International Scholars Journals

African Journal of Crop Science ISSN: 2375-1231 Vol. 12 (8), pp. 001-012, August, 2024. Available online at https://internationalscholarsjournals.org/journal/ajcr/table-of-contents © International Scholars Journals

Full Length Research Paper

Comparative Analysis of Pepper Cultivar Resistance and Inducer Treatments for Mitigating Gray Mould (Botrytis cinerea) in *Capsicum annuum L.*

A. Kamara[1], E. El-Argawy[2]*, A. El. Korany[2] and G. Amer[2]

[1]Plant Pathology Department, Faculty of Agriculture, Alexandria University, Egypt.
[2]Plant Pathology Department, Faculty of Agriculture, Damanhour University, Egypt.

Received 23 October 2023, Accepted 22 April, 2024

Four different highly economic value pepper cultivars Trezaa, Emberu, Mazurka and Taranto usually grown in Egypt were used to evaluate the efficacy of certain resistance inducers to enhance resistance of pepper fruits against gray mould fungus. Pathogenicity tests with *Botrytis cinerea* (BC-3 isolate) revealed that cv. Mazurka (Red Colour) was most tolerant and exhibited 40.15% rot severity, while cv. Emberu (Yellow Colour) was highly susceptible and showed 79.12% rot severity. The other two pepper cultivars, that is, cv. Trezaa and Taranto showed severity of 50.23 and 52.75%, respectively. Treatment of pepper fruits with resistance inducers salicylic acid (SA); abscisic acid, methyl jasmonate and calcium chloride significantly decreased gray mould development under laboratory conditions. Calcium chloride was the most effective on all pepper cvs. mentioned earlier and inhibited diameter of rotting area with overmean of 40.6%. This was followed by SA as 34.01%. Abscisic acid and methyl jasmonate, however, showed lower mean inhibition of 16.8 and 18.8%, respectively. Meantime, treatment of pepper fruits with such resistance inducers and calcium chloride increased activity of the defense related enzymes, that is, polyphenol oxidase (PPO), peroxidase (POD), phenylalanine ammonia lyase (PAL) as well as the total phenols where SA and calcium chloride were the most effective. This could explain potentiality of such compounds to enhance pepper resistance to control *B. cinerea* of the gray mould of pepper.

Key words: Pepper, gray mould, *Botrytis cinerea*, resistance inducers, calcium chloride, defence enzymes, phenolic content.

INTRODUCTION

Sweet pepper (*Capsicum annuum L.*) is one of most popular and favorite vegetable crops cultivated in Egypt for local market and exportation. Such high cash crop has occupied an important rank in the Egyptian and world

*Corresponding author. E-mail: dreman_elarawy@yahoo.com.

agriculture due to its high profit and nutritional value (El-Hifny and El-Sayed, 2011). Pepper is grown extensively both in the field and in the greenhouse. Heavy losses in yield and quality occur in pepper, because of a number of diseases (Zitter, 2011). Gray mould fungus, *Botrytis cinerea* is an ubiquitous fungus distributed worldwide and reported to be a pathogen of plants in more than 200 genera, including pepper, which grown in enclosures that maintain high relative humidity and causes postharvest decay (Pernezny et al., 2003). No pepper cultivars were recorded so far to be resistant to gray mould, while, little number of cultivars were reported to be tolerant to different degree in different parts of the world (Elad and Shtienherg, 1995; Elad et al., 2004). Control of this disease is especially important during storage, because it develops at low temperature and spreads quickly among fruits and vegetables (Karabulut et al., 2004). Losses can be ameliorated by fungicide treatments (Rosslenbroich and Stuebler, 2000). However, public concerns about the negative impact of the synthetic fungicide residues on human health and environment have prompted the search for safer alternatives (Houeto et al., 1995; Meng et al., 2008). Resistance inducers such salicylic acid (SA), jasmonates, and abscisic acid as well as the inorganic resistance inducer elicitor calcium chloride have been reported to play an important role in systemic signaling systems triggering expression of various defense-responsive genes (Leon and Daryl, 2004; Denancé et al., 2013; Yang et al., 2013; Alazem et al., 2014; Kim and Hwang, 2014). During defense communication, SA plays a role in both local and systemic resistance reactions and it combats invading pathogens due to its natural antimicrobial properties (Murphy and Carr, 2002).

Recently, it has been observed that SA treatment could be used to reduce deterioration and chilling injury symptoms in some fruit (Sayyari et al., 2009). Both pre- and post-harvest SA treatments have been reported as being effective in fruit quality maintenance and storage life extension of strawberry (Babalare et al., 2007). Preharvest application of SA has induced resistance against pathogens in pear (Jiankang et al., 2006) and decreased disease development in cherry (Yao and Tian, 2005). Application of exogenous SA at non-toxic concentrations to fruit has been shown to delay the ripening and softening of banana (Srivastava and Dwivedi, 2000), reduce lipid peroxidation of navel orange (Huang et al., 2008), and increase host resistance to postharvest diseases of sweet cherry (Qin et al., 2003). A few studies have reported the effects of SA on chilling injury, showing that SA and methyl salicylate (MeSA) treatments increase resistance to postharvest chilling injury in horticultural crops, including tomato (Ding et al., 2001), peach (Wang et al ., 2006) and (Cao et al., 2010), pomegranate (Sayyari et al., 2009), and pineapple (Lu et al., 2010). On the other hand, SA application either preharvest (Yao and Tian, 2005) or postharvest reduced fungal decay in sweet cherry through induction of the defense resistance system (Chan and Tian, 2006) and stimulation of antioxidant enzymes (Xu and Tian, 2008).

