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Suppression of Powdery Mildew in Okra and Sunflower Plants under Natural Infection through Peroxyacetic Acid Foliar Application

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Application of an eco-friendly hydrogen peroxide-based compound peroxyacetic acid (PAA), to leaves of okra and sunflower plants significantly reduced infection with powdery mildew. The maximum percent disease control was recorded with acetic acid (AA) + hydrogen peroxide (H_2O_2) mixture at 0.2g/l AA + 2.0g/l H_2O_2 concentration, being 69.28 and 70.07 %, when okra and sunflower plants were sprayed three times, respectively along 2 growing seasons. Decreasing sprayings and mixture concentration reduced percent of powdery mildew control. Accordingly, PAA showed a beneficial role to control powdery mildew of either okra or sunflower plants. Thus, PAA could be recommended for integrated disease management program (IDM) against powdery mildew of okra and sunflower.

Keywords: *Abelmoschus esculentus*, *Erysiphe cichoracearum*, *Golovinomyces cichoracearum*, *Helianthus annuus*, Okra, peroxyacetic acid, powdery mildew and sunflower.

Powdery mildew is one of the most common fungal diseases affecting wide range of host plants. Disease symptoms are easily spotted; however, it can spread to the point of being out of control quickly. The term "powdery mildew" encompasses a range of related fungal species which all possess similar damage symptoms with each targeting specific hosts. Powdery mildew is a serious disease of okra and sunflower plants in Assuit and Minia governorates, Egypt (Ismail *et al*., 2012 & Younes and Abo-Elyouser, 2012). The disease is caused by the fungus *Golovinomyces cichoracearum*, formerly named *Erysiphe cichoracearum* DC ex Merat (Ale-Agha *et al*., 2008; Shah *et al*., 2016; Dahivelkar *et al*., 2017 and Madhusudhan *et al*., 2017). This disease is one of the most commonly occurring diseases on okra (*Abelmoschus esculentus* Moench) and sunflower (*Helianthus annunnus* L.). Most of their cultivars are susceptible to powdery mildew, and depending in most cases, upon the age of the plant at the time of infection. The pathogen is easily recognized by the abundant production of mycelia, conidiophores and conidia on the surface of leaves (Gogol *et al*., 2013; Shah *et al*., 2016; Dahivelkar *et al*., 2017 and Madhusudhan *et al*., 2017).

Powdery mildew reduces the photosynthetic rate of plants, causes yellowing and premature death of leaves and, in severe infections, may kill the plant. The reduction in leaf area and changes in the carbohydrate contents of infected leaves can reduce plant yield as well (Karuna *et al*., 2011; Gogol *et al*., 2013; Dahivelkar *et al*., 2017; Suresha *et al*., 2017 and Madhusudhan *et al*., 2017). Several approaches such as fungicides (Naik and Nagaraja, 2000; Kumar *et al*., 2008; Dhutraj, 2011; Madhusudhan *et al*., 2017 and Dahivelkar *et al*., 2017), plant extracts (El-Fahar and Mostafa, 2009; Gado, 2013 and Zhang *et al*., 2016), bioagents (Kumar *et al*., 2008 and Khalikar *et al*., 2011), resistance inducers (Abada *et al*., 2008 and Ismail *et al*., 2012), algae extracts (Zhang *et al*., 2016), nanoparicles of sulphur (Gogol *et al*., 2013 and Reuveni *et al*., 2017) and potassium silicate (Liang *et al*., 2005; Kanto et al., 2006; Yanar *et al*. 2011 and Dallagnol *et al*., 2012) were approved to managing powdery mildews in various plants. Unfortunately, powdery mildew pathogens are still a complex issue to be controlled, due to raising fungicide resistance strains, repeat infection frequently and a few field resistant cultivars produced (McGrath, 2001; Gogol *et al*., 2013; Suresha *et al*., 2017; Dahivelkar *et al*., 2017 and Madhusudhan *et al*., 2017). However, the genetic resistance against powdery mildew is not durable because of rapid genetic changes within the pathogen population (Shah *et al*., 2016). For this reason, chemicals are also used as a control method. However, there are several reports on the selection of isolates either resistant or insensitive to different groups of fungicides (McGrath, 2001; Gogol *et al*., 2013; Kulkarni *et al*., 2015; Dahivelkar *et al*., 2017 and Madhusudhan *et al*., 2017). Thus, the development of additional control measures with low environmental impact represents an important research topic for this disease.

