliflower   | 2.13±0.14        | 0.98±0.07         |
| Significance                | 5.94*            | 1.94 ns           |

**Sample size (n) =** 16; ns, * and ** are insignificant, significant at 0.05 and 0.01, respectively.

### 3.4. Mushroom Nutritional Composition

As illustrated in (Table 5), oyster mushroom fruiting bodies obtained from intercropping with cabbage and cauliflower had a protein content of (20.12% and 18.37%), respectively, based on fresh weight. No significant difference was observed between the two crops in this regard. No significance was observed in *Pleurotus* fruiting bodies' total antioxidant activity (Table 5) which reached high percentage of 72.20% and 70.90% in cabbage and cauliflower, respectively. Total flavonoids content of *Pleurotus* also was not affected by the intercropping between the two crops. However, total phenolic content was found to be higher in fruiting bodies grown between cabbage plants than cauliflower plants. (Table 5).

### Table 5. Means of some bioactive compounds contents (protein %, total antioxidant activity %, total phenols (mg/100g) and total flavonoids (μg /100g)) of oyster mushroom (*Pleurotus columbinus*) fruiting bodies grown between cabbage and cauliflower plants.

| Treatments                  | Protein (%) | Total antioxidant activity (%) |
|-----------------------------|-------------|-------------------------------|
| (2017)                      |             |                               |
| Mushroom with cabbage       | 20.12±0.068 | 72.25±1.21                    |
| Mushroom with cauliflower   | 18.37±0.31  | 70.90±2.77                    |
| Significance                | 1.70 ns     | 0.76 ns                       |
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### Treatments

| Treatments                  | Total phenols (mg/100g) (2017) | Total flavonoids (μg/100g) |
|-----------------------------|---------------------------------|-----------------------------|
| Mushroom with cabbage       | 16.41±0.61                      | 8.39±0.58                   |
| Mushroom with cauliflower   | 13.35±0.77                      | 7.83±1.42                   |
| **Significance**            | **5.38**                        | **0.55** ns                 |

Sample size (n) =16, ns, and ** are insignificant, significant at 0.01, respectively.

### 4. Discussion

Fascinatingly, intercropping of oyster mushroom *Pleurotus columbinus* consistently elevated the crop yield of both cauliflower and cabbage. These findings are in agreement with [14] and [15] who observed an increase in crop yield of cabbage and faba bean, respectively using oyster mushroom *Pleurotus ostreatus* and *Pleurotus columbinus*. [14] Mentioned that cabbage/mushroom interpolating increased cabbage head yield by about 21.8% compared with the sole crop which is closely similar to our results. We estimated the increase in the head weight of cabbage and cauliflower crops by 18.10% and 20.40%, respectively, as an average of the two years of study.

[15] Referred to the regular light hand watering to maintain the moisture of the intercropped mushroom substrate as a possible factor that enhanced water availability for the field crops. In general, soil fertility is one of the major factors affecting plant growth and crop yield. Cabbages and cauliflowers, Brassicaceae, are known to be highly nutrients consuming plants. In this regard, soil analysis of the intercropped cultivated area clearly showed that soil organic matter was higher after growing mushroom intercrop. This can be attributed to rice straw substrate decomposition by oyster mushroom. Nutrients likely leak while decomposing the straw occurred and was ready for the cabbage and cauliflower to use. Obviously, the analysis revealed a magnificent increase in phosphorus uptake level compared to the control. It is known that most P in soil with high pH is in an unavailable form. Remarkably, mushrooms produce extensive belowground fungal hyphae and we could detect these hyphae clearly at the end of the season. These hyphal roots have no competition with plant roots and seem to play a symbiotic role with the plant as an exo-mychorizal effect. Therefore, it is reasonable to assume that these hyphal roots would result in an increased phosphorous solubility via exo-enzymatic processes.

