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Control of Sugarcane Smut Disease Incited by *Sporisorium scitamineum* Syd. Using Peroxyacetic Acid (PAA)

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An eco-friendly agent, hydrogen peroxide (H_2O_2)-based compound, peroxyacetic acid (PAA) gave efficiency against *Sporisorium scitamineum* teliospore viability. A significant sporocidal effects for all AA + H_2O_2 combinations tested against *S. scitamineum* teliospores germination was explored. The highest inhibitory effect (69.9% inhibition) was exhibited at 0.2 AA + 4.0 H_2O_2 g/L followed by 0.1 AA + 4.0 H_2O_2 g/L (51.9% inhibition) and 0.2 AA + 2.0 H_2O_2 g/L (48.9% inhibition).

Under field experiments, most of AA + H_2O_2 treatments were reduced smut disease incidence (SDI) significantly. However, SDI values showed insignificant variances among four treatments, 0.1 AA + 1.0 H_2O_2 g/L, 0.1 AA + 2.0 H_2O_2 g/L, 0.1 AA + 4.0 H_2O_2 g/L and 0.2 AA + 1.0 H_2O_2 g/L, in two planting dates. While the two treatments, viz. 0.2 AA + 2.0 H_2O_2 g/L and 0.2 AA + 4.0 H_2O_2 g/L, showed significant variances values in SDI as they significantly reduced SDI values in May more than in October. Meantime, means of protection values (MPV) of 0.2 AA + 2.0 H_2O_2 g/L and 0.2 AA + 4.0 H_2O_2 g/L, showed significant variances in MPV values as they were significantly raised in May being 86.55% MPV more than in October, 70.85% MPV. In May planting date, MPV was significantly higher than in October planting date particularly in the check plants.

Keywords: Peroxyacetic acid, H_2O_2-based compound, Eco-friendly agent, sugarcane smut, *Sporisorium scitamineum.*

Sugarcane (*Saccharium officinarium* L.) is not only a cash crop for the growers, but it is also the main source of white crystal sugar. It is grown in Upper Egypt for sugar production as well as used in the industrial fermentation that necessary for the alcohol, active yeast (bread yeast), citric acid, acetic acid and dextrin (Abd El Fattah, 1996). Of 240 diseases attacked sugarcane plants (Rott*et al*., 2000), smut is a major disease of sugarcane caused by *Sporisorium scitamineum= Ustilago scitaminea* (Sydow, 1924). The disease spread is worldwide covering most of the sugarcane producing areas. The wide spread of this disease prompted a great deal of experimental work on it (Heinz, 1987; Akalach, 1994 and Banihashemi, 1995). Lovick (1978) comprehensively reviewed on various aspects of sugarcane smut *viz*.

symptoms, yield reduction, causal organism, and physiological races of the smut fungus, epidemiology, host resistance and management. It was reported that severe smut outbreak in the Caribbean has created an impact amongst cane growers and sugar industry. Consequently, the plantations are undertaking different smut management practices such as continued monitoring and rouging of smut affected stools, hot water treatment (at 50°C for 2 hours for initial seed cane nursery) of seed sets, chemical treatment of sets, use of resistant varieties, and avoidance of ratooning of affected fields (Abdou *et al*., 1990; Wada *et al*., 1999; Abera *et al*., 2009; Firehun*et al*., 2009; Mansour *et al*., 2016; Carvalho *et al*., 2016; Sánchez-Elordi *et al*., 2016 and Liu *et al*., 2017)

Due to their core role in plant health, reactive oxygen species (ROS) and antioxidants Sutherland (1991) and Levine *et al*. (1994) have shown a major role in plant pathogen interactions (Galal and Abdou, 1996; Adam *et al.*, 2000; Galal, 2017 and EL-Ashmony *et al.*, 2017). Both acetic acid and hydrogen peroxide reacted as bactericides and fungicides against many phytopathogenic bacteria and fungi (Narciso*et al.*, 2007 and Osório*et al.*, 2013; Ayoub *et al*., 2017 and Galal, 2017).

