J. Agric. & Env. Sci. (Damanhour University) 2024, 23(1): 1-24
Print: ISSN 1687-1464 Online: 2735-5098

EFFECT OF METHYL JASMONATE IN CONTROLLING *ALTERNARIA ALTERNATA* AND ENHANCING THE DEFENSE MECHANISM ACTIVITY OF TOMATO

Ghoname M. Amer*, Sawsan S. El-Shamy and Mahmoud H. Ghozlan

Department of Plant Pathology, Faculty of Agriculture, Damanhur University, El-Beheira, Egypt.

***Corresponding Author:** Ghoname_2012@agr.dmu.edu.eg

ABSTRACT

This study investigated the effect of methyl jasmonate (MeJA) treatment on the control of Alternaria leaf spot caused by *Alternaria alternata* in tomato. *In vitro* and *in vivo* experiments were performed to evaluate the antifungal efficacy of MeJA through its effect on defense enzyme activity. MeJA at concentrations of 50, 100, and 150µl/l significantly inhibited Alternaria mycelial growth and spore germination compared with the control. The strongest inhibitory effect was observed at 150µl/l. Furthermore, the application of 150µl/l MeJA was found to be the most effective solution in reducing the incidence and necrotic areas in tomato leaves. MeJA treatment resulted in higher activities of peroxidase (POD), polyphenol oxidase (PPO), and total phenolic compounds in the treated and inoculated plant leaves compared to untreated plants. These findings suggest that MeJA has potential as an environmentally friendly method for controlling Alternaria leaf spot in tomato.

Keywords: *Alternaria alternata,* Methyl jasmonate (MeJA), Tomato, Defense enzyme

https://doi.10.21608/jaesj.2024.280355.1158

INTRODUCTION

Tomato (*Solanum lycopersicum*) is a globally used horticulture crop, both fresh and processed **(Del Giudice *et al*., 2017)**. Tomato production faces major challenges due to diseases caused by pathogens

resistant to conventional agrochemicals, such as the early blight-causing fungus *Alternaria alternata* (Fr.) Keissler **(Yang *et al*., 2019)**. It is one of the most important tomato diseases, causing significant loss of economic and nutritional value to the tomato crop. This disease is especially prevalent in tomato-growing regions with high humidity and relatively high temperatures in semi-arid climates with frequent and extended night dews **(Chaerani and Voorrips, 2006)**.

Alternaria species are common plant pathogens that have a global impact on numerous plant species, including plants from the solanaceous family, such as tomato **(Agrios, 2005)**. *A. alternata* can infect tomato crops at any stage of growth, resulting in significant losses at harvest **(Bashir *et al*., 2016)**. In recent years, brown spots on tomatoes and potatoes in Egypt have been seen, which can occasionally lead to blight in severe infections. *A. solani* and *A. alternata* are two major Alternaria species **(Bashir *et al*., 2016; El-Gobashy *et al*., 2018)**. He concluded that *A. alternata* causes leaf blight on tomatoes in Egypt based on pathological, phenotypic, and genotypic studies. *Alternaria* infections have also been observed to exceed 30% in greenhouse-grown plants and approximately 80% in open-field-grown plants **(Van der Waals *et al*., 2001; Soleimani and Kirk, 2012)**.

Fungal diseases are often controlled with chemical fungicides. However, conventional fungicides frequently cause toxicity or residues in plants and the environment. As a result, other ways are being investigated to improve plant resistance to diseases. Plants have intrinsic structural and biochemical defenses against pathogen attacks that can be used to increase their resistance **(Agrios, 2005; Van Loon *et al*., 2006)**.

Jasmonic acid (JA) and its volatile derivative, methyl jasmonate (MeJA), are cyclic molecules formed from linolenic acid. These chemicals exist naturally and operate as endogenous regulators, influencing plant stress response, growth, and development **(Creelman and Mullet, 1997)**. Exogenous treatment with methyl jasmonate (MeJA) has been shown to effectively decrease gray mold rot in strawberries caused by *Botrytis cinerea*, as well as prevent

Colletotrichum acutatum infection in loquat fruit **(Cao *et al*., 2008)**. Furthermore, the use of MeJA increased the efficiency of the antagonistic yeast *Cryptococcus laurentii* in controlling brown rot and blue mold produced by *Monilinia fructicola* and *Penicillium expansum* in peach fruit **(Yao and Tian, 2005)**. Additionally, treated cucumber plants with Jasmonic acid decrease *Pythium aphanidermatum* infection **(Sabbagh *et al*., 2018)**.

Induced resistance is a biochemical defensive mechanism found in plants that allows them to establish non-specific or long-lasting systemic resistance to a variety of pathogens, including fungi and bacteria (**Heil and Bostock, 2002)**. This process is known as induced systemic resistance (ISR). The use of plant hormones such as methyl jasmonate (MeJA) or jasmonic acid (JA) has been shown to successfully induce systemic resistance against fungal pathogens in a variety of plant species, including Arabidopsis, potato, tomato, and grapefruit **(Thomma *et al*., 2000; Pozo *et al*., 2005; El-Khallal, 2007)**.

Many plant species have been shown to have higher levels of jasmonates after being infected with pathogens or treated with elicitors **(Creelman and Mullet, 1995)**. Plants treated with JA and MeJA showed increased resistance to diseases **(Cohen *et al*., 1993)**. MeJA has been shown to be particularly important for resistance to necrotrophic fungi, including *A. brassicicola* **(Thomma *et al*., 1999)**.

The importance of oxidative enzymes and their metabolic byproducts in the defensive mechanisms of diseased plants has been widely researched **(Waheed and Tehmina, 2011)**. Antioxidant enzymes, such as polyphenol oxidase (PPO) and peroxidase (POD), participate in the oxidation of phenols and have a role in plant defense against pathogens **(Maffei *et al*., 2007)**. Furthermore, phenolic compounds in plants are crucial for development and defensive responses against invading pathogens. Plants contain a range of phenolic chemicals that have negative impacts on pathogens **(Lattanzio and Cardinali, 2006)**.

Therefore, this work intended to investigate the efficiency of MeJA against *A. alternata* in both *in vitro* and *in vivo* trials. Additionally, the study attempted to examine the influence of MeJA on tomato defensive mechanisms, especially enzymes involved in host defense and total phenolic compounds.

