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Controlling powdery mildew using Fe_3O_4 NPs, yeast, and Bio-Arc and their effects on the performance of lettuce (*Lactuca sativa* L)

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Abstract

The present study used different applications as Fe_3O_4 NPs, yeast, *Bacillus megaterium* (Bio-Arc® 6% WP), Azoxystrobin, (Amistar® 25% SC), and Penaconazole (Topas® 100 10%EC) to control the natural infection of powdery mildew on lettuce growing under field conditions during winter growing seasons (2020 and 2021). The obtained data showed that disease severity was significantly decreased in all treated lettuce plants compared to the untreated plants. In addition, all applications enhanced plant height, number of leaves/plant, and weight of each plant. The different applications significantly increased chlorophyll, carotene, phenolic, and protein contents compared to the control. In addition, the above applications increased the activities of catalase (CAT) and polyphenol oxidase (PPO) in treated plants. Finally, Azoxystrobin fungicide had the best effect compared to the different applications and the control while Bio-Arc was less effective.

Keywords: lettuce, powdery mildew, Fe NPs, yeast, Bio-Arc, Azoxystrobin, Penaconazole

1. Introduction

Around the world, lettuce (*Lactuca sativa* L) is one of the most consumed vegetables because its leaves are used in salads, wraps, soups, and sandwiches [1]. It's rich in sodium, potassium, iron, calcium, magnesium, and vitamins such as C and A [2]. In the growing season, lettuce is attacked by bacteria, fungi, and viral diseases [3]. The losses in lettuce crops may reach from 1 % to 75 % [4], while in other cases, the loss of all the crops may occur [5].
One of the most important fungi that infect lettuce and caused a big loss is the powdery mildew fungus. Information about powdery mildew in *Lactuca* spp has been published [6,7 & 8].

Powdery mildew disease distribution and economic importance are related to dry, hot weather conditions. The powdery mildew fungus appears as a gray-white, powdery growth on both the lower and upper sides of the leaves, and infections cause deformity bucking stages, the leaves begin to turn brown and dry.
It was necessary to intervene to control powdery mildew, whether by traditional methods or using recent trends in the control process. Fungicides are still the major means to control plant diseases under field conditions, but they accumulate in the soil, eliminating beneficial microflora in the soil. In addition to the risks of remaining fungicides or their derivatives in animals, humans, plants, or wastes of agriculture [9]. In addition to using fungicide, herbicide, pesticide, and climate change due to crop losses and soil degradation [10,11].
Recently, there are relationship between nanoparticles (NPs) applications and plants as nanonutrition in agricultural production. Therefore, nanoparticles can improve the controlling of disease, losses crop, increasing plant resistance to microorganism, reducing the use of chemicals [12,13]. Fe3O4 nanoparticles had properties as superparamagnetic, biodegradability and low toxicity [14]. In many previous studies, Fe_3O_4 nanoparticles increased germination and growth of plants [15,16], quality and production of crops [17], chlorophyll and photosynthesis [18].
Nowadays, biological control focuses on the possibility of using safe and natural agents to

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promote plant growth and decrease resistance to different diseases. Yeast (*Saccharomyces cerevisiae*) is considered a new promising stimulant for plant growth for different crops. It has been used as a substitute for chemical fertilizers in the last few years and it's safe for the environment, humans, and animals [19].

This study investigates the effects of different applications *i.e.,* Fe_3O_4 NPs, Bio-Arc, yeast, Penaconazole, and Azoxystrobin in controlling lettuce powdery mildew, chlorophyll, carotenes, proteins, total phenols, enzymes as CAT and PPO on lettuce crop.

