Effect of Fusaric acid on seed germination and seedling growth of rice through *Fusarium proliferatum*inoculation and artificial application

ABSTRACT

Fusaric acid (FA) is a phytotoxin compound produced by many Fusarium species including *F. proliferatum*. The virulence of *F. proliferatum* depends on FA production. In the present study, the effect of FA produced by *F. proliferatum* in seeds germination and seedlings growth of rice was investigated. In addition, the specific role of FA was confirmed through synthetic FA application that produce disease symptoms in seed germination and seedlings growth. The FA produced by *F. proliferatum* was detected using Ultra High Performance Liquid Chromatography (UPLC) and thus, *F. proliferatum* was used as inoculum whereas synthetic FA was used for seed treatment. ~~Seed germination was assayed by counting germination percent and the seedling growth was assessed by measuring stem and root length.~~ Results ~~found~~showed that the seed germination and root length were significantly reduced when the seed were inoculated with *F. proliferatum.* The effect of FA application on seed germination and seedling growth were the same as the symptoms developed by *F. proliferatum* inoculated rice. Thus, the specific role of FA was confirmed by comparing both of the FA treatment with control condition in where normal seed germination and seedling growth were observed.

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Keywords: F. proliferatum, Fusaric acid (FA), inoculation, Root length

1. INTRODUCTION

Fusaric acid (FA) is potentially toxic to both animals and plants. FA is a host non-specific mycotoxin compound having phytotoxin effect on plants. FA was initially identified from *Fusarium heterosporum* culture in the laboratory by Yabuta et al. [1]. FA was one of the first fungal metabolites to be concerned in the pathogenesis of tomato wilt disease caused by *F. oxysporum*f. sp. *lycopersici* [1-2]. It is believed that FA is directly associated with the pathogenesis of vascular wilt, damping off, and root rot diseases in a variety of vegetable crops [3-4]. In addition, FA is directly related with stunting, wilting and root rot symptoms of rice, tomato, banana [3, 5-6]. Several *Fusarium* species, including *F. proliferatum*, produce FA, a broad-spectrum phytotoxin. *Fusarium oxysporum* and its special forms (f. sp.) *lycopersic*i produced FA most extensively [7]. The virulence of plant pathogenic *Fusarium* spp. has been associated with a high production of FA.

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F. proliferatum is a globally distributed fungal pathogen that affects a variety of agriculturally significant host plants, such as rice [8], maize [9], asparagus [10], date palm [11] and ornamental palms [12]. *F. proliferatum* was identified as the most prevalent species responsible for disease transmission in rice fields [13]. For instance, *F. proliferatum* has

been responsible for the development of rice diseases such as bakanae [14], sheath rot [15], and spikelet rot [16]. *F. proliferatum* is a toxigenic species that produces a diverse array of toxins, including fusaric acid [7], fumonisin B1 [17], moniliformin [18], beauvericin [9] and fusaproliferin [19]. Certain toxins are widely recognized for their phytotoxic characteristics. For instance, fusaric acid has been linked to the development of wilt symptoms in tomatoes and bananas [2]. Moniliformin was found to be harmful to tobacco plants [20]. Additionally, fumonisin B1 has been shown to have a toxic effect on maize and tomato plants [21]. Reverberi et al. [22] reported that mycotoxins generated by *Fusarium* species are associated with pathogenesis during infection and aid the fungi in competing with other organisms.

The effects of fusaric acid produced by *Fusarium* were investigated on several crops including rice and*Striga hermonthica*. FA produced by *F. nygamai*, have strongly inhibited seed germination of *S. hermonthica* [23]. According to Yadav et al. [24] rice seeds germination was reduced and caused rotting of rice plants by *F. fujikuroi* inoculation. In contrast, artificial application of FA was investigated on corn seedlings. The root length of corn seedlings was reduced at 0.2 mM FA and 0.5 mM FA that was directly influencing the cell differentiation process [25]. Idris et al. [26] also reported that FA strongly inhibited Striga seed germination. Matysiak & Samyn [27] also registered a complete inhibition of *Aechmeafasciat*a seedling growth at 1 mM fusaric acid. Similar result was found in the study on *Sorghum bicolor* (L.) observed by Rodella [28].

The phytotoxin FA is known to play crucial role in symptoms development by *Fusarium* species. The development of different kinds of symptoms as well as the pathogenicity of *Fusarium* species depends on the production of FA [29]. In rice, FA produced by *F.commune* could contribute root rotting symptoms [30]. However, FA produced by *F. proliferatum* and its specific role in symptom development in rice is still unknown. The present study, therefore, was conducted to assess the role of FA on seeds germination and seedlings growth of rice through *F. proliferatum* inoculation and synthetic FA application. Also, the specific role of FA on seeds germination and seedlings growth of rice produced by *F. proliferatum* in comparison with synthetic application of FA.