MATERIALS AND METHODS

The tested fungal isolate

The highly virulent isolate of *Alternaria alternata* used in the present study was obtained from the culture collection of The Plant Pathology Department of Damanhur University, Egypt. The isolate was maintained on potato dextrose agar (PDA) plates at 25°C. The identity of the acquired *A. alternata* isolate was validated using the appropriate primer-PCR method as follows:

DNA extraction

A pure culture of the obtained *A. alternata* isolate was cultured in potato dextrose broth (PDB) for four days at 25 °C. The mycelium was collected and dried using filter paper under suction. DNA was extracted using the CTAB procedure described by **Doyle and Doyle (1990)**.

A. alternata-specific PCR identification

A. alternata-specific primers Aalt- F (5' GTG CCT TCC CCC AAG GTC TCC G 3') and Aalt- R (5' CGG AAA CGA GGT GGT TCA GGT C 3') were used to amplify the β-tubulin gene sequence according to **Kordalewska *et al*. (2015)**. The PCR amplification was conducted in a 25µl reaction mixture containing 3µl of template DNA, 12.5µl PCR Green Master Mix (Thermo Scientific™), 0.5µl of forward and reverse primers (10pmol), and 8.5µl of molecular grade water. The amplification cycle consisted of an initial denaturation at 95 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30s, annealing at 55 °C for 2 min, and extension at 72 °C for 1 min. A final extension step at 72 °C for 1 min was performed. The presence of amplicons was assessed by electrophoresis on 2% agarose gels stained with ethidium

bromide and visualized using a UV-Transilluminator (Cleaver Scientific, model CSLUVTSDUO365L).

The *in vitro* effect of methyl jasmonate (MeJA) on *A. alternata*

Effect on *A. alternata* radial growth

A 5-mm diameter disc of 7-day-old culture was transferred to a PDA medium adjusted before pouring the plates with MeJA (Sigma Aldrich, USA) to form the proper concentrations of 0 (control), 50, 100, and 150µl/l according to **Kepczynska and Kepczynski (2005)**. The plates were then incubated in the dark at 25°C for five days. The radial growth of the fungus was measured by determining the colony diameter. The inhibition of fungal growth was calculated as a percentage relative to the control. This experiment was repeated three times.

Effect on *A. alternata* spore germination

Five-day-old cultures of *A. alternata* were gently brushed to separate the spores from the mycelial. The spores were then rinsed with a 0.01% Tween 20 solution, and spore suspension was filtered through a fine nylon mesh to remove any mycelial debris. The concentration of spores was quantified using a hemocytometer and adjusted to 10^6 spores ml^{-1}. A spore suspension of 5 ml was mixed with either water (control) or MeJA solutions to achieve final concentrations of 50, 100, and 150µl/l. After that, 50µl of the spore suspension of each treatment was placed on a glass slide in a Petri dish (10 cm) lined with filter paper moistened with distilled water. After 24 hours, the germinated spores were counted under a light microscope. Each treatment was replicated three times **(Kępczyńska and Kępczyński, 2005)**.

The *in vivo* effect of MeJA on *A. alternata* leaf necrosis

In vivo MeJA treatments were applied (50, 100, and 150µl/l) according to the detached leaf approach proposed by **Johnson *et al.* (2000)**, with some modifications. Prior to inoculation, five tomato leaves were selected from 6-week-old plants of the susceptible cv. MicroToms. Each leaf was individually sprayed with 2 ml of MeJA solution at the specified concentrations and allowed to dry for 60 minutes. A 5-mm diameter disc, obtained from the active margin of a 7-day-old *A. alternata* culture, was placed at the center of each tomato

leaflet. The inoculated tomato leaves were then arranged on moist filter papers in plastic boxes, sealed to maintain a humidity level of approximately 90%, and kept in darkness at a temperature of 20 °C. The control group consisted of un-inoculated untreated tomato leaves (sprayed with distilled water and free from the fungus on a PDA disc), as well as inoculated untreated tomato leaves. Each treatment was replicated three times. After 72 hours, the extent of leaf necrosis was evaluated by determining the percentage of necrotic area relative to the total leaf area. This evaluation was conducted using the trypan blue staining method, as described by **Egusa *et al*. (2013)**. The inoculated leaves were briefly boiled in a staining solution containing lactophenol, ethanol, and trypan blue (2.5 mg/ml) for one minute, followed by overnight incubation at room temperature. Subsequently, the stained leaves were immersed in a chloral hydrate solution, photographed, and analyzed using the leaf area measurement software version 1.3 (Sheffield University, England).

Effect of MeJA on tomato defense enzymes and total phenolic content

This experiment was conducted as previously mentioned in the previous experiment. The enzyme activities of peroxidase (POD) and polyphenol oxidase (PPO), and the total phenolic content, were assessed in tomato leaves collected at different time points (0, 12, 24, 48, and 75 hours after inoculation). A one-gram sample of fresh leaves was homogenized in 2 ml of 0.1M phosphate buffer (pH 7.0) at 4°C. Subsequently, the homogenate was centrifuged at 5000xg at 4°C for 15 minutes. The resulting supernatant was used for enzyme measurement.

Determination of Peroxidase (POD) activity

This was conducted according to **Hammerschmidt *et al.* (1982)**. The reaction mixture included 1.5 ml of 0.05M pyrogallol, 0.5 ml of the enzyme extract, and 0.5 ml of 1% H_2O_2. The reaction was carried out at room temperature (28±2°C), and the changes in absorbance at 420 nm were measured every 30 seconds for 3 minutes. Enzyme activity was calculated as changes in absorbance $min^{-1}g^{-1}$ of fresh tissue.

Polyphenol oxidase (PPO) enzyme activity

This was conducted according to **Mayer *et al.* (1965)**. The reaction mixture consisted of 200 µl of the enzyme extract and 1.5 ml of 0.1M sodium phosphate buffer (pH 6.5). The reaction was started by adding 200 µl of 0.01M catechol, and the activity was evaluated by measuring the changes in absorbance at 495 nm.