2. Material and methods

Applications used:

Azoxystrobin (Amistar® 25% SC)
Penaconazole (Topas® 100 10%EC)
Bacillus megaterium (Bio-Arc® 6% WP)
Yeast (*Saccharomyces cerevisiae*)
Fe_3O_4 NPs: obtained from College of Biotechnology, Misr University for Science andTechnology, Egypt

Field experiments were conducted during two winter growing seasons (2020 and 2021) in the experimental farm of Itay-El-Baroud Agricultural Research Station, El-Behera Governorate, Egypt to evaluate the effects of spraying Lettuce plants with Iron nanoparticles, yeast, Bio-Arc, Azoxystrobin and Penaconazole to control the natural infection of powdery mildew.

The soil was prepared for sowing lettuce plants (cv. balady) in the January 2020 and 2021 growing seasons. The experiment was designed in plots in the Randomized Complete Block Design (RCBD). The plot size is 3 x 4 m each ridge had 20 hills. Lettuce seedlings were planted at both sides of the ridges at 25 cm hill spacing with one seedling per hill. Each plot with three replicates. Three plots were left without treatment to serve as a control. In this regard, the growing plants were left to the natural infection by the causal fungus *Golovinomyces cichoracearum* and then sprayed at the first appearance of powdery mildew symptoms with Azoxystrobin (25 cm/100 L water), Penaconazole (50 cm/100 L water), and Bio–Arc (2.5 gm/L water) as recommended by the Ministry of Agriculture and Land Reclamation, Egypt. In addition, the yeast® was sprayed at 5 µl/ml according to [19]. The Fe_3O_4 NPs was sprayed at a concentration of 50 µg/ml. All treatments were sprayed three times on 10-day terms. Plants were sprayed with water served as control.

Disease assessment

The naturally inoculated plants were examined to estimate the severity of the infection by powdery mildew depending on the devised and modified scale (0-5) by [20] using the following formula:

Disease severity (%) = $\frac{\sum(n*v)}{5N} * 100$

Whereas n = number of infected leaves in each category

v = numerical values of each category
N = Total number of the infected leaves

Disease incidence = (Number of diseased plants/number of total plants) ×100

Agronomic trials:

A random sample of 5 lettuce plants was taken from each plot, 100 days after transplanting to estimate the following growth characters:

1-Plant height (cm)
2-Weight of each plant (g)
3-Number of leaves/plant

Determination of chlorophyll

Lettuce leaves of 250 mg homogenized with 5 ml of acetone 80% by using a hand glass homogenizer and filtered using the Buckner funnel with Whatman filter paper. Then the filtrate was completed to 50 ml acetone and measured spectrophotometric at 663 and 645 nm according to [21]. Chlorophyll a, b and total (mg chlorophyll/ g fresh weight) calculated by following equations:

Chlorophyll a (Ch a) = $((12.7*O.D._{663}) - (2.69*O.D._{645})) *0.2$

Chlorophyll b (Ch. b) = $((22.9*O.D._{645}) – (4.68* O.D._{663})) * 0.2$

Total Chlorophyll (Ch T) = Ch. a+ Ch. b

Determination of carotenes

Determination of carotenes contents according to the method of [22 and 23] as follows: Fresh lettuce leaves of 0.25 g were homogenized with acetone by homogenizer until leaves were completely decolorized and filtrated the extract. Then, the filtrate was completed to 50 ml acetone and the absorbance (A) was measured by spectrophotometry at 450 nm. The carotene mg/g fresh weight was calculated using the formula

F.Wt. = $\left(\frac{A}{K}\right) * \left(\frac{1}{0.25}\right)$

F. Wt.: fresh weight of lettuce leaves
A: absorbance
K: the coefficient of extraction

Determination of protein content

The content of protein was determined according to the method of [24] as follows:

Lettuce leaves of 0.5 g were homogenized with 30 mL of 0.1 M sodium hydroxide in 3.5% sodium

chloride. The homogenates were incubated for 90 minutes at 60°C before centrifugation for 30 minutes at 4000× *g* at 4°C. After that, the extracts diluting to 1 mL with H_2O, and adding 0.9 mL of solution A before incubating for 10 min at 50°C. After this, 1 mL of solution B was added and left for 10 min. Finally, 3 mL of solution C was added before incubation for 10 min at 50°C. The absorbance was measured at 650 nm.

