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ORIGINAL ARTICLE

Suppression of electrolyzed fertiliser solution (EFS) on Panama disease caused by *Fusarium oxysporum* f. sp. *cubense* on banana plantlets

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Abstract

Electrolyzed fertilizer solution (EFS) was produced by passing an irrigation solution through an electrolization chamber in order to suppress fungal disease caused by *Fusarium oxysporum* f. sp. *cubense* race 4 (Foc4) infecting banana plantlets. In the laboratory, EFS was prepared by electrolyzing solutions containing different amounts of potassium chloride and potassium nitrate. The results indicated a significant reduction in the conidial densities of Foc4 which was from 10^6 spores · ml^{-1} to a maximum of $10^{1.3}$ spores · ml^{-1} and depended on the concentration of components in the input flow. Eventually the EFS produced from the lowest one was chosen to treat banana plantlets. Greenhouse experiments gave contradictory results of inoculated plantlets irrigated with or without EFS. The untreated banana plantlets virtually showed symptoms of infection such as discoloration of cross-cut corms, rapid wilting and dying within 60 days, while the treated ones kept their shapes and grew normally. The drastic fall in the microbial population in the rhizosphere of treated plants confirmed the activity of oxidation agents which is the major mechanism of disease suppression. The results suggest that further studies of EFS in the field as a potential technique in fighting Panama wilt in the banana industry are necessary.

Keywords: banana, electrolyzed fertilizer solution, Fusarium wilt, Panama disease

Introduction

Banana is a critical fruit of the agricultural global market (FAO 2016). During the mid 50s of the 20th century, banana producers encountered Panama wilt – a destructive disease caused by the pathogenic fungus *Fusarium oxysporum* f. sp. *cubense* (Foc) which had wiped out the once-famous "Grosmichel" banana internationally. After replacement with the highly resistant Cavendish cultivar, the global banana industry entered a long stable period. However, recently it has been facing an emerged threat from Tropical Race 4 (Foc4) – an evolved race of *F. oxysporum* f. sp. *cubense* which can infect a wide range of banana species including Cavendish. Hung *et al.* (2018) studied and announced the existence of Foc4 in Vietnam, where most of the positive samples originally were located in Red River catchments such as Laocai, Hanoi and Hungyen provinces which are geographically adjacent to infested site in China. However, there are still no warnings or regulations for farmers and small rural households who growthe major share of banana production. Although eradication-confinement is the first recommended measure to prevent TR4 (Ploetz 2015)

from spreading widely, the lack of farmers' awareness, and governmental guidance as well as the fact that in Vietnam the areas where bananas are cultivated are very scattered, limit its success. It was hoped that biocontrol, the usage of TR4 antagonists such as non-pathogenic *Fusarium oxysporum* isolates (Thangavalu and Jayanthi 2009) or *Pseudomonas fluorescens* (Sivamani *et al.* 1988; Belgrove *et al.* 2011), would be a major solution but the results are still below expectation due to high percentages of annual losses (12 to 20%) (Raguchander *et al.* 1997). The cause might be the continuous penetration of TR4 hyphae through antagonist layers surrounding the rhizospheric soil of banana plants. Furthermore, it is impossible to strengthen this critical defense as soon as the banana plantlets are planted on infected soil. Overall, there is still no solution for saving existent banana trees in infected or infection-threatened soil.

Electroactivation might be a potential technique by providing a unique electrolyzed fertilizer solution (EFS) with double roles: **fertilizer** but also **fungicide** which aim to expel harmful fungus from the rhizosphere without any harm to bananas. It produces electrolyzed water which is made by flowing an input solution containing a small amount of salt (sodium chloride) through an electrolytic chamber. In this study, sodium chloride was replaced by a mixture of fertilizer: potassium chloride, potassium nitrate or monopotassium phosphate to create an electrolyzed fertilizer solution (EFS). The output solution was designed to possess special properties: (i) bactericidal and fungicidal abilities by high reduction potential (ORP) and free chlorine (Buck *et al.* 2002; Xiong *et al.* 2010), (ii) to return to normal water after a short period of time, (iii) to contain less than 5 g of fertilizer per liter to avoid increasing soil salinity (Hoang *et al.* 2012). Based on the same idea, the electroactivation technology was developed independently in Russia (Bakhir 1979) and in Japan (Shimizu 1992). The idea was to create electrolyzed water containing a small amount of salt (sodium chloride) in an electrolytic chamber in order to establish a special stable state. This solution then has a series of remarkable qualities including fungi and bacteria suppression (Buck *et al.* 2002; Xiong*et al.* 2010) but it is still safe for humans and the environment. The aqueous solution produced from the electroactivation process has strong fungicidal activity (Buck *et al.* 2002) and has been successfully applied to fruit post-harvest procedures (Al-Haq *et al.* 2001; Abbasi *et al.* 2006; Guentzel *et al.* 2010). This ability stems from high ORP regardless of the chlorine remaining in the solution (Hung *et al.* 2000) which destroys fungal cellular membranes by oxidation agents (OA) produced from the electrolyzing process (Tang *et al.* 2011). Therefore, if sodium chloride is replaced by other salts like inorganic fertilizers, the output solution – EFS would still have fungicidal ability (Aider *et al.* 2017). During the experiments, microbial populations in treated and control groups were measured as an affirmation for the activities of OA contained in the irrigation solution.