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2. MATERIAL AND METHODS

2.1 Rice seeds source

One sSusceptible rice variety, MR 211.wasused for the pathogenicity test, .was provided bytThe Malaysian Agricultural Research and Development Institute (MARDI), located in SeberangPerai, Pulau Penang, Malaysia, provided the rice variety MR 211.

2.2 *F. proliferatum*inoculum preparation

F. proliferatum was obtained from infected rice plants in Selangor, Malaysia. For fungal isolation, a 1 cm tissue segment was cut and subjected to surface sterilizationed withby immersing it in a solution of 1% sodium hypochlorite (NaOCl) solution for 1 min.ute, followed by a 3-minute immersion in 70% ethanol, Tthensegment was then rinsed three times with for one minute each in sterile distilled water, (SDW) and placed on sterile filter paper to dry. The sterilized tissue segment was subsequently incubated for five days at a temperature of 25±1°C on plates containing peptone pentachloronitrobenzene agar (PPA). Once the mycelia had grown on the plates, they were transferred to potato dextrose agar (PDA) and left there for 5-7 days. Finally, a single spore culture was performed to obtain a pure culture. The isolate was cultured on PDA plates at 25±1°C for 7 days, with a 12 hr light and 12 hr dark cycle. Afterward, the plates were immersed with 5 ml of sterile distilled water and then spread using a spreader (a hockey stick-like glass rod). The haemocytometer was employed

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to adjust the concentration of the conidial suspensions to ~~1 x~~ 10^6 conidia/ml after they were pooled. Rice seeds (MR 211) were subjected to heat sterilization at 50°C for 10 minutes, followed by surface sterilization with 1% NaOCl for 1 min~~ute~~, 70% ethanol for 3 min~~utes~~, and three times with sterile distilled water for 1 min~~ute, three times~~. The seeds were immersed in a 10 ml spore suspension of *F. proliferatum* for 24 hours.

2.3 Fusaric acid (FA) production by *F. proliferatum*

The extraction ~~FA production by~~of FA produced by ~~*F. proliferatum* was detected and quantified *invitro* through UPLC analysis. The FA was produced by~~ *F. proliferatum*was conducted based on the method described ~~in accordance with the methodology outlined~~ by Husna et al. [30]. In brief, the *F. proliferatum* isolates were cultured on PDA ~~plates~~ and the spore suspension of the isolates were injected into Czapek-Dox media. The isolates were grown in the medium and the mycelial mat was isolated with filter paper (Whatman No. 1). The filtrate was extracted with ethyl acetate and well agitated in a separatory funnel. The top layer of ethyl acetate was collected in a conical flask. The extracts were pooled together. The suspended residue was diluted in ethanol and kept for UPLC analysis.

All samples, mobile phases, and working standard solutions were filtered using a 0.2 µm filter before UPLC analysis. An Ultra UPLC system with a Waters Acquity UPLC® binary pump and a Waters Acquity UPLC® photodiode array (PDA) detector set at 268 nm was used to quantify FA. An AWS C18 reversed-phase column was used for the chromatographic separations. By comparing the UV spectrum and retention time to the FA standard, FA was identified. By comparing the peak height of FA to a calibration curve created using standard solutions, FA was quantified. This experiment was conducted three times independently.

The concentration of FA was quantified as 252.68 µg/g through this *invitro* production.

2.4 FA application

The FA (ACROS ORGANICS, 99%) was weighed and mixed in distilled water to make solution of concentrations used for treatment (0.5 ppm). Rice seeds were heat sterilized and surface sterilized according to the abovementioned condition. Seeds were immersed in a 10 ml solution of FA for 24 hours. Control (non-treated) seeds were immersed in an equivalent volume of sterile distilled water.

2.5 Rice seed germination test

Twenty-five rice seeds were steeped in a 10 ml spore suspension of *F. proliferatum* ~~isolate~~ and 10 ml of FA solution. The inoculated seeds were spread on three layers of sterile water-moistened filter paper in petri dishes. Thereafter, the petri dishes were incubated in incubators at a temperature of 25-26°C for 12 hours of light and 12 hours of darkness [31]. The control seeds were subjected to treatment with sterile distilled water and the test was independently replicated thrice. The seed germination rate was compared to the untreated control and calculated by counting the number of germinated seeds at 7 days after incubation.

