Effect of fusaric acid on riceseed germination and seedling growth 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 on rice seed germination and seedlings growth through*F. proliferatum*inoculation and FA application was investigated. During investigation, thedisease symptoms in seed germination and seedlings growth produced by *F. proliferatum* inoculum and synthetic FA were observed.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. Results showed that the seed germination and root length were significantly reduced when the seeds were inoculated with *F. proliferatum.*In case of FA application,theseeds germination and seedling growth were also hampered and disease symptoms were developed due to the effect of FA.Significant reductions in seed germination, root and shoot length of rice seedlings were recorded when the seeds were inoculated with *F. proliferatum* compared to FA application. 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.The adverse effect of FA on rice seeds germination and seedling growthneeds to overcome by developing resistant rice varieties through modern genetic technology.

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.

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 noticed a complete inhibition of *Aechmea fasciat*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, the effect of FA produced by *F. proliferatum*in rice seeds germination and seedlings growth 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, to investigate thecomparative effect ofFA produced by *F. proliferatum*andFA application on rice seed germination and seedlings growth.

2. MATERIAL AND METHODS

2.1 Rice seeds source

Susceptible rice variety MR 211, used for the pathogenicity test, was provided by the Malaysian Agricultural Research and Development Institute (MARDI), located in Seberang Perai, Pulau Penang, Malaysia.

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 surface sterilized with 1% sodium hypochlorite (NaOCl) solution for 1 min., followed by a 3-minute immersion in 70% ethanol,then rinsed three times with sterile distilled water, and placed on sterile filter paper for drying. Subsequently, the sterilized tissue segment was placed on the plates containing peptone pentachloronitrobenzene agar (PPA) and incubated in 12 hr light and 12 hr dark regime for 5 days at a temperature of 25±1°C.Once the mycelia grown on the plates, they were transferred to potato dextrose agar (PDA) and kept for 5-7 days. Finally, a single spore culture was performed to obtain a pure culture according to the method described by Husna et. at. [31].Then, the pure culture was identified as*F. proliferatum* through morphological and molecular methods described byHusna et. at. [31].The *F. proliferatum* 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 with a spreader (hockey stick glass rod). The conidial suspensions were pooled and the concentration was adjusted to 10^6 conidia/ml by usinghaemocytometer.Rice seeds (MR 211) were heat sterilized at 50°C for 10 minutes, followed by surface sterilization with 1% NaOCl for 1 min, 70% ethanol for 3 min, and three times with sterile distilled water for 1 min. The seeds were immersed in a 10 ml spore suspension of *F. proliferatum* for 12 hours.

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

The extraction of FA produced by *F. proliferatum*was conducted based on the method described by Husna et al. [30]. In brief, the *F. proliferatum* isolates were cultured on PDA plates and the spore suspension of the isolates were inoculated into Czapek-Dox media. Then, the mycelial mat was separated with Whatman No. 1filter paper after10 days of fungal growth on Czapek-Dox medium. The filtrate was extracted with equal volume of ethyl acetate and shaken well in a separatory funnel. The top layer of ethyl acetate was collected in a conical flask. The extracts were pooled. The suspended residue was dissolved in ethanol and kept at 4°Cfor 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 12 hours. Non-treated seeds (control) were immersed in an equal volume of sterile distilled water.

2.5 Rice seed germination test

Twenty-five rice seeds were immersed in a 10 ml spore suspension of *F. proliferatum* 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 petridishes were incubated at a temperature of 25-26°C for 12 hours of light and 12 hours of darkness [32]. The control seeds were treated 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 inoculation.

2.6 Rice seedling growth test

The rice seeds were immersed in 10 ml of *F. proliferatum*spore suspension and FA solution for 12 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 under a 12hr light and12 hr dark, 25-26°C regime. The control seeds were treated with sterile distilled water. The seedlings elongation, stunting and other symptoms were compared to the untreated control and assessed by measuring the shoot and root length ofseedlings at 15 days after inoculation. The test was independently replicated thrice.