The use of peroxyacetic acid (PAA) is gaining attention due to their proven strong oxidizer, bio degradability, low toxicity and no residual toxicity in the ecosystem. So, PAA which has broad-spectrum antimicrobial properties was used in the present study. The present study was under taken to find out the most suitable PAA concentration for controlling the disease.

Materials and Methods

Preparation of PAA:

Three combinations (g/liter distilled water) for hydrogen peroxide and acetic acid which led to form peroxyacetic acid (PAA), as previously reported (EPA, 2004; Buschmann and Del Negro, 2012 and EPA, 2012) e.g., 0.05AA + 1.0 H_2O_2, 0.1 AA + 1.0 H_2O_2 and 0.2 + 2 H_2O_2 were tested and distilled water was served as control.

Field experiments:

Disease severity of powdery mildew was rated weekly beginning at 2 weeks after the last foliar spraying. Each plant was visually rated for disease severity based on estimation of the percentage of the leaf surfaces covered with powdery mildew lesions on fully expanded 5 leaves (from leaf number 4 to leaf number 8 per plant). Values of AUDPC were calculated based on the formula as follows:

$$\Sigma[\{(xi + xi–1)/2\}(ti – ti–1)]$$

Where xi is the rating at each evaluation time and (ti – ti–1) is the time between evaluations as described by Zhang *et al.* (2016).

Statistical analysis:

The least significant difference (LSD) at *P* = 0.05 for AUDPC values of all treatments along 2 seasons per each plant individually was calculated (Gomez and Gomez, 1994).

Results

Okra powdery mildew:

Among acetic acid + H_2O_2 foliar applications, a significant reduction in powdery mildew AUDPC values was obtained as compared to untreated okra plants (Table 1). The least percentage (6.96 % on the average) in powdery mildew protection under natural infection was provided when okra plants were sprayed once only by the tested solutions. The highest protection percentage, being 43.74 % on the average was exhibited when the foliar was sprayed three times by AA + H_2O_2 mixture followed by the two sprays program (30.91 % protection on the average). However, increasing AA + H_2O_2 concentrations significantly increased powdery mildew protection. To be noticed, one time foliar spray by lower AA + H_2O_2 concentrations explored insignificant powdery mildew protection, since single foliar spraying by either 0.05 g/l AA + 1.0 g/l H_2O_2 or 0.1 g/l AA + 1.0 g/l H_2O_2 caused the lowest protection percentage (0.73 %). The highest concentration tested 0.2 g/l acetic acid + 2.0g/l H_2O_2 significantly decreased powdery mildew even when it was applied one time spray (19.42 % protection). On the other hand, significant powdery mildew reduction was achieved when all AA + H_2O_2 concentrations were applied twice or thrice to okra foliar. The three times foliar spray program by AA + H_2O_2 showed the highest protection percentages. At 0.2 g/l AA + 2.0 g/l H_2O_2 the least AUDPC values for powdery mildew along the two growing seasons tested (199 and 220 in 2016 and 2017 growing seasons, respectively). As for growing season, AUDPC values of okra powdery mildew under natural infection were significantly higher for untreated plants in 2016 (712 AUDPC values) than in 2017 (652 AUDPC values). Furthermore, in most cases effectiveness of AA+ H_2O_2 to reduce okra powdery mildew was more in 2016 than in 2017 growing season.

Sunflower powdery mildew:

Generally, all AA + H_2O_2 treatments tested showed significant reduction in sunflower powdery mildew as compared to untreated plants, under natural infection throughout the two growing seasons (Table 2). Among spraying times, the three times foliar sprays program by AA + H_2O_2 expressed the highest protection percentages (37.81 % on the average) followed by the two sprays program (29.29 % protection on the average) against sunflower powdery mildew. Single foliar spray program by AA + H_2O_2 concentrations tested showed the lowest protection percentage (22.27 %). As for AA + H_2O_2 combinations tested, all of them exhibited significant reduction in sunflower powdery mildew. Increasing AA + H_2O_2 concentration increased powdery mildew reduction. Concomitantly raising spray times enhanced AA + H_2O_2 efficiency to reduce AUDPC values for sunflower powdery mildew, since the highest protection percentage (70.07 %) was provided by the three times foliar spray program with 0.2 g/l AA + 2.0 g/l H_2O_2 followed by the two application program of the same concentration (56.5%). During the two growing seasons, AUDPC values of sunflower powdery mildew on the untreated plants were significantly higher in 2017 (698 AUDPC values) than in 2016 (654 AUDPC values).