Determination of total phenolic content

Lettuce leaves 2 g were homogenized in 80% ethanol and centrifuged at 10 000×*g* for 15 min in the cold, saving the supernatant. The residue was again extracted twice with 80% ethanol, and the supernatants were pooled and evaporated to dryness. After that, it dissolved in 5mL of distilled water. 100 µl of the extract was added to 0.5 mL of Folin-Ciocalteu reagent and 3 mL of the water. After 3 min, 2 mL of 20% sodium carbonate was added, then the absorbance was measured at 650 nm. According to the method of [25]

Determination enzymes activity

Determination of catalase (CAT) activity by Homogenizing 1 g of lettuce leaves with 100 mM phosphate buffer (pH 7.5), 1% PVP-40, and 1 mM EDTA. Afterward, the homogenates were centrifuged at 5 ˚C for 15 min at 4500 rpm. Supernatants were collected and centrifuged for 10 min at 10 000 rpm. The extract is analytical according to [26].

Determined the polyphenol oxidase (PPO) activity of lettuce leaves ground with 0.2 mM phosphate buffer at pH 7. The ground sample was transferred to a volumetric flask and added 0.05 mM phosphate buffer after that, put the flask in the refrigerator for 2 hours. The extract was mixed with a catechol solution (0.07 mM) and phosphate buffer solution (0.05 mM) then the absorbance was measured at 420 nm.

Statistical analysis

The statistical analysis system used to analyze the data by variance (ANOVA) Gomes, K. A. and A. A. Gomes (1984). Statistical Procedures for Agricultural Research. 2nd ed., Wiley, New York. Means were compared by the least significant difference (LSD.) test at $P \leq 0.05$ levels.

3. Results and Discussion

Data presented in Table (1) showed that all treatments significantly decreased powdery mildew and disease severity in lettuce compared with non-treated as control under field conditions during two growing seasons (2019-2020 and 2020-2021). Azoxystrobin was the most effective in both seasons with mean disease incidence and severity of 8.7, and 2.9% for both seasons which was reflected in disease reductions of 85.7, and 90.8% for the above parameters, respectively. This was followed by Penaconazole which exhibited 10.0 and 5.6% with reduction being 83.6 and 82.2% for disease incidence and severity, respectively. Meanwhile, low reductions in disease incidence were recorded with the yeast treatment with yeast spray in both seasons while the lowest disease severity was obtained after being treated with Bio-Arc. The mode of action of iron nanoparticles is due to direct interference with fungal cells, which affects the permeability of the membrane and ultimately inhibits growth and death of fungal cells [27]. The results obtained are consistent with the results obtained by [28] who found that treated lettuce plants with aqueous extracts of moringa, neem, eucalyptus, basil, garlic, and Dithane M-45 fungicide significantly decreased disease incidence and disease severity compared to untreated plants. [29] Also found that the use of silver nanoparticles reduced disease severity in pumpkin and cucumber leaves. Potassium silicate significantly reduced downy mildew incidence and severity of lettuce [30].

Table (1): Effect of different applications to control powdery mildew on lettuce during two seasons 2020 and 2021.

Treatments	Disease incidence %		Mean	Reduction %	Disease severity		Mean	Reduction %
	2020	2021			2020	2021		
Control	62.7 a	58.7 a	60.8 a	--	28.2 a	34.7 a	31.5 a	--
Fe (NPs)	18.7 bc	10.7 d	14.7 c	75.8	5.7 cd	7.3 d	6.5 d	79.4
Bio-Arc	17.3 c	16.0 b	16.7 b	72.5	7.0 c	11.3 c	9.2 c	70.8
Yeast	20.0 b	13.0 c	16.5 b	72.9	10.3 b	14.0 b	12.2 b	61.3
Azoxystrobin	9.3 d	8.0 e	8.7 e	85.7	2.7 e	3.0 f	2.9 e	90.8
Penaconazole	10.7 d	9.3 de	10 d	83.6	5.1 d	6.0 e	5.6 d	82.2

Effect of different applications on growth characteristics of lettuce growing under field conditions during winter seasons 2020 and 2021.