The present study was planned to test the efficacy of H_2O_2-based compound PAA on *S. scitamineum* infectivity either to sugarcane plants at two planting dates during two growing seasons of 2016/2017 and 2017/2018.

Materials and Methods

1. Teliospores:

Teliospores of the pathogen *Sporisorium scitamineum* Syd. and plants of *Saccharum officinarum* L., field grown in Mallawy county, Minia governorate, Egypt were used throughout this work. Sugarcane genotype C9 that is commonly cultivated at Minia governorate regions was used in the assays in order to monitor its responses to smut disease.

2. Pathogenicity tests:

For pathogenicity tests, teliospores of *S. scitamineum*, collected from an infected field of cv. C9 (susceptible to smut) were sterilized and incubated as previously described by Santiago *et al*. (2010).One node-cuttings of sugarcane were surface disinfected using 0.5% sodium hypochlorite for 5mn then washed thoroughly by sterilized distilled water then inoculated by paste teliospore (Olweny *et al*., 2008). Inoculum and inoculation were conducted by suspend teleospores (65% viable spores) in 0.8% soluble starch solution after melting it and cooled, became semi solid (jelly like), before adding teleospores to obtain 10^5 spores/ml. The waxy layer covered buds was removed gently then buds were inoculated by panting them with about 50uL spore paste then air dried under ambient temperature for 2h. Forty artificially inoculated cuttings were planted at 1st of May 2015 in one plot 3×3m (3 rows/plot) with 2 m length and 70 cm width. New whip formed in pathogenicity test

was used as a source of inoculation for the subsequent experiments. However, fresh whips formed in check plants of the previous experiment were used as a source of inoculation for subsequent experiments along four planting dates of two years (two planting dates per year).

3. Preparation of test solutions:

Six mixtures of acetic acid (AA) and hydrogen peroxide (H_2O_2), 0.1 AA + 1.0 H_2O_2 g/L, 0.1 AA + 2.0 H_2O_2 g/L, 0.1 AA + 4.0 H_2O_2 g/L, 0.2 AA + 1.0 H_2O_2 g/L, 0.2 AA + 2.0 H_2O_2 g/L and 0.2 AA + 4.0 H_2O_2 g/L, were prepared with distilled water then left for at least 10 days before the test (Buschmann and Del Negro, 2012 and Anonymous, 2012).

4. Effect of PAA on teliospores germination:

Teliospore germination of sugarcane smut fungus *S. scitamineum* was evaluated after 12h incubation at 25°C onto sterilized water agar plates supplemented by the tested solutions as described before (El-Ashmony *et al*., 2017), check plates contained unamended water agar.

5. Field experiments:

Field experiments were conducted at two planting dates, May and October, during two growing seasons 2016/2017 and 2017/2018 on the field of Plant Pathology Dept., Faculty of Agriculture, Minia University, Minia governorate, Egypt. The attempts were made to evaluate the effect of different combinations of AA + H_2O_2 as cuttings wetting on the disease incidence (SDI) of sugarcane smut considering the effect of SDI on percent disease control (protection %). The experiments were laid out in randomized complete design with three replications and seven treatments. One-node cuttings of sugarcane cultivar C9 were used throughout this study. Each replicate was one plot 3×3m (3 rows/plot) with 2 m length and 70 cm width. Cuttings were sown at 20 cm distance at rate of 10 plants/row and 30 plants/plot. Inoculum and inoculation of sugarcane cuttings were conducted as described in the pathogenicity trail and cuttings were treated by the prepared concentrations of AA + H_2O_2 2h post inoculation then planted two hours later. All the recommended agronomical practices were adopted for raising the crops.

Disease assessment:

Sugarcane smut incidence (SDI) was monitored 6 months after sowing (Firehun *et al*., 2009).

