

## Determination of the Effect of Indole Acetic Acid (IAA) Produced from Edible Mushrooms on Plant Growth and Development

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### Abstract

In this study; IAA production amounts of edible mushrooms *Pleurotus eryngii*, *Pleurotus ostreatus*, *Pleurotus florida*, *Pleurotus sajor-caju* and *Pleurotus citrinopleatus* were determined and the highest production was observed in *Pleurotus eryngii*, *Pleurotus sajor-caju* and *Pleurotus citrinopleatus*. The obtained IAAs were applied to bread wheat (*Triticum aestivum* cv.Kirik) and their effects on growth and development were investigated. The plants were grown at 22/20 °C for a total of 15 days. IAA (1g/L) was applied to roots and harvested on the 15th day to be used as a trial material. Then, root-stem length, fresh-dry weight, protein, chlorophyll and sugar contents were determined. As a result of the measurements, it was observed that IAAs belonging to the genus *Pleurotus* applied exogenously affected plant growth and development positively.

**Keywords:** IAA; *Pleurotus*; Plant Growth; *Triticum aestivum*.

### 1. Introduction

Plant hormones are chemical molecules that promote growth from embryo development to adulthood, as well as create stress tolerance and resistance to pathogens. Plant hormones regulate the division of plant cells and also the metabolic activities of plants [1].

One of the hormones affecting plant growth and development is the auxin group indole acetic (IAA) acid. The role of IAA in plant physiology is among the important plant hormones that stimulate the plant defense mechanism against environmental stresses by influencing tryptophan metabolism, root thickness and number, increasing the plant's nutrient and mineral uptake, promoting plant growth [2]. It is known that IAA is produced not only by plants but also by many microorganisms. Due to the increasing world population and rapidly changing climatic conditions, there is a decrease in agricultural production due to the decrease in agricultural lands. This situation will prevent humanity from reaching protein sources in the coming years and humanity will face famine. To combat this, many sustainable farming activities have been developed. One of these activities is the use of microorganisms that produce plant hormones that are

not harmful to the environment and humans (PGPR bacteria and AB Fungus) [3,4]. Along with biotechnological developments, it has been observed in many studies in which the isolation and diagnosis of microorganisms that produce plant hormones, especially IAA. It has been observed that especially filamentous fungi are the leading organisms in IAA production, and it has been stated that plant-fungus relationships will increase IAA production and thus promote plant growth [5,6].

For this purpose, in our study, it was determined that IAA production of edible fungal species and whether the produced IAA stimulated plant growth when applied to the plant exogenously.

### 2. Materials and Methods

#### 2.1. Growing plants

In this study, Kirik variety of wheat (*Triticum aestivum*), which is a monocot plant, was used. Seed sterilization was carried out by washing the dry seeds several times with tap water, then soaking them in 10% commercial bleach for 5 minutes and finally washing them thoroughly with distilled water. The plants were grown in sand culture. 25 g seeds were planted in each pot. Plants were grown in a climate cabinet at 22/20°C and 12/12 hours light-dark period (20,000 lux, 70% humidity) for 15 days. 1g/L IAA was administered on the 7th and 12th days.

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## 2.2. Production IAA from *Pleurotus* sp.

The effect of L-tryptophan concentrations on IAA production was studied using potato dextrose broth medium supplemented with L-tryptophan at concentrations of 0.1%. The culture was incubated at 28°C in a shaker at 120 rpm for 7 days.

IAA quantification. Following the incubation time, culture was centrifuged and the supernatant was mixed with Salkowski's reagent (150 ml of concentrated H<sub>2</sub>SO<sub>4</sub>, 250 ml of distilled water, 7.5 ml of 0.5 M FeCl<sub>3</sub>·6H<sub>2</sub>O) with a 1:2 (v/v) ratio, and was allowed to stand at room temperature for 20 min. The pink color developed, indicating IAA production, was measured at 530 nm with a spectrophotometer. The concentration of IAA was calculated using a standard curve prepared with standard IAA. For the exogenous application of IAA, plants were grown. Twice in a fermentation liquid solution, standard IAA at a final concentration was applied through roots on plants [2].

## 2.3. Determination of protein amount

Protein quantification was performed using the method of Bradford (1976) [7]. The dye reagent solution prepared with Coomassie Brilliant Blue (CBB) G-250 is used for the color reaction. In the determination process, 5 ml of dye reagent (CBB) and 0.1 ml of supernatant are mixed. For the blank, 5 ml of dye reagent and 0.1 ml of distilled water are used. After the prepared samples are kept in the dark for 10 minutes, 595 nm spectrophotometric measurements are made. The standard chart used to determine the protein amounts is prepared by measuring the absorption values at 595 nm after different concentrations of bovine serum albumin (BSA) are treated in the same way. Total soluble protein amounts are given in µg protein/g tissue.

## 2.4. Determination of chlorophyll amount

After the plant leaves (0.1 g) are thoroughly homogenized in liquid nitrogen, 10 ml of cold acetone (80% v/v) is added to the homogenate. The samples are then centrifuged at 12000xg for 15 minutes. After centrifugation, the decrease in the acetone level of the samples is completed and the final volume is completed to 10 ml. The absorbance values of the obtained pigment extracts at 645 and 663 nm are read in the spectrophotometer against the blank. Acetone was used as a blank. Total chlorophyll and carotenoid values are determined by substituting the determined absorbance values in the formulas below [8].

$$\text{mg total chlorophyll / g tissue} = ((20.2 * (D645) + 8.02 (D663)) * (V / 1000 * W))$$

$$\text{mg total carotenoid / g tissue} = (4.07 * D450) - (0.0435 * \text{kl a amount} + 0.367 * \text{kl b amount})$$

## 2.5. Statistical analysis

All experiments were performed 6 times and the average of values was presented. The data were

analyzed by analysis of variance, and means were compared by using Duncan's Multiple Range Test at < 0.05 significance level.