Data in Table (2) showed that the different treatment applications significantly enhanced growth characteristics, i.e. plant height (cm), the number of leaves /plant, and weight/plant of lettuce balady cv. growing in the field naturally infested with the powdery mildew fungus. In the 2020 season, the effect was most exhibited with Azoxystrobin fungicide treatment spraying at 20 days after transplanting which exhibited 40.8 cm, 27.6 and 700 g of plant height, number of leaves/plant and plant weight, respectively. This was compared to 33.2 cm, 20.2 leaves/plant, and 560 g of plant weight for the untreated plant. In the 2021 season, the highest plant was recorded at 42.6 cm after being treated with Azoxystrobin while the lowest value was 32.8 cm for the untreated plant. Regarding the number of leaves/plants, the highest value is 27.3 after Azoxystrobin treatment, while the lowest value was 21.9 for the untreated plants. As for the weight of each plant, it was found that the highest value was 684g recorded after Azoxystrobin treatment, but in contrast, the lowest value was 547g for untreated plants. This line agrees with [31] who found that treatment with Cadmium (Cd) at different concentrations increased the growth parameters over the control treatment in lettuce plants. The same trend was recorded in terms of No. of leaves/plants increased after spraying lettuce plants with eucalyptus, moringa, and garlic extracts compared to the control [32]. Also, [33] used different concentrations of Fe_3O_4 NP significantly increased root number, root length, and shoot length of barley cultivars. Other studies indicated that using different concentrations of Fe_3O_4 NPs increased growth parameters of oak plants [34], enhanced the parameters of growth of tomato [35], increased number of leaves and root length of seedling of Yellow medick [36] and increased root and shoot length of garden rockets [37].

Table (2): Effect of different applications on growth characterizes and yield of lettuce during winter seasons 2020 and 2021

Treatments	2020			2021		
	Plant high (cm)	No. of leaves/plant	Weight/plant (g)	Plant high (cm)	No. of leaves/plant	Weight/plant (g)
Control	33.2 d	20.2 d	560 e	32.8 d	21.9 d	547 e
Fe (NPs)	38.0 b	24.2 c	648 c	37.1 b	26.7 bc	656 bc
Bio-Arc	36.4 c	25.2 bc	622 d	36.0 bc	27.2 b	640 d
Yeast	38.4 b	26.2 ab	680 b	35.3 c	28.0 a	668 b
Azoxystrobin	40.8 a	27.6 a	700 a	42.6 a	26.3 c	684 a
Penaconazole	38.2 b	24.6 c	615 d	37.5 b	26.9 bc	645 cd

Effect of different applications on carotenoid and chlorophyll content in lettuce leaves growing under field conditions during winter seasons 2020 and 2021.

The data showed that lettuce leaves had a variety of carotenoid and chlorophyll a, b, and total chlorophyll presented in Figure (1). Also, results showed that carotenoid and chlorophyll concentrations in lettuce leaves were influenced by different applications after being treated. The highest carotenoid content was obtained after being treated with Azoxystrobin while the lowest content with Bio-Arc. The chlorophyll amount was higher than carotenoids and chlorophyll b and lowest than total chlorophyll in all applications, which is in agreement with [38, 39]. Also, [40, 41, 42] found that used Fe_3O_4 NPs increased chlorophyll and photosynthesis in soybeans, maize, yellow medick, barley and rice. The same researchers revered to chlorophyll contents increased after treated with Fe_3O_4 NPs in barley seedlings [43], in oak plants [44]. Also, [45] found that Fe_3O_4 NPs at low concentration enhanced Chl *a* and *b* content. As a result of treatment with Mancozeb, the contents of chlorophyll and carotene were less affected because they were more stable photosynthetic [46, 47]. Also, recorded data by [48] revealed increased values of the photosynthetic pigments in lettuce leaves after being treated with eucalyptus moringa, neem extracts, and Dithane M-45 compared to untreated plants. The total chlorophyll and carotenoid contents of lettuce leaves improved after 30 days of different applications of Cd under different concentrations [49].

