Evaluation The Efficiency of Some of Bio - Control Fungi on The Content of Rice Leaves (Oryza Sativa L,) Class Jassmine of Phenols, Alkaloids, And Some of Hormones

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Abstract:
This study was conducted to evaluate the effect of using biocontrol fungi - Trichoderma harzianum. Australian (T.h.a). and Trichoderma harzianum, tahadi(T.h.t) and Chaetumium elatum( C.e) isolates on contents of rice leaves ( Oryza sativa L.)class jasmine of phenols, alkaloids, and hormones( zeatin, gibberellic acid,indol acitic acid) .To attaining these aims , two experements were carried out in two regions at Rice Research Center(RRC) , and Agriculture college - AL-Najaf province. The results of this study can be summarized as follows: The suspension (10)-4 of biocontrol agent T.h.a gave a significant difference in concentrations of phenols in rice leaves for treatment: Soil + hay + NP + T.h.a which reached 0.378 ,0.363 ppm in RRC and college of agriculture fields, in compare with control treatment which gave 0.251,0.245 ppm, respectively. T.h.a. gave a significant differences in concentrations of alkaloids in rice leaves for treatment: Soil + hay + NP + T.h.a which reached 1.67,1.51 µg/ml in RRC and college of agriculture fields ,in compare with control treatment which gave 1.19,1.15 µg/ml , respectively T.h.a attained the highest concentrations of hormones ( zeatin, gibberllic acid,indol acitic acid) in rice leaves for treatment: Soil + hay + NP + T.h.a which reached (0.0941, 53.84, 0.287) ppm, at RRC and (0.0835, 44.52, 0.268) ppm for college of agriculture fields ,in compare with control treatment which gave ( 0.0712, 51.12, 0.210) ppm with RRC and (0.0523, 42.10, 0.174)ppm for college of agriculture ,respectively.

KeyWords: biocontrol fungi, Trichoderma harzianum, phenols alkaloids zeatin, gibberllic acid, indol acitic acid.

