

Peroxyacetic Acid (PAA): an eco-friendly Agent for Reducing *Sclerotinia sclerotiorum* Growth, Sclerotia Carpogenic Germination and Infectivity

Ranya M.S. El-Ashmony, M.R. Abdel-Latif, EL.S. Abdou and A.A. Galal

Plant Pathology Department, Faculty of Agriculture, Minia University, Egypt.

An eco-friendly agent, hydrogen peroxide (H₂O₂)-based compound, peroxyacetic acid (PAA) gave efficiency against *Sclerotinia sclerotiorum* growth and infectivity. Significant inhibitory effect for PAA against linear growth (LG), sclerotial formation (SF) and mycelial dry weight (MDW) by 0.4 g/l acetic acid +0.8 g/l H₂O₂ was recorded. Inhibitory effects were increased by enhancing H₂O₂ concentration (0.4 g/l AA +1.2 g/l H₂O₂) that caused 85, 91 and 83% inhibition for LG, SF and MDW, respectively. In addition, carpogenic germination measures of *S. sclerotiorum* sclerotia were significantly reduced upon PAA treatments. Carpogenic germination was reduced from 100% to 62% and 50% by 38% and 50% inhibition when *S. sclerotiorum* sclerotia were treated by 0.4 g/l AA + 0.8 g/l H₂O₂ and 0.4 g/l AA+ 1.2 g/l H₂O₂, respectively. Reduction of carpogenic germination by H₂O₂ based compound was accompanied with significant lowering in number of stipes and apothecia/ sclerotium. Drenching sclerotinia-infested soil with PAA, seven days pre-planting provided significant reduction in root and crown rot diseases of bean plants. When soil was singly drenched by 0.4 g/l acetic acid +0.8 g/l H₂O₂ or 0.4 g/l acetic acid+1.2 g/l H₂O₂ protected bean plants against root and crown rot by 47 and 67%, respectively. A substantial protection was pronounced against white rot of bean pods. Post-treatment by PAA was more effective to reduce white rot than pre-one.

Keywords: Peroxy acetic acid, H₂O₂-based compound, Eco-friendly agent and *Sclerotinia sclerotiorum*.

Sclerotinia sclerotiorum (Lib.) de Bary is one of the most devastating and cosmopolitan plant pathogens. More than 60 names have been used to refer to diseases caused by this fungal pathogen (Purdy, 1979) including cottony rot, watery soft rot, stem rot, drop, crown rot, blossom blight and, perhaps most common, white mould. The fungus infects over 400 species of plants worldwide including important crops and numerous weeds (Boland and Hall, 1994). Severe losses due to *Sclerotinia* diseases can occur when the pathogen attacks the stem and entire plants are killed. Wilting due to infection reduce plant vigor and yield. However, pods infection reduces marketability and yield. Extensive crop damage, lack of high levels of host resistance and the general difficulty of controlling diseases caused by *S. sclerotiorum* have been the impetus for sustained research on this pathogen (Bolton *et al.*, 2006).

Either active oxygen species or antioxidants compounds were considered as a secret of life for all living organisms (Levine, 1985) and play a core role in plant health (Sutherland, 1991). Moreover, these compounds have major role in plant pathogen interactions (Adam *et al.*, 1993; Galal and Abdou, 1996; Abdou and Galal, 1997 and EL-Ashmony *et al.*, 2017). Both acetic acid and hydrogen peroxide are reacted as bactericides and fungicides against many phytopathogenic bacteria and fungi (Kang *et al.*, 2003, Narciso *et al.*, 2007 and Osório *et al.*, 2013). To increase oxidizer effects of H₂O₂, mixing acetic acid with H₂O₂ resulted in a new strong and stable oxidizer called peroxyacetic acid (PAA) which approved in food industry as sanitizer (Buschmann and Del Negro, 2012). PAA has been involved in plant disease management (Hopkins *et al.*, 2003; Wessels and Ingmer, 2013; Ayoub *et al.*, 2017 and Galal, 2017). Recently, Galal, (2017) found that PAA was effective to reduce bacterial soft rot caused by *Erwinia carotovora* subsp. *carotovora* of some vegetable fruits (cucumber, pepper, tomato, eggplant, squash, okra) plus potato tubers.

The present study was planned to test the efficacy of H₂O₂ based compound PAA on *S. sclerotiorum* growth and infectivity either *in vitro* and in plant, respectively.