6. Statistical analysis:

The least significant difference (LSD) at P = 0.05 for SDI values of all treatments along two growing seasons per each sowing date individually was calculated (Gomez and Gomez, 1994).

Results

1. Effect of PAA on S. scitamineum teliospores germination:

Obvious significant inhibitory effects for all AA + H2O2 combinations tested were explored against teliospores germination (Table 1). Increasing AA or H_2O_2 increased teliospores germination inhibition. Lowest inhibitory effect (18.9% inhibition) was provided at 0.1 AA + 1.0 H_2O_2 g/L followed by 0.1 AA + 2.0 H_2O_2 g/L that caused 21.95 inhibition. The highest inhibitory effect (69.9% inhibition) was exhibited at 0.2 AA + 4.0 H_2O_2 g/L while using 0.1 AA + 4.0 H_2O_2 g/L expressed 51.9%inhibition. Meanwhile, insignificant difference was recorded with using 0.2 AA + 2.0 H_2O_2 g/L which explored 48.9% inhibition.

Table 1: Germination% of *Sporisorium scitamineum* teliospores as affected by various combinations of acetic acid (AA) + hydrogen peroxide (H_2O_2) incubated at 25°C for 12 hr.

Treatments and Conc. (g/l)	Germination %	Inhibition %
Untreated	66.6 a	0.00
AA + H_2O_2		
0.1 + 1.0	54 ab	18.90
0.1 + 2.0	42 c	21.95
0.1 + 4.0	34 d	51.90
0.2 + 1.0	48 b	27.90
0.2 + 2.0	36 d	48.90
0.2 + 4.0	22 e	69.90

Values with the same letters are not significantly differed

2. Effect of PAA on sugarcane smut incidence (SDI) under field experiments:

2.1. May planting date:

Along two growing seasons for May planting date, all treatments (except 0.1 AA + 1.0 H_2O_2 g/L) showed significant protection values against smut infection as compared to untreated inoculated sugarcane cuttings (Table 2). Increasing AA or H_2O_2 resulted in protection enhancement. The highest protection value (86.55%) was obtained by using 0.2 AA + 4.0 H_2O_2 g/L followed by 0.2 AA + 2.0 H_2O_2 g/L which caused 60.61% protection. The least 0.1 AA + 1.0 H_2O_2 g/L combination showed insignificant SDI reduction (5.59%) of protection while using 0.1 AA + 2.0 H_2O_2 g/L caused 27.69% protection. At 0.2 AA + 1.0 H_2O_2 g/L, SDI was significantly decreased, recording 37.58% protection while 0.1 AA + 4.0 H_2O_2 g/L exhibited 42.80% protection. Untreated inoculated sugarcane cuttings resulted in SDI at May 2016 higher than May 2017 as well as treatments were most effective by May 2016 than May 2017.

Table 2: Sugarcane smut incidence (SDI) as influenced by treatment of post inoculated sugarcane cuttings cv., C9 with the tested solutions of various acetic acid (AA) and hydrogen peroxide (H_2O_2) combinations. Smut incidence was monitored six months after planting in May 1st of 2016 and 2017.

Treatments and Conc. (g/l)	Smut incidence during		Mean	Protection %
	2016	2017		
Untreated	58.7	52.5	55.6	0.00
AA + H_2O_2				
0.1 + 1.0	53.4	49.7	51.5	5.59
0.1 + 2.0	41.8	38.6	40.2	27.69
0.1 + 4.0	32.7	31.8	32.3	42.80
0.2 + 1.0	36.3	33.2	34.7	37.58
0.2 + 2.0	24.2	19.6	21.9	60.61
0.2 + 4.0	5.4	9.6	7.5	86.55
Mean	36.1	33.6	35.0	
LSD at 0.05 for Treatments (A) 4.8, Growing seasons (B) 3.6, A×B 7.8				

2.2. October planting date:

Generally, all treatments showed significant SDI reduction at October planting date even at the lowest concentration, 0.1 AA + 1.0 H_2O_2 g/L, which caused 10.85% protection (Table 3). Increasing AA or H_2O_2 enhanced protection where 0.2 AA + 4.0 H_2O_2 g/L combination expressed the highest protection value (70.80% protection) followed by 0.2 AA + 2.0 H_2O_2 g/L that provided 52.8% protection and 0.1 AA + 4.0 H_2O_2 g/L which showed 41.96% protection.