Jasmonic acid signaling is crucial in regulating defense responses against necrotrophic pathogens and it also plays an important role through the induced systemic resistance (Pieterse et al., 2009). Abscisic acid (ABA) is called the hormone controlling plant response to fungal, bacterial, and viral stress (Mohr and Cahill, 2003, 2007). The role of abscisic acid (ABA) in plant defense is not well understood and even controversial. ABA mostly studied as a global regulator of abiotic stress adaptation, and it has recently emerged as a key determinant in the outcome of plant-pathogen interactions (Thaler and Bostock, 2004; Achuo et al., 2006; Asselbergh et al., 2007). Calcium was found to reduce plant susceptibility to fungal infection in some pathosystems (Vale et al., 2000). Calcium plays an important role in the defense of plants against *Sclerotinia sclerotiorum* since it is essential in the structure of the middle lamellae of plant cells and in maintaining selectivity of cell plasma lemma (Maxwell and Lumsden, 1970; Godoy et al., 1990; Zhou and Boland, 1999). Moreover, it has been frequently reported that $CaCl_2$ and SA extended shelf life of several vegetables and fruits (Dimitrios and Pavlina, 2005; Hernandez-Munoz et al., 2006; Ramezanian and Rahemi, 2007; Arhtar et al., 2010; Chen et al., 2011; Rao et al., 2011). Plant enzymes and phenols were found to be involved in defense reactions against plant pathogens, this include oxidative enzymes such polyphenol oxidase and peroxidase (Thilagavathi et al., 2007) which involved in oxidization of phenols and lignification of plant cells during the microbial invasion (Kolattukudy et al., 1992; Chittoor et al., 1999). Other enzymes such phenylalanine ammonia-lyase were involved in phytoalexin or phenolic compound biosynthesis (Yedidia et al., 2000; Anesini et al., 2008). The present study therefore was conducted to: (a) reveal the causal fungus responsible for gray mould and to reveal amount of variation existed in its population in four major regions for pepper cultivation in EL-Beheira governorate, (b) to study potential of four widely grown pepper cultivars to control the gray mould fungus and, (c) to evaluate the efficacy of certain resistance inducers as alternative for fungicides to control post-harvest gray mould of pepper.

MATERIALS AND METHODS

Sampling, isolation and identification of the causal fungus

Samples of diseased pepper (*C. annuum* L.) fruits showing symptoms of gray mould were collected from greenhouses in four major regions for pepper cultivation in El-Beheira governorate, that is, Hosh Issa, EL-Dillingate, EL-Nubaria and Wadi El-Natrun during the 2012 to 2013 growing seasons. Samples were separately kept in polyethylene bags and directly transferred to laboratory for

isolation of the causal fungus. Diseased fruits were washed in running tap-water, and then surface disinfested with 70% ethanol. A small portion of symptomatic tissues was plated on potato dextrose agar (PDA) and incubated at 22 to 25°C for 3 to 6 days. A pure culture was established using hypal tip and single spore isolation techniques. Cultures were maintained on PDA and stored for short periods in refrigerator. The isolates obtained were identified based on their morphological and cultural characters according to Raposo et al. (1995).

Pathogenicity tests

Pathogenicity tests of the recovered isolates were conducted on the susceptible cv. Emberu (yellow) of pepper. Pepper fruits were washed with 1% sodium hypochlorite for 1 min, rinsed with sterilized distilled water and air-dried at room temperature (25°C) for 03 min and then placed in sterile plastic containers containing moist cotton. Fruits were wounded at one side to a depth of 2 mm and inoculated with a 0.5 cm disc taken from the edge of PDA culture of the causal fungus or free disc of PDA as a control. Five replicates for each treatment were conducted. Diameters of rotting area were measured seven days after inoculation and incubated at 22 to 25°C, and taken as a criterion for virulence of the recovered isolates (Ozdemir and Floros, 2004).

Susceptibility of different pepper cultivars to gray mould fungus

Under laboratory conditions, healthy pepper fruits of four widely grown cultivars, that is, Taranto and Emberu (yellow fruits) and Mazurka and Trezaa red fruits were used to study their susceptibility to gray mould fungus isolates. A highly virulent isolate, according pathogenicity test, of the gray mould fungus was used in the study. Pepper fruits were inoculated as previously mentioned in the pathogenicity test. Five replicates of each cultivar were conducted. The diameters of rotting area were estimated in centimeters, seven days after inoculation and incubation at 22 to 25°C.

Effect of certain resistance inducers on gray mould of pepper

Four pepper cultivars previously tested for their susceptibility were used to evaluate the efficacy of certain resistance inducers to enhance resistance of pepper fruits against gray mould fungus. A highly virulent *B. cinerea* isolate was used in the study. Pepper plants of the four cultivars were planted in a commercial greenhouse at EL-Dillingate region in El-Beheira governorate during 2014 growing season. The cultural practices were carried out according to the recommended practices followed in this area. Plants were never treated with any fungicide. The plants were sprayed with solutions of SA (2-hydroxybenzoic acid; C7 H_6O_3) at 1 mM, methyl jasmonate (MeJA) with concentration of 100 µM and abscisic acid (ABA) with concentration of 0.1 mM as organic compounds and calcium chloride as non-organic resistance inducers compound at 1.5 g/L, after 45 days of cultivation. Treatments were repeated weekly for four weeks until fruit harvest. Plants sprayed by distilled water were used as a control. All chemicals were sigma products. At harvest, fruits of pepper of the same four cultivars were collected in polyethylene bags and transferred to laboratory, washed in running tap-water, then disinfested with sodium hypochlorite 1% for 1 h and finally rinsed in sterile water before being allowed to dry. Fruits were then sprayed with solutions of the resistance inducers at the same concentration used in the greenhouse and kept to dry at room temperature for 30 min. Pepper fruits were then inoculated with a highly virulent gray mould isolate as mentioned in the pathogenicity test. Five replicates for each treatment were conducted. Diameters of rotting area were estimated in centimeters seven days after inoculation and incubation at 22 to 25°C.