In the battle against Panama wilt disease, eradication-confinement is commonly recommended (FAO 2014; Queensland Government 2022), there by limiting available remediesfor already infested farms. This research was aimed to create of a new chemical technique based on the following idea: Application of EFS into the banana root zone to wipe TR4 hyphae out of the rhizospheric soil and the adjacent soil zone. The technique also expected to soften the damage by TR4 until a new resistant cultivar is commercially accepted world wide.

Materials and Methods

Fusarium oxysporum f. sp. *cubense* race 4 (Foc4) inoculum preparation

Fungal isolation was provided by Hung *et al.* (2018) from an infected site in PhuTho Province, Vietnam where Foc4 existence was already confirmed. The inoculum was prepared by the method described by Sudarsono *et al.* (2008) in which fungal samples were grown on potato dextrose agar (PDA) medium and incubated for 10 days at 27°C. Then the potato dextrose broth (PDB) medium was used to contain harvested Foc4 spores after filtration to remove the fungal mycelia. The final conidial density was 10^7 spores · ml^{-1}.

Electrolyzed fertilizer solution (EFS) preparation and effectiveness against banana wilting disease

The EFS solutions were produced from input mixtures which contained two basic components: potassium chloride and potassium nitrate. Three initial mixtures with distilled water (control) and electrolyzed water made from sodium chloride were processed through electrolyzed chambers from a GIAVANG machine (Gia Nguyen Ltd., Vietnam) to produce different testing solutions. Concentrations of each input solution are given in Table 1. Basic physical chemical properties of output solutions such as pH, free chlorine concentration, ORP and temperature were also measured. The first solution was used as the control while the last one represented common "traditional" electrolyzed water which has previously been studied worldwide. Numbers 2–4 would be the major candidates for the next phase – the greenhouse experiments.

After the electrolyzed process, five input solutions were processed which corresponded to five output

Table 1. Concentrations of different input solutions to produce electrolyzed fertilizer solution)

Input solution	Components concentration [g · l^{-1}]		
	potassium chloride	potassium nitrate	sodium chloride
1 (Control)	0	0	0
2	5	15	0
3	10	25	0
4	15	35	0
5	0	0	50

solutions whose effectiveness against Foc4 spores was studied. Five test tubes containing 9 ml freshly produced output solutions were mixed with 1 ml Foc4 inoculum with a density of 10^7 spores · ml^{-1}. After 1 h, the density of spores which remained were measured by the most probable number (MPN) method. The experiment was repeated five times. The results indicated not only the effectiveness but also the proper concentration of input solution which would be applied for the banana plantlet test.

Banana plantlet experiments

The greenhouse experiment took place in the Soils and Fertilizers Research Institute (Hanoi, Vietnam) during winter months with the average temperature ranging from 16 to 22°C. There was a total three repeated experiments. Cocopeat-based organic compost was used as the primary medium for banana growth and inoculation tests. Before being placed in the greenhouse, the compost was sterilized in an autoclave at 200°C before storage and hydrated 14 days prior to the experiments.

Inoculation

Before being transferred to plastic buckets, the selected plantlets were inoculated with Foc4 spores by dipping banana corms into inoculum with 10^5 spores · ml^{-1} for 30 min. Every plantlet was then nursed equally for the next 5 days to reach their stabilities before treatments. After wards, 80 plantlets were used in each repetition and categorized into four groups of 20 based on the treatment they received: (i) control – no inoculation, irrigated with distilled water (ii) no inoculation, irrigated with EFS (iii) inoculation, irrigated with distilled water (iv) inoculation, irrigated with EFS. The concentration of EFS was selected from previous experiments. The priorities for EFS ratification were the determining effects against Foc and the suitable fertilizer concentration to support banana plantlets. The condition of the

