# The Role of Trichoderma in The Early Growth of Rice and Soybean in Saline Soils

*Sutarman*[*], *Andriani* E. Prihatiningrum, *Noviana* Indarwati, *Risalatul* Hasanah, and *Agus* Miftahurrohmat

Department of Agrotechnology, Faculty of Science and Technology, Universitas Muhammadiyah Sidoarjo, Sidoarjo, Indonesia, 61215

**Abstract.** Optimizing the use of marginal saline soils to improve food security requires solutions that involve exploiting local potential resources, including the use of biological agents. This research aims to determine the ability of *Trichoderma* sp. Tc-31 and *Aspergillus* sp. As-27 to overcome salinity stress in rice and soybean seedlings. Both isolates were tested for their ability to grow in vitro in saline soil. Next, the ability to overcome saline soil stress during germination and initial growth of rice and soybean seedlings was tested. The experiment consisted of three types of treatment, namely the use of As-27, Tc-31, and the use of husks treated with complete basic fertilizer. The average inhibitory power of saline soil against As-27 and Tc-31 was (-)62.50±16.68% and (-) 52±3.54% respectively at 48 days of incubation. The application of biological agents had an effect on germination in rice 94.10-94.46% and soybeans 74.49-77.04%. Biological agents also influence the height growth of rice and soybean seedlings. These two fungal isolates have the potential to be used as biological agents to help rice and soybean seeds overcome salinity stress.

## 1 Introduction

Some consider saline land to be marginal land, yet it has the potential to be used for agricultural expansion while also posing a threat to crop production and food security [1], including rice and soybean commodities grown on saline land. The amount of saline land in Indonesia is 12.02 million hectares, or 6.20% of total land area [2], and it is part of a global saline soil with a depth acceptable for ploughing layers for food crops of 4,240,000 km2 [3]. Meanwhile, salinity is typically produced by natural and human forces such as wind-borne salt, sea level rise or tidal inundation, and seawater intrusion [4-5], as well as bad agricultural practises [6-7]. Moderate to high salinity has been proven to result in yield losses of up to 50% of agricultural productivity, threatening global food security [8]. To avoid further losses, measures must be made to rehabilitate the soil, including the addition of organic matter from agricultural waste, such as rice husks, in the form of biochar [9-10].

Biological agents such as *Trichoderma* and *Aspergillus*, cosmopolitan fungi, on the other hand, have been shown to be able to act as biofertilizers to help plant growth, including in conditions of facing biotic and abiotic stresses [11-12] and provide protection

[*] Corresponding author: sutarman@umsida.ac.id

© The Authors, published by EDP Sciences. This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (https://creativecommons.org/licenses/by/4.0/).

from pathogenic disturbances [13-14]. *Aspergillus* sp. has the ability to decompose organic matter, remediate pollutants in fields, and can also be used as a biocontrol agent for certain disease-causing pathogens due to its ability to produce various secondary metabolites including organic acids and various enzymes [15], in addition to assisting plants in overcoming abiotic stresses and remediating land [16 -17].

Meanwhile, little progress has been made in overcoming salinity stress by the application of efficient fungus in lowland rice cultivation in conditions of water availability and in soybean production as crop rotation during limited water or dry season. Among the applications are the use of rhizobacteria microbes, which can produce PGPR-mediated growth promotion that is beneficial for plants to increase plant resistance in overcoming salinity stress [18-19] and can produce one or more auxin compounds and their derivatives, cytokinins, gibberellins, and abscisic acid [20]. Some *Trichoderma* isolates, on the other hand, have the potential to overcome salinity in plants [21-22], while some *Aspergillus* isolates can aid plant growth in salinity stress [23-24].