Determination of the defense related enzyme activity and total phenols

The four pepper cultivars of different degrees of susceptibility to gray mould, that is, Taranto, Mazurka, Emberu and Trezaa were used to reveal the differences in defense enzyme activities and total phenols between pepper cultivars and after treatment with resistance inducers. This included polyphenol oxidase (PPO), peroxidase (POD), phenylalanine ammonia lyase (PAL) and total phenolic content. Also pepper fruits were sprayed with the resistance inducers compounds and inoculated with a highly virulent isolate of gray mould fungus as previously mentioned. Fruits sprayed with water were used as a control. Enzyme activities and total phnoles were evaluated in pepper after 0, 12, 24, 72 and 168 h after inoculation. Each treatment was represented by five replicates.

Estimation of polyphenol oxidase (PPO) activity

One gram of the pepper fruits was homogenized in 2 ml of 0.1 M sodium phosphate buffer (pH 6.5) in a pre-chilled pestle and mortar. The homogenate was centrifuged at 12,000 g for 15 min at 4°C and the supernatant served as enzyme source. Polyphenol oxidase activity was determined as the procedure given by Mayer et al. (1965) with some modification. The reaction mixture consisted of 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5) and 200 µl of the enzyme extract. To start the reaction, 200 µl of 0.01 M catechol was added. The reaction mixture was incubated at room temperature and the absorbance was set to zero at 398 nm. The changes in absorbance were recorded at 30 s interval for 2 min and the activity was expressed as change in absorbance min^{-1} g^{-1} of fresh tissue.

Estimation of peroxidase (POD) activity

The peroxidase activity was assayed as described by Chen et al. (2000). Extraction was carried out by homogenizing 1 g of the pepper fruit in 2 ml of 0.1 M sodium phosphate buffer (pH 6.5) using pre chilled pestle and mortar (4°C). The homogenate was centrifuged at 12,000 g for 15 min at 4°C. The supernatant served as enzyme source and the reaction mixture consisted of 1.5 ml of 0.05 M Pyrogallol, 0.5 ml of enzyme extract, and 0.5 ml of 1% H_2O_2. The reaction mixture was incubated at 28±2°C. At the start of enzyme reaction, the absorbance of the mixture was set to zero at 460 nm in the spectrophotometer and the change in the absorbance was recorded at 20 s interval for 3 min. The peroxidase activity was expressed as change in the absorbance of the reaction mixture min^{-1} g^{-1} of fresh tissue.

Estimation of phenylalanine ammonia lyase (PAL) activity

One gram of the pepper fruits was homogenized in 3 ml of ice-cold 0.1 M sodium borate buffer, pH 7.0 containing 1.4 mM of 2-mercaptoethanol and 0.1 g of insoluble polyvinylpyrrolidone (PVP). The extract was filtered through cheese cloth and the filtrate was centrifuged at 15,000 g for 15 min at 4°C. The supernatant was

Table 1. Frequency of virulence of *Botrytis cinerea* isolates recovered from pepper fruits showed gray mould symptoms collected during 2012-2013 growing seasons from four regions in EL-Beheira governorate.

Region	Total number of isolates	Frequency of virulence			
		Low	Moderate	High	Very high
El-Delengat	8	1	3	3	1
Wadi El -Natrun	6	1	2	3	-
EL-Nubaria	5	1	1	3	-
Haush Essa	5	-	3	2	-
Total	24	3	9	11	1
Ratting (%)	-	12.5%	37.5%	45.83%	4.17%

Low virulence= gray mould rot covered less than 25% of the fruit surface, moderate= rot covered 25- 50% of the fruit surface, High = rot covered > 50-75% and Very high= rot covered > 75% of the fruit surface.

used as enzyme source. Phenylalanine ammonia lyase activity was determined as the rate of conversion of L-phenylalanine to trans-cinnamic acid at 290 nm (Dickerson et al., 1984). Sample containing 0.4 ml of enzyme extract was incubated with 0.5 ml of 0.1 M borate buffer, pH 8.8 and 0.5 ml of 12 mM L-phenylalanine in the same buffer for 30 min at 30°C. The amount of trans-cinnamic acid synthesized was calculated using its extinction coefficient of 9630 M^{-1} cm^{-1}. Enzyme activity was expressed as synthesis of trans-cinnamic acid (in µmol quantities) min^{-1} g^{-1} fresh weight.

Estimation of total phenols

Total phenolic content of pepper fruit was estimated by Folin Ciocateau method (Zieslin and Ben Zaken, 1993) with some modification. One gram of sample was homogenized in 10 ml of 80% methanol and agitated for 15 min at 70°C, then 1 ml of methanolic extract was added to 5 ml of distilled water and 250 µl of Folin-Ciocateau reagent and incubated at 25°C; after 3 min, 1 ml of the saturated solution of sodium carbonate and 1 ml of distilled water were added and the reaction mixtures were incubated further for 1 h at 25°C. The absorption of the developed blue color was measured using spectrophotometer at 725 nm. The total soluble phenol content was calculated according to a standard curve obtained from a Folin-Ciocateau reagent with a phenol solution and expressed as catechol equivalent g of fresh tissue.