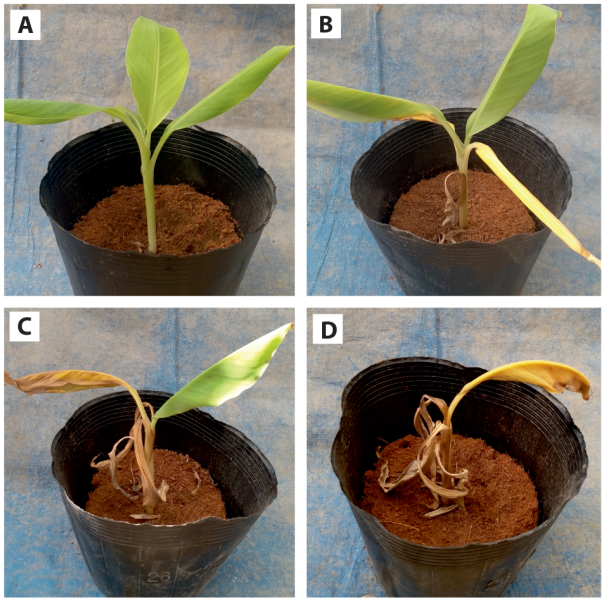

Fig. 1. Appearance of plantlets corresponded to wilting index scores: totally healthy plantlet (A); only one wilting leaf (B); only one green leaf remaining (C); dead plant (D)

plantlets was observed everyday following the wilting index (WI) described by Muthusamy *et al.* (2003) in which every plantlet was observed and scored from 1–4 (1 indicates a dead plantlet, 4 totally healthy ones). The appearance of plantlets in each WI score is given in Figure 1.

The average value of WI points out the overall condition of each examined group during a period of 65 days. After the experiments, the corms of deceased plantlets were cross-cut to confirm the discolored stems which indicated infection caused by fungal disease.

Throughout the experiments, before irrigation, samples of compost from the rhizospheric zone of plantlets were collected randomly with a total of three samples per day in inoculated bananas with (treated) and without treatment (control) groups. Total microbial density of each sample was determined by the MPN method resulting in the logarithm of CFU per gram of compost. This data would indicate any change in the population of microorganisms which was acutely affected by OA contained in the irrigation solution.

Statistics

One-way ANOVA was applied to detect initial differences between all of four groups while the t-test was used to perform comparisons between two single groups. Statistics tests were performed by R (R Core Team, Austria) and figures were created by the SPSS version 16.0 (IBM, Armonk, New York, USA).

Results

Electrolyzed fertilizer solution (EFS) properties

After electrolyzation, the indexes of the output solution were measured and shown in Table 2.

The pH values of all solutions execpt No. 5 were kept in the range between 6 and 7. Meanwhile, ORP and free chlorine increased according to component concentration in each input solution from No. 1 to No. 5. This result correlated with many previous studies reviewed by Oh *et al.* (2016) which employed sodium chloride as the electrolyzed agent. Due to the lowest concentration of fertilizers, input solution No. 2 was chosen for the greenhouse experiments in which banana plantlets were the study object.

Effect against *Fusarium oxysporum* f. sp. *cubense*race 4 spores

The concentrations of remaining spores after treatments with each of the output solutions were presented in a boxplot graph (Fig. 2).

The value of $\log_{10}$ spores · ml^{-1} correlated with free chlorine concentration and ORP ($p < 0.01$) which also indicated no deterioration in fungicidal effects of the output solutions caused by the replacement of sodium chloride by potassium chloride. As the concentration of salts increased, the remaining spores reduced sharply. While the original electrolyzed water – No. 5 had the most effective solutions, the others (excluding No. 1 – control) also presented a reasonable ability to destroy Foc4 spores. Despite their components being feasible to become a proper irrigation solution for banana plantlets, No. 2 was chosen for the next tasks due to its low concentration which would be appropriate for banana plantlets – the protagonists in greenhouse experiments.

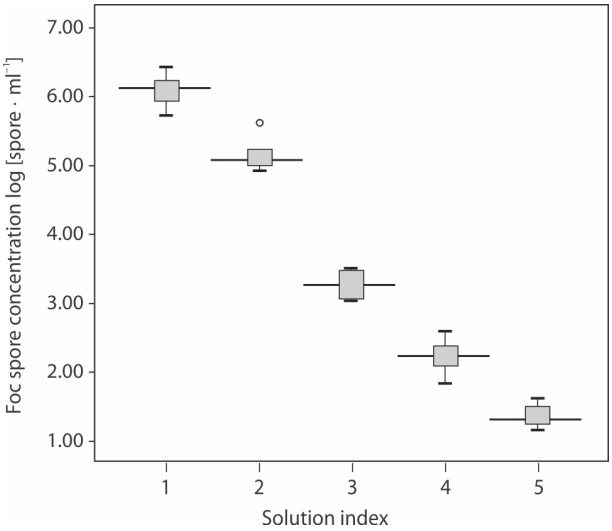

Fig. 2. Concentrations of inoculums after treatments with electrolyzed fertilizer solution