## 3. Results and Discussion

In the study, 5 different *Pleurotus* species were used and IAA production amounts were determined after 10 days of fermentation. The three species with the best production were used. The results are given in Table 1.

**Table 1.** IAA and wet biomass amounts of fungal species

Fungal Species	IAA (mg/L)	Wet biomass g/L
<i>Pleurotus eryngii</i>	75	23
<i>Pleurotus ostreatus</i>	70	32
<i>Pleurotus florida</i>	48	24,8
<i>Pleurotus sajor-kaju</i>	84	36
<i>Pleurotus citrinopileatus</i>	98	41

In the study, root, stem and fresh-dry weight amounts were determined as a result of the application of exogenous application to wheat plants for plant growth and development of the most IAA producing species. The longest root stem length was observed in *P.citrinopleatus* application. This is thought to be due to the fact that the highest amount of IAA produced is in this type. At the same time, similar results were observed in the amount of fresh-dry weight (Table 2).

The amount of protein, chlorophyll and carotenoid content, and total sugar content, which are accepted as indicators of growth and development, were also examined and the results are summarized in Table 3. When compared with the results given in Table 2, the protein amounts increased in parallel with the amount of IAA. The amount of chlorophyll, which is the factor that allows the photosynthesis to take place, was similar to the amount applied exogenously, and the highest was measured as 3.52 (Table 3). The amount of carotenoid, which is an auxiliary pigment for photosynthesis, was also increased compared to the control (Table 3).

Fungi are known to secrete hormones such as auxin, gibberellic acid and IAA that affect plant growth and development [9,10]. It has been seen in the study that capped mushrooms of the genus *Pleurotus*, which are members of the Basidiomycetes class of the fungal kingdom, also produce IAA. (Table 1). Studies have shown that exogenously applied IAA affects plant root and stem growth positively [11,12]. In this study, IAA applied exogenously increased the root stem lengths (Table 2). Many studies have reported that IAA produced by fungi increases the fresh and dry weight of plants [9]. In the study, it was determined that wheat increased dry weight with IAA applied (Table 2).

Wheat is one of the cereals with high economic value and protein content. The amount of protein, which is one of the most important parameters of plant growth, has been found to increase in IAA applications in various studies [13]. It was observed that the protein amounts of the wheat plant, which were applied 25 ppm and 50 ppm IAA, increased significantly compared to the control in both applications [14]. In our study, the protein content of the control group was measured as 9.24mg/g, and it was observed that the administered IAA increased the protein amount between 10.62 - 13.17 mg/g (Table 3).

At the same time, it has been seen in studies that IAA, which is a plant growth regulator, positively affects the amount of chlorophyll [15]. In another study, it was found that exogenously administered IAA (90-

120mg/L) increased the chlorophyll content compared to the control, but low concentration increased this content more [16]. It has been observed that the amount of chlorophyll content in our study is in parallel with the studies done.

In addition, it is known that exogenously applied IAA positively affects the growth and development of plants under normal and stress conditions [11]. IAA; It has been reported that it supports the release of amino acids, sugars and lipids [17,18] emphasized in his study that IAA did not affect the amount of sugar positively or negatively, but when applied together with GA, it increased the amount of sugar significantly. In our study, it was observed that when IAA was applied alone, it increased the amount of sugar and was a preliminary information in the literature in this sense.

**Table 2.** Root stem length and wet-dry weight amounts in IAA applications

Species	Root length (cm)	Stem length (cm)	Wet weight (g)	Dry weight (g)
<b>Control</b>	12 d	21.5 d	2,25 d	0,32 d
<i>P. eryngii</i>	15,5 c	24 c	3,4 c	0,56 c
<i>P. sajor-kaju</i>	18.75 b	28 b	4,2 b	0,62 b
<i>P. citrinopleatus</i>	<b>20,5 a</b>	<b>30 a</b>	<b>5,65 a</b>	<b>0,75 a</b>

Differences between groups with the same letters in a column are insignificant at  $P < 0.05$  significance level

**Table 3.** The effect of exogenously administered IAA on growth parameters

Species	Protein (mg/doku)	Chlorophyll mg/g <sub>1</sub>	Carotenoid mg/ g <sup>-1</sup>	Total sugar content (mg/g <sup>-1</sup> )
<b>Control</b>	9,24 d	2,8 d	7,12 c	7.75 d
<i>P. eryngii</i>	10.62 c	3 c	7.64 b	8.37 c
<i>P. sajor-kaju</i>	12 b	3,33 b	7.5 b	9,24 b
<i>P. citrinopleatus</i>	<b>13,17 a</b>	<b>3.52 a</b>	<b>8.24 a</b>	<b>10,57 a</b>

Differences between groups with the same letters in a column are insignificant at  $P < 0.05$  significance level

#### 4. Conclusions

As a result; It has been determined that IAA produced by edible mushrooms has a positive effect on plant growth. It is usability against abiotic and biotic stress conditions can be investigated in future studies.

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#### Conflict of Interest

The authors declare no conflict of interest.

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