Total phenolic content

According to **Saikia *et al.* (2004)**, total phenol contents were conducted as follows: One gram of leaves was crushed with 10 ml of 80% methanol in a mortar, then transferred to a flask and heated in a water bath at 70 °C for 15 minutes with continuous stirring. One mL of the filtered solution was placed in a sterile glass tube, followed by adding 5 mL of sterilized distilled water and 250µl of Folin Reagent. The resulting solution was incubated at 25 °C for 30 minutes, and the absorbance was measured with a spectrophotometer at 725 nm. The total phenol content was calculated as mg of phenols per gram of fresh leaf tissue.

Statistical analysis

Statistical analysis was performed on the data using analysis of variance (ANOVA), and significant differences among the means were determined at a significance level of $p \leq 0.05$ using the revised LSD test with the SAS statistical system (SAS, 1997).

RESULTS

Alternaria alternata-specific PCR assay

The *A. alternata*-specific PCR demonstrated 100% sensitivity and specificity, as shown in Fig (1). A 184 bp band specific to *A. alternata* was successfully generated in the analyzed DNA sample of *A. alternata* tested isolate and no PCR product was found for the negative reaction, which was DNA extracted from an *Alternaria* but non-*alternata* isolate.

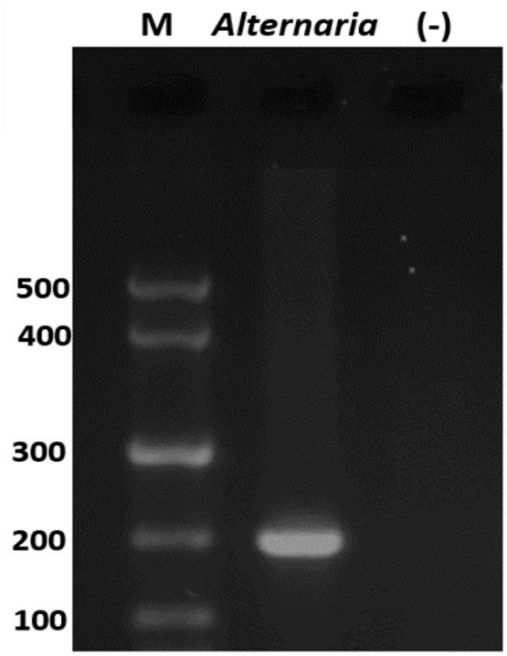

Fig. 1. *Alternaria alternata* isolate specific PCR assay. M, molecular marker (fragment sizes 500, 400, 300, 200, and 100 bp); results of *A. alternata*-specific PCR performed for *A. alternata* isolate. (-) negative control.

In vitro, antifungal activity of MeJA against *A. alternata*

Data presented in Fig. (2) revealed that MeJA Significantly inhibited radial growth (colony diameter) of the tested *A. alternata* isolate at all tested MeJA concentrations compared to the untreated control. However, the inhibitory effect increased with increasing the MeJA concentration where the highest inhibition (83%) was observed with the 150µl/l MeJA treatment (Fig. 2). Meanwhile, Fig. (3) showed that applying MeJA at 50, 100, and 150µl/l significantly inhibited the spore (conidia) germination of the *A. alternata* isolate. The inhibition ranged from 58% to 88%, with the highest inhibition (88%) observed with the 150µl/l MeJA treatment (Fig. 3).

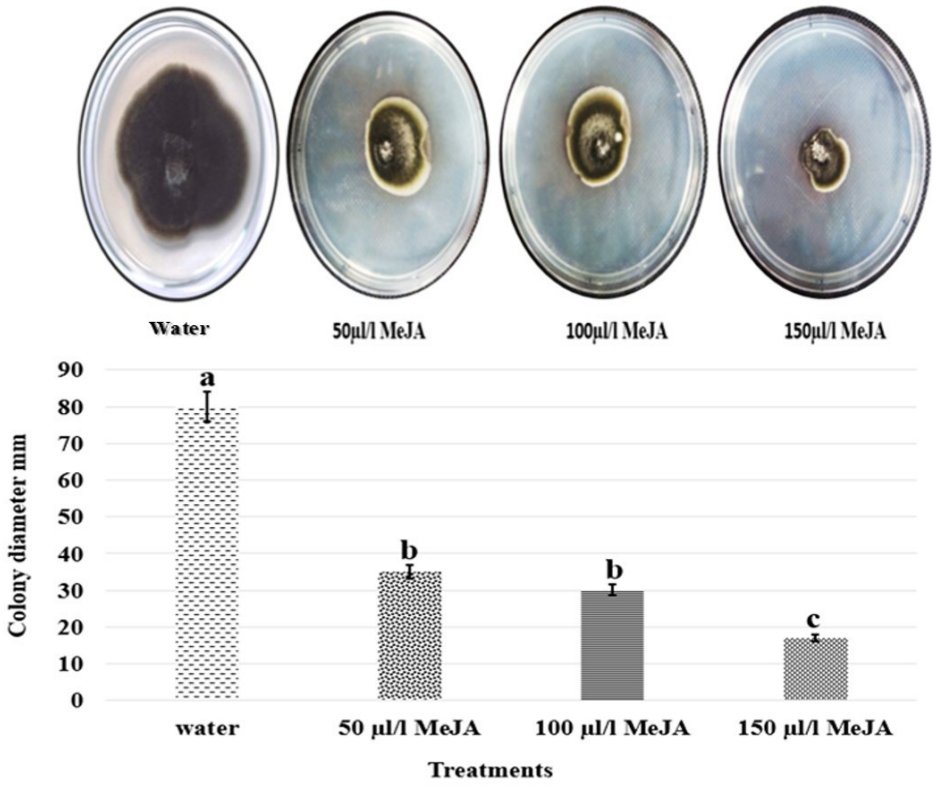

Fig. 2. The effect of MeJA on *A. alternata* isolate mycelium radial growth (colony diameter). Vertical bars indicate mean± SD. Columns with different letters are significantly different at $p<0.05$