Materials and Methods

1- Causal agent:

An aggressive *Sclerotinia sclerotiorum* isolate SS3 isolated by EL-Bana *et al.*, (2006) was used throughout this study. However, pathogenicity test was confirmed on bean pods before use.

2- Preparation of the peroxyacetic acid (PAA):

Stock solutions of two mixtures of acetic acid (AA) and hydrogen peroxide (H₂O₂) were prepared with distilled water then left for at least 10 days before they tested (EPA, 1993 and 2004). Different concentrations of AA+ H₂O₂ mixtures, *i.e.* (0.1 g/l AA+ 1.0 g/l H₂O₂, 0.2 g/l AA+ 2.0g/l H₂O₂ and 0.5 g/l AA+5.0 g/l H₂O₂) were prepared for bean pods treatment, while 0.4 g/l AA +0.8 g/l H₂O₂ and 0.4g/l AA +1.2 g/l H₂O₂ were used against *Sclerotinia sclerotiorum* growth parameters and root and crown rot infection.

3- Effects of peroxyacetic acid (PAA) on *Sclerotinia sclerotiorum* growth parameters *in vitro*:

3.1. Linear growth and sclerotia production:

Test solutions were added to the autoclaved nutrient agar medium in conical flasks and then dispensed to Petri dishes (15 ml medium/plate) and left to solidify. Plates were then inoculated with the desired isolate (SS3) of *S. sclerotiorum* by placing agar disks (10 mm) taken from the periphery of fungal colonies grown 7 days after incubation at 20°C. Plates containing nutrient agar medium without test chemicals were inoculated similarly to be taken for comparison (Abdou and Galal, 1997).

Three plates for each treatment were used, and then incubated at 20°C. Data were recorded by measuring the diameter of the fungal growth (two diameters) and their means when the mycelial growth of any plate reached the edge of the plate. When the sclerotia covered any plate of treated or untreated, after 14 days, sclerotia from each plate were harvested, and their numbers were recorded (EL-Bana *et al.*, 2006).

3.2. Mycelial dry weight (MDW):

The toxicity of the mixture of hydrogen peroxide and acetic acid, with different concentrations, on growth of *S. sclerotiorum* isolate SS3 in liquid nutrient medium was studied. Equal disks (10 mm in diameter) were taken from actively edges of 7 days old cultures of the tested *S. sclerotiorum* isolate grown on nutrient agar medium at 20°C and used for inoculation of 250 ml. Erlenmeyer flasks containing 50 ml sterilized nutrient medium amended with the tested concentration of the tested chemicals. Concentrations were prepared in sterile distilled water and aliquots were pipetted to nutrient broth (NB) medium to obtain final concentration of 0.4 g/l acetic acid+0.8g/l H₂O₂ and 0.4 g/l acetic acid+1.2 g/l H₂O₂. For control treatment conical flasks containing medium without test chemicals were inoculated similarly to be taken for comparison. All flasks were incubated at 20°C for 7 days. Mycelial dry weight (MDW) of different treatments was measured (mg MDW per 50 ml liquid medium) after separation of fungal mass by filtration through Whatman No-1 filter paper and dried at 60°C for 48 hrs. The following equation was used to calculate percentage inhibition of fungal growth (Sutton and Starzyk, 1972).

$$\text{Inhibition \%} = (\text{MDW of the control} - \text{MDW of the treatment} / \text{MDW of the control}) \times 100$$

3.3. Carpogenic germination:

Based on the highest inhibitory effects of the tested solutions against *S. sclerotiorum* growth parameters 0.8 and 1.2g/l for hydrogen peroxide were individually combined with 0.4 g/l acetic acid and used for test on carpogenic germination. The tested solutions at the rate of 50 ml tested solution/plate were added to sandy soil containing Petri plates. Sclerotia of *S. sclerotiorum* were removed from *S. sclerotiorum* SS3 infested barely grains incubated at 20°C for 30 days (Galal and EL-Bana, 2002) and transferred immediately (under aseptic conditions) to autoclaved sandy soil in 16-cm Petri plates.