However, there hasn't been much testing of beneficial microorganisms as single isolates or consortia on saline soils, particularly those with lowland rice and soybean production potential. As a result, the purpose of this study was to test the adaptability of *Trichoderma* sp. and the *Aspergillus* sp. isolates obtained from suboptimal wetlands in coastal areas for saline soil conditions in vitro, as well as their ability to promote germination and early growth of plants as a critical phase of plants. The ability of plant seeds and sprouts to build tolerance and resist salt stress is critical to rice plant performance in surviving environmental stress [25] during the germination phase and subsequent stages of production in rice plants [26] and soybeans [27]. The findings of this test are expected to offer preliminary data on the possibility of these two isolates to be utilised in a formula intended as a biofertilizer to assist plants in overcoming saline soil stress.

## 2 Method

### 2.1 Preparation of test isolates

In this experiment, two biofertilizer candidate isolates from the LBM-UMSIDA collection were used: *Trichoderma* sp. Tc-27 (from Trimulyo village, Juwana District, Pati Regency, Central Java) and *Aspergillus* sp. 31 (from Tanjungsari village, Jabon District, Sidoarjo Regency, East Java). These two potential fungal isolates for biofertilizer biological agents were reproduced by cultivating them on Potato Dextrose Agar-chloramphenicol (PDA-c) media for 10 days before being used in in vitro and in vivo experiments [28].

### 2.2 Test for growth in sludge media

With a 96-hour incubation period, the growth ability test on medium containing saline soil was performed to determine the percentage of inhibition of the media on the activity of the two candidate fungal isolates. Soil samples were collected and used as growing media materials from land in Tlocor, Jabon District, Sidoarjo that had never been planted with rice or soybeans. The suspension formed by combining 500 grammes of diluted soil with 500 ml of distilled water was filtered through a vacuum funnel using Whattman No 1 filter paper. The filter products were mixed with 500 ml of PDA-c media in a 1:1 ratio, and the pH and salinity [29] were measured, yielding an average of 4.3 dS/m and a pH of 6.5-7.0. The ability of both fungal isolates to respond to growth media in saline soil was examined. The testing mechanism is comparable to the inhibition test, which is often performed by

growing the isolate in a dual culture model and growing one isolate alone as a control. Samples of propagule isolates collected with a 5 mm cork borer were placed in a cup with PDA-c-soil saline 1:1, 3 cm from either side of the petri dish, and cultured in an incubator at 25°C for 4 24 hours. Every 24 hours, the percentage of inhibition is computed using formula (1).

$$IP = (r1-r2)/r1 \times 100\% \quad (1)$$

With the provisions: *IP* is the percentage of inhibition, r1 and r2 are the colony radii of biological agents on PDA-c media and PDA-c media containing saline soil at a 1:1 concentration ratio, respectively; IP is positive, and if it is negative (-), it indicates the percentage of support for biological agent growth.

### 2.3 Germination support test

This experiment was conducted in Tlocor, Jabon-Sidoarjo District, in a pond region with an average salinity of 4.3 dS/m and a pH of 6.5-7.2. The rice seed germination test was performed by placing 20 seeds of the Trisakti variety in polybags that had been treated with: (i) complete NPK fertiliser, (ii) previously treated soil mixed with 400 g of biofertilizer *Trichoderma* sp. per polybag for two weeks before conditioning to become macak-macak, and (iii) previously treated soil mixed with 400 g of biofertilizer *Aspergillus sp.* For ten days, the percentage of germination was measured. Furthermore, each polybag with a capacity of 10 kg was kept alive by two sprouts until 14 days after planting, or until the rice seedlings sprouted new shoots. The soybean seed germination test was carried out by planting up to 20 Detap 1 rice seeds in polybags with a capacity of 8 kg that had been treated in the same way as the rice experiment. The polybags were then kept alive by two sprouts until 21 days following planting, or when the plants formed their first trifoliate leaves.

## 3 Result and discussion

### 3.1 Test the ability to grow in the sludge media

The results of growing two candidate isolates of biological agents projected as biofertilizer active ingredients on saline soil growth media and on PDA-c for 96 hours of incubation period are shown in Figures 1 and 2.

In the three isolates tested it appears that since 21 HAI the growth of colonies grown on muddy media is faster than that grown-on PDA-c media. Thus, the inhibition of saline soil sludge on colony growth becomes negative (Table 1).