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) (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**).

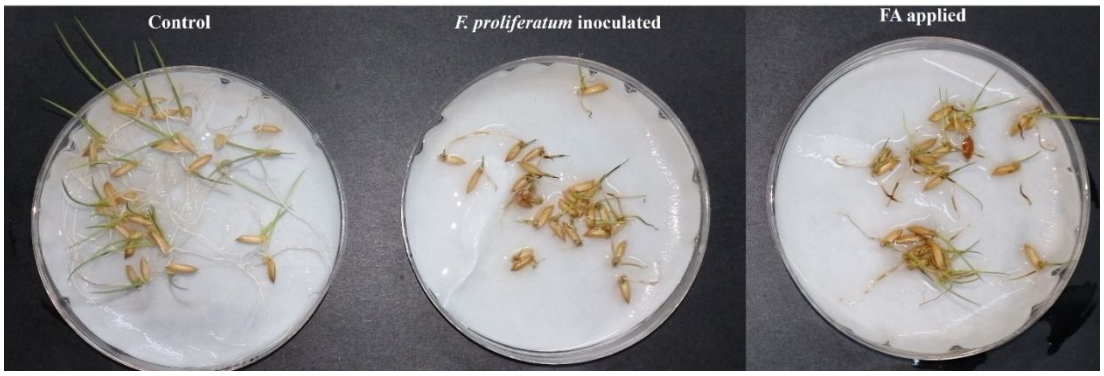

Fig. 1.Evaluation of rice seed germination inoculated with *F. proliferatum* and treated with FA

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 root length of rice seedlings inoculated by *F. proliferatum*was shorter than the seedlings applied by FA. No significant differences were found in shoot lengthof rice seedlings inoculated by *F. proliferatum* and 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).

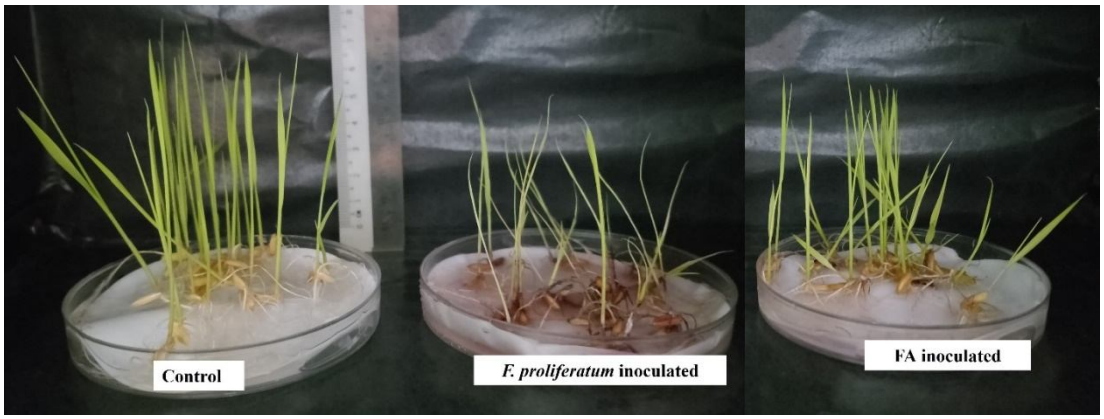

Fig. 2. The effect of FA on rice seedlings inoculated with *F. proliferatum* and treated with FA

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)

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 ofFA 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.* [33]. 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 root rot symptoms were found in the seedling inoculated with *F. proliferatum*in accordance with the findings observed by Li *et al.* [34]. It was also reported that stunted seedlings were produced when inoculated with *F. proliferatum* [32, 35]. 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. [36] and Jeon et al. [37].