To each Petri plate, 20 sclerotia were set on the surface of sand and 5 plates were used as replicates for each treatment. After 35 days incubation at 15°C under darkness, the plates were placed under fluorescent light (2800 Lux, 14hr photoperiod) at 15°C for 18 days (Casale and Hart, 1986). After that, the sclerotia in each replicate were washed 3 times by distilled water then placed in a Petri plate (9- cm diameter) with 15 ml distilled water. The water was replaced with fresh distilled water after 24, 48, 96 hr, and at 3 days intervals till the end of experiment. All sclerotia were incubated under fluorescent light at 15°C. After 28 days, percentages of carpogenic germination, number of stipes and apothecia per sclerotium were assessed (Galal and EL-Bana, 2002).

4- Efficacy of peroxyacetic acid on *S. sclerotiorum* infectivity:

4.1. Root and crown rot severity:

Test solutions of acetic acid and hydrogen peroxide were added, as soil drenching (1.0 L/3 kg soil), to steam sterilized soils potted in 20-cm diameter sterilized pots, to obtain a final concentration of 0.0 (control) and hydrogen peroxide combined with acetic acid as previously mentioned. Soil was mixed with *S. sclerotiorum* infested barely grains at 3.0 % w/w, which fungus free sterilized barley grains were added to serve as control. All pots were irrigated regularly and kept under the greenhouse conditions. Seven days later, pots were cultivated by seeds of bean cv. Giza 6 after which had been surface sterilized. Ten seeds were planted per pot, three pots were used per replicate and each treatment consisted of three replicates. After 10, 20 and 40 days of planting, percentages of root and crown rot severity were assayed.

4.2. Disease assessment:

Damped off bean seedlings, either pre- or post- emergence damping off, and root rot severity were calculated as follows:

$$\text{Root rot \%} = (\text{N-DP} / \text{N}) \times 100$$

Where N is the total number of plants and Dp is the number of damped off seedlings.

To assess the crown rot severity, modified disease rating (0-4) scale (Lesovoi *et al.*, 1987 and Sansford, 1995) was used as follow: numerical scale description /lesion length on stem: 0= healthy (no visible lesion), 1= 0.1- 2 cm lesion length on stem, 2= 2.1-4 cm lesion length on stem, 3=4.1 - 6 cm lesion length on stem, 4=> 6.1 cm lesion length on stem or complete dried plant.

The length of lesion on infected stem was considered for recording the disease severity (Sharma *et al.*, 2016). The infected area was calculated from plants in each pot and then average for each treatment was worked out. The disease severity was calculated using the formula of (Wheeler, 1969):

Disease severity = [Sum of individual ratings/ No. of plants observed x Maximum disease rating] x 100.

4.3. Pod rot severity:

4.3.1. Pre-inoculation treatment:

Three concentrations of AA+ H₂O₂ *i.e.*, 0.5 g/l AA +5 g/l H₂O₂, 0.2 g/l AA+ 2.0 g/l H₂O₂ and 0.1g/l AA + 1 g/l H₂O₂ were tested. Apparently healthy uniformed size sample of bean pods were wetted in solutions individually for 30 min prior to inoculation with the white rotting fungus *Sclerotinia sclerotiorum*. After wetting, samples were air dried at room temperature and each sample was divided into 2 major groups, 1st group was wounded (punctured with sharp needle) and 2nd group was left sound similarly as described by Galal (2017). Either wounded or unwounded samples were divided into 2 parts, one inoculated and the other was left non-inoculated. Treated and untreated fruits were inoculated by spraying of mycelial fragments inoculum. Fungal inoculum was prepared from fresh fungal cultures grown on PDA at 20°C for 4 days suspended in sterilized distilled water at 104

mycelial propagule/ml. Untreated control was maintained for each pod, which was inoculated with the fungal inoculum but not treated with the tested solutions. The severity was measured in terms of percentage of rotted weight of infected pods by white rot pathogen (EL- Bana *et al.*, 2006), when the untreated inoculated fruits gave 100% of white rot incidence, the bean white rot severity was recorded 4 days after inoculation.

4.3.2. Post-inoculation treatment:

Inoculated and non-inoculated samples were left one hour then were wetted by PAA at concentrations of 0.0 (control), 0.5 g/l AA +5 g/l H₂O₂, 0.2 g/l AA+ 2.0 g/l H₂O₂ and 0.1 AA g/l +1.0 g/l H₂O₂. Each sample contains 30 bean pods were sprayed by 10ml test solution in plastic bag, then bags were closed tightly for 30 min. After that, plastic bags were opened, samples were dried and retagged into new plastic bags. Each treatment contained 3 replicates.

