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Effect of Peroxyacetic Acid (PAA) On Controlling Maize Late Wilt Disease Caused by *Magnaporthiopses maydis*

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ABSTRACT

Late-wilt disease of maize caused by *Magnaporthiopsis maydis* is the most serious disease of duet corn in many parts of the world. A strong oxidizing compound, peroxyacetic acid (PAA) as mixed of acetic acid (AA) and hydrogen peroxide (H_2O_2), gave a promising control approach against late wilt disease. All treatments of acetic acid (AA), H_2O_2 and peroxyacetic acid (PAA) at different concentrations reduced the linear growth of *M. maydis* fungus compared with control *in vitro*. 0.1 AA + 4.0 H_2O_2 m/L and 0.2 AA + 4.0 H_2O_2 m/L were the most effective treatments in reducing *M. maydis* linear growth which showed the highest growth reduction, while 0.1 AA m/L was the lowest effective treatment. All maize grain coated with AA, H_2O_2 and PAA alone or in combinations reduced the disease infection of maize late wilt compared with infected control *in vivo*. Treated by 0.2 AA+4.0 H_2O_2 m/L and 0.1 AA+4.0 H_2O_2 m/L were the most efficient treatments in reducing disease incidence, while 0.1 AA m/L and 2.0 H_2O_2 m/L were the lowest effective treatment in this respect. In field trails, treated grains with all PAA combinations, some growth and yield parameters increased (plant length, stem diameter, weight of the ear, length of ear and weight of 100 grains) and late wilt disease was significantly decreased, of maize plant compared with control under field condition during 2021/2022 and 2022/2023 growing seasons. 0.1 AA+4.0 H_2O_2 m/L and 0.2 AA+4.0 H_2O_2 m/L were the most effective treatments for decreasing late wilt disease incidence and increasing disease reduction, growth parameters and yield component during the two-growing season. On the other hand, 0.1 AA+1.0 H_2O_2 m/L was the least effective treatment against late wilt disease incidence and growth parameters in both seasons. The effect of peroxyacetic acid on the oxidative enzymes in leaves of maize plants was evaluated. All PAA combinations resulted an increase in peroxidase and polyphenol oxidase activity. The highest increase in peroxidase and polyphenol oxidase activity was recorded due to treated grain maize by 0.2AA+4.0 H_2O_2 m/L and 0.1AA+4.0 H_2O_2 m/L compared to uninfected and infected control. Meanwhile, 0.1 AA + 1.0 H_2O_2 m/L was the lowest treatment in peroxidase and polyphenol oxidase activity.

Keywords: Peroxyacetic acid, maize late wilt, *Cephalosporium maydis, Magnaporthiopsis maydis*. growth parameters, oxidative enzymes

1. Introduction

After rice and wheat, maize is regarded as the third most important food plant. It is crucial that we look for ways to maximize food plant productivity and reduce yield losses. In many regions of the world, late-wilt disease of maize is generally one of the most dangerous diseases of the corn. It was detected for the first time in Egypt by Samra *et al.* (1962). *Cephalosporium maydis* mostly infects plants through their roots and is thought to be a soil-borne pathogen (Sabet *et al.*, 1966b) or by infected grains. As a new genus in the Magnaporthaceae (Ascomycota) family, Magnaporthiopsis was introduced by Luo and Zhang in 2013. Ultimately, *Magnaporthiopsis maydis* replaces *C. maydis* in the classification (Klaubauf *et al.,* 2014) synonymous: *Harpophora maydis* (Games, 2000) and one of the most major diseases affecting maize in Egypt is *Cephalosporium maydis* (Samra *et al.,* 1963) (Sabet *et al.,* 1966b; Ali, 2000 and Saleh & Leslie, 2004). Furthermore, the Egyptian isolates of *C. maydis* differ in terms of their genetic profile, morphological characters, and capacity for infection (Saleh *et al.,* 2003).

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The pathogen reduced grain yield of infected maize plants by about 37% (Abdel-Rahim, 1971). Moreover, naturally infested crops may have yield losses of up to 40% and infection rates of up to 80% (El-Shafey and Clafline, 1999). El-Shafey and Claflin (1999) describe late wilt as a fast wilting of the lower leaves that occurs during tasseling and develops to hollow and shrunken stalks with a dark yellow-to-brown or black-stained pith. Primarily a soil-borne fungus, the pathogen can penetrate root tissue and colonize in the xylem (Sabet *et al.,* 1970). The best way to combat this disease is through breeding resistant varieties of maize (El-Shafey *et al.,* 1988). Numerous attempts were crafted to use chemical and biological methods to control the pathogen (El-Mehalowy *et al.,* 2004; Ashour *et al.,* 2013; El-Moghazy *et al.,* 2017 and Elshahawy and El-Sayed, 2018).