Effect of storage temperature on development of gray mould in pepper fruits treated with SA and calcium chloride

Fruits of peppers cv. Mazurka and cv. Emberu were washed in running tap-water, disinfested with sodium hypochlorite (1%) for 5 min and finally rinsed in sterile water before being allowed to dry. Then, peppers were immersed in aqueous solutions of calcium chloride at 1.5 g/L, and SA at 1 mM for 60 min, and were left to dry at room temperature for one day before being inoculated (Hajhamed et al., 2007). Fruits immersed in water were used as negative control. Inoculated fruits with (*B. cinerea*, *BC-3)* isolate were used as positive control. After three, seven and eleven days of incubation at 8, 12 and 25°C, the diameters of rotting area were estimated in centimeters. Each treatment was represented by five replicates.

Virulence and disease assessments

Virulence of the recovered isolates and severity of the developed gray mould on pepper fruits in the different experiment were conducted according to Balogun et al. (2005) as follows:

Low virulence= gray mould covered ≤25% of fruit surface;
Moderat virulence= gray mould covered >25-50% of fruit surface;
High virulence= gray mould covered >50-75% of fruit surface;
Very high virulence= gray mould covered >75% of fruit surface

$$\text{Severity (\%)} = \frac{\text{Mean diameter of rotting aera}}{\text{Mean diameter of fruit surface}} \times 100$$

Statistical analysis

The obtained data were statistically analyzed according to Gomez and Gomez (1984) using the SAS program version 9.2 (SAS Inc., 2009). Means were compared using the least significant difference (LSD) test at 0.05 level of probability.

RESULTS

Fungi associated with gray mould of pepper

Twenty four *B. cinerea* isolates were recovered from diseased pepper fruits showed gray mould symptoms collected from four major regions for pepper cultivation in El-Behera governorate, that is, Wadi El-Natrun, EL-Nubaria, El Delengat and Haush Essa during the 2012 to 2013 growing season (Table 1). Eight of the *B. cineria* isolates were recovered from El Delengat pepper samples, while six isolates were isolated from Wadi El Natrun and also five isolates were isolated from each of EL-Nubaria and Haush Essa regions (Table 1).

Pathogenicity tests

Pathogenicity tests of the twenty four *B. cinerea* recovered in the survey were conducted on the susceptible pepper cv. Emberu (yellow pepper). The obtained data presented in Table 1 showed that all the

Table 2. Susceptibility of four pepper cultivars to the artificial infection with *Botrytis cinerea* (*BC-3* isolate).

Cultivars	Fruit Colour	Diameter of rotting area (cm)*	Rot severity (%)
Emberu	Yellow	5.250[a]	79.12
Trezaa	Red	3.500[b]	52.75
Taranto	Yellow	3.333[b]	50.23
Mazurka	Red	2.667[c]	40.15

*Data are average of five replicates.

tested isolates were virulent to different degrees on the tested peppers cultivar. However, only one isolate (that is, 4.17% of the total) was recognized as very highly virulent as incited gray mould on pepper more than 75% of the fruit surface, seven days after inoculation. Meantime, eleven isolates out of the 24 analyzed (that is, 45.83% of the total) were highly virulent and incited gray mould of > 50%-75% on the fruit surface. Also, nine isolates (37.5%) were moderate and incited gray mould of 25 to 50% on the fruit surface, while three isolates (that is, 12.5% of the total) showed low virulence as gray mould covered less than 25% of the fruit surface (Table 1).

Susceptibility of pepper cultivars to *B. cinerea*

Data in Table 2 shows that all tested pepper cultivars, that is, Emberu, Trezaa, Mazurka and Taranto were susceptible to the infection with *B. cinerea* (*BC-3* isolate). The cv. Emberu (yellow) was highly susceptible as gray mould developed with artificial inoculation covered 79.12% of the fruit surface, seven days after inculation. However, cv. Mazurka (Red) showed the least susceptibility (most tolerant) as gray mould covered only 40.15% of the fruits surface. Meanwhile, cv. Trezaa (Red) and cv. Taranto (yellow) cultivars exhibited intermediate susceptibility as gray mould developed with artificial inoculation covered 52.75 and 50.23% of the fruits surface, respectively (Table 2). Values followed by different letters are significantly different at p= 0.05 of probability.

$$\text{Rot severity (\%)} = \frac{\text{Mean diameter of rotting aera}}{\text{Mean diameter of fruit surface}} x100$$

Enzyme activity and total phenols in relation to susceptibility of pepper cultivars to *B. cinerea*

The defense related enzyme activity and the total phenols contents were determined in pepper fruits of the four tested cultivars for their susceptibility to *B. cinerea* (*BC-3* isolate). Data illustrated in Figure 1A and B showed that the enzyme activities of PPO, POD, PAL as well as total phenols constantly increased in pepper fruits of the four cvs. after inoculation with *B. cinerea* (*BC-3* isolate) compared with non -inoculated control. Meantime, it was obvious that the most tolerant cv. Mazurka showed the highest activity for all tested enzymes and total phenols in both the uninoculated and inoculated pepper cvs. The highest peak is as early as 24 h after inoculation. On the contrary, the most susceptible cultivar, that is, cv. Emberu showed the lowest enzymes activities and total phenols with highest peack as late as 72 h after inoculation. The two other cultivars, that is, cv. Taranto and cv. Trezaa of the intermediate susceptibility showed intermediate enzyme activities and total phenols values.

Effect of certain resistance inducers on gray mould of pepper

SA, methyl jasmonate)MeJA(, abscisic acid (ABA) and calcium chloride(Ca Cl_2), were tested for their potentiality to enhance resistance of pepper fruits of different cultivars, that is, Trezaa, Emberu ,Mazurka and Taranto against the highly virulent *B. cinerea* (*BC-3* isolate).