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 *Aechmea fasciat*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. [38] 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. [35] and Qiu et al. [32] reported rice seedlings were stunted when inoculated with *F. proliferatum*. Quazi et al., [14] and Eğerci et al., [39] 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

This study aimed to explore the influence of FA causing disease symptoms on seeds and seedlings inoculated with *F. proliferatum*and FA application. The specific role of FA was investigated through the effect of *F. proliferatum*inoculation andFA application in seed germination and seedlings growth test.Low seed germination, and reducing root and shoot length of rice seedling were observed 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.Resistant rice varieties need to develop to overcome the effect of FA in symptoms developmentas well as for effective management of fungal disease.For FA prevention, gene editing tool CRISPR/Cas9 can be used to modify susceptibility genes in rice to introduce resistant high-yielding varieties and RNAi technology can be used to silence specific genes in *F. proliferatum*that are critical for FA production.

ACKNOWLEDGEMENTS

This study has been funded by USM Research University Grant: 1001.PBIOLOGI.8011097.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

The authorshereby declare that no generative ai technologies such as large language models (chatgpt, copilot, etc) and text-to-image generators have been used during writing or editing of manuscript.

COMPETING INTERESTS

The authors declare that they have no known competing financial interests or conflict of interest.

AUTHORS' CONTRIBUTIONS

'AsmaulHusna'designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript.'Md. AsaduzzamanMiah' managed the analyses of the study and edited manuscript.'Nik Mohd IzhamMohamed Nor'concepted and editedmanuscript.All authors read and approved the final manuscript.

REFERENCES

1. Yabuta T, Kambe K, HayashiT. Biochemistry of the bakanae fungus. I. Fusarinic acid, a new product of the bakanae fungus. J. Agric. Chem. Soc. Jpn. 1937;10:1059–1068.
2. Gaeumann E. Fusaric acid as a wilt toxin. Phytopathology. (1957);47:342–357.
3. Ghag SB, Shekhawat UK, Ganapathi TR. Native cell-death genes as candidates for developing wilt resistance in transgenic banana plants. AoB Plants. 2014;6:1037–1049. doi: 10.1093/aobpla/plu037
4. Wang M, Sun Y, Sun G, Liu X, Zhai L, Shen Q, Guo S. Water balance altered in cucumber plants infected with *Fusarium oxysporum* f. sp. *cucumerinum*. Sci. Rep. 2015;5:7722–7729. doi:10.1038/srep07722
5. Quazi SAJ, Sariah M, Ahmad ZABM, Hawa J. Detection of Fungal Metabolites from Bakanae Diseased Plants and Their Relationship with Bakanae Disease Symptoms Expression. American Journal of Bioscience and Bioengineering. (2016);4(6):77. https://doi.org/10.11648/j.bio.20160406.14
6. Singh VK, Singh HB, Upadhyay RS. Role of fusaric acid in the development of 'Fusarium wilt' symptoms in tomato: Physiological, biochemical and proteomic perspectives. Plant physiology and biochemistry. 2017;118:320-332.
7. Bacon CW, Porter JK, Norred WP, Leslie JF. Production of fusaric acid by *Fusarium*. Applied and Environmental Microbiology. 1996;62:4039–4043.
8. Desjardins AE, Plattner RD, Nelson PE. Production of fumonisin B1 and moniliformin by *Gibberellafujikuroi* from rice from various geographic areas. Applied and Environmental Microbiology. 1997;63:1838–1842.
9. Logrieco A, Moretti A, Ritieni A, Bottalico A, Corda P. Occurrence and toxigenicity of *Fusarium proliferatum* from preharvest maize ear rot, and associated mycotoxins in Italy. Plant Disease. 1995;79:727–731.
10. Elmer WH. *Fusarium proliferatum*, as causal agent in Fusarium crown and root rot of asparagus. Plant Disease. 1990;74:938.
11. Abdalla MY, Al-Rokibah A, Moretti A, Mule`G. Pathogenicity of toxigenic *Fusarium proliferatum* from date palm in Saudi Arabia. Plant Disease. 2000;84:321–324.
12. Armengol J, Moretti A, Perrone G, Vicent A, Bengoechea JA, Garcia-Jemenez J. Identification, incidence and characterization of *Fusarium proliferatum* on