Table 3: Sugarcane smut incidence (SDI) as influenced by treatment of post inoculated sugarcane cuttings cv, C9 with the tested solutions of various acetic acid (AA) and hydrogen peroxide (H_2O_2) combinations. Smut incidence was monitored six months after planting in October 1st of 2016 and 2017.

Treatments and Conc. (g/l)	Smut incidence during		Mean	Protection %
	2016	2017		
Untreated	48.3	43.6	45.9	0.00
AA + H_2O_2				
0.1 + 1.0	38.4	41.4	39.9	10.85
0.1 + 2.0	36.5	36.3	36.4	20.69
0.1 + 4.0	31.3	22.2	26.7	41.96
0.2 + 1.0	28.8	33.3	31.05	32.45
0.2 + 2.0	23.6	21.4	22.5	52.8
0.2 + 4.0	13.7	12.6	13.15	70.80
Mean	31.5	30.1	30.7	
LSD at 0.05 for Treatments (A) 3.4, Growing seasons (B) 2.9, A×B 6.2				

2.3. Comparison between planting dates:

Generally, SDI in May planting date was significantly higher than that in October planting date particularly in check plants (control) as shown in Table (4), as the check plants showed mean values of SDI 55.6% by May planting date compared to 45.9% in October planting date. However, SDI values showed insignificant differences among four treatments, 0.1 AA + 1.0 H_2O_2 g/L, 0.1 AA + 2.0 H_2O_2 g/L, 0.1 AA + 4.0 H_2O_2 g/L and 0.2 AA + 1.0 H_2O_2 g/L, during the two planting dates. While the two treatments, viz. 0.2 AA + 2.0 H_2O_2 g/L and 0.2 AA + 4.0 H_2O_2 g/L, showed significant differences in SDI values, since they significantly decreased SDI values in May more than in October. Meantime, means of protection values (MPV) of 0.2 AA + 2.0 H_2O_2 g/L and 0.2 AA + 4.0 H_2O_2 g/L, showed significant differences since they significantly rised MPV values in May (86.55% MPV more than in October (70.*%MPV).

Table 4: Comparison between May and October sowing dates means values of smut incidence (SDI) and protection (MPV) as influenced by treatment of post inoculated sugarcane cuttings cv, C9 with the test solutions of various acetic acid (AA) and hydrogen peroxide (H_2O_2) combinations.

Treatments and Conc. (g/l)	Means of SDI for Plating date (Mean Values of two seasons)		MPV of planting date,	
	May	October	May	October
Untreated	55.6 a	45.9 b	0.0 g	0.0 g
AA + H_2O_2				
0.1 + 1.0	51.5 a	39.4 c	5.59 g	10.85 f
0.1 + 2.0	40.2 c	36.4 cd	27.69 e	20.69 e
0.1 + 4.0	32.3 d	26.7 e	42.80 c	41.96 c
0.2 + 1.0	34.7 cd	31.1 d	37.58 d	32.45 d
0.2 + 2.0	21.9 e	22.5 e	60.61 c	52.8 d
0.2 + 4.0	7.5 f	13.15 f	86.55 a	70.80 b

