Efficiency of some Chemical factors in Stimulating systemic Resistance of Barley plant against Fusarium wilt disease caused by the fungus *Fusarium oxysporum*

Jasim Mahmood Abed, Theyab Abd Al-wahed Farhan and Rashid Mushrif Theer

College of Agriculture/ University of Anbar/ Republic of Iraq

Corresponding author Email: ag.jasim.mahmood@uoanbar.edu.iq

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Abstract

This study aimed to evaluate the efficiency of chemical-inducing factors (melatonin, beta amino butyric acid, and salicylic acid) in inducing the systemic resistance of barley against the *Fusarium oxysporum.* Results of the collection sample that the *Fusarium oxysporum* was associated with collection samples. The results of the test of the inhibitory ability of the second inducing factors (Melatonin at a concentration of 200 mg L^{-1} and Beta Amino Butyric Acid (BABA) at a concentration of 2000 mg L^{-1} and SA at a concentration of (200 mg L^{-1}) showed that all factors significantly reduced the colony growth rate of *Fusarium oxysporum* (4.33, 4.66, 5.66)cm compared with control treatment (9.00) cm. The treatment using Melatonin was significantly superior to the rest of the treatments in reducing the dry weight of the biomass, which reach 0.13 mg, followed by the treatment of SA and BABA, which reached (0.15, 0.16) mg respectively. The same induction factors also significantly decreased some of the studied growth parameters (germination percentage, plant height, number of stems, and grain weight). The results showed an increase (49.33 gm) in the activity of the peroxidase enzyme and the rate (319.33 $mg.g^{-1}$)of accumulation of phenols as a result of using all inducing factors.

Keywords: Stimulate, *Fusarium oxysporum* , Systemic resistance, Barley

Introduction

Barley (*Hordeum vulgare* L.) is one of the most important cereal crops in the world. The crop is grown in large parts of the world. As its cultivation does not require large amounts of irrigation water, as well as its tolerance to soil salinity. The main barley-growing countries are the United States of America, Russia, China, Canada, India, France, Australia, the United Kingdom, Spain and Turkey (20). The cultivation of the crop faces many challenges, the most prominent of which is the biological stress caused by pathogens that lead to the loss of economically important cereal crops. The cultivation of the crop is exposed to many challenges, perhaps the most prominent of which is the infection of the types of fungus Fusarium spp., which causes a reduction in the quantity and quality of the crop. This effect of the fungus Fusarium spp is due to the production of a group mycotoxins that cause harmful to humans, animals and plants. (4 and 19) Fusarium oxysporum represents one of the most soil-born fungi that cause wilt and root rot diseases. (11 and 1).

Types of *Fusarium* spp. negatively affects the crop, quantitatively and qualitatively, as well as producing high concentrations of mycotoxins (3). There are more than 100 Format specials of *F. oxysporum* found to infect different plant hosts (11). Several diseases caused by pathogenic fungi in order to reduce of pathogen damage used many of chemical pesticides but negative effects health and the environment, it is necessary to find alternative means to control this disease and reduce the losses caused by it. Research and studies tended to stimulate systemic resistance with high-efficiency materials that are safe for the consumer and the environment, including melatonin, beta-aminobutyric acid and salicylic acid. Melatonin is an indole molecule based on tryptophan. It performs various functions in plants including promoting plant growth, seed germination, biomass and melatonin from the antioxidant compounds (5). Thus, it acts as an anti-stress agent against various biotic and abiotic stresses such as drought, salinity, toxic metals and pathogens. In addition, gene regulation related to the main role of melatonin stands out as an antioxidant agent that can control the interaction of oxygen and nitrogen species in plants. It's improving the parameter growth and quality of fruits and vegetables (18) Salicylic acid is one of the chemical-inducing factors that have the ability to stimulate resistance in plant cells and it is one of the signaling compounds as it stimulates systemic acquired resistance in plants (14). The plant quantities of proteins related to plant pathogenicity are doubled to 10 times, which helps to enhance the stimulation of plant resistance genes to produce proteins of an enzymatic nature or enzymes such as Glucanase and Chitinase, which have defense mechanisms against various pathogens such as fungi, viruses and bacteria when use SA (16). The laboratory results showed a high efficiency of salicylic acid in inhibiting the growth of fungi, reaching 100% when using the concentration of 1.5%. And a significant decrease in the severity of early blight on treated plants (2). It was discovered in the twentieth century the presence of some chemical compounds that stimulate the resistance of the host plant against

pathogens, and these compounds include β-aminobutyric acid (9). It was found that BABA stimulates many physical and biochemical defense mechanisms in plants.. Plant defense mechanisms depend not only on the type of plant but on the stimulus or stimuli released by the pathogen (7 and 17). In view of the seriousness of the disease and its spread, this study was conducted to evaluate the role of chemical-inducing factors (Melatonin, BABA and SA) in reducing the incidence of the disease and their efficiency in stimulating plant resistance against the fungus *Fusarium oxysporum.*