Hydrogen peroxide (H_2O_2) and acetic acid are combined to make peroxyacetic acid (PAA). One biodegradable product is PAA (Acetic acid, hydrogen peroxide, water, and oxygen are the degradation products), very effective biocide, not pollutant during manufacturing process, it meets all the requirements of green chemistry because it is non-toxic to the environment after usage (Carrasco and Urrestarazu, 2010; Ayoub *et al.,* 2017; Jo *et al.,* 2019 and Kakade *et al.,* 2020). Apart from its remarkable antimicrobial activity (Baldry, 1983). PAA has demonstrated efficacy in numerous applications in agriculture such as protecting the surface of fruits and vegetables and increasing their postharvest life (Alvaro *et al.,* 2009). 412 Listeria monocytogenes populations in lettuce were decreased by PAA (Beuchat *et al.,* 2004), reduced tomato bacterial wilt (Hong *et al.,* 2018), and demonstrated antimicrobial effectiveness against fungal sheath blight and bacterial leaf blight in rice (Jo *et al.,* 2019). Furthermore, according to Carrasco *et al.,* (2011), PAA increased the growth and yield of watercress plants without producing any harmful compounds during its breakdown. Because peroxyacetic acid is a strong oxidant, biodegradable, low-toxicity, and has no residual toxicity in the ecosystem, its application is gaining attention. Hence, PAA, which possesses broad-spectrum antimicrobial properties was applied in the current research.

The application of H_2O_2 produces no residues since it decomposes to water and oxygen (Afek *et al.,* 1999). Strong oxidizers like H2O2 are formed when water in the atmosphere mixes with ozone (Slesak *et al.,* 2007). An organic acid and H_2O_2 are mixed in an activated peroxyacetic acid disinfection (most commonly acetic acid), it functions as a "activator" to create peroxyacetic acid (Fig.1) so that the H_2O_2 and peroxyacetic acid in these disinfectants are in solution.

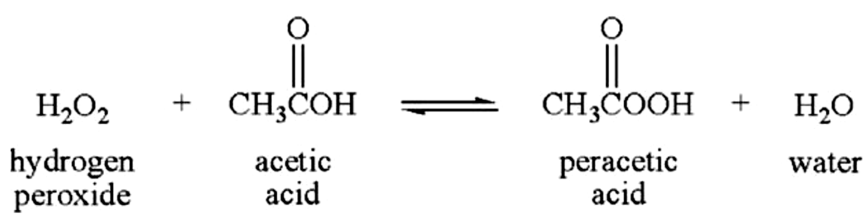

Fig. 1: Production of peracetic acid (Buschmann and Del Negro 2012).

Hydrogen peroxide (H_2O_2)-mediated oxidative burst has been identified as a crucial element of the plant defense mechanism in the event of an incompatible contact. Elevated levels of H_2O_2 also activated the expression of many genes that provide protection against fungus and bacteria pathogens (Kachroo *et al.,* 2003). H2O2 plays a role in numerous resistance mechanesms, which includes phytoalexin production, plant cell wall fortification, and enhanced resistance to various stresses (Shetty *et al.,* 2007; Quan *et al.,* 2008). According to Kang *et al.,* (2003) and Osório *et al.,* (2013), acetic acid has demonstrated fungicidal and bactericidal effects on many kinds of phytopathogenic fungi and bacteria. Reactive oxygen species (ROS) and antioxidants have demonstrated an important role in plant-pathogen interactions (Sutherland, 1991; Levine *et al.,* 1994; Galal, 2017 and EL-Ashmony *et al.,* 2017). According to Abdulrazek *et al.,* (2022) and Warren *et al.,* (2023), acetic acid and hydrogen peroxide both functioned as bactericides and fungicides against a variety of phytopathogenic bacteria and fungi.

2. Material and Methods

2.1. Pathogen 1.

Diseased corn plants showed typical late wilt symptoms grown in the open fields were collected from, El-Minia Governorate, Egypt during 2020 growing season. The collected specimens were washed gently with tap water, blotted between two sterilized filter papers after being surface sterilized for 4 minutes using a 0.25% sodium hypochlorite solution and repeatedly washed in sterilized distilled water. These pieces were grown for three to seven days at 28±2°C on potato dextrose agar medium supplemented with 0.1% yeast extract (PDAY). According to Booth (1977), the hyphal tip technique was used to purify the cultures, examined under a microscope and kept for further experiments on PDAY slants at 4 oC using the Khalifa (1991) method. Barnett (1960) and Singh (1982) identified this pathogenic isolate as *Cephalosporium maydis*. The pathogenicity of *M. maydis*, the method of soil infestation,and the preparation of the inoculums were followed by the instructions of El-Shafey et al.'s (1988) at Dept. of Maize, Sugar and Foliages crop diseases, Plant Pathology Research Institute, Giza, Egypt.