1. Introduction
Treating plant waste such as wheat with fungi is a vital means of raising the nutritional value of this waste (Van Wyk and Mohulatsi, 2003). T. harzianum has been used in the treatment of waste of a number of plants, including saw dust, barley and wheat straw, millet, rice bran and wheat (Windham et al., 1986) (Mutlag et al., 2011), which leads to increased protein in the residues on which these fungi grow (Zeilinger, 2003) The strains of the Trichoderma spp.fungus affect Significantly, in plant development and biochemical processes in it, as these strains occupy root tissues and produce phenotypic and biochemical
changes that are part of the defensive response of the plant, which ultimately leads to the induction of systemic resistance (ISR) in all parts of the plant (Harman et al., 2004). When the chromographic separation method (HPLC) of plant leaf extract which treated with Trichoderma, was observed the separation of effective phenolic compounds against many pathogens (Yedidia et al., 1999) Phenolic compounds are common compounds in plant tissue. These compounds begin as a result of external catalysts such as ultraviolet radiation, microbial infections and chemical transformations and are considered as phytochemical compounds and range from 4000 compounds. These compounds do not contribute to plant growth, but some of them contribute to protecting plants from predators, pathogens and reproductive processes by attracting pollination factors and spreading seeds, and some also help in producing toxins that protect the plant. Most of these compounds in plants include three types are (alkaloids, Phenylpropanoids Terpenoids) where the second represents phenolic compounds (Emanuela L.Santos, et al (2017). Phenolic compounds contribute to protecting plants from herbivorous animals and are also pharmacologically beneficial. Phenolic compounds are created by cleavage of shikimic acid products (Nello et al 2010). These compounds include tannins, lignans and flavonoids. The phenols are found in nature and can be synthetically prepared from chemicals that compose a number of associated hydroxylated clusters with hydrocarbon elements, it is generally found in some materials such as pentachlorophenol, antiseptics, washing materials, cosmetics, foods, beverages, and medicines. Many of the vital vegetative ingredients that protect the plant from its natural enemies include: tannins, phenolic amalgams, flavones, coumarins, anthraquinones, glycosides contain phenolic molecules (Hopkins, W.G. 1999). The alkaloids are basic organic compounds whose molecule contains one or more nitrogen atoms and are generally crystalline materials without color and odor and are sensitive to the high temperatures that they decompose and it is rare for a plant to contain a single alkaline compound, but generally contains a group of active substances chemically bonded (Facchini, et al. 2004). Alkaloids are found as one of the secondary metabolic compounds in various plants as salts of some acids such as acetic, malic, oxalic, tartaric, succinic and some alkaloids are combined with sugars to form what is called gluco-alkaldoid (Shmeller and Wink 1988). Alkaloids give important benefits to the plant that exists In it, as defensive materials to protect the plant from other organisms because most of it has a bitter taste as well as its importance in the ionic balance in the plant as well as being nitrogen storage materials or other elements to supply the plant when needed. Alkaloids represent mainly the final products in the process of synthesized nitrogen in plants. And it is linked according to the nature of its role in protecting the plant, as the aporphine compound helps protect the tulip plant from parasitism by mushroom, and alkaloids prevent insects from getting close to the plant and even keep the animals from eating them. It also contributes to regulating plant growth. Most alkaloids contain oxygen, and therefore these compounds are non-colored crystals, as well as volatile and oily liquids. They are weak bases, but some are neutral, such as theobromine. It is also poorly soluble in water, but it is rapidly soluble in organic solvents such as chloroform. It tastes bitter and contains some toxic substances in it in order to protect it from natural enemies Zhang Det al (2012) . Blanchard and Bjorkman (1996) also showed that rates of increasing in the growth of white maize plants that treated with T.h fungi are due to the activity of the oxin-like compounds that this isolation may produce from the fungus and that this mechanism works in conjunction with other mechanisms to stimulate plant growth (Dewan et al., 1994). The production of plant growth regulators by the fungus. T.h stimulates the growth of corn root, and in a study to compare the effectiveness of the fungus T.h T22isolate and hormone experiments preparations to root the tomato plant, it was found that, although thick tissue from callus was not formed at the base of the plant that treated with this isolate, its effectiveness in rooting was equivalent to the effectiveness of the rooting hormone. Oxins are organic acids that have the ability to influence biological processes within plants with very few concentrations. Oxine (IAA) is one of the first plant hormones to be discovered that the role of oxins in tissue culture is to
stimulate callus stimulation or encourage the formation of roots in tissue-rich branches (Vasil, 1985). In addition, oxins are important in stimulating the ductility of the cell wall by breaking the cell wall ties and returning them to new locations under the influence of mitotic diffusion pressure which contributes to increasing cell size and amplitude (Hamid, 2002), in addition to that oxins may affect enzymes responsible for the construction, decomposition, and influence of cellular wall components for mechanical properties of walls (Yassin, 2001). Cytokines are organic bases with high molecular weights that are used in low concentrations to produce physiological effects on the cultivated plant part. Balancing with oxins, they help in the development and growth of callus and increase its ability to differentiate into vegetative growths (Wei and Xu, 1990) and cytokines are organic compounds produced by the plant or industrially prepared to perform many regulatory processes within the plant tissue, it plays a major role in stimulating cells to divide and break the apical dominance of the branches, which helps to form branches of the axillary buds and encourages the formation of transverse buds from callus tissue and Leaves, roots, and stem pieces (Hopkins, 1999). The most popular compounds used in agriculture are zeatins. As for the gibberelins, they are Terpenoides of Gibbane structure, naturally made in the plant and produced new leaves and fruits. The newly developed seed embryos are an essential source for them (Goldi, et al., 2001). Gabrielsins are concentrated in the fastest growing and developed regions, so they are modern hormones that stimulate cell division and elongation. Cytokines stimulate cell division and elongation and affect the distribution of hemicellulose fibrils to the cell walls, reducing their hardness and increasing their elasticity and ductility, facilitating the process of cell expansion, and gibberelins have a role in cell division and increasing their absorption of water and thereby increasing their size, protoplasmic content and surface size (Byers et al., 1990), the gibberelins work to elongate the stem and plant height in two physiologically different processes, the first is the cell division and the other is cellular elongation of the plant tissue cells internally (Hunter and Keith, 2002), i.e. the parent cell, in which the division occurs, giving its seeds many cells, which grow in size and then divide, which also leads to an increase in growth length and then an increase in the weight of the vegetative group. And there appears another role for gibberelin in its effect on the genetic level in the cell as it affects the synthesized of nuclear acid DNA, RNA and protein, and then the enzymes which necessary for vital activities in the cell (Wilkins, 1984). The gibberelin leads to an increase in the rate of formation of free oxine, and this increase in the level of oxine works to accumulate this hormone in the flower ovary to the extent required, which in turn helps in holding fruits later (Al-Manisi, 1975). Also, studies conducted by researchers indicate the effect of gibberellin acid in flower and fruit growth through its ability to push plants to bloom by stimulating cells to divide and elongating the flowering peduncles and their subsequent contribution to the fruit holding process (Auerswald, 1991 and Shokur, 1989). The process of growth and development of fruits is affected by gibberelins, which leads to various physiological effects, as many studies indicate the effect of gibberellic acid in the yield and its components (Goldyet al., 2001).