Data in Table 3 showed that gray mould developed on the fruits mostly significantly decreased with the treatment of the tested resistance inducers on the four pepper cvs compared with the untreated inoculated control. SA and calcium chloride were the most effective on all tested cultivars and significantly decreased mean developed rot by 34.01 and 40.6%, respectively. However, abscisic acid and methyl jasmonate inhibited the mean gray mould developed by 16.8 and 18.8%, respectively.

Effect of resistance inducer on defense related enzyme activity and total phenols

Pepper cv. Emberu was used to determine the effect of resistance inducers and calcium chloride on the defense related enzyme activity and total phenols in treated fruits and then inoculated with *B. cinerea (BC*-3). It was evident

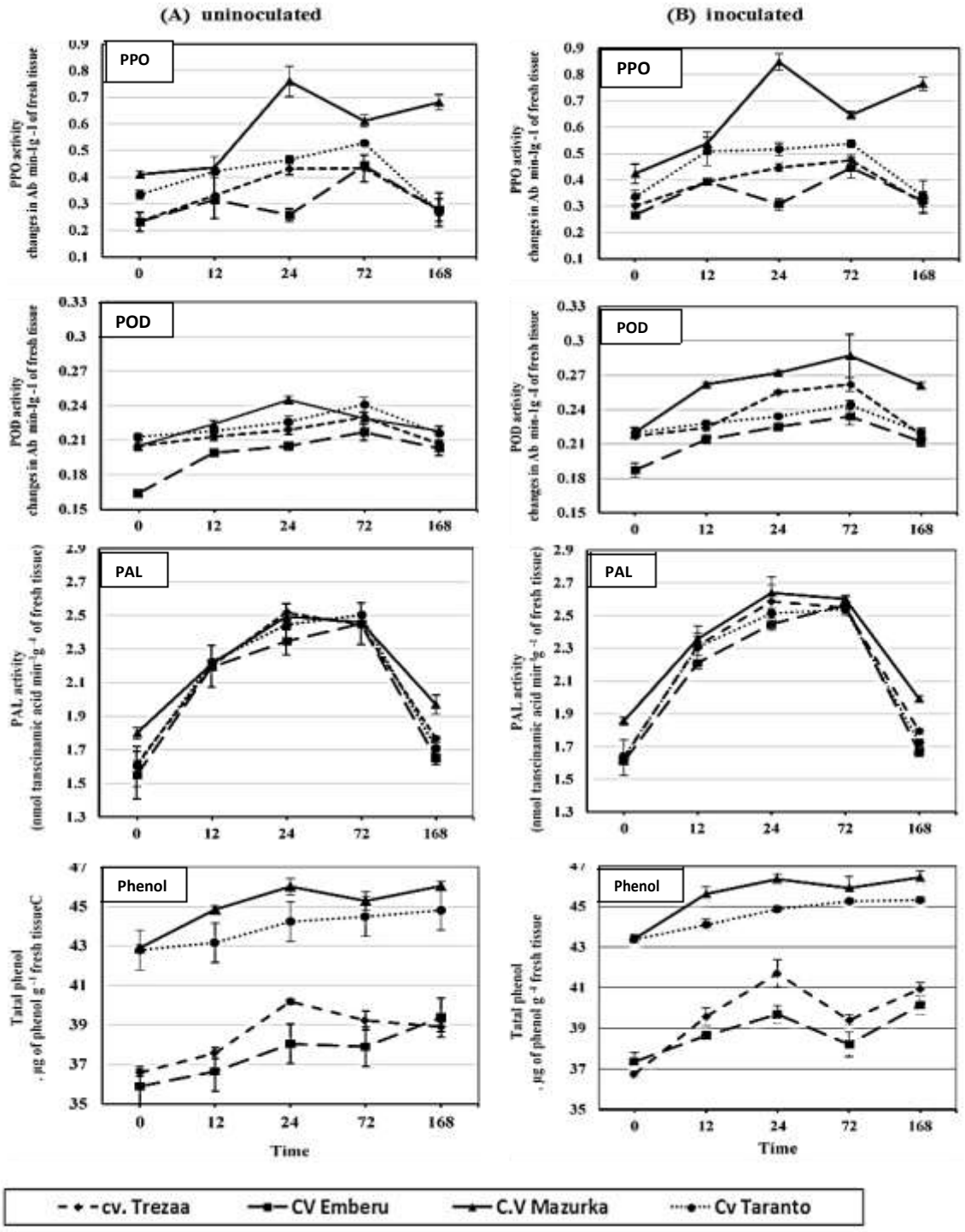

Figure 1. Enzyme activity of polyphenol oxidase (PPO), peroxidase (POD), and phenylalanine ammonia lyase (PAL) and the total phenol content in pepper fruits of four pepper cultivars, uninoculated (A) and inoculated (B) with *B. cinerea* (*BC-3* isolate).

(Figure 2) that all treatments obviously increased enzyme activity of the tested defence related enzymes as well as the total phenols content compared with the inoculated untreated control. This was steady over the 168 h period

Table 3. Effect of treatment with certain resistance inducers and calcium chloride on diameter of gray mould developed on pepper fruits of four tested cultivars treated and then inoculated with *B. cinerea (BC-3)* under laboratory conditions, seven days after inoculation.

