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Short Articles

Stimulation of systemic resistance in strawberries against gray mold disease caused by *Botrytis Cinerea* using amino butyric acid and melatonin

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ABSTRACT

The study aimed to isolate and diagnose the fungus that causes gray mold disease in strawberries, and the study showed the spread of the fungus *Botrytis cinerea* in all areas of sample collection (Baghdad - Anbar - Babylon - Salah al-Din). Reducing the infection rate to (28%, and 32.33%) in the strawberry plant compared to the control treatment contaminated with the pathogenic fungus (82.67). The results showed a significant effect of the above induction factors in reducing the diameter growth rate and the dry weight rate of the biomass of the fungus, which reached (2.80 cm, 4.67 cm) and (0.253, 0.357 mg) respectively, compared to the control treatment with a diameter of (9 cm, 0.423 mg). For the inducing factors BABA and Melatonin, the results showed a significant increase in the rate of peroxidase enzyme activity, as it reached Melatonin (48.248 minutes gm fresh weight^{-1}) and BABA (37.330 minutes gm fresh weight-1). Accumulated total phenols achieved BABA (3.203 mg fresh weight^{-1}) and Melatonin (2.635 mg fresh weight^{-1}).

Keywords: Stimulate; *Botrytis cinerea*; BABA; Melatonin; Strawberry

INTRODUCTION

The strawberry, *Fragaria ananassa* Duch, belongs to the Rosaceae family. It is a perennial plant that has a wide adaptation to temperatures and grows as a wild or cultivated plant [1]. Strawberries are characterized by high nutritional value and flavor by increasing their content of dietary compounds [2]. Strawberry plants are infected with many plant diseases, including gray mold disease, by the pathogen *Botrytis cinerea*, the second most crucial fungal pathogen in molecular plant pathology from an economic and scientific point of view [3]. The fungus kills plant tissues and then feeds on them, and all types throw away nutrition, so most of the cells that infect it die during the infection. [4]. Symptoms differ from one plant to another and from one tissue to another, as the symptoms of the leaves acquire Gray, then brown and then lead to the fall of infected leaves on the soil; as for the stem, it appears in the form of ulcers in the form of a mass of combined fungal hyphae, and it is possible to find sclerotium in the affected tissue.
In contrast, a gray color appears in the area of infestation on the fruits and then turns to brown after the progression of the infection; then, the fruits fall off and are considered the secondary source of infection. Flowers are a good source of infection, as the petals are affected first, and their color is gray, then turns brown, and the area where the plant contacts the soil (the stem) is more susceptible to infection with fungi and flowers. Most plant species can be contaminated, yet they do not clearly show infection symptoms (disguised symptoms) [5]. The fungus forms sclerotium and growth to give apothecia, and each contains several sacs, each containing eight ascospores [6, 7]. Recently, inducing factors have begun to eliminate and reduce the use of chemical pesticides polluting the environment. Induced resistance is the process of generating or stimulating plants to activate the role of chemical and physical defenses that are naturally present in the plant against plant pathogens by using biological or abiotic stimulating factors, which are usually accompanied by the production of Disease-related enzymes, phytoalexins, or proteins that kill or inhibit the pathogen [8]. The induced resistance stimulates the plant to produce the substances responsible for resistance before and after infection. Its advantages are that it does not affect humans and the environment, and the plant acquires protection against viral, fungal or bacterial diseases 9. Some studies have shown the role of endogenous melatonin in resisting pathogens, including the causative of Botrytis cinerea, by

enhancing plant resistance and raising the content of anti-enzymes such as peroxidase (POD), antioxidant enzyme superoxide dismutase (SOD) and jasmonic acid (JA). To resist plant stress and reduce fungal infection [10], studies indicated that BABA has an essential role in lowering biotic and abiotic stress and enhancing plant protection from fungal diseases by stimulating stimuli within the plant and its resistance [11, 12]. Spraying BABA on the plant significantly increases nitrogen oxide accumulation and resistance against the pathogenic fungus Botrytis cinerea in tomato plants. A study indicated that BABA leads to the collection of nitrogen peroxide and is essential in resisting gray mold disease against B. cinerea in tomato plants [13].