Disease assessment was monitored when samples of inoculated water treated pods showed 100% white rot incidence at incubation temperature 20°C. The severity was measured in terms of percentage of rotted weight of infected pods by white rot pathogen as mentioned before.

5- Statistical analysis:

Tukey test for multiple comparisons among means was utilized (Neler *et al.*, 1985).

Results

1- Efficiency of PAA against *S. sclerotiorum* growth parameters:

Generally, all measured growth parameters of *S. sclerotiorum* were significantly affected by the tested solutions (Table 1). A substantial linear growth (LG) inhibition was recognized even at 0.4 g/l AA+ 0.8 g/l H₂O₂ and the inhibitory effect was increased by increasing H₂O₂ concentration (0.4 g/l AA+1.2 g/l H₂O₂). Since 0.4 g/l AA+0.8 g/l H₂O₂ caused 75% inhibition and 0.4 g/l AA+1.2 g/l H₂O₂ exhibited 85% inhibition in *S. sclerotiorum* linear growth.

Sclerotial formation for *S. sclerotiorum* was significantly suppressed when the fungus was grown onto nutrient agar medium amended with the tested solutions of AA and H₂O₂ mixture as compared with control. The highest suppression value in sclerotia formation was pronounced (93% inhibition) by 0.4 g/l AA+1.2 g/l H₂O₂ followed by (91% inhibition) when the medium was amended with 0.4 g/l AA+0.8 g/l H₂O₂ (Table 1).

Adding AA+H₂O₂ mixture to nutrient broth medium caused significant MDW reduction as compared to unamended medium (Table 1). The 0.4 g/l AA + 0.8 g/l H₂O₂ concentration caused 72% inhibitory effect to MDW of *S. sclerotiorum*, however inhibitory effect increased (83% inhibition) at 0.4 g/l AA+1.2 g/l H₂O₂ concentration.

Table 1. Linear growth, sclerotia formation (SF) and mycelial dry weight (MDW) of *Sclerotinia sclerotiorum* as influenced by various concentrations of acetic acid (AA) + hydrogen peroxide (H₂O₂)

Treatments and Conc. (g/l)	Linear growth		Sclerotia formation		Mycelial dry weight	
	mm/plate	Inhibition %	no. sclerotia /plate	Inhibition %	mg/50 ml liquid media	Inhibition %
Untreated	80*	0.0	47	0.0	898	0.0
AA+H ₂ O ₂ (0.4+0.8)	21	75	4	91	244	72
AA+H ₂ O ₂ (0.4+1.2)	13	85	3	93	145	83
L.S.D at 0.05	5.9	---	1.4	---	77.8	---

*Data are means of 3 replicates of *S. sclerotiorum* cultures grown for 7 days incubation at 20°C for linear growth and MDW, while sclerotia were counted at 14 days incubation at 20°C.

2- Efficiency of PAA against carpogenic germination of *S. sclerotiorum* sclerotia:

Under in vitro conditions, the tested solutions of AA+ H₂O₂ mixture significantly reduced carpogenic germination of *S. sclerotiorum* sclerotia (Table 2). At 0.4 g/l AA +0.8 g/l H₂O₂ combination, significant carpogenic germination inhibition was expressed by 38% and increased to 50% inhibition at 0.4 g/l AA +1.2 g/l H₂O₂ (Table 2). Moreover, number of stipes/sclerotium was significantly reduced by AA+ H₂O₂ mixture (Table 2).

Adding 0.4 g/l AA+0.8 g/l H₂O₂ showed 60% reduction in the number of stipes and greater reduction (64 % reduction) was recorded at 0.4 g/l AA+1.2g/l H₂O₂. In addition to significant reduction, carpogenic germination and number of stipes/sclerotium, number of apothecia per sclerotium were also significantly decreased upon these treatments (Table 2). Since 0.4 g/l AA + 0.8 g/l H₂O₂ per liter gave 52% reduction while 0.4 g/l AA+ 1.2g/l H₂O₂ per liter increased reduction percentage to 64%.

Table 2. Carpogenic germination measures, i.e. germination%, number of stipes/sclerotium and number of apothecia/sclerotium of *Sclerotinia sclerotiorum* sclerotia as affected by H₂O₂+acetic acid.