Treatment	Pepper cultivars									
	Cv. Emberu		Cv. Trezaa		Cv. Taranto		Cv. Mazurka		Mean	
	Diameter (cm) *	% Inhibition	Diameter (cm) *	% Inhibition	Diameter (cm) *	% Inhibition	Diameter (cm) *	% Inhibition	Diameter (cm) *	% Inhibition
Jasmonate (MeJA)	4.250^{bc}	23.32	3.103^{ab}	16.36	2.977^{a}	7.55	2.000^{ab}	27.98	3.082^{b}	18.80
Abscisic acid (ABA)	4.330^{b}	21.88	3.12^{a}	15.71	2.877^{a}	10.65	2.250^{ab}	18.98	3.146^{b}	16.80
Salicylic acid (SA)	3.667^{cd}	34.60	2.410^{bc}	35.04	2.427^{b}	24.63	1.617^{b}	41.77	2.529^{c}	34.01
Calcium chloride ($Ca Cl_2$)	3.627^{d}	34.57	1.980^{b}	46.63	2.330^{b}	38.20	1.583^{b}	42.99	2.379^{c}	40.60
Infected -un treated (control)	5.543^{a}	-	3.710^{a}	-	3.220^{a}	-	2.777^{a}	-	3.811^{a}	-
Mean	4.283^{a}	-	2.864^{b}	-	2.764^{b}	-	2.045^{c}	-		-

*Values are means of five replications. Values for each parameter followed by different letters are significantly different at p=0.05 of probability.

of the investigation. The highest peak was recognized 24 h after inoculation for PAL and total phenols, while it was relatively later at 72 h for PPO and POD. Meantime, SA was the most effective to induce activity of PPO and POD, while $CaCl_2$ was most effective to induce PAL activity and total phenols.

Effect of storage temperature on development of gray mould

Data illustrated in Figure 3 showed that a strong positive correlation (r = 0.6 to 0.9) was revealed between storage degrees of temperature and the developed diameter of gray mould on both tested pepper cultivrs. Diameter of the rotting area decreased with decreasing storage temperature and vice versa where 8°C exhibited the least diameter of gray mould. Meanwhile, treatment of pepper fruits with calcium chloride and SA before storage led to a further decrease in the developed gray mould during storage and calcium chloride (1.5 g/L) exhibited the highest effect (Table 4). On the other hand, data tabulated in Table 4 also showed that, calcium chloride effectively delayed time of appearance of the first signs of gray mould (that is, increased shelf life) on both tested cultivars as there were no gray mould symptoms up to seven days after inoculation with *B. cinerea* (*BC-3* isolate).

DISCUSSION

Sweet pepper (*C. annum L*.) is one of the most important high value crop grown extensively throughout the world, especially in the temperate region. Gray mould of pepper caused by the fungus *Botrytis cinerea* is the most important disease of pepper worldwide in the field and under post-harvest conditions (Vagelas et al., 2009). Twenty four isolates of *B. cinerea* were isolated from diseased pepper fruits showed gray mould symptoms collected from four major regions for pepper cultivation in El-Behera governorate, Egypt. All fungal isolates were virulent to the susceptible cv. Emberu to different degrees. On the other hand, all pepper cultivars, that is, Taranto, Emberu, Mazurka and Trezaa were susceptible to the gray mould fungus *B. cinerea* (*BC-3* isolate). Searching for resistant pepper cultivars against post-harvest disease is restricted (Kiran et al., 2006). Plant pathologists worldwide are more concerned about dealing with threats of using synthetic fungicides (Okigbo, 2009). Finding safe alternative methods of pathogen control is obligatory (Ijato et al., 2011; Nsabiyera et al., 2012). One of the most well-known methods of plant prevention to plant pathogenic fungi is chemical control by deploying different groups of fungicides (Yaqub and Shahzad, 2006). Chemical control can generate resistance of fungi to fungicides and can cause environment pollution with direct effect of human health (Zhang et al., 2007; Damalas and Eleftherohorinos, 2011). In recent years, the trend of using different chemicals in plant production has been minimized. Thus, there is need for an efficient,

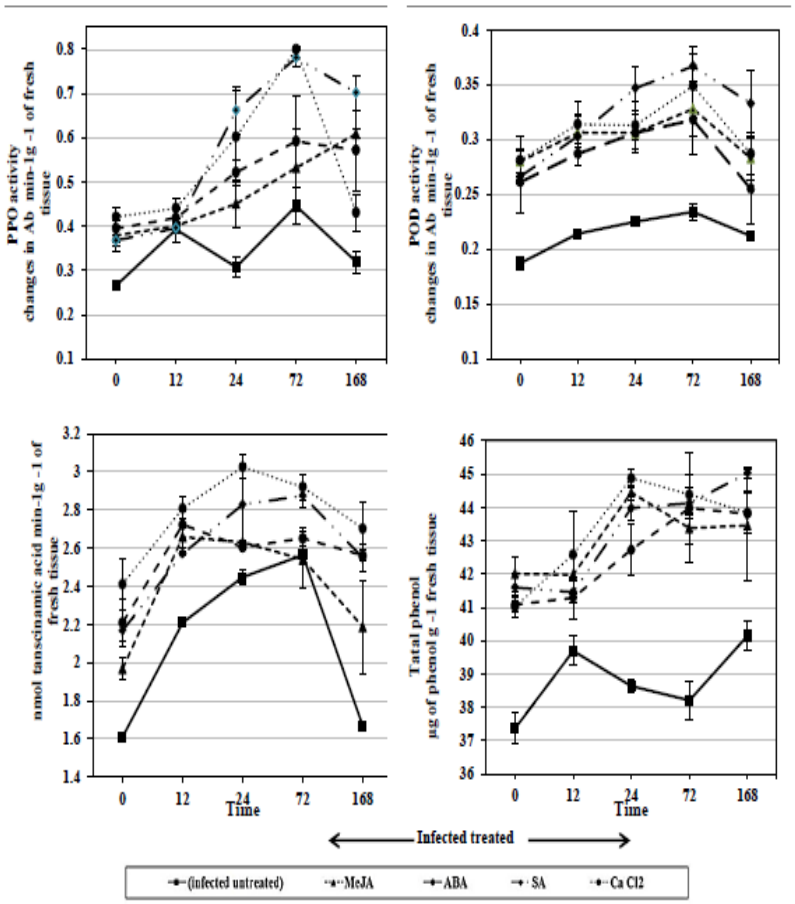

Figure 2. Enzymes activity in pepper fruits of cv. Emberu treated with jasmonate (MeJA), Abscisic acid (ABA), salicylic acid (SA) and calcium chloride (Ca Cl_2) and inoculation with *B. cinerea* (*BC-3)* isolate.