**Table 1**. Percentage of inhibition of PDA-c media – 1:1 saline soil against two candidate isolates of biological agents during 96 hours of incubation.

| Treatments | Observation time | | | |
|---|---|---|---|---|
| | 24 HAI | 48 HAI | 72 HAI | 96 HAI |
| *Aspergillus* sp. As-27 | (-)11.90±1.98 | (-)62.50±16.68 | 0 | 0 |
| *Trichoderma* sp. Tc-31 | (-)36.50±4.95 | (-)47.50±10.61 | (-)34.50±13.44 | (-)52.50±3.54 |

All colonies grew perfectly on PDA-c growth medium saline soil 1:1, and the colony's diameter reached the border of the petri dish faster than normal conditions, which took 96 hours (Figure 1-2). However, the media's reaction to the mud material was not the same (Table 1). The negative indication suggests that saline soil sludge does not limit colony formation but rather stimulates it. In this situation, both types of isolates have taken use of

the mud's resources, which are promptly converted for the growth of their colonies. These three varieties of fungi appear to benefit from the mineral content of the mud in order to expand their colonies faster than those cultivated solely on PDA-c. Trichoderma Tc-31, in particular, appeared until the last day of the incubation period, when the colonies had filled the plate and showed a strong growth rate with growth support ranging from 34.50 to 52.50 to 96 HAI.

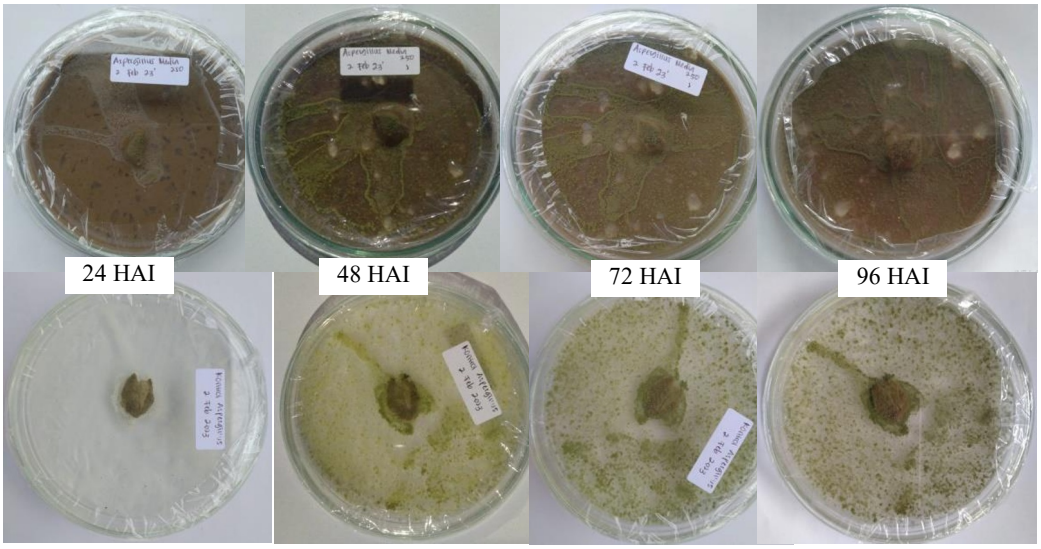


**Fig. 1**. Colony growth of *Aspergillus* sp. As-031 on PDA-mud media 1:1 (top) and on PDA-c media (bottom) for 96 hours incubation