Greenhouse experiments

After 65 days, the average WI values of group 1a (control) and 1b showed no difference and all plantlets stayed healthy throughout the testing period ($p < 0.01$). On the other hand, WI values from remaining groups contrasted over time with the control group (Fig. 2). The control began with an initially stable period up to day 20, while group 3 (infected w/o treatment) WI fell sharply to reach almost 1 on day 67. Group 4 (infected and treated with EFS) only decreased by a narrow margin and became similar to the control group. The differences between highlighted groups are visualized in Figure 3 as from day 01 to day 67.

Apparent states of plantlets in the two infected groups were in direct contrast to each other. Treated plantlets were green and remained healthy whereas untreated plantlets wilted seriously and died out (Fig. 4). Assessment of plantlet corms after the experiments by cross-cutting slides also found the

Table 2. Physical chemical properties of output electrolyzed fertilizer solution (EFS)

Solution	EFS solutions' properties			
	pH	free chlorine [mg · l^{-1}]	ORP [mV]	temperature [°C]
1 (Control)	7.02	0	289	23
2	6.29	25	864	23
3	6.25	45	928	23
4	6.15	60	>1000	23
5	2.65	81	>1000	23

ORP – oxidation reduction potential

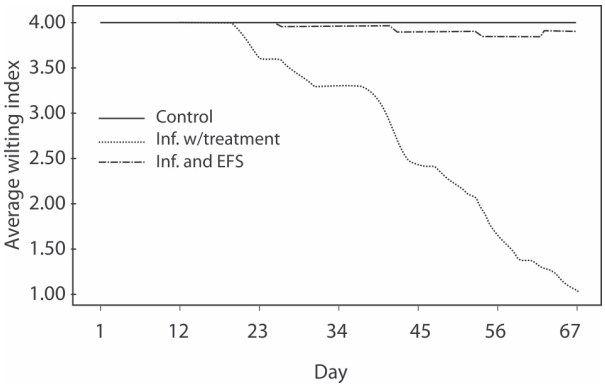

Fig. 3. Time series of wilting index value of three differently treated groups of banana plantlets. EFS – electrolyzed fertilizer solution

Fig. 4. The contrast between treated and untreated plantlets with electrolyzed fertilizer solution (EFS) on day 10 and day 56

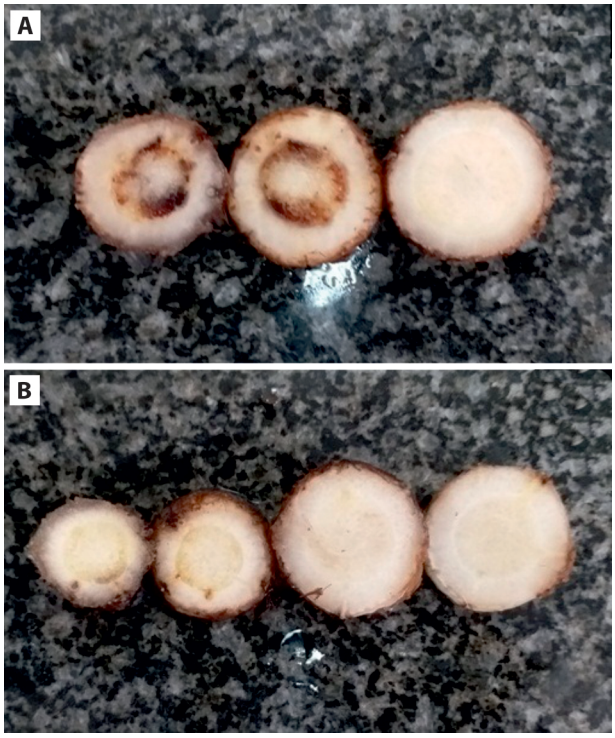

Fig. 5. Cross-cutting sections of banana corms from untreated (A) to treated (B) with electrolyzed fertilizer solution

discoloration appeared in infected plantlets, which could not be found in normal ones (Fig. 5).