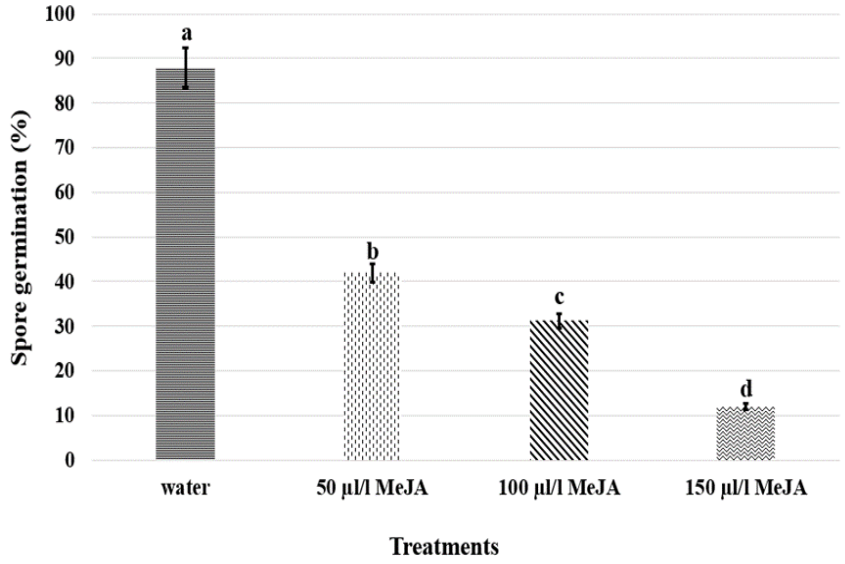

Fig. 3. Effect of MeJA on spore germination of *A. alternata* isolate. Vertical bars indicate mean± SD. Columns with different letters are significantly different at $p<0.05$

In vivo* antifungal effect of MeJA against *A. alternata.

It is evident in Fig. (4) that a large and dark necrotic area (88% of the leaf) was detected in the untreated *A. alternata*-inoculated tomato leaves while no necrotic lesions were detected on the uninoculated leaves (negative control). Meanwhile, tomato leaves treated with MeJA exhibited more tolerance to infection than untreated ones. All MeJA concentrations significantly decreased necrotic area on inoculated leaves, with the most significant reduction effect recorded with the 150µl/l MeJA which showed necrosis as low as 18% of the leaf area compared to 88% for the untreated inoculated control (Fig. 4).

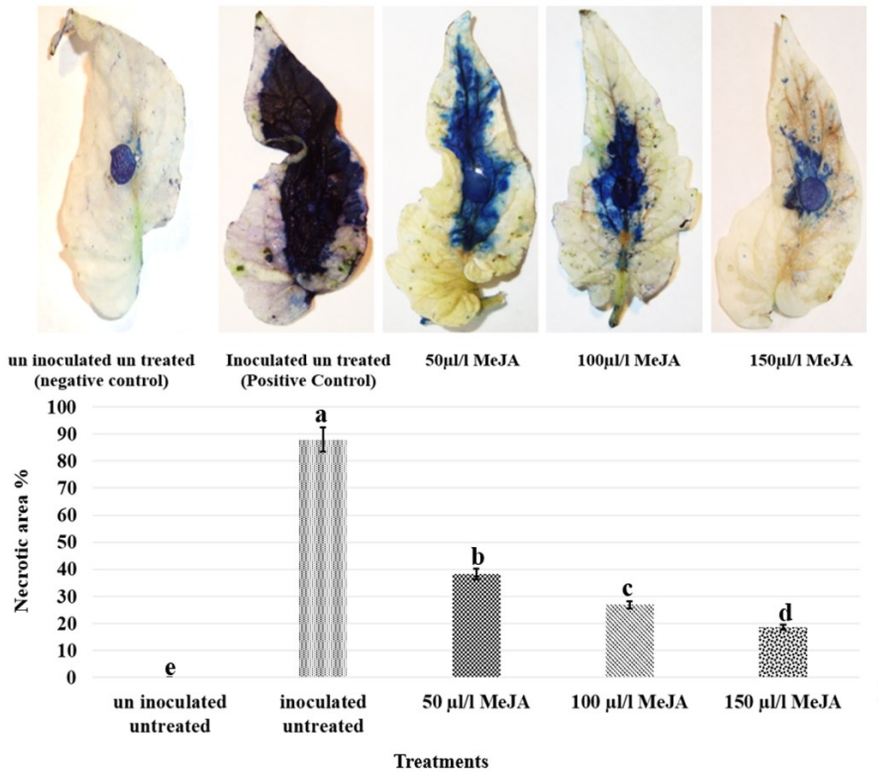

Fig.4 The effect of MeJA on *A. alternata* isolate disease severity. Vertical bars indicate mean± SD. Columns with different letters are significantly different at $p<0.05$

Peroxidase (POD) enzyme activity:

Data in Table (1) demonstrated that inoculated untreated tomato plants (positive control) had greater POD activities than uninoculated untreated plants (negative control). However, tomato plants treated with MeJA had a higher POD activity compared to both control groups. The MeJA treatment at 150µl/l was the most effective and significantly increased POD activity to an overall mean of 0.423 (changes in absorbance $min^{-1}g^{-1}$ fresh tissue). POD activity peaked at 48 hours after inoculation, with 0.419 changes in absorbance $min^{-1}g^{-1}$ of fresh tissue (Table 1).

Table 1: Peroxidase (POD) activity in tomato leaves of cv. MicroTom treated with different concentrations of methyl jasmonate (MeJA) and inoculated with the *A. alternata*

Treatment	POD activity (changes in absorbance /min/g of fresh tissue)					
	Time (hour)					Mean
	0	12	24	48	72	
Un-inoculated - Untreated	0.287	0.314	0.325	0.334	0.312	0.314d
Inoculated - Untreated	0.361	0.387	0.406	0.418	0.355	0.385c
MeJA 50 µl/l	0.38	0.406	0.406	0.428	0.383	0.401b
MeJA 100 µl/l	0.381	0.414	0.413	0.449	0.387	0.409b
MeJA 150 µl/l	0.366	0.403	0.447	0.467	0.433	0.423a
Mean	0.355 d	0.384 c	0.399 b	0.419 a	0.374 c	

Values are means of five replicates.
Means for each parameter followed by different letters are significantly different at $p<0.05$ probability.

Polyphenol oxidase (PPO) enzyme activity.