Values with the same letters are not significantly differed

Discussion

Sugarcane smut continues to be a serious threat to sugarcane production in different countries. Integrated disease management strategy is the viable option in smut disease control, rather than resorting to a single method. Recommended phytosanitary practices like seed selection, roguing of infected clumps etc is the best possible way to reduce smut inoculum levels (Sundar *et al*., 2012). The current work showed a significant sporocidal effects for all AA + H_2O_2 combinations tested against *S. scitamineum* teliospores germination. The highest inhibitory effect (69.9% inhibition) was exhibited at 0.2 AA + 4.0 H_2O_2 g/L while using 0.1 AA + 4.0 H_2O_2

g/L expressed 51.9%inhibition that showed insignificant effect with using 0.2 AA + 2.0 H_2O_2 g/L which explored 48.9% inhibition. Antifungal effects for PAA which resulted from mixed AA + H_2O_2 (Buschmann and Del Negro, 2012) were confirmed against several phytopathogenic fungi ((Mari *et al*., 2004; Feliziani *et al*., 2016; Ayoub *et al*., 2017 and El-Ashmony *et al*., 2017).

In plants, reactive oxygen species (ROS) play a crucial role in growth, development and in plant defense against the pathogens (Foreman *et al*., 2003 and Kawano, 2003). For example there are at least 152 genes involved in ROS secretion, function and signaling in *Arabidopsis thaliana* (Mittler *et al*., 2004). During plant pathogen interactions, ROS function in the formation of physical defense components (such as cell wall appositions) and in the activation of the R gene-mediated cell wall appositions (Delledonne *et al*., 2001 and Collinge, 2009). The recent study for May planting date explored that all treatments (except 0.1 AA + 1.0 H_2O_2 g/L) showed significant protection values against smut infection as compared to untreated inoculated sugar cane cuttings. The highest protection value (86.55% protection) was pronounced by using 0.2 AA + 4.0 H_2O_2 g/L followed by 0.2 AA + 2.0 H_2O_2 g/L which caused 60.61% protection. The least 0.1 AA + 1.0 H_2O_2 g/L combination showed insignificant SDI reduction, 5.59% protection. Untreated inoculated sugarcane cuttings resulted in SDI at May 2016 higher than May 2017 as well as treatments were most effective by May 2016 than May 2017. By October planting date, all treatments showed significant SDI reduction even at the lowest concentration, 0.1 AA + 1.0 H_2O_2 g/L, which caused 10.85% protection. The highest protection value (70.80% protection) was caused by 0.2 AA + 4.0 H_2O_2 g/L followed by 0.2 AA + 2.0 H_2O_2 g/L that provided 52.8% protection and 0.1 AA + 4.0 H_2O_2 g/L which showed 41.96% protection.

Generally, SDI in May planting date was significantly higher than in October planting date particularly in check plants (control), as check plants showed mean values of SDI 55.6% by May planting date and 45.9% in October planting date. However, SDI values showed insignificant differences among the four treatments, 0.1 AA + 1.0 H_2O_2 g/L, 0.1 AA + 2.0 H_2O_2 g/L, 0.1 AA + 4.0 H_2O_2 g/L and 0.2 AA + 1.0 H_2O_2 g/L, during the two planting dates. While the two treatments, viz. 0.2 AA + 2.0 H_2O_2 g/L and 0.2 AA + 4.0 H_2O_2 g/L, showed significant variances in SDI values, since they significantly decreased SDI values in May more than in October. Meantime, means of protection values (MPV) of 0.2 AA + 2.0 H_2O_2 g/L and 0.2 AA + 4.0 H_2O_2 g/L, showed significant variances in MPV values, since they significantly raised MPV values in May (86.55% MPV more than in October (70.85%MPV). Condition(s) are critically important in the development and spread of the pathogen causing smut of sugarcane. Some of these can be utilized to form the basis of disease prediction model. They may vary in their combinations in different agro climatic zones and influence not only the pathogen but also the host (Mansoor *et al*., 2016). Narciso *et al*. (2007) reported that sensitivity of *Botrytis cinerea* hyphae and conidia to PAA was shown by the presence of a zone of inhibition using