2.2. Effect of peroxyacetic acid on *M. maydis* linier growth *in vitro*

2.2.1. Preparation of test solutions

Before testing, stock solutions of two mixtures containing hydrogen peroxide (H2O2) and acetic acid (AA) were made with distilled water and kept for at least ten days (EPA, 1993 and 2004). Different concentrations of AA and H_2O_2 individually or in combination at different concentrations, *i.e.* 0.1 AA + 1.0 H_2O_2 m/L, 0.1 AA + 2.0 H_2O_2 m/L, 0.1 AA + 4.0 H_2O_2 m/L, 0.2 AA + 1.0 H_2O_2 m/L, 0.2 AA + 2.0 H_2O_2 m/L and 0.2 AA + 4.0 H_2O_2 m/L, were ready to assess the late-wilt disease caused by *Magnaporthiopsis maydis* both in vitro and in vivo (Buschmann and Del Negro, 2012; Anonymous, 2012). Each of prepared chemical solutions of acetic acid (AA) and hydrogen peroxide (H_2O_2) individually or in combination at different concentrations was added to conical flasks 125 mL containing sterilized PDAY medium (100 ml), before solidification to obtain the desired concentration and mixed thoroughly, After that, it was put into petri dishes (20 ml medium/plate) and let solidify. Plates containing untreated PDAY was used as control (Abdou and Galal, 1997). For every treatment, three plates were used. The desired isolate of *M. maydis* 8-day-old cultures was injected into each plate using a disc with a diameter of 0.5 cm. The plates were then incubated at 28±2°C until the control plates reached complete growth. After incubation period of 8 days at 28±2°C the plates were inspected, and the following formula was used to assess the linear growth of each treatment in order to estimate the reduction in linear growth in accordance with Yeh and Sinclair (1980):

$$G = C\text{-}T/C \times 100$$

Where:
G = Percentage of fungal growth reduction.
C = Fungal growth in the control (Pathogen alone)
T = Fungal growth in the treatment (Pathogen with the tested chemical).

2.3. Effect of peroxyacetic acid on controlling maize late wilt disease *in vivo*

2.3.1. Greenhouse conditions

A pot experiment was conducted to study the effect of grain coatings at the examined concentrations of AA, H_2O_2, and all AA and H_2O_2 combinations against *M. maydis*, the casual organism of maize late wilt.

2.3.1.1. Inoculum preparation

Water was used to soak 100 grams of sorghum grain seeds in a 500-ml glass glucose bottle for the entire night and after that, the excess water was decanted before an hour-long autoclave. To test for soil infestation, one piece from *M. maydis* 8-days old cultures grown on PDAY (grown on PDA + 0.2% yeast extract at 28ºC) was placed in each bottle and incubated for fifteen days at 28oC±2 (Sabet *et al.,* 1966; El-Shabrawy and Shehata., 2018; El-Gazzar *et al.,* 2018).

2.3.1.2. Soil infestation

Magnaporthiopsis maydis inoculum was added to the autoclaved clay loam soil at a rate of 5%, and the mixture was well mixed (Samra *et al.,* 1966). Sterilized pots (30-cmdiameter) were filled with infested soil, and irrigated and left for a week before sowing. However, sorghum grains that have been autoclaved and sterilized were thoroughly mixed with soil as control treatment. Using a 2.5% sodium hypochlorite solution, maize grains (Boushy cv.) were surface sterilized for three minutes, after which they were washed for five minutes in distilled water that had been sterilized. In every pots, ten grains were planted. For every treatment, three pots were utilized as a replicate.

2. Field experiment

The purpose of this study was to evaluate the effectiveness of H_2O_2-based compound peroxyacetic acid (PAA) at different concentrations (0.1 AA + 1.0 H_2O_2 m/L, 0.1 AA + 2.0 H_2O_2 m/L, 0.1 AA + 4.0 H_2O_2 m/L, 0.2 AA + 1.0 H_2O_2 m/L, 0.2 AA + 2.0 H_2O_2 m/L and 0.2 AA + 4.0 H_2O_2 m/L) against *M. maydis* which caused late-wilt disease in maize plants under field conditions in disease nursery at Mallawy Research Stations, ARC during two growing seasons of 2021/2022 and 2022/2023. In this study, maize seeds of Boushy CV, which are susceptible to *M. maydis*, were used. PAA with their six varied concentrations were used as seed coating treatment, the check treatment was using untreated seeds.