The findings in Table 2 clearly show that treating tomato plants with different concentrations of MeJA resulted in a significant increase in polyphenol oxidase (PPO) activity. The treatment with 150 µl/l MeJA resulted in the greatest rise in PPO activity, with a value of 3.774 (changes in absorbance/min/g fresh tissue). This was closely followed by the treatments with 100 µl/l and 50 µl/l MeJA, which significantly elevated PPO activity to 3.6392 and 3.509 changes in absorbance $min^{-1}g^{-1}$ of fresh tissue, respectively. Furthermore, the maximal mean peak of PPO activity was detected as early as 24 hours after inoculation and continued to increase until 48 hours after inoculation. However, after 72 hours, PPO activity started to decline.

Table 2: Polyphenol oxidase (PPO) activity in tomato leaves of cv. MicroTom treated with different concentrations of methyl jasmonate (MeJA) and inoculated with the *A. alternata*.

Treatment	PPO activity (changes in absorbance /min/g of fresh tissue)					
	Time (hour)					Mean
	0	12	24	48	72	
(Un-inoculated - Untreated)	2.549	3.192	3.346	3.451	2.651	3.038 d
(Inoculated - Untreated)	2.968	3.659	3.631	3.541	3.187	3.397 c
MeJA 50 µl/l	3.167	3.571	3.605	3.651	3.549	3.509 b
MeJA 100 µl/l	3.209	3.723	3.828	3.874	3.562	3.639 b
MeJA 150 µl/l	3.411	3.809	4.025	3.920	3.703	3.774 a
Mean	3.060 d	3.590 b	3.687 a	3.687 a	3.330 c	

Values are means of five replicates.
Means for each parameter followed by different letters are significantly different at $p<0.05$ probability.

Total phenolic content

The data presented in Table 3 indicate the impact of MeJA treatments on the total phenolic content in tomato leaves. Inoculated untreated plants have considerably greater total phenolic content than uninoculated untreated plants. However, when tomato plants were treated with various concentrations of MeJA, there was a significant increase in total phenolic content. Treatments with MeJA at 100 and 150 µl/l yielded the same total phenolic content of 42.076 and 42.456µg of phenol/g fresh tissue, respectively. Furthermore, the highest increase in total phenolic content was detected as late as 72 hours after inoculation.

Table 3: Total phenolic content in tomato leaves of cv. MicroTom treated with different concentrations of methyl jasmonate and then inoculated with the ***A. alternata*.**

Treatment	µg of phenol/ g fresh tissue					
	Time (hour)					Mean
	0	12	24	48	72	
(Un-inoculated - Untreated)	34.88	36.23	37.03	36.89	38.37	36.68 d
(Inoculated - Untreated)	36.36	38.69	37.64	37.21	39.15	37.81 c
MeJA 50 µl/l	40.02	40.29	41.73	42.38	42.81	41.45 b
MeJA 100 µl/l	40.08	40.98	43.88	42.98	42.46	42.08 a
MeJA 150 µl/l	41.01	41.59	43.45	43.39	42.84	42.46 a
Mean	38.47 d	39.56 c	40.75	40.57 b	41.13 a	

Values are means of five replicates.
Means for each parameter followed by different letters are significantly different at $p<0.05$ probability.

DISCUSSIONS

Alternaria leaf spot, caused by *Alternaria alternata*, is a severe and widespread disease that damages tomato plants worldwide **(Soleimani *et al*., 2008; Somma *et al*., 2011)**. This fungal pathogen causes diseases on tomato stems, leaves, and fruit by producing AAL toxin **(Akamatsu *et al*., 1997)**. While many commercial tomato cultivars are resistant to Alternaria leaf spot, susceptible varieties can still be affected if planted **(Vakalounakis, 1988)**. This disease is generally managed by the use of resistant cultivars and fungicides **(Singh *et al*., 2015)**. Resistance-inducing agents like methyl jasmonate (MeJA) have been proposed as an alternative to fungicides for managing Alternaria species **(Thomma *et al*., 1999; Kępczyńska and**

Kępczyński, 2005; Kępczyńska and Król, 2012; Tiwari *et al*., 2017; Pan *et al*., 2022; Zhang *et al*., 2023).

To validate the molecular identity of the fungal isolate under examination, the PCR method was used using appropriate beta-tubulin gene primers, resulting in the isolate being identified and confirmed as *A. alternata*. Our findings are consistent with previous research by **Konstantinova *et al*. (2002), Kordalewska *et al*. (2015),** and **Mirkova and Konstantinova (2003)**, which identified *A. alternata* based on beta-tubulin gene sequence. The sequencing of the beta-tubulin gene was found to be successful in producing amplification fragments unique to *A. alternata* but not to other *Alternaria* fungus.

Our investigation found that MeJA concentrations of 50, 100, and 150µl/l inhibited mycelium radial growth and spore germination in the *A. alternata* isolate. Using 150µl/l of MeJA resulted in the highest percentage of inhibition. MeJA has been shown to reduce mycelial growth and spore germination in plant pathogenic fungi such as *Colletotrichum acutatum* **(Cao *et al*., 2008)**, *A. alternata* **(Kępczyńska and Kępczyńska, 2005)**, and *Erysiphe graminis* f.sp. *hordei* **(Schweizer *et al*., 1993)**.

The severity and extent of *A. alternata* spread and plant cell death in MeJA-treated and inoculated tomato leaves were examined using trypan blue staining. The concept of trypan blue staining is that dead cells with broken membranes enable the dye to penetrate, resulting in a blue appearance under a light microscope. In contrast, living cells with undamaged cell membranes avoid the dye, therefore looking unstained **(Uzuner, 2018)**.

The Current findings reveal that MeJA concentrations decreased the necrotic area (blue area) in inoculated tomato leaves, indicating that MeJA plays an important role in conferring resistance in the tomato plants against A. *alternata* infection under greenhouse conditions. **Kępczyńska and Król (2012)** found that pretreating tomato seeds with MeJA at a concentration of 0.1mM efficiently inhibited the development of disease caused by *Alternaria*. **Sabbagh *et al*. (2018)**

reported that jasmonic acid induced systemic resistance in infected cucumber by *Pythium aphanidermatum*. Recent studies on chrysanthemum leaves **(Zhang *et al*., 2023)** and cherry fruit **(Pan *et al*., 2022)** have similarly demonstrated significant decreases in susceptibility and the induction of resistance to *A. alternata* by MeJA pretreatment. MeJA application increased plant resistance to *A. alternata* by activating the jasmonic acid (JA) signaling pathway and up-regulating the expression and activities of antioxidant enzymes and disease resistance-related enzymes, such as catalase, peroxidase, superoxide dismutase, polyphenol oxidase, phenylalanine ammonia lyase, chitinase, and β-1,3-glucanase.