Table 1. Area under powdery mildew progress curve values to okra plants as affected by different sprayings by PAA

Spray treatments and Cons. (g/l)	Growing season,		Mean	Protection %
	2016	2017		
One spray AA+ H_2O_2				
0.05 + 1.0	702	652	677	0.73
0.1 + 1.0	702	652	677	0.73
0.2 +2.0	594	505	549.5	19.42
Mean	666	603	634.5	6.96
Twice sprays AA+ H_2O_2				
0.05 + 1.0	572	529	550.5	19.28
0.1 + 1.0	500	481	490.5	28.08
0.2 +2.0	369	376	372.5	45.38
Mean	480.33	462	471.16	30.91
Triple sprays AA+ H_2O_2				
0.05 + 1.0	545	524	534.5	21.62
0.1 + 1.0	424	390	407	40.32
0.2 +2.0	199	220	209.5	69.28
Mean	389.33	378	383.66	43.74
Untreated	712	652	682	0.0
Grand Mean	561.9	523.75	542.83	

LSD at 0.05 for Spraying numbers = 9.11 Growing seasons = 12.5
PAA concentrations = 14.8

Table 2. Area under powdery mildew progress curve values to sunflower plants as affected by different sprayings by PAA

Spray treatments and Cons. (g/l)	Growing season,		Mean	Protection %
	2016	2017		
One spray AA+ H_2O_2				
0.05 + 1.0	644	637	640.5	6.97
0.1 + 1.0	476	556	516	25.05
0.2 +2.0	394	504	449	34.78
Mean	504.66	565.66	535.16	22.27
Two sprays AA+ H_2O_2				
0.05 + 1.0	644	623	633.5	7.98
0.1 + 1.0	560	444	502	27.08
0.2 +2.0	280	371	325.5	52.79
Mean	494.66	479.33	486.83	29.29
Three sprays AA+ H_2O_2				
0.05 + 1.0	616	518	567	17.64
0.1 + 1.0	546	476	511	25.78
0.2 +2.0	231	182	206.5	70.07
Mean	464.33	392	428.16	37.81
Untreated	679	698	688.5	
Grand Mean	535.66	533.74	534.66	

LSD at 0.05 for Sprayings = 12.6 Growing seasons = 13.7
PAA concentrations = 13.65

Discussion

The present study is concerned to test the efficiency of AA+ H_2O_2 combinations that form an eco-friendly H_2O_2 -based compound named peroxyacetic acid (PAA) as reported by Buschmann and Del Negro (2012). This PAA is successfully reacted as a strong oxidizer disinfectant, fungicide and bactericide compound (EPA, 2004; Hopkins *et al*., 2003; Hopkins *et al*., 2009 and Ayoub *et al*., 2017).

The results of the present study, along 2 growing seasons tested revealed that treatments with acetic acid + H_2O_2 were effective to reduce AUDPC values of powdery mildew in either okra or sunflower plants significantly. Least percentages (6.96 and 22.27 %) in powdery mildew protection under natural infection were provided when okra and sunflower plants, respectively were sprayed one time by the tested solutions. The highest protection percentages, 43.74 % for okra and 37.81 % for sunflower, were exhibited when the foliar was sprayed three times by the tested solutions where the two sprays program exhibited 30.91 and 29.29 % protection in case of okra and sunflower, respectively. However, increasing AA + H_2O_2 concentrations significantly enhanced powdery mildew protection. To be noticed, one time foliar spray program by lower AA + H_2O_2 concentrations explored insignificant powdery mildew protection, particularly in case of okra plants. Since one time foliar spraying program by either 0.05 g/l AA + 1.0 g/l H_2O_2 or 0.1 g/l AA + 1.0 g/l H_2O_2 caused more or less 0.73 % protection, (AUDPC values) merely to check ones, while sunflower plants provided 6.97 and 25.05 % powdery mildew protection by one time program using 0.05 g/l AA + 1.0 g/l H_2O_2 or 0.1 g/l AA + 1.0 g/l H_2O_2 foliar spray, respectively.