2.4. Disease assessment

Sabet *et al.,* (1966a) reported disease incidence as the proportion of infection 90 days after sowing. There is now only one plant per hill and a space of 25 cm between hills. El-Shafey *et al.,* (1988) documented the disease incidence levels of the surviving plants as a percentage of infected plants 35 days after silking as follow:

$$\textit{Disease incidence (DI \%)} = \frac{\textit{No. of infected plants}}{\textit{No. of total plants}} \times 100$$

Utilizing the following formula, disease incidence data were reused to calculate percentages of disease reduction for each treatment.

$$\textit{Reduction \%} = \frac{\textit{DI \% of control} - \textit{DI \% of treatment}}{\textit{DI \% of control}} \times 100$$

However, some growth and yield parameters, including plant length, stem diameter, weight of 100 grains, weight of the ear and length of ear were determined.

The following formula was used to determine each treatment's effectiveness on grain yield productivity relative to control plants, according El-Assiuty *et al.,* (1986):

$$\textit{Efficacy \%} = \frac{\textit{Yield of treatment} - \textit{Yield of control}}{\textit{Yield of control}} \times 100$$

2.5. Effect of peroxyacetic acid on the oxidative enzymes in maize plants

The effect of peroxyacetic acid (PAA) at different concentrations against the organism causing maize late wilt, *M. maydis*, after 45 days from replication, the activity of defense enzymes including peroxidase and polyphenoloxidase was measured in maize leaves (Boushy cv.), as previously mentioned (Sathiyabama and Manikandan, 2016).

2.5.1. Enzymes extraction and bioassay

Using 0.1 M sodium phosphate buffer at pH 7.1, 1g of maize leaf tissues from each treatment were individually homogenized in a mortar for 1 minute at a rate of 2 ml/g fresh weight leaves. After the tissues had been triturated, they were filtered through four layers of cheesecloth, and the filtrates were centrifuged for 15 minutes at 6 ^{o}C at 3000 rpm.

2.5.1.1. Polyphenoloxidase activity

The activity of polyphenoloxidase was measured in accordance with Esterbaner *et al.,* (1977). One milliliter of enzyme extract, one milliliter of 0.2 M sodium phosphate buffer at pH 7.0, and one milliliter of catechol made up the reaction mixture. With distilled water, the reaction mixture was reduced to a final volume of 6.0 ml. The activity of polyphenoloxidase was measured as a change in absorbance/min at 495 nm.

2.5.1.2. Peroxidase activity

According to Allam and Hollis (1972), the oxidation of pyrogallol to pyrogallin in the presence of H_2O_2 was used for measuring peroxidase activity at 425 nm. The reaction mixture included 0.3 mL of pyrogallol, 0.5 mL of enzyme extract, 0.5 mL of 0.1 M sodium phosphate buffer at pH 7.0, and 0.1 mL of 1.0% H2O2 distilled to a final volume of 3.0 mL.

2.6. Statistical analysis

Using the SPSS program version 15, the data were statistically analyzed for analysis of variance (ANOVA). In the present study, a complete randomized design was utilized. Least significant differences (LSD).

3. Results

3.1. Effect of peroxyacetic acid on *M. maydis* linier growth *in vitro*

Data shown in Table (1), demonstrate that all treatments of acetic acid, H_2O_2 and peroxyacetic acid at different concentrations decreased the linear growth of *M. maydis* fungus compared with control. Increasing the concentration of AA or H_2O_2 generally increases the inhibitory effect on linear growth. The highest inhibitory effect in fungus growth was exhibited at 0.1 AA + 4.0 H_2O_2 m/L followed by 0.2 AA + 4.0 H_2O_2 m/L being (81.11% and 76.89%) growth reduction, respectively. Meanwhile, 0.1 AA m/L recorded the lowest inhibitory effect in linear growth being 23.11% growth reduction.

Table 1: Effect of acetic acid, H_2O_2 and peroxyacetic acid at different concentrations against *M. maydis* linier growth *in vitro*.

Treatments	Linear growth after 8 days	Growth reduction (%)
0.1 AA	6.92	23.11
0.2 AA	5.17	42.56
1.0 H_2O_2	5.72	36.45
2.0 H_2O_2	5.68	36.89
4.0 H_2O_2	5.11	43.23
0.1 AA + 1.0 H_2O_2	4.80	46.67
0.1 AA + 2.0 H_2O_2	4.86	46.0
0.1 AA + 4.0 H_2O_2	1.70	81.11
0.2 AA + 1.0 H_2O_2	4.07	54.78
0.2 AA + 2.0 H_2O_2	4.08	54.67
0.2 AA + 4.0 H_2O_2	2.08	76.89
Infected control	9.0	-
LSD 0.05	0.495	

3.2. Effect of peroxyacetic acid on controlling maize late wilt disease *in vivo*

3.2.1. Greenhouse conditions

The efficiency of tested chemical i.e., AA, H_2O_2 and AA + H_2O_2 combination at different concentrations as grain coated against *M. maydis* was evaluated under greenhouse condition.