Treatments and Conc. (g/l)	Carpogenic germination		Stipes/sclerotia		Apothecia/sclerotia	
	%	Inhibition %	No.	Inhibition %	No.	Inhibition %
Untreated	100	0.0	4.8	0.0	3.4	0.0
AA+H ₂ O ₂ (0.4+0.8)	62	38	1.9	60	1.6	52
AA+H ₂ O ₂ (0.4+1.2)	50	50	1.7	64	1.2	64
L.S.D at 0.05	7.5	---	1.2	---	0.9	---

3- Efficiency of PAA to control *Sclerotinia sclerotiorum* infection:

3.1. Root and crown rot:

Upon soil drenching by AA+H₂O₂ mixture, a significant protection against root rot and crown rot was pronounced (Table 3). Both root rot and crown rot severity was significantly reduced when bean seeds of cultivar Giza 6 were sown in *Sclerotinia* infested soil drenched by AA+H₂O₂ solution 7 days before planting. The least root rot (12%) and crown rot severity (18%) were obtained by 0.4 g/l AA+1.2 g/l H₂O₂ treatment which exhibited the highest protection percent (67%).

Table 3. Root rot and crown rot severity on bean plants, cv. Giza 6, due to *Sclerotinia sclerotiorum* infection as influenced by hydrogen peroxide (H₂O₂) + acetic acid (AA) combination soil as drench treatment.

Treatments and Conc. (g/l)	Root rot severity %	Crown blight severity %	Total disease severity	Protection %
Untreated	33	60	93	0.0
AA+H ₂ O ₂ (0.4+0.8)	16	33	49	47
AA+H ₂ O ₂ (0.4+1.2)	12	18	30	67
L.S.D at 0.05	2.1	2.8	4.7	--

3.2. *Sclerotinia* pod rot:

Either wounded or unwounded, untreated or treated with AA+ H₂O₂ at pre- and post-inoculated bean pods showed significant white rot reduction (Table 4). Unwounded post-inoculated treated pods provided the lowest white rot values (8%) at 0.5 g/l AA+5.0 g/l H₂O₂ combination, followed by unwounded pre-inoculated fruits under the same concentration of AA+ H₂O₂ combination. Wounded fruits showed significant white rot reduction when they were treated by AA+ H₂O₂ combination, when it was applied either pre- or post- inoculation with *S. sclerotiorum*, but lesser than unwounded fruits.

Table 4. *Sclerotinia* fruit rot severity caused by *S. sclerotiorum* to bean (*Phaseolus vulgaris*, cv. Giza 6) pods as influenced by peroxyacetic treatments.

Treatments and Conc.(g/l)	Sclerotinia fruit rot severity %			
	Unwounded		Wounded	
	Pre-inoculation	Post-inoculation	Pre-Inoculation	Post-inoculation
Untreated	100	100	100	100
AA+H ₂ O ₂ (0.1+0.1)	53	46	90	83
AA+H ₂ O ₂ (0.2+0.2)	28	23	56	46
AA+H ₂ O ₂ (0.5+0.5)	10	8	16	16
L.S.D at 0.05	12	9	14	13

Discussion

Fungicide alternatives eco-friendly approaches to control plant diseases became a must to avoid side effects of such hazardous fungicides from environmental pollution and human health problems. Among fungicide alternatives H₂O₂ based compounds are worldwide as a disinfectant (Gómez-López, 2012), fungicides (Abdou and Galal, 1997 and Adam *et al.*, 1993) and bactericides (Kachroo *et al.*, 2003). The present study showed significant antifungal effects against certain growth parameters of *S. sclerotiorum*. H₂O₂ combined with acetic acid led to form strong oxidizer compound, peroxy acetic acid (PAA), is reported elsewhere (Kitis, 2004; Thipaksorn *et al.*, 2012; Wessels and Ingmer, 2013 and Galal, 2017). The present study showed that adding PAA to growth medium exhibited significant reduction (75% inhibition) in linear growth, (91% inhibition) in sclerotia formation and (72% inhibition) in mycelial dry weight at 0.4 g/l AA +0.8 g/l H₂O₂. The inhibitory effects were increased by increasing H₂O₂ concentration. Since 0.4 g/l AA +1.2 g/l H₂O₂ showed greater inhibitory reduction effects, being 85, 91 and 83% in the linear growth, sclerotia formation and MDW, respectively. Our results are in line with several researches reports on otherwise phytopathogenic fungi *i.e.*, *Rhizopus stolonifer* and *Botrytis cinerea* (Narciso *et al.*, 2007), *Fusarium oxysporum* and *Didymella bryoniae* (Hopkins *et al.*, 2003).