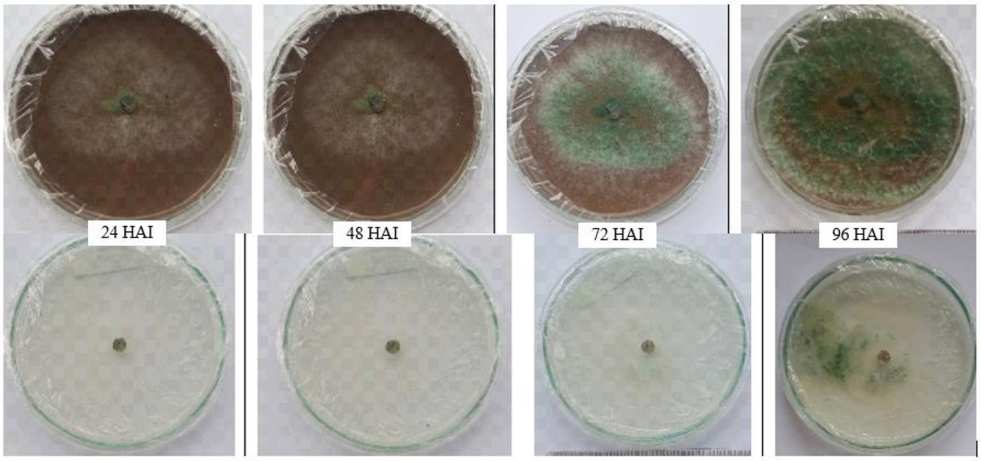


**Fig. 2**. Colony growth of *Trichoderma* sp. Tc-027 on PDA-c-mud media 1:1 (top) and on PDA-c media (bottom) for 96 hours incubation

From colony samples, each isolate grown on PDA-c mixed media and saline soil with a ratio of 1:1 was observed under a microscope, especially for the shape and dimensions of the spores, as shown in Fig. 3.

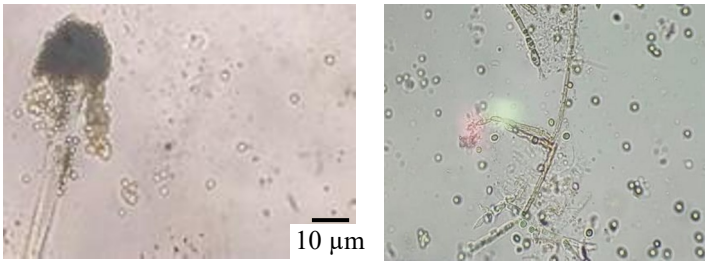


**Fig. 3**. Observations of post-growth biological agent spores on PDA-c medium saline soil 1:1: *Aspergillus* sp. As-27 (left), *Trichoderma* sp. Tc-31 (right)

Morphological observations (Fig.3) of isolates cultured on PDA-c-saline soil media 1:1 revealed no change in form or size. *Aspergillus* sp. discovered along the shore of Sidoarjo (East Java) had a constant form with spore dimensions of 2.37±0.51 µm. *Trichoderma* sp. isolates from Trimulyo village, Juwana District, Pati Regency (Central Java) originating from the rhizosphere of soybean plants during the dry period of suboptimal wetlands with an average size of 3.22 µm for hyphae diameter, 3.38 µm for phialid hyaline oval, and 2.45± 0.73 µm for spore diameter.

The results of observations of Tc-31 isolates showed morphological characteristics including septa on hyphae, conidiophores, fialids and oval conidia similar to isolates determined as *T. asperellum* [30-31]. For *Aspergillus* sp. As-27 has similarities with *Aspergillus tamarii* [32] which effectively acts as a biofertilizer [33]. It is necessary to determine the Tc-31 and As-27 isolates using molecular markers that are enhanced by their phylogenetic arrangement [34] to see their closeness in kinship with known species.

The lack of changes in shape and size implies that the developing environment, in this case the growth media in the petri dish, is not exerting any pressure. Rapid growth indicates that the sludge has more nutrients than PDA-c. This result (Fig. 1-3 and Table 1) indicates that the candidates for biofertilizer biological agents in the two fungal isolates were not only able to adapt to salinity, but were also able to utilise the nutrients present in the sludge.

### 3.2 In vivo test

The two fungal isolates as candidates for biological agents did not significantly affect the germination percentage of both rice and soybean seeds. The average percentage of germination of rice and soybean seeds is shown in Table 2.