2.6 Rice seedling growth test

The rice seeds were immersed in 10 ml of *F. proliferatum*~~inoculum~~spore suspension and FA solution for 24 hr. The 25 inoculated seeds were grown on three layers of sterile water-moistened filter paper in petri dishes, and then the petri dishes were incubated in incubators

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under a 12hr light and12 hr dark, 25-26° C regime. The control seeds were subjected to treatment with sterile distilled water. The seedlings elongation. Stunting and other symptoms were compared to the untreated control and assessed by measuring the seedlings shoot and root length at 15 days after incubation. The test was independently replicated thrice.

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2.7 Statistical analysis

IBM SPSS v. 26 was employed to analyze the root length and shoot length data. The level of statistical significance was determined to be $p < 0.05$. The significance of the difference between the *F. proliferatum*, FA and the control was estimated through analysis of variance (ANOVA) in the statistical analysis. The means of root and shoot length were evaluated using Turkey's multiple range test.

3. RESULTS

3.1 Role of FA on seed germination

The role of FA on seed germination was assayed through *F. proliferatum*inoculation and FA application separately with control. Results showed that the lowest seed germination (70.66%) was recorded in seeds inoculated with *F. proliferatum* whereas 100% seed germination was observed in rice seeds treated with distill water (control ~~condition~~) (Table 1). In FA treated seeds, 87.2% seed germination was observed. The germination percentage of rice seeds inoculated by *F. proliferatum*was significantly lower than the seeds applied by FA (**Fig. 1**).

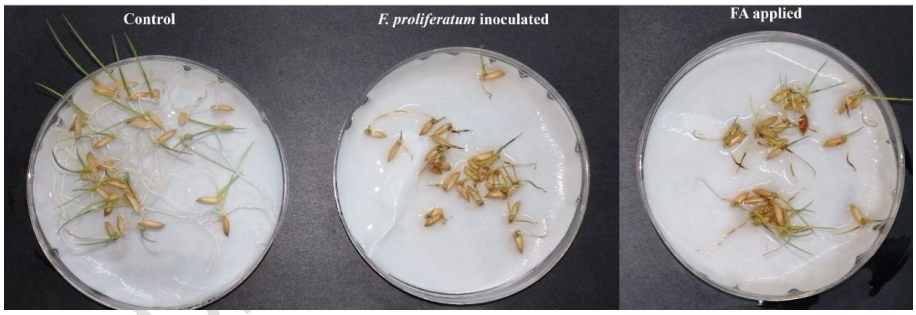

Fig. 1.Evaluation of rice seed germination inoculated with *F. proliferatum* and treated with FA

3.2 Role of FA on seedling growth

The role of FA on seedling growthwas assayed through *F. proliferatum*inoculation and FA application separately with control. The shoot and root length were considered as seedling growth in assay. Results showed that *F. proliferatum* inoculated rice seedlings turned yellowish leaves, stunted and finally wilted. The root of inoculated rice seedling by *F.proliferatum* were significantly reduced, discolored and rotted (Fig. 2). The shoot and root length of rice seedlings inoculated by *F. proliferatum* were shorter than the seedlings applied by FA (Table 1). The rice seedlings applied by FA were also stunted with yellow leaves. The root of rice seedlings was also reduced and rotted by the application of FA (Fig. 2).

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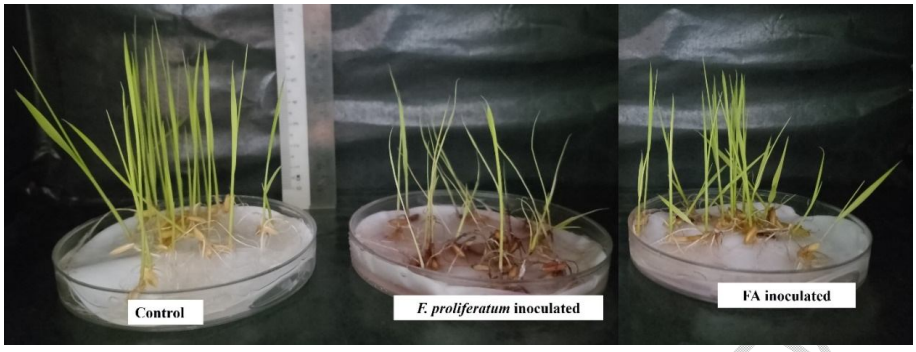

Fig. 2. The effect of FA on rice seedlings inoculated with *F. proliferatum* and treated with FA