In a different trend, [50] found a decrease in the contents of pigment after being treated with Fludioxonil and Carbendazim. The previous studies agree with our findings of decreased chlorophyll content in infected plants such as lime crops [51], Chinese jujube [52], corn beans [53], and lettuce leaves [54].

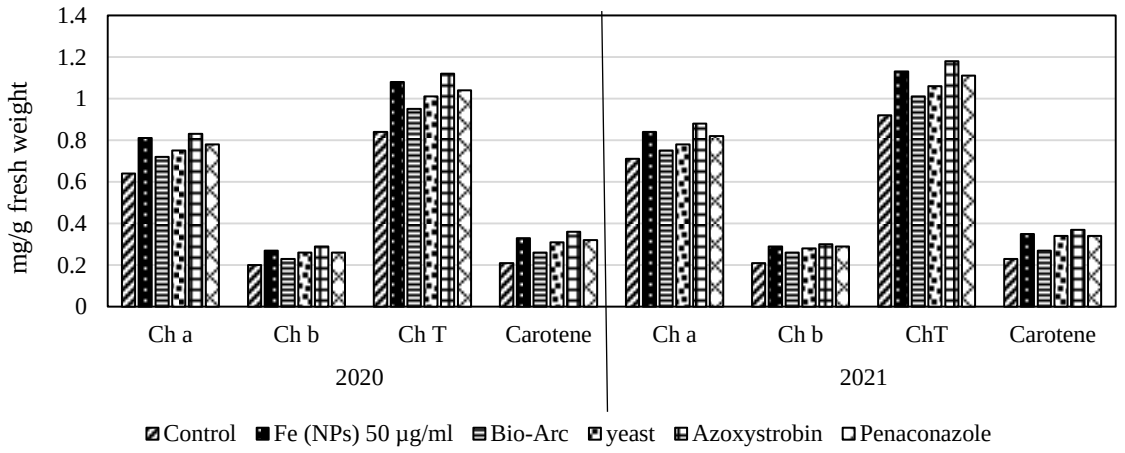

Figure (1): Effect of different applications on carotene and chlorophyll contents in lettuce during two seasons 2020 and 2021.

Effect of different applications on protein and phenolic contents in lettuce leaves growing under field conditions during winter seasons 2020 and 2021.

The results of protein in *Lactuca sativa* samples were presented in Figure (2). The soluble protein content in leaves was significantly lower in untreated compared to treated plants. In comparison to the other application, the highest content of total soluble proteins was observed in lettuce leaves after being treated with Azoxystrobin. Similar results were obtained for protein deficiency in infected lettuce leaves by [55], also, [56], who indicate a decrease in protein content in infected chickpea plants. In this respect, [57] spraying potato plants with Bio-Arc increased protein contents.

The phenolic content in lettuce leaves after being treated with different applications is shown in Figure (2). Generally, all applications enhanced the total phenols content in lettuce compared to the untreated plants. The statistical analysis showed a significant increase total phenols in lettuce after being treated with Azoxystrobin followed by Fe_3O_4 NPs treatment. However, the lowest concentration of total phenols in lettuce after being treated with Bio-Arc compared to other applications. In lettuce leaves, the high content of phenolic compounds makes it a vegetable with high antioxidant activity. Phenolic acids are regarded as one of the secondary metabolites groups as natural antioxidants in plants. Phenolic compounds had biological activity, including anticancer, antioxidant activities, cytotoxic antidepressants, and inflammatory [58]. Also, increased levels of polyphenol compounds contribute to the strengthening of cell walls which responsible for plant protection against pathogenic microorganism's penetration [59]. Also, [60] found that increased polyphenolic compounds in lettuce leaves after treated with iodosalicylic acids.