**Table 2**. Germination percentage of rice and soybean seeds grown on saline soil

| Treatments | Rice seed germination percentage | Soybean seed germination percentage |
|---|---|---|
| Saline soil + basic fertilizer | 91.14±6.05 | 76.28±7.20 |
| Saline soil + *Trichoderma* | 94.46±6.95 | 74.49±8.91 |
| Saline soil + *Aspergillus* | 94.10±7.41 | 77.04±6.14 |
| Laboratory conditions | 96.57±3.26 | 92.71±2.87 |

In terms of rice seeds, it appears that the germination percentage of seeds planted on soil planting media containing the biological agent candidate fungal isolate formula (91.14-94.10%) is nearly identical to laboratory germination, with an average of 96.57±3.26. In contrast, the percentage of germination of soybean seeds treated with biological agents was substantially lower (74.49-77.04%) than in laboratory settings. This suggests that the two biological agents, which are potential biofertilizer agents, were less able to maintain high

germination rates. The use of biological agent fungus from saline soils had a substantial influence on rice shoot growth in 14 DAS but had no effect in 21 DAS. Meanwhile, Tc-31 and As-27 isolates had a substantial effect on the growth of soybean sprouts until the appearance of the first trifoliate leaves, although As-27 had the lowest reaction from the sprouts. Tables 3 and 4 indicate the average growth of each sprout.

**Table 3**. The average effect of the application of biological agents on the length of rice sprouts grown on saline soil media until tillers appeared

| Treatments | 14 DAS | 21 DAS |
|---|---|---|
| Saline soil + basic fertilizer | 13.92±2.19 b | 28.15±4.51 |
| Saline soil + *Trichoderma* | 16.46±1.57 a | 27.86±4.27 |
| Saline soil + *Aspergillus* | 14.08±1.74 b | 26.39±2.88 |
| HSD 5% | 1.56 | ns |

Note: letters accompanying the mean value of different treatments in one column indicate different effects; ns = not significant

**Table 4**. The average effect of the application of biological agents on the length of rice sprouts grown on saline soil media until the first trifoliate leaves appear

| Treatments | 14 DAS | 21 DAS | 28 DAS |
|---|---|---|---|
| Saline soil + basic fertilizer | 11.64±1.25 b | 13.97±0.72 b | 26.43±4.13 a |
| Saline soil + *Trichoderma* | 13.64±1.74 a | 18.14±0.48 a | 25.60±1.01 a |
| Saline soil + *Aspergillus* | 12.86±2.17 b | 12.70±0.96 c | 18.73±1.13 b |
| HSD 5% | 1.40 | 0.59 | 0.87 |

Note: letters accompanying the mean value of different treatments in one column indicate different effects

Isolate Tc-31 exhibits the general characteristics of Trichoderma, including the ability to produce extracellular compounds such as acid phosphatase, cellulolytic enzymes, and encouraging microbial activity that aids plant growth [35], the ability to produce various plant growth regulators such as IAA and siderophore [36], and the ability to help plants cope with stress in the environment, including overcoming salinity [37]. One of the A. niger strains can manufacture secondary metabolites and compete for space and nutrients [40], in addition to creating antimetabolites against pathogenic fungus [41].

Tc-31 isolate improved the performance of rice sprouts up to 14 DAS, but had little influence on growth at 21 DAS when compared to no biological agents and As-27 therapy. The same thing happened to soybean sprouts till 21 DAS. This demonstrates that the extracellular chemicals produced by Tc-31 benefit both types of sprouts. However, when new shoots grow in rice and trifoliate leaves appear in soybeans, this effect does not provide a consistent response.

Trichoderma swiftly exploits the saline soil's resources, but the mineral pressure in the saline soil puts more strain on the sprouts. In saline soils, roots will have reduced water intake, as well as increased osmotic pressure and Na+ and Cl- ions, which will damage and poison root cells [42-43]. Mechanical impedance to sprout emergence, decreased soil aeration around roots, and suppression of root growth are all unfavourable impacts of saline soils on seedlings [44-45].