ornamental palms in Spain. European Journal of Plant Pathology. 2005;112:123–131.
13. Park JW, Choi SY, Hwang HJ, Kim YB. Fungal mycoflora and mycotoxins in Korean polished rice destined for humans. International journal of food microbiology. (2005); 103(3):305-314.
14. Quazi SJ, Meon S, Jaafar H, Ahmad ZABMM. Characterization of *Fusarium proliferatum* through species specific primers and its virulence on rice seeds. International Journal of Agriculture and Biology. 2013;15(4):649–656.
15. Abbas HK, Cartwright RD, Xie W et al. Mycotoxin production by *Fusarium proliferatum* isolates from rice with Fusarium sheath rot disease. Mycopathologia. 1999;147:97-104.
16. Wang L, Ge S, Liang W et al. Genome-Wide Characterization Reveals Variation Potentially Involved in Pathogenicity and Mycotoxins Biosynthesis of *Fusarium proliferatum* Causing Spikelet Rot Disease in Rice. Toxins. 2022;14(8). https://doi.org/10.3390/toxins14080568
17. Leslie JF, Marasas WFO, Shephard GS, Sydenham EW, Stockenstrom S, Thiel PG. Duckling toxicity and the production of fumonisin and moniliformin by isolates in the A and F mating populations of *Gibberellafujikuroi* (*Fusarium moniliforme*). Applied Environmental Microbiology. 1996:62:1182–1187
18. Marasas WFO, Nelson PE, Toussoun TA. Toxigenic *Fusarium* species: Identity and mycotoxicology. University Park: Pennsylvania State University Press. 1984.
19. Ritieni A, Fogliano V, Randazzo G, Scarallo A, Logrieco A, Moretti A, Mannina L, Bottalico A. Isolation and characterization of fusaproliferin, a new toxic metabolite from *Fusarium proliferatum*. Natural Toxins. 1995;3:17–20.
20. Cole RJ, Kirskey JW, Cutler HG, Doupnik BL, Peckam JC. Toxin from *Fusarium moniliforme*: Effects on plants and animals. Science 1973;179:1324–1326.
21. Lamprecht SC, Marasas WFO, Alberts JF, Cawood ME, Gelderblom WCA, Shephard G S, Thiel PG, Calitz FJ. Phytotoxicity of fumonisins and TA toxin to corn and tomato. Phytopathology. 1994;84:383–391.
22. Reverberi M, Ricelli A, Zjalic S, Fabbri AA, Fanelli C. Natural functions of mycotoxins and control of their biosynthesis in fungi. Applied microbiology and biotechnology. 2010;87:899-911.
23. Zonno MC, Vurro M, Evidente A, Capasso R, Cutignano A, Sauerborn J. Phytotoxic metabolites produced by *Fusarium nygamai* from *Striga hermonthica*. In: Proceedings of the IXth International Symposium on Biological Control of Weeds, January 19–26, 1996, Stellenbosch, South Africa (V.C. Moran, J.H. Hoffmann, ed.) University of Stellenbosch, South Africa, 223–226.
24. Yadav J, Mahawer AK, Bashyal BM, Mahawer AK, Prashantha ST, Bashyal BM. Confirming the role of fungal secondary metabolites and mycotoxins in bakanae disease incidence through artificial inoculation. The Pharma Innovation Journal. 2022;11(2):2317–2330. http://www.thepharmajournal.com
25. Diniz SPSS, Oliveira RC. Effects of fusaric acid on *Zea mays* L. seedlings. International Journal of Experimental Botany. 2009;78:155–160.
26. Idris AE, Abouzeid MA, Boari A, Vurro M, Evidente A. Identification of phytotoxic metabolites of a new *Fusarium* sp. inhibiting germination of *Striga hermonthica* seeds. Phytopathology Mediterr.2003;42:65–70.
27. Matysiak B, Samyn G. Growth of young *Aechmea fasciata* seedlings on a medium supplemented with fusaric acid. Med. Fac. Landbouww. Univ. Gent. 1996;61:333-336.
28. Rodella RA. Efeitos de herbicidassobreaanatomia da plântula de *Sorghum bicolor* (L.) Moench. Agro-Ciência. 1991;7:11.
29. Zainudin NAIM, Razak AA, Baharuddin S. Secondary metabolite profiles and mating populations of *Fusarium* species in section Liseola associated with bakanae disease