MATERIALS AND METHODS

Sample Collection

The strawberry fruits that show symptoms and signs of infection with the fungus Botrytis cinerea were collected from markets and fields from (Baghdad - Babil - Anbar - Salah al-Din) in Iraq and placed in polyethylene bags and transferred to the pathology laboratory in the plant protection department to isolate the fungus by taking part of the growth. The fungi were cultured in the center of a petri dish with a diameter of 9 cm. The culture medium contained sterilized PDA medium with oxidizer at a temperature of 121°C and a pressure of 1.5 kg/cm2 for 20 minutes, supplemented with the antibiotic Tetracycline at a rate of 200 mg/L; the plates were incubated at ±25°C. 2 a.m. for seven days, then examined and characterized, molecularly and phenotypically, based on the taxonomic key [14].

Pathogenicity test

The strawberry fruits were obtained from the market fields intact and were selected in equal and homogeneous sizes to conduct pathogenicity experiments. The fruits were washed with water for 3 minutes, then placed in free chlorine at a rate of 10%, then soaked in sterile water for 3 minutes to get rid of chlorine residues three times, and placed on blotting paper inside the isolation room for 45 minutes, turning them from time to time to ensure the dryness of the fruits and get rid of traces of water as much as possible. Sealed plastic dishes with a diameter of 15 cm were brought. Two layers of filter paper were placed at the base of the container to ensure internal moisture absorption. After that, strawberries were placed in the containers, each containing five fruits. The fruits were inoculated with mushroom isolates (10 weeping isolates were the strongest) Separately by placing a 5 mm disc from the edge of each colony in the center of the plate and the comparison treatment, The dishes were placed in the incubator at 25 ± 2 °C according to a complete randomized design (CRD) and were monitored every 24 hours. The results were taken after 7 days. The percentage and severity of infection were calculated according to equation [15].

Effect of BABA and Melatonin in Inhibiting the Growth of Botrytis cinerea isolates

I prepared the chemical agent beta-amino butyric acid BABA from Sigma Aldrich company-Germany using a concentration of 2000 mg l-1, melatonin from Pure Bulk Company USA at a concentration of 200 mg l-1, and the pesticide Pristine from the German producer BASF at a concentration of 0.75 gm l-1. The sterile PDA was placed in glass flasks containing 100 ml of the medium, then the materials were added according to the concentrations, mixed well until homogeneity, and the medium was poured into Petri dishes and left to solidify in the isolation chamber. Placed upside down on the surface of the medium, the plates were incubated at a temperature of 25± 2C, while the dishes were monitored every 24 hours with three replicates for each treatment. As for the control treatment, it was inoculated with a 5 mm disc of pure PDA only. The average length of two perpendicular diameters was recorded for each colony of mushrooms for each isolation after the completion of the growth of the control treatment after five days at a temperature of 25±2C. The percentage of inhibition was calculated for all treatments according to the following equation:

$$\% \text{ inhibit} = \frac{The\ average\ diameter\ comparison - The\ diameter\ of\ the\ treatment\ is\ average}{Diametercomparison\ average} \times 100$$

Effect of BABA and Melatonin on the dry weight of the biomass of *Botrytis cinerea*

One tablet with a diameter of 0.5 cm was taken from a pure culture of Botrytis cinerea at the age of 7 days and added to a 250 ml glass flask containing 60 ml of sterile liquid culture medium, potato Dextrose Broth, which was prepared simultaneously, and the chemicals were added according to the required concentration and the comparison treatment was left Without addition, and incubated at 25 ± 2° C. The culture medium was filtered through Wattman no. filter paper. 1 The biomass was dried at 80 °C for 24 hours, the dry weight of the pathogenic mushroom biomass was taken, and the results were compared with the comparison treatment.

RESULTS

Phenotypic Diagnosis of *Botrytis cinerea*

The isolates of the pathogenic fungus Botrytis cinerea that were isolated and purified from strawberry fruits were diagnosed using the taxonomic key [15] based on the appearance and shape of the divided mycelium and spores, which were collected from the areas shown in (Table 1).