Materials and Methods

Isolation of Fungus

Isolation of the pathogenic fungus *Fusarium oxysporum*, preparation and industrial infection. Samples were collected from barley plants that showed symptoms of wilt disease, and the diagnosis was based on the characteristics mentioned by (12) Samples were prepared from plants that showed symptoms of wilt, then the affected roots and stems were washed with water and sterilized with a commercial sodium hypochlorite solution 2% (containing 4.5%). The active ingredient was for 3 minutes, then washed twice with sterile distilled water and dried on a blotting paper. The edges were cut at the base of the affected leg with a thickness of 2-4mm, and 4-5 pieces were placed in a Petri dish containing Potato Dextrose Agar (PDA), (Tetracycline was added to prevent bacterial growth). The plates were incubated at 25±2°C for 5 - 7 days, and the colonies of the pathogen *F. oxysporum* were purified depending on the fungal colony shape and color regardless of Forma specialist for their similarity when examined by light microscope), and applied Koch's hypothesis to ascertain the pathogen after the appearance of symptoms of infection, and it was re-isolated in dishes containing the same aforementioned culture and incubated for 10 - 14 days at 25±2°C).

Chemical inducing

Chemical catalysts used in this study (Melatonin at a concentration of 200 mg L^{-1}, BABA at a concentration of 2000 mg L^{-1} and SA at a concentration of 200 mg L^{-1} were getting from the plant protection lab. - Agriculture College –University of Anbar

Effect of chemical stimuli on the growth of the *F. oxysporum* Stimuli

The PDA medium treated with chemical stimuli (Melatonin, BABA and SA in the above concentrations) was used separately. The medium was distributed in glass Petri dishes with a diameter of 9cm at a rate of 20 ml. Plate-1. After the medium solidified, the center of each dish was inoculated with one 0.5cm diameter disc from a pure culture at the age of 7 days of *F. oxysporum* growing on PDA culture medium. The dishes were incubated at 25±2°C, and then the fungal growth rate of the different treatments was calculated for 14 days of inoculation with the fungus. Used 3 replicates to any treatment.

Effect of chemical stimuli on the dry weight of the biomass of *F.* oxysporu*m*

(Melatonin, BABA and SA in the above concentrations) was used separately. Add in liquid dextrose PDB while the control treatment used liquid dextrose PDB only. One 0.5cm diameter tablet was taken from a pure culture of *F. oxysporum* at the age of 7

days and added to a 250 ml glass flask equipped with 100 mL of the culture medium, potato extract and liquid dextrose PDB.25 ±2°C. The culture medium was filtered through Wattman no1 filter paper. The biomass was dried at 80°C until the weight was stable

Test treatment of seeds with chemical control agents and their role in controlling Fusarium wilt disease and their effect on some growth parameters

After preparing the above research factors, the pathogenic fungus grown on millet seeds was added at 5% to 5 kg to sterile field soil in plastic pots three days before planting the seeds, which were soaked in chemical catalysts solutions for 3 hours before planting (Melatonin, BABA and SA). The experiment was carried out by RCBD design and it included 3 replicates. The treatments were carried out as follows:

1- Treatment with the stimulator Melatonin + *F. oxysporium*
2- Treatment with the stimulator BABA + *F. oxysporium*
3- Treatment with SA catalyst + *F. oxysporium*
4- Treatment with the fungus+ *F. oxysporium*
5- Treatment without pathogenic fungi.

Determination of peroxidase enzyme activity

Peroxides activity was determined according to Hammerschmidt *et al* (1982).

By measuring the oxidation of pyrogallol to purogallin in the presence of H2 O2 at 425 nm. The reaction mixture consists of 0.5 ml of 0.1 M sodium phosphate buffer solution at Ph. 7.0, 0.5 ml enzyme extract, 0.3 pyrogallol, 0.1 ml 1.0 H2 O2 brought to final volume of 3.0 ml with distilled water.

Results and Discussion

Table (1) shows that the effect of Melatonin, BABA and SA treatment on the growth of the fungus *F. oxysporum* under laboratory conditions, as it was found that all the tested substances caused a significant inhibition of the growth rate of the fungus and the dry weight of the live mass of the pathogenic fungus compared to the control treatment in the presence of the pathogenic fungus only, and the transactions were recorded And BABA, SA and Melatonin (4.33, 4.66, 5.66) cm respectively, compared to the control treatment, which amounted to 9.00 cm, with an inhibition rate of (51, 47, 37)%. The treatment using BABA was significantly superior to the rest of the treatments in reducing the dry weight of the live mass, which amounted to 0.16 mg and an inhibition rate of 51%, followed by the treatment of SA and Melatonin, which amounted to (0.15, 0.13) mg, with an inhibition rate of (44,40), respectively.