In addition, H₂O₂ combined with acetic acid showed significant inhibitory effects to carpogenic germination of *S. sclerotiorum* sclerotia. Carpogenic germination was reduced from 100% to 62% and 50% by 38% and 50% inhibition when *S. sclerotiorum* sclerotia were treated by 0.4 g/l AA + 0.8 g/l H₂O₂ and 0.4 g/l AA+ 1.2 g/l H₂O₂, respectively. Reduction of carpogenic germination by H₂O₂ based compound was accompanied with significant lowering in number of stipes and apothecia/sclerotium.

In vivo trials, the results of the present study clearly revealed significant reduction of *Sclerotinia sclerotiorum* infectivity upon PAA application as soil drenching or pod spraying. Drenching soil with PAA significantly reduced root rot /crown blight severity from 93% in control to 49 and 30% when soil was drenched by 0.4 g/l AA + 0.8 g/l H₂O₂ and 0.4 g/l AA+1.2 g/l H₂O₂, respectively.

Furthermore, PAA successfully decreased sclerotinia pod rot severity. H₂O₂-based compound PAA as pod treatment had significantly reduced sclerotinia pod rot of either wounded or unwounded pods. However, post-inoculation treatment was more effective to suppress sclerotinia pod rot than pre-inoculation one. Additional fact was that unwounded pods gave lower sclerotinia pod rot than wounded one. The present data demonstrate that PAA is a promising eco-friendly H₂O₂-based compound to be used against bean white rot exportable pods which cover Europe GAP for exportation.

References

- Abdou, E. and Galal, A.A. 1997. Sensitivity of *Fusarium moniliforme*, *F. oxysporum* and *F. solani* to superoxide anion and hydrogen peroxide *in vitro*. *Egypt. J. Microbiol.*, **32**: 523-536.
- Adam, A.L.; Bestwick, C.S.; Galal, A.A.; Manninger, K. and Barna, B. 1993. What is the putative source of free radical generation during hypersensitive response in plants? In: Mozsik, G. Y.; Emerit, I.; Feher, J.; Mathovics, B. and Vincz, A. (Eds.). Oxygen free radicals and scavengers in the natural sciences. Akademiai Kido, Budapest, pp. 35-43.
- Ayoub, F.; Oujji, N.B.; Chebli, B.; Ayoub, M.; Hafidi, A.; Salghi, R. and Jodeh, S. 2017. Antifungal effectiveness of fungicide and peroxyacetic acid mixture on the growth of *Botrytis cinerea*. *Microbial Pathogenesis*, **105**: 74-80.
- Boland, G.J. and Hall, R. 1994. Index of plant hosts of *Sclerotinia sclerotiorum*. *Can. J. Plant Pathol.*, **16**: 93-108.
- Bolton, M.D.; Thomma, B.P. and Nelson, B.D. 2006. *Sclerotinia sclerotiorum* (Lib.) de Bary: biology and molecular traits of a cosmopolitan pathogen. *Mol. Plant Pathol.*, **7**:1-16.
- Buschmann, W.E. and Del Negro, A.S. 2012. Production of peroxyacetic acids. USA Patent US8318972B2. 845 November 27, 2012.
- Casale, W.L. and Hart, L.P. 1986. Influence of four herbicides on carpogenic germination and apothecium development of *Sclerotinia sclerotiorum*. *Phytopathology*, **76**(10): 980-984.
- El-Ashmony, R.M.S.; Abdel-Latif, R.M.; Abdou, E. and Galal, A.A. 2017. Influence of hydrogen peroxide (H₂O₂) on *Trichoderma harzianum* potentiality to control sunflower root/collar rot pathogen *Sclerotium rolfsii*. *Egypt. J. Phytopathol.*, **45**(2). In press.
- EL-Bana, A.A.; Hassan, H.M.M.; Abdou, E. and Galal, A.A. 2006. Effect of calcium salts on growth, sclerotia and infectivity of *Sclerotinia sclerotiorum*. *Assiut J. Agri. Sci.*, **37**(1):175-187.
- EPA (Environmental Protection Agency). 1993. R.E.D. Facts. peroxy compounds. prevention, pesticides and toxic substances. EPA-738-F-93-026.
- EPA, 2004. Registration eligibility decision (RED) PAKTM 27 (sodium carbonate peroxyhydrate with active ingredient hydrogen peroxide), Human and Ecological Risk Assessment for Section 3 Registration of the end-use product PAKTM 27 for application to lakes, ponds, and drinking water reservoirs, DP#301201, PC#000595, EPA File Symbol No. 68660-O; US EPA, Office of Pesticide Program, November 9, 2004.14 Massachusetts Department of Environmental Protection Massachusetts Department of Agricultural Resources.