On the other hand, [2] found decrease in brussels sprout yield and [16] found a negative effect of mushroom interpolating with kale and radish crops. We can suppose that a source of difference between our study and [2] study was the substrate used to grow oyster mushroom. Rice straw is used in this study and is also recommended by [14], [15], and [16], while [2] used sawdust substrate mixed with rice straw. [16] Suggested that *Pleurotus* intercropping with field crops is dependent on substrates inoculation rate and fungal strain. In our study, the inoculation rate of 5% was used and produced basidiocarp yield much similar to the controlled room production. Otherwise, the mushroom strain used in the present assessment may be more adapted to our conditions in Upper Egypt.

Since no significant differences were observed for mushroom fruiting bodies' number, the higher yield of mushroom fruiting bodies obtained from cauliflower/mushroom intercropping was a result of larger fruiting bodies' weight. Mushroom is a saprophytic organism; however, low-intensity light enhances its production. In our laboratory, we observed oyster mushrooms to produce smaller fruiting body sizes with longer and thinner stems when grown under darkness as compared to weakly intense light (unpublished data). The data of the current study (cab diameter and thickness, stem length and diameter) largely support these observations. The reduced mushroom fruiting bodies' yield and average fruiting bodies' weight of the mushroom intercropped with cabbage as compared to cauliflower can be attributed to a forceful covering of cabbage outer leaves. In contrast, cauliflower outer leaves grow rather vertically allowing more optimal illumination circumstances besides humidity for mushroom growth. [16] Also found differences about mushroom yield between kale and radish where the former gave higher mushroom yield than the latter.
Mushroom fruiting bodies' cab diameter and thickness as well as their stem length and diameter are important quality attributes in the marketing of mushrooms. Thus, fruiting bodies' quantity and quality of mushroom intercropped with cauliflower are superior to those of mushroom intercropped with cabbage. However, both of them are still on the acceptable marketable scale. Mushrooms are rich in protein of high [17] biological quality and it is considered to be of similar quality to animal protein [18]. In our study, mushroom fruiting bodies' crude protein ranged between 18.37 and 20.12% and did not seem to be affected by plants intercropped with mushroom. Similar results for fresh oyster mushroom analysis with protein content have been reported by [19] and [20]. Further, in this assessment, the antioxidant potential of methanolic extract of oyster mushroom was investigated and found to be high but without significant difference between treatments. It ranged between 70.90 % and 72.20% and according to review given by [21] similar amounts of total antioxidant 56.20% and 60.02% were in Pleurotus ostreatus from the different treatments. Furthermore, there was no significant difference between the treatments regarding flavonoids content that recorded 8.39 and 7.83μg/100g) based on a fresh basis in cabbages and cauliflowers, respectively.

Phenolic compounds which are recognized as potent antioxidants significantly varied between the studied treatments. The most important finding in (Table 5) refers to the higher phenolic content in mushroom fruiting bodies grew with cabbages than cauliflowers. It can be observed that total phenolic contents were 16.41 and 13.35 mg/100g fresh weight in the mushroom intercropped with cabbages and cauliflowers, respectively. Phenolic compounds act as reactive oxygen species (ROS) scavenging agents and their synthesis is triggered in response to biotic and abiotic stresses [22]. Most likely some mushroom bags were hidden under slouching cabbage leaves and consequently subjected to water deficit.

5. Conclusion
Increasing crop efficiency is the ultimate goal of every agricultural research; however, sustainable utilization of resources has an intensive concern nowadays. In the present study, cabbage and cauliflower intercropped with oyster mushroom produced a magnificent crop increase as compared to their sole cultivation. Meanwhile, mushroom intercropping with cauliflower was more productive than with cabbage. Thus, mushroom outdoor cultivation paired with field crops such as cabbages and cauliflowers could be a viable practice to increase their production efficiency as well as obtaining additional unconventional nutritious food of mushroom. Further, oyster mushroom spent can add organic content and plant nutrient elements as a result of lignocellulosic wastes decomposition.

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