In view of the "scarcity of studies using fungi in analyzing plant residues and their effects on the growth of rice plants, the study aimed to test some biological control fungi in the analysis of wheat residues and the content of rice leaves sativa Oryza Anbar-33 of phenols, alkaloids and some hormones. To achieve this goal, the following items were followed:

1. Treating wheat residues with biological control fungi and adding them to the soil before planting
2. Estimating the concentration of phenols, alkaloids and some hormones (zatin, gibberellic acid and indole acetic acid) in the leaves of the plant before ripening
2. Materials and methods

This study was carried out in:

1- Rice fields in the College of Agriculture which occupy an area of 3750 m².

2- Rice fields in RRC. The two sites are cultivated in succession of wheat-rice crops.

The soil was plowed smoothed, and divided into panels, where the area was divided into three replicating lines (R1, R2, R3) that includes (16) experimental units (boards) The area of each board (3 x 3) m² and separates each one with another distance of (70) m. The seeds of rice were dispersed (Jassmine cultivar) according to the method and the recommended amount (120) g per experimental unit and according to the scheme prepared for the experiment using sporadic irrigation method (2-3) days from one irrigation to another. After selecting fungi that promotes growth, T.h.a., T.h.t. and C.e were loaded onto the sterilized wheat residue (straw powder) with autoclave device (Dewan, 1989), and the these fungi suspensions were sprayed into the soil with the same percentage of addition without straw. Treatment fields were planted with rice seeds of jassamine and according to the recommended seed quantity, the treatments were watered. The concentrations of phenols, alkaloids and some hormones (zeatin, gibberelic acid and indole acetic acid) were measured before the flowering period. The treatments that were used (16) were treated with three replicates as follows:

1- Soil only
2- Soil + Straw
3- Soil + NP
4- Soil + Straw + NP
5- Soil + T.h.t.
6- Soil + T.h.a.
7- Soil + C.e.
8- Soil + Straw + T.h.t.
9- Soil + Straw + T.h.a.
10- Soil + Straw + C.e.
11- Soil + NP + T.h.t.
12- Soil + NP + T.h.a.
13- Soil + NP + C.e
14- Soil + Straw + NP + T.h.t.
15- Soil + Straw + NP + T.h.a.
16- Soil + Straw + NP + C.e

2.1. Determination of phenols in rice leaves:

Phenols in rice leaves were measured before flowering by a UV Spectro photometer and wavelength of nm765 and using absorbance of Gallic Acid (Dhar and Bath, 1982)

2.2. Determination of alkaloids in rice leaves Alkaloids were measured in the leaves of the flowering plant before flowering by (HPLC), where the relative area was recorded, from which the concentration was calculated from the relative area chart and the standard concentration (Dhar and Bath, 1982).