When compared to an infected control under artificial infestation, Table (2)'s results show that all grain treatments with all AA and H2O2 combinations significantly decreased the disease infection percentage of late wilt.

The best treatments for decreasing disease incidence were 0.2 AA+4.0 H_2O_2 m/Land 0.1 AA+4.0 H_2O_2 m/L being (100% and 100%) disease reduction, respectively followed by 0.2 AA+2.0 H_2O_2 m/L, 0.2 AA+1.0 H_2O_2 m/L and 0.1 AA+2.0 H_2O_2 m/L (92.38%, 84.61% and 84.61%) disease reduction, respectively. While the lowest effective treatment was 0.1 AA m/L being (46.15%) disease reductions.

Table 2: Effect of peroxyacetic acid on maize late wilt disease under greenhouse conditions.

Treatments	Disease infection %	Disease reduction %
0.1 AA	46.66	46.15
0.2 AA	26.66	69.23
1.0 H_2O_2	40	53.84
2.0 H_2O_2	33.33	61.53
4.0 H_2O_2	26.66	69.23
0.1 AA + 1.0 H_2O_2	20	76.92
0.1 AA + 2.0 H_2O_2	13.33	84.61
0.1 AA + 4.0 H_2O_2	0.00	100
0.2 AA + 1.0 H_2O_2	13.33	84.61
0.2 AA + 2.0 H_2O_2	6.60	92.38
0.2 AA + 4.0 H_2O_2	0.00	100
Infected control	86.66	-
LSD 0.05	18.63	

3.3. Effect of peroxyacetic acid on controlling maize late wilt disease under field condition.

3.3.1. Field experiments

The influence of AA + H_2O_2 combination at different concentrations against maize late wilt disease incidence, some growth parameters and yield were studied under field condition.

Data shown in Table (3) indicates that grain treatment with all AA and H_2O_2 combinations significantly reduced late wilt infection in maize when compared with check plants which recorded (35.02% and 39.01%) disease incidence at the first season (2022) and the second season (2023), respectively. It has been noted that the 2023 season had a higher disease incidence than the 2022 season. The disease reduction was increased by increasing concentrations of AA and H_2O_2. The 0.1 AA+4.0 H_2O_2 m/L and 0.2 AA+4.0 H_2O_2 m/L were the most effective treatments in decreasing late wilt disease infection being (1.4% and 1.39%) and (1.46% and 1.4%) disease incidence percentage at the first and the second season, respectively. Conversely though, 0.1 AA+1.0 H_2O_2 m/L was the lowest treatment in reducing disease incidence percentage being (6.22% and 9.09%) throughout the two growing seasons, respectively.

Table 3: Effect of treated maize grains with PAA at different concentrations on late wilt disease incidence at disease nursery, Mallawy under field condition during 2021/2022 and 2022/2023 growing seasons.

Treatments	2021/2022		2022/2023	
	Disease Incidence %	Disease reduction %	Disease Incidence %	Disease reduction %
0.1 AA + 1.0 H_2O_2	6.22	82.0	9.09	76.7
0.1 AA + 2.0 H_2O_2	4.86	86.1	6.43	83.5
0.1 AA + 4.0 H_2O_2	1.4	96.0	1.39	96.4
0.2 AA + 1.0 H_2O_2	4.96	85.8	6.33	83.8
0.2 AA + 2.0 H_2O_2	3.64	89.6	4.17	89.3
0.2 AA + 4.0 H_2O_2	1.46	95.8	1.4	96.4
Control	35.02	-	39.01	-
LSD 0.05	3.746		4.835	

Data demonstrated in Table (4 and 5) show that all grain treatments of AA + H_2O_2 combinations at different concentrations were effective for increasing growth and yield parameters (Plant length, stem diameter, weight of the ear, length of ear and weight of 100 grains), of maize plant under field condition during 2021/2022 and 2022/2023 growing seasons.

Obtained data indicate that 0.2 AA + 4.0 H_2O_2 m/L and 0.1 AA + 4.0 H_2O_2 m/L were the most effective treatment for increasing plant length, stem diameter, weight of the ear and length of ear being (247.3, 6.3, 354.6 & 20.8), (247.3, 6.3, 326.7 & 19.6) and (252.7, 6.5, 247.1 &17.4), (250, 6.2, 243.1 & 17.0) at the first and the second seasons, respectively. On the other hand, 0.1 AA + 1.0 H_2O_2 m/L showed the lowest effect treatment for increasing plant length, stem diameter, weight of the ear and length of ear being (243, 5.4, 273.7 & 17.1) and ((243.7, 5.2, 192 & 14.5) during the two growing seasons, respectively compared with control which recorded (229.7, 4.13, 259.5 & 16.6) and (227.7, 4.0, 172.6 & 13.5) of plant length, stem diameter, weight of the ear and length of ear in both growing seasons, respectively.