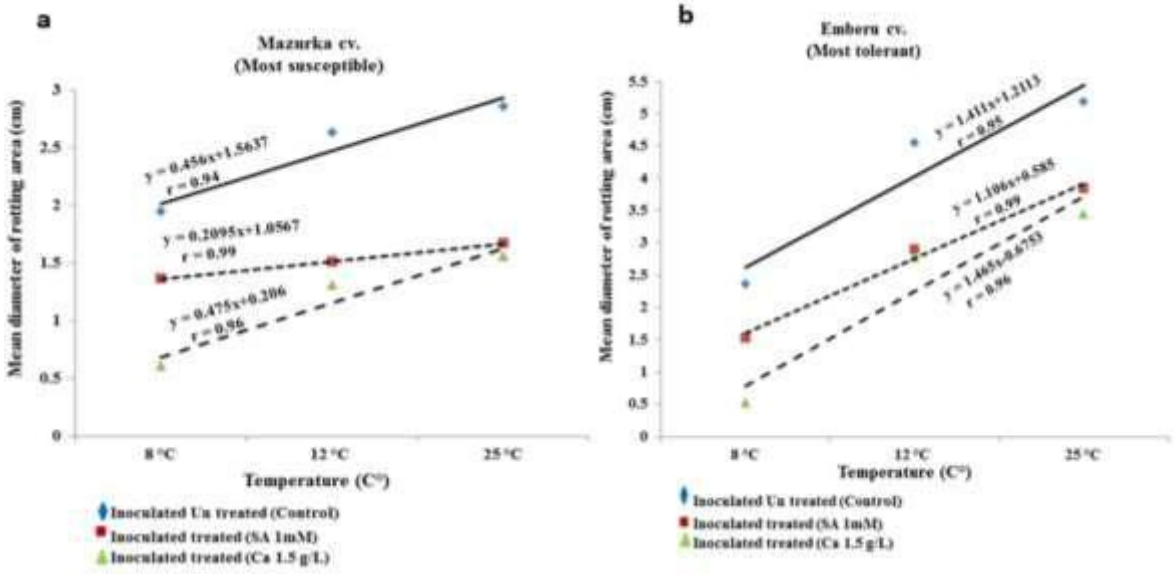

Figure 3.Correlation between storage temperature and development of gray mould on pepper cv. Mazurka (a) and cv. Mazurka (a) Emberu (b) treated with salicylic acid and calcium chloride and inoculated with *B. cinerea* (*BC-3*).

Table 4. Effect of storage at 8°C on development of gray mould on pepper fruits treated with salicylic acid and calcium chloride and inoculated with *B. cinerea* (*BC-3*).

Treatment	Diameter of rotting area (cm)*							
	Cv. Mazurka				Cv. Emberu			
	3 days	7 days	11 days	Mean	3 days	7 days	11 days	Mean
Fungus (*BC-3*) Control	0.333	2.250	3.250	1.944^{a}	0.417	2.667	4.000	2.361^{a}
SA (1 mM) + Fungus	0.000	1.667	2.083	1.250^{b}	0.333	1.9167	2.667	1.638^{b}
Ca Cl_2 (1.5 g/L) + Fungus	0.000	0.000	1.816	0.606^{c}	0.000	0.000	1.583	0.528^{c}
Mean	0.139^{c}	1.306^{b}	2.383^{a}	-	0.139^{c}	1.528^{b}	2.750^{a}	-

*Values are means of three replications.

environmental-safe method to control plant diseases. SA, methyl jasmonate (MeJA) and calcium chloride is a natural compound that plays a central role in disease resistance (Delaney et al., 1994; El-Ghouth et al., 2000; Darras et al., 2005; Yao and Tan, 2005; Jin et al., 2009). SA, methyl jasmonate (MeJA), Abscisic acid (ABA) and calcium chloride were considered as systemic acquired resistance (SAR) inducers (Mengiste et al., 2010) were tested in the present study for their potential to enhance resistance of pepper cvs. against gray mould fungus *B. cinerea.* Calcium chloride was the most effective and decreased diameter of rotting area by 46.63, 42.99, 38.20 and 34.57% on the tested pepper cvs. Trezaa, Mazurka, Taranto and Emberu, respectively, with over mean of 40.6%. This was followed by SA which inhibited diameter of rotting area by 35.04, 41.77, 24.63 and 34.60% on the mentioned cvs. respectively, with over mean of 34.01%. Abscisic acid and methyl jasmonate however showed lower mean inhibition of 16.8 and 18.8%, respectively. The obtained results could be explained in view that application of $CaCl_2$ at postharvest stage successfully inhibited spore germination and thus provided a good control for *B. cinerea* on apple (El-Gali, 2008). Also fruits containing high concentration of Ca were found to be less susceptible to the fungal infection (Naradisorn, 2013). On the other hand, SA was involved in reducing the damage caused by various pathogens such bacteria, fungi and viruses (Raskin, 1992). It is considered an important factor in systemic acquired resistance (SAR) against many pathogens (Nie, 2006). Meanwhile, jasmonic acid signaling molecules in plants make the plant responsive to various biotic and abiotic stresses (Wasternack and Hanse, 2013; Zhou et al., 2013). Jasmonic acid was also involved in fruit ripening and plant response to injury as well as resistance to insects and pathogens (Meng et al., 2009; Jin et al., 2009). However, the role of abscisic acid (ABA) in plant defense is not well understood and even controversial (Mohr and Cahill, 2003, 2007). Exogenous application of ABA increased the susceptibility of various plant species to bacterial and fungal pathogens (Thaler et al., 2004; Achuo et al., 2006; Asselbergh et al., 2007). Disruption of ABA biosynthesis was shown to confer resistance to the necrotroph *B. cinerea* (Audenaert et al., 2002).