2.3. Estimation of plant hormones in rice leaves Hormones were measured in the rice leaves before the flowering date, where the leaves of the rice plants were taken and estimated by a UV Spectro photometer at wavelengths of 254 nm, 222-280 nm and 269 nm for gibberelin, indole acetic lion and zeatin, respectively.
3. Results and discussion

3.1. Phenols

Figure (1,2) shows that *T.h.a.*, *T.h.t.* and *C.e.* isolates which were loaded on wheat residues with or without compost (NP) and added to the soil in the form of suspensions, achieved an increase in the concentration of phenols in the rice leaves of the treatments: Soil + hay + NP + *T.h.a*, Soil + hay + NP + *T.h.t*, Soil + hay + *T.h.a*, Soil + Hay + NP + *C.e*, Soil + hay + *T.h.t*, Soil + NP + *T.h.a*, Soil + hay + *C.e* a significant increase in the concentration of phenols that amounted 0.378, 0.368, 0.362, 0.339, 0.325, 0.322 and 0.319 ppm, respectively, in the fields of RRC, while in the fields of the faculty of agriculture were 0.363, 0.338, 0.327, 0.325, 0.319 and 0.315 ppm, respectively, in comparison with the treatment (soil only), which amounted to 0.251 and 0.245 ppm in Al Hirah fields and the College of Agriculture, respectively, this is indicated by (Nello et al 2010), that the leaves which treated with *Trichoderma*, a separation of effective phenolic compounds against pathogens was observed.

![Figure 1](image1.png)

**Figure (1)** Effect of addition of biological control fungi in the concentration of phenols (ppm) in the leaves of rice plant before flowering date in RRC fields.

![Figure 2](image2.png)

**Figure (2)** Effect of addition of biological control fungi in the concentration of phenols (ppm) in the leaves of rice plant before flowering date in the College of Agriculture fields.
3.2. Alkaloids

The results shown in Figure (3, 4) indicated that *T. h. a.*, *T. h. t.*, and *C. e.* isolates led to an increase in the concentration of alkaloids, as it was observed that the vital role played by fungi encouraging growth, especially those that were carried on wheat residues, achieved a significant differences in RRC fields that amounting 1.67, 1.57, 1.58, 1.48, and 1.48 micro gm/ml of the treatments: Soil + hay + NP + *T. h. a.*, Soil + Hay + *T. h. a.*, Soil + hay + NP + *T. h. t.*, Soil + hay + NP + *C. e.*, Soil + NP + *T. h. a.*, and Soil + NP + *T. h. t.*. While it amounted to 1.51, 1.44, 1.39, 1.38, and 1.32 micro gm / ml in the field of the faculty of agriculture and for the treatments Soil + hay + NP + *T. h. a.*, Soil + hay + NP + *T. h. t.*, Soil + Hay + *T. h. a.*, Soil + Hay + NP + *C. e.*, Soil + NP + *T. h. a.*, and Soil + NP + *T. h. t.*, in compared with control treatment (soil only), which amounted 1.19 and 1.15 micro gm / ml for RRC and College of Agriculture fields respectively, this consistent with Verma et al (2012) that indicate to an increasing in the concentration of alkaloids when adding bio-control fungi as organic fertilizers.

![Figure 3](image1.jpg)

**Figure (3)** Effect of addition of biological control fungi in the concentration of alkaloids (micro gm/ml) in the leaves of rice plant before flowering date in RRC fields

![Figure 4](image2.jpg)

**Figure (4)** Effect of addition of biological control fungi in the concentration of alkaloids (micro gm/ml) in the leaves of rice plant before flowering date in the College of Agriculture fields
3.3. Zeatin

The results shown in figure (3) indicate that *T. h. a.*, *T. h. t.* and *C. e.* isolates gave a significant increasing in the concentration of zeatin (ppm) that reach 0.0941, 0.0897, 0.0895, 0.0833, and 0.0833 ppm, respectively, in the RRC fields of the treatments: Soil + Hay + NP + *T. h. a.*, Soil + Hay + NP + *T. h. t.*, Soil + Hay + *T. h. a.*, Soil + Hay + *T. h. t.*, Soil + Hay + NP + *C. e.* While in the field of the College of Agriculture reached 0.0835, 0.0783, 0.0782, 0.0757 and 0.0749 ppm, respectively, and for Soil + Hay + NP + *T. h. a.*, Soil + Hay + NP + *T. h. t.*, Soil + Hay + *T. h. a.*, Soil + Hay + NP + *C. e.* treatments, in compared with control treatment (soil only), that amounted 0.0712 and 0.0523 ppm, respectively, as indicate by (Degani, O et al, 2015), that the positive effect of some isolates on stimulating plant growth has led to the secretion of plant growth regulators working in conjunction with other mechanisms including increased availability and absorption of plant nutrients.