The data of microbial populations from treated and untreated plantlets also indicated an undeniable gap between them (Fig. 6). While the control showed

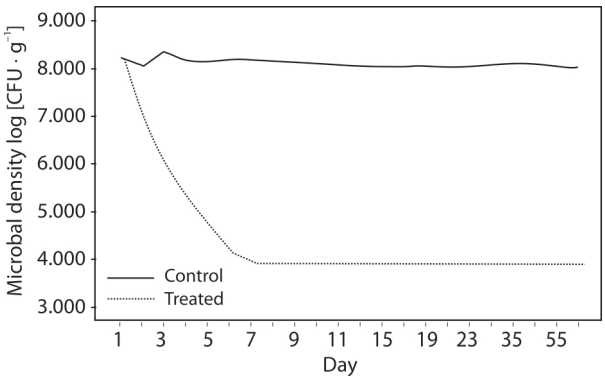

Fig. 6. Microbial population from untreated inoculated plantlets (control) group and irrigated with electrolyzed fertilizer solution (treated) group

stability throughout the experiments at approximately 10^8 CFU · g^{-1}, the treated fell sharply in just only 7 consecutive days irrigated with EFS toward the threshold of 10^4 CFU · g^{-1}.

Discussion

Before this study, only chemical control of Fusarium wilt in bananas was effective in disinfecting tools and farming environment in order to prevent the spread of fungus (Ganapathi *et al.* 2015). For direct involvement in banana *in vivo* protection, limited types of fungicides (especially demethylation-inhibiting fungicides) were utilized (Viljoen *et al.* 2007). The disadvantage of this approach is that these chemicals could not be applied *en masse* but were restricted to one-time treatment. In general, the usage of fungicides to prevent Foc4 was considered uneconomic and environmentally harmful (Zheng and Siamak 2018). Meanwhile, in contrast, EFS operated as a type of regular irrigation with a multiple-treating function corresponding to each time infected plantlets were irrigated. Therefore, the *in vitro* experiments were not carried out with PDA dishes which are prevalent in evaluating fungicidal effects of objective substances. Instead, the efficiency of EFS on Foc4 disease was assessed by the reduction of spores or microbial population after each treatment.

Electrolyzed fertilizer solution (EFS) in laboratory

Despite replacement of sodium chloride with potassium chloride as the main agent in input solution, physical chemical criteria listed in Table 2 indicated that EFS shared the same pattern with "traditional" electrolyzed water, in which, free chlorine con-

centrations and ORP values strongly correlated ($p < 0.01$) with potassium chlorine content in inflow. They played the key roles in fungicidal performance demonstrated in laboratory experiments as observed in previous studies (Hwang *et al.* 2008) which included a series of different types of bacteria and fungi. Therefore, it is reasonable that the suppression of Foc4 spores by EFS is feasible as was demonstrated in this laboratory experiment. Overall, the replacement by potassium chloride did not change the pattern of physical chemical indexes of the output solution as well as the fungicidal ability of the studied EFS which confirmed the results studied by Tamaki *et al.* (2005). Thus, EFS is a reliable solution to have a direct contact with Foc4 disease in greenhouse experiments when banana plantlets are the study object. The No. 2 solution was chosen due to its low content of salt, which is appropriate for nursing young plants.

Electrolyzed fertilizer solution (EFS) in greenhouse experiments

The immutability of WI values between healthy plantlets irrigated with water (group 1a) and EFS (group 1b) determined that EFS can be used for nursing banana plantlets despite the existence of electroactive agents such as free chlorine, $OH^{\bullet}$ formed during the electrolyzing process. Like "traditional" electrolyzed water, EFS maintained the environmental safety because its components consisted of only fertilizers and the low-but-effective concentration of electroactive agents which suppressed to fungal disease but did not harm the banana plantlets. The gradual decrease of the microbial population in growing media from day 1 to day 7 (Fig. 6) confirmed the primary mechanism of suppression caused by EFS: the activity of AO over microorganisms. The stable threshold of 10^4 CFU $\cdot$ g^{-1} from day 8 to the end of the experiments showed the balancing point between suppression and recovery of the micro community in the rhizosphere of plantlets. It also indicated that the continuous supplement of AO into growing media disturbed the development of disease which saved most of the treated plantlets.

Effectiveness on fungal diseases was proved by the difference in WI of treated and untreated plants ($p > 0.1$). After day 20, the plant health of the untreated plantlets plunged quickly due to fungal development which caused the discoloration in banana corm (Fig. 5) and died at a very high rate of 95% by day 65. Meanwhile, the wilting value of treated plants was barely reduced and gradually recovered from day 50 which indicated the long-term suppression of disease despite a low concentration of AO in EFS. The result of 95% cured plants surpassed the best performance recorded for the usage of fungicides as 80% (Viljoen *et al.* 2007; Ganapathi *et al.* 2015) which indicated that this technique would be considered as a potential chemical control applied to infected banana fields with bigger and more mature plants.

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