Table 1. Sample collection areas infected with a gray mold of Botrytis cinerea

Sequence	Place of collection	Source of collection	Number of samples
1-	Anbar – Fallujah	the strawberry	**7**
2-	Anbar – Aramadi	the strawberry	**9**
3-	Anbar – khalidia	the strawberry	**4**
4-	Anbar – Heat	the strawberry	**6**
5-	Anbar - College of Agriculture	the strawberry	**5**
6-	Baghdad - Abu Ghraib	the strawberry	**3**
7-	Waset – Essaouira	the strawberry	**9**
8-	Salah al-Din – Samarra	the strawberry	**7**
9-	Anbar – Amria	the strawberry	**4**

Pathogenicity test of Botrytis cinerea isolates on strawberry fruits.

Table (3) showed significant differences in the pathogenicity of the fungus isolates that were selected based on the speed of their dissemination and growth phenotypically on strawberry fruits after four days of treatment. The results showed that isolate BC5 caused the highest infection severity on fruits, reaching 96.67%, followed by severity The fruits were infected with isolate BC1, which recorded 91.67 %, while the remaining isolates BC3, BC7, BC9, BC10, BC6, BC4, BC2, and BC8 recorded infection severity of 90.00, 85.00, 83.33, 75.00, 73,33, 70.00, 66.67, 66.00%, respectively. The variation in the pathogenicity of isolates may be due to genetic differences between isolates.

Table 2. Pathogenicity test of Botrytis cinerea isolates on strawberry

B.cinerea isolation symbol	Source of isolation	Disease severity %
BC1	Anbar / Ramadi	91.67
BC2	Anbar / Khalidiya	66.67
BC3	Anbar / Ramadi	90.00
BC4	Anbar / Heat	70.00
BC5	Anbar / Fallujah	96.67
BC6	Anbar / Amriya	73.33
BC7	Saladin / Samarra	85.00
BC8	Baghdad / Abu Ghraib	66.67
BC9	Baghdad / Yusufiyah	83.33
BC10	Waset / Essaouira	75.00
control	Anbar / College of Agriculture	00.00
LSD5%		16.27**

Effect of BABA and Melatonin on Inhibition and Dry Weight of Growth of *Botrytis cinerea* isolates in Vitro

The statistical analysis results (Table 2) showed a significant effect of the substances (BABA, Melatonin and Pristine) in inhibiting the fungus growth on the PDA medium. After four days of incubation at 25 °C, 2.03, 2.80, and 4.67 cm) the diameter of the colony for the treatments Perstin,

BABA, Melaton sequentially and with inhibition percentage (77.4%, 68%, 48%) compared to the control treatment with the colony diameter of 9 cm, In the study of the dry weight of the biomass of the mushroom used.

Table 3. Effect of chemicals on fungal growth rate and dry biomass on Botrytis cinerea in vitro.

Treatment	average colony diameter (cm)	inhibition ratio%	mass weight mg	inhibition ratio%
BABA	2.80	68%	0.253	**40.4%**
Melatonin	4.67	48%	0.357	**16.6%**
Pristine	2.03	77.4%	0.173	**59.5%**
control	9.00	00	0.423	**00**
LSD 0.05%	0.5128 **		**0.124	

The effect of BABA and Melatonin in reducing the incidence and severity of infection on the fungus Botrytis cinerea

The results of Table (4) showed a significant effect on the rate and severity of infection using induced substances BABA and Melatonin. The results showed the superiority of treatments using preventive use, as it gave the lowest infection rate (28, 32.33) and injury severity (23.91, 25.74) for BABA and Melatonin treatments, respectively, as for the control treatment with the presence of the pathogen. Moreover, the witness without a nurse (0, 82.67) and the severity of the injury (57.16, 0), respectively, were set for comparison. In therapeutic use, the lowest injury rate (31.33, 37.67) and injury severity (28.33, 35.6) were found for BABA and Melaton treatments, respectively.