Table 1. Effect of Melatonin, BABA and SA treatment on the growth of *F.oxysporum* under laboratory conditions

Treatment	Rate of Growth (cm)	% Inhibition	Weight of biomass (mg)	% Inhibition)
F.oxysporum + BABA	*4.33	51	0.13	51
F.oxysporum + SA	4.66	47	0.15	44
F.oxysporum + Melatonin	5.66	37	0.16	40

F.oxysporum	9.00	00	0.27	00
Control	9.00	00	0.27	00
LSD α at 5%	1.47		0.067	

*Notes: Each value is an average of three replicates

The results in Table 2 indicate that the effect of inducing factors (Melatonin, BABA and SA) on some growth parameters of barley plant grown in 5 capacity pots, all treatments were significantly superior in most of the studied growth parameters compared to the treatment of the infected control, and the treatments were significantly superior in increasing (germination percentage , Plant height, number of strands) where the percentage of germination of the treatments, BABA, SA and Melatonin were recorded (86.67, 93.33, 86.67, 46.67), plant height (54.67, 53.33, 56.00, 44.00, 54.67) cm and the number of branches (5.00, 6.33, 4.67) The weight of the grains was (40,48,39)gm respectively compared to the treatment of the infected witness whose height was 44.00cm and the number of stalks,2.67) and the weight of the grains(27,46)gm.

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Table 2. Effect of Melatonin, BABA and SA treatment on some growth parameters of barley infected with the fungus *F.oxysporum*

Treatment	Germination percentage	Plant height (cm)	Number of branches	Grain Weight (gm)
F.oxysporum + BABA	*86.67	54.67	5.00	40
F.oxysporum + SA	93.33	53.33	6.33	48
F.oxysporum + Melatonin	86.67	56.00	4.67	39
F.oxysporum	46.67	44.00	2.67	27
Control	100.00	54.67	6.00	46
LSD α at 5%	17.53	6.72	1.24	7.63

*Notes: Each value is an average of three replicates

The results in Table (3) indicate an increase in the rate of change in absorption in all plants spraying treatments on the shoots, and it led to a significant increase in the activity of peroxidase enzyme in the treated plants based on the change in light absorption. min^{-1}.g fresh weight-1 (Fw-1 g.min-1) compared to the control treatment, as it reached the highest. The rate of change in the uptake of peroxidase enzyme after treatment with the pathogenic fungus, the treatment of spraying with BABA acid at a concentration of 2000 mg was superior. L-1, which amounted to 49.33, followed by treatment BABA and Melatonin, which amounted to 47.67, 45.33. While the highest rate of phenol accumulation when plants were treated with BABA was 319.33 µg.gm fresh weight-1, superior to The rest of the treatments, while all of the treatments outperformed the two comparison

treatments with the presence and absence of the pathogen, which amounted to (308.67, 313.63, 150.00, 199.00) for the treatments (SA, Melatonin, infected and healthy control). The peroxidase enzyme works to catalyze the oxidation of hydrogen donors in the presence of hydrogen peroxide H2O2 to more toxic substances for pathogens called quinones, and these substances may contribute to and a reduction in the incidence of infection. This induction was accompanied by an increase in the accumulation of phenolic compounds in the treated plants, and this was considered in many studies a standard in activating defense mechanisms in plants against pathogens. Melatonin regulates various aspects of plant physiological processes, and improves plant growth and development under multiple biotic and abiotic stress conditions (8). Using melatonin in storing fruits and vegetables after harvest to extend their shelf life by delaying the process Maturation, mitigation of cold damage, and influence on some vitality of plant pathogenic fungi (15). pathogen activation as well as to stimulating resistance (10). The effect of peroxidase enzyme is due to its role in forming defensive barriers and increasing the hardness of plant cell walls, which increases plant resistance to invasion by pathogens (6). From the results, it is clear that the use of various inducing factors on the vegetative system led to the stimulation of systemic resistance in the treated plants

Table 3. Effect of Melatonin, BABA and SA treatment, peroxidase enzyme

Treatment	Plant content of peroxidase (Fresh weight min.gm)		Plant content of phenols (wet weight mg/g)
F.oxysporum+BABA	49.33	*	319.33
F.oxysporum+SA	47.67		308.67
F.oxysporum+Melatonin	45.33		313.67
F.oxysporum	43.00		150.00
Control	28.67		199.00
LSD α at 5%	4 .30		3.06

*Notes: Each value is an average of three replicates

Conclusion

Wilt disease of barley cause by *F. oxysporium* and all inducing factors (Melatonin at a concentration of 200 mg L-1, BABA at a concentration of 2000 mg L-1 and SA at a concentration of 200 mg L-1) given decreasing in colony growth rate, biomass of colony and increasing in parameter of growth.

Conflict of interest

The authors have no conflict of interest.

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