Plant enzymes were shown to be involved in defense reactions against plant pathogens (Lebda et al., 2001; Elad et al., 2004). This included oxidative enzymes such polyphenol oxidase (PPO), peroxidase (POD) and phenylalanine ammonia lyase (PAL) (Thilagavathi et al., 2007). Also plant Phenols as secondary metabolites were recorded to initiate a number of defensive reactions against plant pathogens (Lattanzio et al., 2006). In the present study, the highest levels in enzyme activity of PPO, POD, and PAL as well as the total phenols were revealed in the most tolerant pepper cv. Mazurka while the lowest levels were recorded in the most susceptible cv. Emberu of pepper. Our findings were in agreement with earlier studies of Chittoor et al. (1997) and Li and Steffens (2002). The present study however, showed that treatment of pepper fruits with such resistance inducers and calcium chlorides increased activity of the defense related enzymes, that is, PPO, POD and PAL as well as the total phenols where SA and calcium chloride were the most effective. This could explain potentiality of such compounds to enhance pepper resistance to control *B. cinerea* of the gray mould of pepper (Chen, 2010; Lijuan and Yuxing, 2013). Qin et al. (2003) and Yao and Tian (2005) found that exogenous SA could significantly induce -1, 3-glucanase, phenylalanine ammonia-lyase (PAL) and peroxidase (POD) and effectively restrain pathogen expansion of *Monilinia fructicola* in harvested sweet cherry fruit. These studies proved the important role of exogenous SA in activating fruit defense responses against fungal pathogen infection. However, up to date, most experiments of SA-mediated defense response of fruit are more concerned in hydrolytic enzymes and PR-proteins (Ding et al., 2001, 2002; Qin et al., 2003; Yao and Tian, 2005). There are some different methods used to reduce discoloration of fruits and inhibition microbial growth by reducing moisture losses, limitation of oxygen uptake, inhibition of respiration, retardant ethylene production, these methods are SA

application, fruit dipping in calcium treatments, safe edible coating and also, modified atmosphere (Fisk et al., 2008; Yao and Tian, 2005; Montanaro et al., 2006). Meantime, diameter of the rotting area decreased with decreasing storage temperature and vice versa where 8°C exhibited the least diameter of gray mould between 8 and 25°C. Meanwhile, calcium chloride (1.5 g/L) and SA (1 mM) exhibited a further effect to decrease the developed gray mould under the different degrees of storage temperature. Moreover, calcium chloride effectively delayed time of appearance of the first signs of gray mould (that is, increased shelf life) of the inoculated pepper fruits of both tested cultivars as there were no gray mould symptoms up to seven days after inoculation with *B. cinerea* (*BC-3* isolate).

The obtained results were in harmony with Sakaldaş and Kaynaş (2010) and Gross et al. (2004). Calcium was employed for harvested fruits in preserving qualities, controlling softening, inhibiting the ratio of rottenness and extending shelf life (Chen et al., 2011). Calcium dips have been employed to improve firmness and extend the postharvest shelf life of a wide range of fruits and vegetables (Hernandez-Munoz et al., 2006). Pre- and postharvest application of calcium may help to reduce senescence during commercial and retail storage of fruit, with no detrimental effect on consumer acceptance (Lester and Grusak, 2001). Pila et al. (2010) reported that the application of calcium prolonged the storage life of strawberries and tomato fruits, respectively, as measured by a delay in accumulation of sugars, decrease in organic acids, increase of colour saturation index and mold development. Further, Lam et al. (1987) stated that SA as an antitranspirant chemical can retard moisture loss associated pericarp browning of fruits. Senescent changes resulting to losses in physicochemical changes and nutritional qualities can also be inhibited. Consequently, fruit storage life could be prolonged. The post-harvest application of $CaCl_2$ was reported to increase the storage shelf life of apricots (Antunes et al., 2003), Kiwi (Dimitrios and Pavlina, 2005; Arhtar et al., 2010), peaches (Manganaris et al., 2007), tomato (Pila et al., 2010) and strawberries (Verdini et al., 2008). This effect was mainly by reducing pectin solubility (Lara et al., 2004), strengthening cell wall (Vicente et al., 2007), maintaining firmness (Manganaris et al., 2007), and delaying fruit ripening and decreasing decay rate (Lara et al., 2004). In the light of these results, we recommend the use of SA (1 mM) and $CaCl_2$ (1.5 g/L) treatments to maintain the quality of peppers during postharvest storage.

Therefore, commercial application of SA (1 mM) and $CaCl_2$ (1.5 g/L) can be considered for the maintenance of quality and the extension of shelf life of sweet peppers during storage and marketing. Also, SA and $CaCl_2$ are environmental-friendly method to control the gray mould disease.

Conflict of Interests

The authors have not declared any conflict of interests.

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