The highest concentration tested 0.2 g/l AA + 2.0 g/l H_2O_2 decreased powdery mildew significantly event even when it applied one time spray which caused 19.42 and 34.78 % powdery mildew reduction in okra and sunflower plants, respectively. On the other hand, all tested solutions of AA + H_2O_2 significantly protected okra and sunflower against powdery mildew when they were applied twice or thrice. At 0.2 g/l AA + 2 g/l H_2O_2 the least AUDPC values for powdery mildew along 2 growing seasons tested recorded 199 and 220 AUDPC values in 2016 and 2017 growing seasons, respectively in case of okra powdery mildew. While in sunflower powdery mildew, the same concentration recorded 231 and 182 AUDPC values in 2-16 and 2017 growing seasons, respectively. As for growing seasons, AUDPC values of okra powdery mildew under natural infection was higher significantly for untreated plants in 2016 (712 AUDPC valuea) than 2017 (652 AUDPC values). Furthermore, effectiveness of AA + H_2O_2 to reduce okra powdery mildew was more significant in 2016 than 2017 growing season.

In the light of the present data, it can be concluded that H_2O_2 -based compound which called PAA has a beneficial effect to suppress powdery mildew development in either okra or sunflower plants tested. Three times PAA foliar sprays program gave the highest protection against powdery mildew specially when okra or sunflower plants were sprayed by the concentration 0.2 g/l AA + 2.0 g/l H_2O_2 that lowered AUDPC values of okra powdery mildew from 682 in case of untreated to 209.5 (69.28 % protection) and from 688.5 to 206.5 (70.07 % protection) of sunflower powdery mildew. Data are in line with those obtained by PAA application against several plant diseases caused by either phytopathogenic fungi (Mari *et al.*, 2004; Pukdee and Sardsud, 2007 Thipaksorn *et al*., 2012.; Feliziani *et al*., 2016 and Ayoub *et al*., 2017) or phytopathogenic bacteria (Hopkins *et al*., 2003 and Hopkins *et al*., 2009). Thus, the present study highly recommended including PAA in any integrated powdery mildew management programs against powdery mildew of okra and sunflower.

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تثبيط الإصابة بالبياض الدقيقي في نباتات الباميا وعبــاد الشمس بـــرش المجموع الخضري بمركــب بيروكسي حمض ألخليك

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أدى رش المجموع الخضري لنباتات الباميا وعباد الشمس كلا على حده بمركب PAA محتواه الأساسي فوق أكسيد الإيدروجين (بيرو كسي حمض ألخليك) إلى خفض معنوي في إصابة تلك النباتات بالبياض الدقيقى. أظهرت الإصابة بالبياض ألدقيقي علي كل من الباميا وعباد الشمس اختلافات معنوية باختلاف عدد رشات وتركيزات بيروكسى حمض الخليك وأمكن الحصول على اعلى نسبة وقاية ضد البياض الدقيقى ٦٩,٢٨ و ٧٠,٠٧ % وذلك برش نباتات الباميا وعباد الشمس على التوالي ثلاث رشات متتالية بالمركب ٠,٢ جرام حامض خليك + ٢,٠ جرام فوق أكسيد الإيدروجين/لتر خلال موسمي التجربة ٢٠١٦ و ٢٠١٧.

أدى خفض عدد مرات الرش وتركيز بيروكسى حمض الخليك الى خفض معنوي لنسب الوقاية ضد الاصابة بالبياض الدقيقى على كل من الباميا وعباد الشمس.

أوضحت الدراسة بان المركب بيروكسى حمض الخليك ذو فعالية فى وقاية نباتات الباميا وعباد الشمس ضد الإصابة بالبياض الدقيقى تحت ظروف العدوى الطبيعية. بناء عليه توصى الدراسة بإدخال المعاملة بمركب PAA بيروكسى حمض ألخليك فى برامج المكافحة المتكاملة لإمراض البياض الدقيقى.