![Figure (5)](image5.png)

**Figure (5)** Effect of addition of biological control fungi to soil in the concentration of zeatin (ppm) in the leaves of rice plant before flowering date in RRC fields.

![Figure (6)](image6.png)

**Figure (6)** Effect of addition of biological control fungi to soil in the concentration of zeatin (ppm) in the leaves of rice plant before flowering date in the College of Agriculture fields.
3.4. Gibberellic acid

Figure (4) shows that \( T.h.a, T.h.t. \) and \( C.e \) isolates, which loaded on wheat residues with or without compound fertilizer (NP) and added to the soil in the form of suspensions, gave a significant increase in the concentration of gibberellic acid (ppm) in rice leaves for a following treatments Soil + Hay + NP + \( T.h.a \), Soil + Hay + \( T.h.t \), Soil + Hay + NP + \( T.h.t \), Soil + Hay + \( T.h.a \), Soil + Hay + NP + \( C.e \), which amounted to 53.84, 53.76, 53.63, 53.54 and 52.67 ppm, respectively, in the fields of RRC, while in the fields of the college of agriculture were 44.52, 44.15, 44.08, 44.02 and 43.66 ppm, respectively, for the Soil + Hay + NP + \( T.h.a \), Soil + Hay + NP + \( T.h.t \), Soil + Hay + \( T.h.t \), Soil + Hay + \( T.h.a \) and Soil + Hay + NP + \( C.e \) treatments, in comparison to the control treatment (soil only), which amounted to 50.12 and 42.10 ppm in RRC fields and the College of Agriculture respectively, this corresponds to what Ewa O et al (2018) indicated that the positive effect of some isolates of \( T.harzianum \) in stimulating growth Plants to secrete growth regulators, including gibberellic acid.

![Figure (7)](image-url)

**Figure (7)** Effect of addition of biological control fungi to soils in the concentration of Gibberellic acid (ppm) in the leaves of rice plant before flowering date in RRC fields
3.5 Indol acetic acid

Figure (5) shown in, that the treatment of wheat residues with *T.h.a*, *T.h.t.* and *C.e.* isolates with or without compound fertilizer (NP) led to an increase in the concentration of indole acetic acid, as the addition of those isolates to RRC soils, achieved a significant increase in that ratio for the following treatments: Soil + Hay + NP + *T.h.a.*, Soil + Hay + *T.h.a.*, Soil + Hay + *T.h.t.*, Soil + Hay + NP + *T.h.t.* and Soil + Hay + NP + *C.e.*, which amounted 0.287, 0.278, 0.271, 0.265, and 0.263 ppm, respectively, while it amounted 0.291, 0.282, 0.281, 0.276, 0.275, 0.270 ppm in the soils of the college of agriculture for treatments Soil + Hay + NP + *T.h.a.*, Soil + Hay + *T.h.a.*, Soil + Hay + *T.h.t.*, Soil + Hay + NP + *C.e.* and Soil + Hay + *C.e.*, respectively, in compared to a control treatment (only soil), which amounted to 0.210 and 0.174 ppm in the RRC and the college of agriculture fields, respectively, while the other treatments achieved a significant increase,( Fu, S.F et al 2015), and others affirmed that the isolates of *T.harzianum* achieved a significant increase in the production of IAA that stimulating the germination of seeds and the growth of seedlings.
Figure (9) Effect of addition of biological control fungi to soils in the concentration of Indol acetic acid (ppm) in the leaves of rice plant before flowering date in RRC field.

Figure (10) Effect of addition of biological control fungi to soils in the concentration of Indol acetic acid (ppm) in the leaves of rice plant before flowering date in the College of Agriculture field.
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