Peroxidase (POD) and polyphenol oxidase (PPO) enzymes play critical roles in conferring plant resistance against various pathogens. These enzymes trigger the phenylpropanoid pathway, which results in the biosynthesis of a variety of plant metabolites such as phenolic compounds, flavonoids, tannins, and lignin, all of which serve to improve plant resistance against pathogenic attacks **(Hahlbrock and Scheel, 1989; Adss *et al*., 2024)**. Numerous studies demonstrated that greater accumulation of phenolics leads to elevated activities of POD and PPO enzymes, which may provide a defense against plant diseases **(Thipyapong *et al*., 2004 and 2007; Rivero Meza *et al*., 2021; Zhu and Tian, 2012)**.

Our findings clearly indicated that treating tomato plants with MeJA at all tested concentrations resulted in a significant increase in POD and PPO activities. The maximum rate of increase was observed with MeJA at a concentration of 150µl/l. In terms of time, POD Peaks at 48 hours, whereas PPO peaks as early as 24 hours after inoculation, continues until 48 hours, and declines at 72 hours after inoculation. Previous studies have also demonstrated that MeJA can significantly elevate POD and PPO levels **(Boughton *et al*., 2006; Pan *et al*., 2022)**.

Phenolic compounds in plants have an important function in both development and defense against diseases. Plants can create a variety of secondary metabolites that are harmful to pathogens **(Lattanzio and Cardinali, 2006)**. These defensive compounds help to

prevent diseases and decrease damages, especially in the early stages after pathogen inoculation, resulting in an effective defense response **(Wittstock and Gershenzon, 2002)**. Numerous studies have found a direct and positive relationship between the quantity and quality of phenolic compounds and the induction of systemic resistance in infected plants treated with resistance activators **(Amzad Hossain and Shah, 2015)**. In this study, we observed that inoculated untreated plants had a greater total phenolic content in tomato leaves than uninoculated untreated plants. However, treating tomato plants with MeJA at all tested concentrations significantly increased the total phenolic content. Total phenolic peaked at 72 hours after inoculation. several researches have indicated that MeJA significantly increases the accumulation of total phenolic compounds, leading to improved plant resistance against pathogens **(El-Khallal, 2007; Sabbagh, 2018; Wang *et al*., 2014)**.

CONCLUSION

Our findings indicated that MeJA's inhibitory impact has a high potential for minimizing *A. alternata* disease in tomatoes. The protective mechanism of action may be related to its capacity to boost tomato defense enzymes such as POD and PPO, resulting in resistance and delaying disease development in tomato plants. In conclusion, our findings imply that MeJA might be used as an environmentally acceptable control option against *A. alternata*.

REFERENCES

Adss, I. A., A. A. A-Hiqail, F. Khan, S. S. El-Shamy, G. M. Amer, E. E. Hafez, O. M. Ibrahim, S. E. Sobhy and A. A. Salah. (2024). Physio-molecular responses of tomato cultivars to biotic stress: Exploring the interplay between *Alternaria alternata* OP881811infection and plant defence mechanisms. Plant Physiology and Biochemistry, 207: 1-11.

Agrios, G. (2005). Plant pathology. 5th ed. San Diego (CA): Academic Press, p. 452-456.

Akamatsu, H., Y. Itoh, M. Kodama, H. Otani, K. Kohmoto (1997). AAL-toxin-deficient mutants of *Alternaria alternata* tomato pathotype by restriction enzyme-mediated integration. Phytopathology, 87: 967-72.

Amzad Hossain M. and M. D. Shah (2015). A study on the total phenols content and antioxidant activity of essential oil and different solvent extracts of endemic plant *Merremia borneensis*. Arabian Journal of Chemistry, 8: 66–71.

Bashir, Z., S. Shafique, A. Ahmad, S. H. Shafique, N. A. Yasin, Y. Ashraf, A. Ibrahim, W. Akram, S. Noreen (2016). Tomato plant proteins actively responding to fungal applications and their role in cell physiology. Front. Physiol., 7: 57.

Boughton, A. J., K. Hoover and G. W. Felton (2006). Impact of chemical elicitor applications on greenhouse tomato plants and population growth of the green peach aphid, *Myzus persicae*. Entomol. Exp. Appl., 120:175-188.

Cao, S., Y. Zheng, Z. Yang, S Tang., P. Jin, K. Wang and, X. Wang (2008). Effect of methyl jasmonate on the inhibition of *Colletotrichum acutatum* infection in loquat fruit and the possible mechanisms. Postharvest Biology and Technology, 49(2): 301-307.

Chaerani, R. and R.E. Voorrips (2006). Tomato early blight (*Alternaria solani*): The pathogen, genetics and breeding for resistance. Journal of General Plant Pathology, (72): 335-347.

Cohen, Y., U. Gysi and T. Niederman (1993). Local and systemic protection against *Phytopthora infestans* induced in potato and tomato plants by jasmonic acid and jasmonic methyl ester. Phytopathology, 83: 1054-1062.

Creelman, R.A. and J.E. Mullet (1997). Biosynthesis and action of jasmonate in plants. Annu. Rev. Plant Physiol. Mol. Biol., 48: 355-381.

Creelman, R.C. and J.E. Mullet (1995). Jasmonic acid distribution and action in plants: regulation during development and response to biotic and abiotic stress. Proc. Natl. Acad. Sci. USA, 92:114-119.

Del Giudice, R., G. Petruk, A. Raiola, A. Barone, D.M Monti. And M.M. Rigano (2017). Carotenoids in fresh and processed tomato (*Solanum lycopersicum*) fruits protect cells from oxidative stress injury. J. Sci. Food Agric., 97: 1616-1623.

Doyle, J. J. and J. L. Doyle (1990). Isolation of plant DNA from fresh tissue. Focus, 12: 13-15.