Table 4: Effect of treated maize grains with PAA at different concentrations on plants growth parameters and yield component at Mallawy setation under field condition during 2021/2022 growing season.

Treatments	Plant length(cm)	Stem diameter(cm)	Ear weight(g)	Ear length(cm)	100 grain weight (g)	Efficacy %
0.1 AA + 1.0 H_2O_2	243	5.4	273.7	17.1	36.1	3.7
0.1 AA + 2.0 H_2O_2	246.3	5.5	289.9	17.6	37.6	8.0
0.1 AA + 4.0 H_2O_2	247.3	6.3	326.7	19.6	40.4	16.1
0.2 AA + 1.0 H_2O_2	241	5.9	300.6	17.8	38.5	10.6
0.2 AA + 2.0 H_2O_2	245.3	6.1	316.1	19.1	39.7	14.1
0.2 AA + 4.0 H_2O_2	247.3	6.3	354.6	20.8	42.0	20.6
Control	229.7	4.13	259.5	16.6	34.8	-
LSD 0.05	3.244	0.30	18.73	1.102	1.702	

Table 5: Effect of treated maize grains with PAA at different concentrations on plants growth parameters and yield component at Mallawy station under field condition during 2022/2023 growing season.

Treatments	Plant length (cm)	Stem diameter (cm)	Ear weight (g)	Ear length (cm)	100 grain weight (g)	Efficacy %
0.1 AA + 1.0 H_2O_2	243.7	5.2	192.0	14.5	33.5	2.4
0.1 AA + 2.0 H_2O_2	244.7	5.5	211.0	15.7	34.6	5.8
0.1 AA + 4.0 H_2O_2	250.0	6.2	243.1	17	37.7	15.2
0.2 AA + 1.0 H_2O_2	244.0	5.7	215.5	16.5	36	10.1
0.2 AA + 2.0 H_2O_2	246.3	6.1	228.1	17	37	13.1
0.2 AA + 4.0 H_2O_2	252.7	6.5	247.1	17.4	39.7	21.4
Control	227.7	4.0	172.6	13.5	32.7	-
LSD 0.05	6.705	0.196	16.27	0.977	1.513	

Also, the results indicated that 0.2 AA + 4.0 H_2O_2 m/L and 0.1 AA + 4.0 H_2O_2 m/L were more effective treatment where they recorded the highest increase in 100 grains weight being (42.0 & 40.4 gm) and (39.7 & 37.7 gm) with high efficacy (20.6% & 16.1%) and (21.4% & 15.2%) in both growing seasons, respectively. However, 0.1 AA + 1.0 H_2O_2 m/L was the lowest effective treatment in this respect being (36.1 & 33.5gm) in 100 grains weight with lower efficacy being (3.7% & 2.4%) during the two growing seasons, respectively.

3.3.2. Effect of peroxyacetic acid on the oxidative enzymes in maize plants

The effect of various concentrations of peroxyacetic acid against *M. maydis* on the activity of polyphenoloxidase and peroxidase was assessed in maize leaves (Boushy cv.).

Data in Table (6) present that all AA and H_2O_2 combinations resulted an increase in peroxidase and polyphenol oxidase activity in leaves of maize plants compared with uninfected and infected control. The highest increase in peroxidase and polyphenol oxidase activity was recorded due to treated grain maize with 0.2AA + 4.0. H_2O_2 m/L followed by 0.1AA + 4.0 H_2O_2 m/L treatments, being (1.049 and 1.032) and (0.704 and 0.633) in both enzymes, respectively. In the meantime, 0.1 AA + 1.0 H_2O_2 m/L was noted the lowest increase in peroxidase and polyphenol oxidase activity being (0.793 and 0.486) in the two enzymes, respectively.

Table 6: Effect of grain treatment with peroxyacetic acid in the presence of *M. maydis* on peroxidase, polyphenol oxidase activities (min/g fresh weight) in maize leaves at 45 days after application.