- Galal, A.A. 2017. Evaluation of peroxyacetic acid (PAA) for controlling bacterial soft rot caused by *Erwinia carotovora* subsp. *carotovora*. *Egypt. J. Phytopathol.*, **45**(2). In press.
- Galal, A.A. and Abdou, E. 1996. Antioxidants for the control of fusarial diseases in cowpea. *Egypt. J. Phytopathol.*, **24**:1-12.
- Galal, A.A. and EL-Bana, A.A. 2002. Inhibition of carpogenic germination of sclerotia of *Sclerotinia sclerotiorum* by cinnamic acid derivatives. *Egypt. J. Phytopathol.*, **30**(1): 67-79.
- Gómez-López, V.M. 2012. Decontamination of Fresh and Minimally Processed Fresh Produce, first edition John Wiley and Sons, Inc.
- Hopkins, D.L.; Hilgren, J.; Lovic, B. and Thompson, C.M. 2003. Wet seed treatment with peroxyacetic acid for the control of bacterial fruit blotch and other seed borne diseases of watermelon. *Plant Disease*, **87**(12):1495-1499.
- Kachroo, A.; He, Z.; Patkar, R.; Zhu, Q.; Zhong, J.; Li, D.; Ronald, P.; Lamb, C. and Chattoo, B.B. 2003. Induction of H₂O₂ in transgenic rice leads to cell death and enhanced resistance to both bacterial and fungal pathogens. *Transgenic Research*, **12**: 577-586.
- Kang, H.C.; Park, Y.H. and Go, S.J. 2003. Growth inhibition of a phytopathogenic fungus, *Colletotrichum* species by acetic acid. *Microbial Res.*, **158**(4):321-6.
- Kitis, M. 2004. Disinfection of wastewater with peracetic acid: a Review. *Environmental International. J.*, **30**(1): 47-55.
- Lesovoi, M.P.; Parfenyuk, A.I. and Kondrafyuk O.K. 1987. A method of identify and selecting sunflower resistant to pathogen of white rot and grey mold. *Mikollogiya and Fitopatologiya*, **21**: 273-278.
- Levine, J.S. 1985. The photochemistry of the early atmosphere. In: Levine, J.S. (ed). *The photochemistry of the atmosphere: Earth the Other Planets and Comets*. Orlando: Academic Press. pp. 3-38.
- Narciso, J.A.; Baldwin E.A.; Plotto, A. and FERENCE, C.M. 2007. Preharvest peroxyacetic acid sprays slow decay and extend shelf life of strawberries. *Hort. Science*, **42**(3): 617-621.
- Neler, J.; Wassermann, W. and Kutner, M.H. 1985. Applied linear statistical models. In: Richard, D. (Ed.) *Regression analysis of variance and experimental design: 2nd Irwin Inc. Homewood Illinois*. pp. 117-155.
- Osório, T.G.; Oliveira, B.S. and Di Piero, R.M. 2013. Effect of fumigants on blue and gray molds of apple fruit. *Tropical Plant Pathology*, **38**(1): 63-67.
- Purdy, L.H. 1979. *Sclerotinia sclerotiorum*: history, disease and symptomology, host range, geographic distribution and impact. *Phytopathology*, **69**(8):875-880.

- Sansford C. 1995. Oil seed rape: development of stem rot (*Sclerotinia sclerotiorum*) and its effect on yield In: Proc.ix. International Rapeseed Congress Today and Tomorrow. Cambridge UK, **2**: 634-636.
- Sharma, J.; Godika, S.; Hasolia, RP.; Goyal, S.K. and Yadav, A.L. 2016. Evaluation of bioagents against *Sclerotinia sclerotiorum* causing sclerotinia rot of indian mustard. *J. Oilseed Brassica*, **7**(2): 194-197.
- Sutherland, M.W. 1991. The generation of oxygen radical during host plant response to infection. *Physiol. Mol. Pl. Pathol.*, **39**: 79-93.
- Sutton, L.M. and Starzyk, M.J. 1972. Procedure and analysis of a useful method in determining mycelial dry weights from agar plates. *Appl. Microbiol.*, **24**(6):1011-1012.
- Thipaksorn, C.N.; Rattanapanone, A. and Boonyakiat, D. 2012. Effects of peroxyacetic acid, peroxydictric acid, sodium bicarbonate, potassium sorbate, and potassium metabisulfite on the control of green mold in Sai Nam phueng tangerine Fruit. *CMU. J. Nat. Sci.*, **11**(2): 203-211.
- Wessels, S. and Ingmer, H. 2013. Modes of action of three disinfectant active substances: A review. *Regulatory Toxicology and Pharmacology*, **67**(3):456-467.
- Wheeler, B.E.J. 1969. An Introduction to Plant Disease. John Wiley and Sons Ltd., London, 301p.