of rice. Malaysian Journal of Microbiology. 2008;4(1):6–13. https://doi.org/10.21161/mjm.01708

30. Husna A, Miah MA, Latiffah Z, Nor NMIM. *In vitro* production of GA_3 and FA by *Fusarium* spp. and their role in bakanae disease development. Journal of Applied Biology & Biotechnology. 2024; (Accepted)
31. Husna A, Zakaria L, Mohamed Nor NM. *Fusarium commune* associated with wilt and root rot disease in rice. Plant Pathology.2021;70(1):123-132.
32. Qiu J, Lu Y, He D, Lee Y W, Ji F, Xu J, Shi J. *Fusarium fujikuroi* Species Complex Associated with Rice, Maize, and Soybean from Jiangsu Province, China: Phylogenetic, Pathogenic, and Toxigenic Analysis. Plant Disease. 2020;104:2193–2201. https://doi.org/10.1094/PDIS-09-19-1909-RE
33. Wu HS, Bao W, Liu DY, Ling N, Ying RR, Raza W, *et al*. Effect of fusaric acid on biomass and photosynthesisof watermelon seedlings leaves. Caryologia.2008;61(3):258-268.
34. Li C, Zuo C, Deng G, Kuang R, Yang Q, Hu C, et al. Contamination of bananas with beauvericin and fusaric acid produced by *Fusarium oxysporum* f. sp. *cubense*. PLoS One. 2013;8(7):e70226.
35. Jiang H, Wu N, Jin S, Ahmed T, Wang H, Li B, Wu X, Bao Y, Liu F, Zhang JZ. Identification of Rice Seed-Derived *Fusarium* spp. and Development of LAMP Assay against *Fusarium fujikuroi*. Pathogens. 2021;10(1):1–22. https://doi.org/10.3390/pathogens10010001
36. Wulff EG, Sørensen JL, Lübeck M, Nielsen KF, Thrane U, Torp J. *Fusarium* spp. associated with rice Bakanae: Ecology, genetic diversity, pathogenicity and toxigenicity. Environmental Microbiology. 2010;12(3):649–657. https://doi.org/10.1111/j.1462-2920.2009.02105.x
37. Jeon YA, Yu SH, Lee YY, Park HJ, Lee S, Sung JS, Kim YG, Lee HS. Incidence, molecular characteristics and pathogenicity of *Gibberellafujikuroi* species complex associated with rice seeds from Asian countries. Mycobiology. 2013;41(4):225–233. https://doi.org/10.5941/MYCO.2013.41.4.225
38. Zainudin NAIM, Razak AA, Salleh B. Bakanae disease of rice in Malaysia and Indonesia: Etiology of the causal agent based on morphological, physiological and pathogenicity characteristics. Journal of Plant Protection Research. 2008;48(4):475–485. https://doi.org/10.2478/v10045-008-0056-z
39. Eğerci Y, Kınay-Teksür P, Uysal-Morca A. First report of Bakanae disease caused by *Fusarium proliferatum* on rice in Turkey. Journal of Plant Diseases and Protection.2021;128:577–582. https://doi.org/10.1007/s41348-020-00369-z