Table 4. Effect of BABA and Melatonin on the rate and severity of infection with the fungus Botrytis cinerea

Treatment	Percentage of infection%		Disease severity %	
	protection	Treatment	protection	Treatment
BABA	28	31.33	23.91	**28.33**
Melatonin	32.33	37.67	25.74	**35.6**
Pristine	23.67	12.33	16.85	**7.69**
Control with pathogenic fungi	82.67	82.67	57.16	**57.16**
control only	0	0	0	**0**
LSD 0.05%	3.333**	3.463**	2.187**	**3.287****

Effect of BABA and Melatonin in the determination of the amount of peroxidase enzymes and phenols in strawberry plants

The statistical analysis results (Table 5) in the experiment showed a significant effect in increasing the peroxidase enzyme activity estimated based on the rate of change in light absorption/min/g fresh weight in strawberry plants. The melatonin treatment excelled at an average of (48,248) in therapeutic use, followed by (37,330) in aminobutyric acid treatment and (28.183 22.243) for the Perstin and comparison + pathogen treatments, respectively. As for the preventive method, the aminobutyric acid treatment outperformed at an average of (28,893) followed by 26,817 for the melatonin treatment and (22.243, 22,217) for the Perstin and comparison + pathogen treatments, respectively, all treatments in both methods (therapeutic, protection) were compared with the control treatment 17,877. This is attributed to the increase in the peroxidase enzyme by the action of the materials used, as a study showed in the induction of enzymes, including peroxidase produced by the action of abiotic components (Surekha et al., 2014). As for the average estimation of the number of phenols by therapeutic use, the highest estimate of the amino butyric acid treatment showed an average of 3.203, followed by 2.357 for the melatonin treatment and (1.596, 1.900) ،For the treatments Perstin and the control + fungus, respectively, and with the preventive use of

aminobutyric acid, 2.884, followed by 2.635 for the melatonin treatment and 1,900, 1.655 for the control treatments + pathogen, Perstin respectively.

Table 5. Effect of BABA and Melatonin on the determination of the amount of peroxidase enzymes and phenols in strawberry plants

Treatment	Peroxidase - min/gm/fresh weight		Phenols - mg/g fresh weight	
	Protection	Treatment	protection	**Treatment**
BABA	28.893	37.330	2.884	**3.203**
Melatonin	26.817	48.248	2.635	**2.357**
Pristine	22.217	28.183	1.655	**1.596**
Control + pathogenic fungi	22.243	22.243	1.900	**1.900**
control only	17.877	17.877	1.199	**1.199**
LSD 0.05%	2.92**	6.204**	0.3577**	**0.9733****

DISCUSSION

It agrees with studies that indicated that the substances used, such as melatonin, have a role in inhibiting the infection of B. cinerea by regulating plant growth and increasing tolerance to stress against pathogens B. cinerea [18,17]. The results showed (0.173, 0.253, and 0.357 g) for the treatments Perstin, BABA, and Melaton, respectively and with an inhibition percentage (59.5%, 40.4%, 16.6%) compared to the comparison treatment with a weight rate of (0.423 g), as A study indicated the extent of mushroom resistance to some compounds and pesticides[16]. Studies showed that strawberry plants treated with melatonin and γ-aminobutyric acid transaminase (GABA-T) lead to an increase in phenols and the accumulation of anthocyanins (PAL). Its effect on ATP production produces a high percentage of unsaturated/saturated fatty acids (unSFA / SFA), which protects fruits even after harvesting. [19]

CONCLUSIONS

This study showed the spread of gray mold disease significantly in strawberries regarding the rate and severity of infection. The inducing factors (BABA and Melatonin) had a role in inhibiting the growth of the fungus in the laboratory, as their use led to the inhibition of the development of colonies and the biomass of the fungus. Their service was in field experiments. A role in stimulating plant resistance, which led to a reduction in the rate and severity of infection by both curative and preventive methods, as well as an increase in the activity of peroxidase enzyme and the accumulation of phenols in treated plants.

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