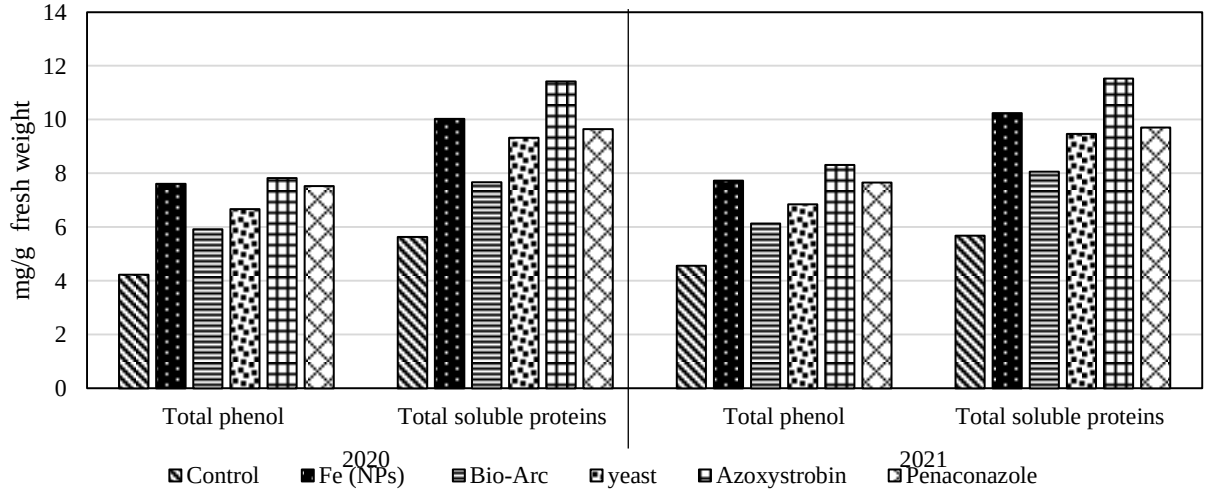

Figure (2): Effect of different applications on total phenol and total soluble proteins in lettuce during two seasons 2020 and 2021.