Treatments	**Enzymes activity (min/g fresh weight of maize leaves)**	
	Peroxidase	**Polyphenol-oxidase**
0.1 AA + 1.0 H_2O_2	0.793	0.486
0.1 AA + 2.0 H_2O_2	0.806	0.519
0.1 AA + 4.0 H_2O_2	1.032	0.633
0.2 AA + 1.0 H_2O_2	0.83	0.512
0.2 AA + 2.0 H_2O_2	0.916	0.537
0.2 AA + 4.0 H_2O_2	1.049	0.704
Infected control	0.654	0.336
Uninfected control	0.504	0.291
LSD 0.05	0.045	0.017

4. Discussion

One of the most significant fungal diseases affecting Egypt's corn crop in terms of economic impact is late wilt disease, which is brought on by *M. maydis*, when sensitive maize cultivars were sown in extensively infested fields with yield losses approaching 40% and infection rates reaching 80–100% (El-Hosary *et al.,* 2015). The best way for controlling this disease is through breeding resistant varieties of maize (El-Shafey *et al.,* 1988). According to El-Hosary *et al.,* (2015), non-chemical control methods are being employed in this work for environmental safety because the pathogen's control is still a significant concern for farmers and Egyptian researchers are paying close attention to it. The use of pesticides to combat plant diseases has created a number of environmental risks in recent years. Chemical and biological methods were used in many attempts to increase resistance to the disease (Abdel-Hamid *et al.,* 1981; Singh and Siradhana, 1989; El-Mehalowy *et al.,* 2004; and Ashour *et al.,* 2013).

In this study, all treatments of acetic acid, H_2O_2 and peroxyacetic acid at different concentrations reduced the linear growth of *M. maydis* fungus compared with control *in vitro*. 0.1 AA + 4.0 H_2O_2 m/L was the best effective treatment, and it was followed by 0.2 AA + 4.0 H_2O_2 m/L in reducing *M. maydis* linear growth and resulted the highest growth reduction, while 0.1 AA was the lowest effective treatment on the fungus linear growth and growth reduction. These results were confirmed against a number of phytopathogenic fungi (Mari *et al.,* 2004; Abd-Alla *et al.,* 2011, Feliziani *et al.,* 2016; Ayoub *et al.,* 2017; El-Ashmony *et al.,* 2017; Galal, 2017& 2018; Tantawy, *et al.,* 2020; Abdelrazek *et al.,* 2022 and Warren *et al.,* 2023). Using the disc assay method, it was possible to determine the sensitivity of *Botrytis cinerea* hyphae and conidia to PAA by looking for a zone of inhibition (Narciso *et al.,* 2007). Additionally, according to Mari *et al.,* (2004) and Elbouchtaoui *et al.,* (2015), PAA significantly reduced the growth of *Monilinia laxa, Rhizopus stolonifer, Penicillium digitatum,* and *Botrytis cinerea.*

In the recent study under greenhouse, all maize grain coated with AA, H_2O_2 and AA + H_2O_2 alone or in combinations significantly decreased the disease infection percentage of *M. maydis* late wilt disease compared with infected control *in vivo*. The most effective treatments in decreasing disease incidence were 0.2 AA+4.0 H_2O_2 m/L and 0.1 AA+4.0 H_2O_2 m/L, whereas the least effective treatment in this respect was 0.1 AA m/L. The study's findings support the conclusions noted by Ahammed *et al.* (2018a, b) and Mostofa *et al.,* 2018) who show that exogenous chemicals, including signaling moleculesmay play an important role in enhancing plant resilience to the adverse effects of ever-changing environmental impacts (Ahammed *et al.,* 2018a, b; Mostofa *et al.,* 2018). Reactive oxygen species (ROS) that plants produce as a messenger molecule for adaptive signaling include hydrogen peroxide (H_2O_2), which initiates tolerance against a range of biotic and abiotic conditions (Sutherland, 1991; Levine *et al.,* 1994; Galal, 2017; EL-Ashmony *et al.,* 2017 and Younes *et al.,* 2019). It plays a key role against O_2 derived cell toxicity (Hasanuzzaman *et al.,* 2020). H_2O_2 participates in an array of resistance mechanisms, such as phytoalexin production, plant cell wall reinforcement, and improved resistance to different stresses (Shetty *et al.,* 2007; Quan *et al.,* 2008. According to Kang *et al.,* (2003) and Osório *et al.,* (2013), acetic acid demonstrated fungicidal and bactericidal effects on a variety of phytopathogenic fungi and bacteria. It is known that the PAA mixture has antifungal activity against many kinds of plant diseases caused by either phytopathogenic fungi or plant pathogenic fungi (Hopkins *et al.,* 2003; Pukdee and Sardsud, 2007; Buschmann and Del Negro, 2012; Elbouchtaoui *et al.,* 2015; El-Ashmony *et al.,* 2017; Tantawy *et al.,* 2020; Abdelrazek *et al.,* 2022 and Warren *et al.,* 2023) or phytopathogenic bacteria (Hong *et al.,* 2018).