the disc assay method. Galal (2017) recommended PAA for integrated disease management program (IDM) against powdery mildew of okra and sunflower plants. Application of PAA against several plant diseases caused by either phytopathogenic fungi (Hopkins *et al*., 2003; Mari *et al*., 2004; Pukdee and Sardsud, 2007; Thipaksorn *et al*., 2012; Feliziani *et al*., 2016 and Ayoub *et al*., 2017; Galal, 2017 and El-shmony *et al*., 2017) or phytopathogenic bacteria (Hopkins *et al*., 2009 and Hong *et al*., 2018). The results of this work confirm the findings recorded by Galal (2017) on okra and sunflower powdery mildew and can be included in an integrated disease management programs for sugarcane smut.

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مكافحة مرض تفحم قصب السكر المتسبب عن الإصابة بفطر *Sporisorium scitamineum* Syd. بإستخدام بيروكسي حمض الخليك

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اظهر المركب بيروكسى حمض الخليك (مكونه الاساسى فوق اوكسيد الهيدروجين) الذى يعتبر صديق للبيئة قدرة على تثبيط حيوية الجراثيم التيليتية للفطر المسبب لمرض تفحم قصب السكر. حيث اظهرت كل التركيزات المختبرة تثبيط معنوىلانبات الجراثيم التيليتية. واعطى التركيز ٠,٢ جرام حمض خليك + ٤ جرام فوق اكسيد الهيدروجين اعلى نسبة تثبيط ٦٩,٩ % تلاه التركيز ٠,١ جرام حمض خليك + ٤ جرام فوق أكسيد الهيدروجين في اللتر حيث اعطى نسبة تثبيط ٥١,٩ % ثم التركيز ٠,٢ جرام حمض خليك + ٢ جرام فوق أكسيد الهيدروجين في اللتر اعطى ٤٨,٩% تثبيط.

بمعاملة عقل قصب السكر (العقلة تحتوي علي برعم واحد) بالمركب بيروكسي حمض الخليك بعد ساعتين من العدوى الصناعية أدي الي حفض معنوي في نسبة الإصابة بالتفحم وكانت قيم نسبة الاصابة ذات فروق غير معنوية لأربع معاملات ٠,١ جرام حمض خليك ، ١ جرام فوق أكسيد هيدروجين للتر و ٠,١ جرام حمض خليك + ٢ جرام فوق اكسيد هيدروجين في اللتر و ٠,١ جرام حمض خليك + ٤ جرام فوق اكسيد رهيدروجين في اللتر و ٠,٢ جرام حمض خليك + ١ جرام فوق اكسيد هيدروجين في اللتر علي مدار موعدين للزراعة في مايو أو اكتوبر . بينما أظهرت معاملتين (٠,٢ جرام حمض خليك + ٢ جرام فوق أكسيد هيدروجين و ٠,٢ حمض خليك + ٤ جرام فوق أكسيد الهيدروجين) أظهرتا تباينا معنويا في قيم نسب الإصابة حيث أنخفضت قيم الإصابة معنوياً في زراعات مايو عن ما هو في أكتوبر. وفي ذات الوقت أظهرت قيم الحماية لتركيز ٠,٢ جرام حمض خليك + ٢ جرام فوق اكسيد هيدروجين للتر ،و جرام حمض خليك + ٤ جرام فوق اكسيد هيدروجين فى اللتر اظهرت تباينا معنويا في قيم الحماية حيث كانت قيم الحماية عالية معنوياً في مايو حيث كانت ٨٦,٥٥% عن ما هو في زراعات أكتوبر بلغ ٧٠,٨٥%. أعطي ميعاد الزراعة في شهر مايو زيادة معنوية بنسب الإصابة بالتفحم مقارنة بشهر أكتوبر خاصة في النباتات الغير معاملة بالمركب .