Table 1. The role of FA on seed germination and seedling growth of rice inoculated with *F. proliferatum* and FA application

Treatments	FA Concentration	Germination (%)	Shoot Length (mm) (mean ±SD)	Root Length(mm) (mean ±SD)
T_1	252.68 µg/g	70.66±1.3c	51.29±1.7b	14.6±2.0c
T_2	0.5 ppm	87.2±1.1b	52.6±0.7b	33.4±1.3b
Control	0.0	100a	82.31±1.1a	128.33±1.4a

Where, T1= seeds inoculated with *F. proliferatum*and T2= seeds treated with FA; Same letters are not significantly different by Turkey's multiple range test (P < .05)

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The shoot length was found more or less the same in the rice seedlings inoculated with *F. proliferatum*and treated with FA but the significant difference was observed in root length of rice seedlings with the same treatment. Both *F. proliferatum* inoculated and FA applied rice seeds showed low germination rate, and reduced shoot and root length. However, seed germination and seedling growth of rice was found normal in control condition.

4. DISCUSSION

*F. proliferatum*could produce variable amounts of FA in rice plants affected by bakanae disease [14]. In this study, the effect of FA on seed germination and seedling growth of rice was assayed through *F. proliferatum*inoculation. Besides, the specific role of FA was confirmed through synthetic FA application that produces disease symptoms in seed germination and seedlings growth.

In the present study, the seed germination and seedling root length were overbed to be reduced when inoculated with *F. proliferatum*and applied by synthetic FA. This result is in accordance with the findings of Wu *et al.* [32]. Similar results were observed by Kumar *etal.* [33] and Balaguera-Lopez *et al*. [34]. The FA produced by *F. nygamai* has exhibited potent inhibition of *S. hermonthica*seed germination [23]. Likewise, FA was produced by the *Fusarium* in diseased plants in different concentrations under different types of symptoms. Therefore, it is crucial to confirm the role of FA in seed germination and seedling growth through inoculated with *F. proliferatum* and FA. In this study, seedling stunting, wilting and

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root rot symptoms were found in the seedling inoculated with *F. proliferatum*in accordance with the findings observed by Li *et al.* [35]. It was also reported that stunted seedlings were produced when inoculated with *F. proliferatum* [36, 31]. Again, the root length was significantly reduced by *F. proliferatum*inoculated rice seeds in this study. The roots became discolored and rotted also. Similar findings were reported by Wulff et al. [37] and Jeon et al. [38].

During FA application, seed germination was reduced and caused rotting of rice plants [24]. Besides, reduced seed germination and root length were found in corm seedlings when treated with FA [25]. Similar findings were observed by Rodella [28] in *Sorghum bicolor*. Idris et al., [26] also reported that FA strongly inhibited Striga seed germination. Matysiak & Samyn [27] also reported that a complete inhibition of *Aechmeafasciat*a seedling growth at 1 mM fusaric acid.

F. proliferatum is one of the FA producing *Fusarium* species. Quazi et al. [5] reported that FA produced in high amount by *F. proliferatum*, which is the one of the causative agents of bakanae disease of rice. Zainuddin et al. [39] also reported *F. proliferatum* isolated from rice bakanae disease was capable of FA production. *Fusarium* species produce FA, which have been demonstrated to play a role in pathogenesis during infection and provide a competitive advantage against other organisms [22]. During disease symptoms development, FA plays an important role in host plant. *F. proliferatum* produced varied symptoms on rice plants. *F. proliferatum* inoculated plants were elongated, sometimes stunted, wilted, root rotted and reduced root length. Jiang et al. [36] and Qiu et al. [31] reported rice seedlings were stunted when inoculated with *F. proliferatum*. Quazi et al., [14] and Eğerci et al., [40] reported seedlings were elongated by *F. proliferatum*inoculation. On the other hand, *F. proliferatum* produced several mycotoxins such as FA, fumonisin, moniliformin and beauvericin etc., these mycotoxins were responsible for their virulence and disease symptoms development.

5. CONCLUSION

In this study, we investigate that *F. proliferatum*inoculated seeds and seedlings produced disease symptoms due to the FA. In addition, the specific role of FA to produce disease symptoms in seed germination and seedlings growth was investigated. In conclusion, low seed germination, and reducing root and shoot length of rice seedling were developed significantly when the seeds were inoculated with *F. proliferatum* compare to FA application. Thus, it is confirmed that seed germination, shoot and root length were affected by FA. Therefore, the adverse effect of FA should be considered during rice seed germination, and seedling growth and appropriate measures should be taken for effective management of fungal disease.

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