Egusa, M., T. Miwa, H. Kaminaka, Y. Takano and M. Kodama (2013). Nonhost resistance of *Arabidopsis thaliana* against *Alternaria alternata* involves both pre-and postinvasive defenses but is collapsed by AAL-toxin in the absence of LOH2. Phytopathology, 103(7): 733-740.

El-Gobashy, S. F., W. Z. A. Mikhail, A. M. Ismail, A. Zekry, A. Moretti, A. Susca, A. S. Soliman (2018). Phylogenetic, toxigenic and virulence profiles of *Alternaria* species causing leaf blight of tomato in Egypt. Mycol. Prog., 17 (11): 1269-1282.

El-Khallal, S. M. (2007). Induction and modulation of resistance in tomato plants against *Fusarium* wilt disease by bioagent fungi (arbuscular mycorrhiza) and/or hormonal elicitors (jasmonic acid and salicylic acid): 2-changes in the antioxidant enzymes, phenolic compounds and pathogen related-proteins. Aust. J. Basic. Appl. Sci., 1(4):717-732.

Hahlbrock, K. and D. Scheel (1989). Physiology and molecular biology of phenylpropanoid metabolism. Annu. Rev. Plant Physiol. Plant Mol. Biol., 40: 347-369.

Hahlbrock, K. and, D. Scheel (1989). Physiology and Molecular Biology of Phenylpropanoid Metabolism. Annual Review of Plant Biology, 40: 347-369.

Hammerschmidt, R., E. M. Nuckles and J. Kuc (1982). Association of enhance activity with induced systematic resistance of cucumber to *Colletotricum lagenrium*. Physiol. plant pathol., 20:73-82.

Heil M. and R. M. Bostock (2002). Induced systemic resistance (ISR) against pathogens in the context of induced plant defences. Annals of Botany, 89(5): 503-512.

Johnson, R. D., L. Johnson, K. Kohmoto, H. Otani, CR. Lane and M. A. Kodama (2000). Polymerase Chain Reaction-Based Method to specifically detect *Alternaria alternata* apple pathotype (*A. mali*), the causal agent of Alternaria blotch of apple. Phytopathology, 90(9): 973-976.

Kępczyńska, E. and J. Kępczyńska (2005). Inhibitory effect of methyl jasmonate on development of phytopathogen *Alternaria alternata* (Fr.) Keissl. and its reversal by ethephon and ACC. Acta Physiol. Plant., 27: 491-496.

Kępczyńska, E. and P. Król (2012). The phytohormone methyl jasmonate as an activator of induced resistance against the necrotroph *Alternaria porri* f. sp. *solani* in tomato plants. Journal of Plant Interactions, 7(4): 307-315.

Konstantinova, P. P., J. M. Bonants, M. Van Gent-Pelzer, P. Van Der Zouwen and R. Van Der Bulk (2002). Development of specific primers for detection and identification of *Alternaria* spp. in carrot material by PCR and comparison with blotter and plating assays. Mycological Research, 106: 23-33.

Kordalewska, M., A. Brillowska-Dąbrowska, T. Jagielski and B. Dworecka-Kaszak (2015). PCR and real-time PCR assay to detect fungi of *Alternaria alternata* species. Acta Biochimica Polonica, 62(4): 707-712.

Lattanzio, V. M. and A Cardinali. (2006). Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. Phytochemistry: Advances in Research, 661(2): 23-67.

Maffei, M. E., A. Mithöfer and W. Boland (2007). Insects feeding on plants: Rapid signals and responses preceding the induction of phytochemical release. Phytochemistry, 68(22): 2946-2959.

Mayer, A. M., E. Harel and R. B. Shaul (1965). Assay of catechol oxidase a critical comparison of methods. Phytochemistry, 5:783-789.

Mirkova, E. and P. Konstantinova (2003). First report of *Alternaria* leaf spot on gerbera (*Gerbera jamesonii* H. Bolux ex J. D. Hook) in Bulgaria. Journal of Phytopathology, 151: 323-328.

Pan, L., X. Chen, W. Xu, S. Fan, T. Wan, J. Zhang and Y. Cai. (2022). Methyl jasmonate induces postharvest disease resistance to decay caused by *Alternaria alternata* in sweet cherry fruit. Scientia Horticulturae, 292: 110624.

Pozo, M. J., L. C. Van Loon and C. M. J. Pieterse (2005). Jasmonates signals in plant microbe interactions. J. Plant Growth Regul., 23: 211- 222.

Rivero Meza, S.L., de Castro Tobaruela, E., G. Benedetti Pascoal, I. Louro Massaretto and E. Purgatto (2021). Post-harvest treatment with methyl jasmonate impacts lipid metabolism in tomato pericarp (*Solanum lycopersicum* L. cv. Grape) at different ripening stages. Foods, 10: 877.

Sabbagh, E., S. K. Sabbagh, N. Panjehkeh, H. R. Bolok-Yazdi (2018). Jasmonic acid induced systemic resistance in infected cucumber by *Pythium aphanidermatum*. Journal of Agricultural Sciences, 24: 143-152.

Saikia, R., R. Kumar, T. Singh, A. K. Srivastava, D. K. Arora and M. Lee (2004). Induction of defense related enzymes and pathogenesis related proteins in *Pseudomonas fluorescens*- treated chickpea in response to infection by *Fusarium oxysporum* f. sp. *Ciceri*. Mycobiology, 32(1): 47-53.

SAS (1997). SAS/STAT User's Guide: Statistics, Version 6.12. SAS Institute Inc. Cary, NC., USA.

Schweizer, P., R. Gees and E. Mosinger (1993). Effect of jasmonic acid on the interaction of barley (*Hordeum vulgare* L.) with the powdery mildew *Erysiphe graminis* f.sp. *hordei*. Plant Physiology, 102(2): 503- 511.

Singh V., A. Shrivastava, S. JAdon, N. Wahi, A. Singh and N. Sharma (2015). Alternaria Diseases of vegetable crops and its management control to reduce the low production. International Journal of Agriculture Sciences, 7(13): 834-840.

Soleimani, M. J. and W. Kirk (2012). Enhance resistance to *Alternaria alternata* causing potato brown leaf spot disease by using some plant defense inducers. J. Pl. Prot., 52: 83-90.

Soleimani, M., M. Esmailzadeh and H. Rouhani (2008). Exogenous Applications of salicylic acid for inducing systemic acquired resistance against tomato stem canker disease. J. Biol. Sci., 8: 1039-1044.