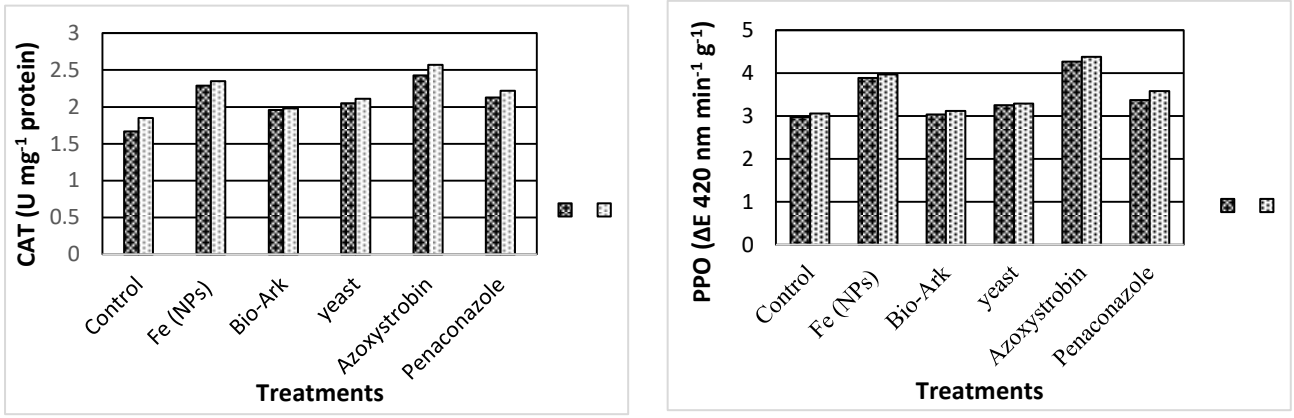

Figure (3): Effect of different applications on activities of CAT and PPO enzymes in lettuce during two seasons 2020 and 2021.

Effect of different applications on CAT and PPO enzymes in lettuce leaves growing under field conditions during winter seasons 2020 and 2021.

The activity of CAT and PPO enzymes in lettuce leaves is enhanced with different applications as shown in Figure (3). Different applications were statistically significant in increasing the activities of CAT and PPO enzymes. In comparison to the other applications, the highest activities of CAT and PPO were observed in lettuce leaves after being treated with Azoxystrobin followed by Fe_3O_4 NPs treatment. Bio-Arc treatment being the lowest effect in enhancing the activity of two enzymes mentioned before. This agree with [61] who found Fe_3O_4 NPs at low concentration enhanced antioxidant enzymes activity in barley plants. The activity of the CAT enzyme elevated against pathogen-attack plants [62]. CAT enzyme is capable of protecting biological systems against free radical attack [63] and reduces H_2O_2 into H_2O and O_2 [64]. In the same line [65, 66] found an application of iodide increased CAT in lettuce. Lettuce plants treated with high concentrations of Cd improved CAT activity compared with untreated plants.

These data disagree with [67] who found that using glutathione, tropolone, aminobenzoic acid, and ascorbic acid inhibits the activity of PPO in lettuce at different concentrations.

4. Conclusion

The present investigation clearly exhibited that although the fungicides used had the best effects, but the alternative safety agents used as Fe_3O_4 NPs and yeast had significant effects and followed Azoxystrobin. Therefore, Fe_3O_4 NPs and yeast could be proposed as alternative, safe fungicide to control powdery mildew disease of lettuce under field conditions in Egypt.

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الملخص العربى

مكافحة البياض الدقيقي باستخدام جزئيات اكسيد الحديد النانوية والخميرة وبيوأرك وتأثيراتها على اداء الخس (لاكتوكا ساتيفا ال)

تم دراسة مدى إستجابة صنف الخس بلدى للرش ببعض بدائل المبيدات الفطريه مثل جزيئات الحديد النانويه والخميره على تحسين بعض صفات النمو والمكونات الكيميائيه وعلاقة ذلك بنسبة وشدة الإصابه بمرض البياض الدقيقى تحت الظروف الحقليه خلال موسمى الزراعه الشتويه (٢٠٢٠ و ٢٠٢١). وقد أظهرت النتائج التي تم الحصول عليها أن معدل الإصابة وشدة المرض انخفضى بشكل معنوي في جميع نباتات الخس المعاملة مقارنة بالنباتات غير المعاملة بالإضافة إلى ذلك عززت جميع المعاملات كل من ارتفاع النبات وعدد الأوراق لكل نبات ووزن النبات. كما أدت التأثيرات المعنوية للمعاملات المختلفة إلى زيادة محتوى الاوراق من الكلوروفيل والكاروتين والفينول والبروتين مقارنة بالنباتات غير المعامله. بالإضافة إلى زيادة نشاط إنزيمى CAT و PPO في النباتات المعاملة. أظهر إستخدام جزيئات الحديد النانوى وكذلك الخميره نتيجه مبشره كبديل للمبيدات سواء فى تحسين بعض صفات النمو أو خفض معدل أو شدة الإصابه بالبياض الدقيقى من أجل استبدال مبيدات الفطريات وبالتالي تكون بيئة أكثر أمانا.