The obtained results in field trial reveal that, grain treatment with all AA + H_2O_2 combinations at different concentrations significantly reduced maize infection with late wilt disease and increased some growth and yield parameters (Plant length, stem diameter, weight of the ear, length of ear and weight of 100 grains), of maize plant compared with control under field condition during 2021/2022 and 2022/2023 growing seasons. The disease reduction and growth parameters were increased by increasing concentrations of AA and H_2O_2. The 0.1 AA+4.0 H_2O_2 m/L and 0.2 AA+4.0 H_2O_2 m/L were the most effective treatments for decreasing late wilt disease incidence, growth and yield parameters during the two-growing season. Conversely, though, 0.1 AA+1.0 H_2O_2 m/L was the lowest effective treatment. These results match those that Buschmann and Del Negro (2012) reported who maintained that the antifungal effects for PAA which resulted from mixed AA + H_2O_2 against *Magnaporthiopsis maydis* late-wilt disease and several pathogenic fungi (Thipaksorn *et al.,* 2012, Ayoub *et al.,* 2017; Tantawy *et al.,* 2020 and Abdelrazek *et al.,* 2022). The biocide capacity of H_2O_2-based compounds on plant pathogens was reported (Wisniewsky *et al.,* 2000; Gurtler *et al.,* 2014). Prior researches revealed the function of PAA in lowering the numbers of microbial spores on fruit surfaces, disinfestation of bulbs and prevention of other horticultural diseases (Narciso *et al.,* 2007 and Alvaro *et al.,* 2009). H_2O_2 treatment at lower level can significantly impact plant growth, growth regulators, antioxidant enzyme activity, fruit yield, and tomato quality (Orabi *et al.,* 2015). In accordance with these results, PAA increases the production and increases the height and length of the watercress plants (Carrasco *et al.,* 2011). In a second study, for older plants and those cultivated on solid substrates, PAA was less toxic (Vines *et al.,* 2003). The efficacy of peroxygen compounds against fungi can be influenced by the peroxygen that was used and fungal genus (Warren *et al.,* 2023).

Peroxidase and polyphenol oxidase activity were increased in leaves of maize plants due to being treated by peroxyacetic acid combinations. The highest increase in peroxidase and polyphenol oxidase activity was recorded due to treated grain maize by 0.2AA + 4.0.H_2O_2 m/L and 0.1AA + 4.0 H_2O_2 m/L treatments compared to uninfected and infected control treatments. Meanwhile, 0.1 AA + 1.0 H_2O_2 m/L was recorded as the lowest increase in peroxidase and polyphenol oxidase activity. Enzymes that activate following infection produce inducible defense responses, which are necessary for protection (Vanitha *et al.,* 2009). Certain alterations are brought about by the pathogen's contact with the host plant in cell metabolism, activity of enzymes including phenylalanine ammonia lyase, peroxidase, polyphenol oxidase, superoxide dismutase and β-1,3 glucanase (Hammerschmidt *et al.,* 1982; Mauch *et al.,* 1988; Ohta *et al.,* 1991; Thipyapong and Steffens, 1992 and Fukasawa-Akada *et al.,* 1996). Oxidation enzymes, as polyphenol oxidase and peroxidase, induce plants to resist pathogens which play an essential role in the cellular defense against the oxidative stresses (Radjacommare *et al.,* 2004 and Sharma and Dubey, 2007. These enzymes are necessary for the oxidation of phenols, which results in the production of quinones, which are more toxic to fungus, and ultimately the control of disease (Bi and Zhang, 1993). The enzyme polyphenol oxidase catalyzes the oxygen-dependent oxidation of phenols to quinones (Vanitha *et al.,* 2009) and is important in the formation of melanin compounds in necrotic tissues (Mayer, 1987). According to Constabel *et al.,* (2000) and Stewart *et al.,* (2001), PPO levels in plants increase in response to wounds or infections, as well as when they are treated with signaling molecules such as salicylic acid, jasmonic acid, and systemin. Oxidoreductive enzymes called peroxidases are crucial for a number of metabolic processes, including the oxidation of phenols (Reuveni *et al.,* 1991; Swami *et al.,* 2018) plant cell wall reinforcement (Dean and Kuc, 1987 and

Bernards *et al.,* 1999) and enhancing lignification in response to infection (Hammerschmidt and Kuc, 1982), which might inhibit the pathogen's ability to penetrate (Ride, 1983).

In conclusion, peroxyacetic acid (PAA) gave a promising control approach against maize late wilt disease *in vitro* and *in vivo*. All PAA combinations decreased late wilt disease incidence and increased growth and yield parameters of maize plants, so that we can use PAA to control the disease and raise the productivity of maize yield. Additionally, PAA could be regarded as an eco-friendly agent for controlling plant diseases as an environmentally safe alternative of synthetic fungicide.

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