(Received 25/10/2017;
in revised form 20/11/2017)

استخدام بيروكسي حمض الخليك كعامل صديق
للبيئة في خفض النمو والإنبات الثمري للأجسام
الحجرية والقدرة المرضية للفطر
Sclerotinia sclerotiorum

رانيا مصطفى الأشموني - مرزوق رجب عبد اللطيف - السيد
عبد السيد أحمد - أنور عبد العزيز جلال

قسم أمراض النبات - كلية الزراعة - جامعة المنيا - المنيا - مصر

أعطى المركب صديق البيئة المكون من فوق أكسيد الهيدروجين كأساس (بيروكسي حمض الخليك) تأثير ضد النمو والقدرة المرضية للفطر *Sclerotinia sclerotiorum* وكانت التأثيرات المثبطة لبيروكسي حمض الخليك معنوية ضد النمو الميسليومي وتكوين الأجسام الحجرية والوزن الجاف لميسليوم الفطر. وكانت التأثيرات المعنوية واضحة عند استخدام التركيز المنخفض ٠,٤ جرام/لتر حمض الخليك + ٠,٨ جرام/لتر فوق أكسيد الهيدروجين وازدادت بزيادة التركيز إلى ٠,٤ جرام/لتر حمض الخليك + ١,٢ جرام/لتر فوق أكسيد الهيدروجين حيث أحدثت ٨٥% و ٩١% و ٨٣% تثبيط لكل من النمو الخطي وتكوين الأجسام الحجرية والوزن الجاف لميسليوم الفطر على التوالي. علاوة على ذلك أظهرت معاملات بيروكسي حمض الخليك تأثير معنوي في خفض قياسات الإنبات الثمري للأجسام الحجرية للفطر حيث انخفض الإنبات الثمري من ١٠٠% في حالة المقارنة إلى ٦٢% و ٥٠% و ٣٨% و ٥٠% تثبيط عند معاملة الأجسام الحجرية بتركيزات ٠,٤ جرام/لتر حمض الخليك + ٠,٨ جرام/لتر فوق أكسيد الهيدروجين وتركيز ٠,٤ جرام/لتر حمض الخليك + ١,٢ جرام/لتر فوق أكسيد الهيدروجين على التوالي. وصاحب انخفاض الإنبات الثمري نتيجة المعاملة بتلك المركبات انخفاضاً معنوياً لعدد الحوامل والأجسام الثمرية لكل جسم حجري.

أدى غمر التربة المعدية بالاسكليروتنيا مرة واحدة ببيروكسي حمض الخليك قبل الزراعة بـ ٧ أيام إلى خفض معنوي في شدة الإصابة بكل من أعفان الجذور وعفن ساق نباتات الفاصوليا المختبرة صنف جيزه ٦ فعند ري التربة بالتركيز الأول ٠,٤ جرام/لتر حمض الخليك + ٠,٨ جرام/لتر فوق أكسيد الهيدروجين والتركيز ٠,٤ جرام/لتر حمض الخليك + ١,٢ جرام/لتر فوق أكسيد الهيدروجين كل على حده أدى إلى خفض معنوي للإصابة لعفن الجذور وعفن الساق بنسبة ٤٧% و ٦٧% على التوالي.

أظهرت معاملة قرون الفاصوليا ببيروكسي حمض الخليك إلى خفض معنوي للإصابة بالعفن الأبيض الذي يسببه فطر *Sclerotinia sclerotiorum* وكانت معاملة القرون بعد العدوى بالفطر أكثر فعالية في خفض الإصابة عند المقارنة بمعاملة القرون قبل إجراء العدوى الصناعية بنفس الفطر.