Somma, S., G. Pose, A. Pardo, G. Mulè, V.F. Pinto, A. Moretti and A.F Logrieco (2011). AFLP variability, toxin production, and pathogenicity of *Alternaria* species from Argentinean tomato fruits and puree. Int. J. Food Microbiol., 145: 414-419.

Thipyapong, P., J. Melkonian, D. W. Wolfe and J. C. Steffens (2004). Suppression of polyphenol oxidases increases stress tolerance in tomato. Plant Sci., 167: 693-703.

Thipyapong, P., J.M. and J. Attajarusit (2007). Functional analysis of polyphenol oxidases by antisense/sense technology. Molecules, 12: 1569-1595.

Thomma, B. P. H. J., K. Eggermont, W. F. Broekaert and B. P. A. Cammue (2000). Disease development of several fungi on *Arabidopsis* can be reduced by treatment with methyl jasmonate. Plant Physiol. Biochem., 38(5): 421-427.

Thomma, B.P.H.J., K. Eggermont, K.F.M.J. Tierens and W.F. Broekaert (1999). Requirement of functional ethylene-insensitive 2 gene for efficient resistance of *Arabidopsis* to infection by *Botrytis cinerea*. Plant Physiol., 121: 1093-1101.

Tiwari, S., D. Pandey, M. Gaur and A. Kumar. (2017). Effect of methyl jasmonate on disease severity and expression of plant defensin gene during *Alternaria brassicae* infection in Arabidopsis. Int. J. Curr. Microbiol. App. Sci, 6(7): 857-865.

Uzuner, S. Ç. (2018). Development of a direct trypan blue exclusion method to detect cell viability of adherent cells into ELISA plates. Celal Bayar University Journal of Science (CBUJOS), 14(1): 99-104.

Vakalounakis, D. J. (1988). Cultivar reactions and the genetic basis of resistance to Alternaria stem canker (*Alternaria alternata* f.sp. *lycopersici*) in tomato. Plant Pathology, 37: 373-376.

Van der Waals, J. E., L. Korsten, T. A. S. Aveling (2001). A review of early blight of potato. Afr. Plant Protect., 7: 91-102.

Van Loon, L. C., M. Rep and, C. M. J. Pieterse (2006). Significance of inducible defense-related proteins in infected plants. Annu Rev Phytopath., 44: 135-162.

Waheed, A. and A. Tehmina (2011). Quantitative changes in defense system of tomato induced by two strains of *Bacillus* against *Fusarium* Wilt. Ind. J. Fund. Appl. Life Sci. 1: 2231-6345.

Wang, K. T., P. Jin, L. Han, H. T. Shang, S. S. Tang, H. J. Rui, Y. F. Duan, F. Y. Kong, X. Kai and Y. H. Zheng (2014). Methyl jasmonate induces resistance against *Penicillium citrinum* in Chinese bayberry by priming of defense responses. Postharvest Biol. Technol., 98: 90-97.

Wittstock, U. and J. Gershenzon (2002). Constitutive plant toxins and their role in defense against herbivores and pathogens. Current Opinion in Plant Biology, 5: 300-307

Yang, L. N., M. H. He And H. B. Ouyang (2019). Cross-resistance of the pathogenic fungus *Alternaria alternata* to fungicides with different modes of action. BMC Microbiol., 19: 205.

Yao, H. J. and S. P. Tian (2005). Effects of a biocontrol agent and methyl jasmonate on postharvest diseases of peach fruit and the possible mechanisms involved. J. Appl. Microbiol., 98: 941-950.

Zhang, S., W. Miao, Y. Liu, J. Jiang, S. Chen, F. Chen and Z. Guan (2023) Jasmonate signaling drives defense responses against *Alternaria alternata* in chrysanthemum. BMC Genomics, 24:553.

Zhu, Z. and S. Tian (2012). Resistant responses of tomato fruit treated with exogenous methyl jasmonate to Botrytis cinerea infection. Sci. Hortic., 142: 38-43.

J. Agric. & Env. Sci. (Damanhour University) 2024, 23(1): 1-24
Print: ISSN 1687-1464 Online: 2735-5098

تأثير إستخدام الميثيل جاسمونات في مكافحة فطر الألترناريا الترناتا وتحفيز آليات الدفاع في نبات الطماطم

غنيم محمد عامر ، سوسن صلاح الشامي ، محمود حلمي غزلان

قسم أمراض النبات ، كلية الزراعة – جامعة دمنهور ، دمنهور ، محافظة البحيرة ، مصر

تهدف هذه الدراسه إلي تقييم تأثير الميثيل جاسمونات فى مكافحة مرض تبقع الأوراق في الطماطم المتسبب عن فطر الالترناريا الترناتا. تم إجراء تجارب معملية وفي الصوبة لتقدير فاعلية الميثيل جاسمونات كمضاد فطري من خلال دوره في تنشيط الانزيمات الدفاعية في النبات. وقد أوضحت النتائج ان الميثيل جاسمونات بتركيزات ٥٠ و١٠٠ و١٥٠ ميكروليتر/لتر أدي الي تثبيط نمو ميسيليوم الفطر وكذلك تثبيط نمو الجراثيم معمليا مقارنه بالكنترول. ولوحظ أعلي معدل تثبيط عند التركيز ١٥٠ ميكروليتر/لتر. كذلك فإن تركيز ١٥٠ ميكروليتر/لتر من الميثيل جاسمونات كان الأكثر تأثيرا في خفض معدل الاصابة بتبقعات اوراق الطماطم بالاضافه الى ان المعامله بالميثيل جاسمونات ادت الى زياده نشاط الانزيمات الدفاعيه ومنها البيروكسيديز والبولى فينيل اوكسيديز ومحتوى الفنيولات الكلى مقارنه بالنباتات الغير معاملة. هذه الدراسه تقترح استخدام الميثيل جاسمونات كمادة صديقة للبيئة للتحكم فى مرض تبقع الاوراق الناتج عن الاصابة بفطر الالترناريا الترناتا على الطماطم.

الكلمات الدلالة: الالترناريا الترناتا ,الميثيل جاسمونات,الطماطم ,الانزيمات الدفاعيه.