Organic husks utilised as carriers in biological agent formulation appear to have little effect on salinity stress. Although husk provides several advantages [46], its large particle size (40 mesh) and low ash content do not assist sprouts resist saline stress very well [47]. Small organic matter particles with a high ash content, such as biochar, have a high cation

exchange capacity and specific surface area, which are beneficial for improving soil quality and reducing the overall effect of salinity stress [48-50] and providing plant protection from salinity stress [51]. The slow growth response in the final phase of sprout growth (Tables 3 and 4) indicates that salinity stress is stronger than the contribution of the two biological agents in this experiment to shoot growth in rice (21 DAS) and early trifoliate leaf growth on soybeans (28 DAS). Except for isolate As-27, it appears that Tc-31 has future development potential, given that a Trichoderma isolate comparable to Tc-31 has demonstrated to be beneficial in saline soils [52-53].

## 4 Conclusion

At 24, 48, 72, and 96 days, the average inhibition of saline soil against *Aspergillus* sp. As-27 was (-)11.90±1.98 and (-)62.50±16.68%, respectively, and against *Trichoderma* sp. Tc-31 was (-) 36.50±4.95%, (-) 47.50±1.61%, (-) 34.50±13.44%, and (-) 52.50±3.54%. Both biological agents can develop in vitro on saline soil substrate, and saline soil promotes the growth of As-27 and Tc-31 isolates. The two biological agents had an effect on rice and soybean germination, however under laboratory settings, it was between 94.10-94.46% for rice and 74.49-77.04% for soybean. These two biological agent candidate isolates had an effect on rice sprout development 14 days after germination (DAS) and soybean growth up to 21 DAS. Rice sprouts reached 16.46±1.57 cm and 27.86±4.27 cm at 14 and 21 DAS, whereas soybean sprouts reached 13.64±1.74, 18.14±0.48, and 25.60±1.01 cm at 14, 21, and 28 DAS. *Trichoderma* sp. Tc-31 and *Aspergillus* sp. As-27 have the potential to be used as biological agents to help rice and soybean plants overcome stress in the early phase of growth.

**Acknowledgment.** We would like to thank the Director General of Higher Education - Ministry of Education and Culture, Research and Technology of the Republic of Indonesia who has funded the research and preparation of this article as one of the outputs according to the contract number 035/SP2H/PT/LL7/2023 in the scheme Basic Research of Higher Education Excellence (PDUPT).

## References

1. F. Yang, Y. Yuan, Q. Liu, X. Zhang, S. Gai, Y. Jin, K. Cheng. Env. Pol. **15**,327:121588 (2023)
2. V. Karolinoerita, W.A. Yusuf. J. S. D. L. **14**(2), 91-99 (2020)
3. FAO. https://www.fao.org/soils-portal/data-hub/soil-maps-and-databases/global-map-of-salt-affected-soils/en/. Accessed May 2023 (2021)
4. E. Ruto, D. Tzemi, I. Gould, G. Bosworth. CRC Press. Taylor & Francis Group. P. 93-114 (2022)
5. Md.A. Rahaman, Md.S. Hossain, Md.I. Hossain. CRC Press. Taylor & Francis Group p. 293-304 (2022)
6. E.M. Hafez, A.E.D. Omara, F.A. Alhumaydhi, M.A. El-Esawi. Physiol. Plant., **172**, 587–602 (2020)
7. Hafez, E.E.-D.M.; El Hassan, W.H.A.; Gaafar, I.A.; Seleiman, M.F. J. Agric. Sci. 7, 208 (2015)
8. N. Ullah, A. Ditta, A.Khalid, S. Mehmood, M.S. Rizwan, M. Ashraf, F. Mubeen, M. Imtiaz, M.M. Iqbal. J. Soil Sci. Plant Nutr. **20**, 346–356 (2019)
9. M. Guo. W. Song, W. Tian. J. Front. Environ. Sci., **8**, 183 (2020)
10. X. Zhang, J. Qu, H. Li, S. La, Y. Tian, L. Gao, L. Geoderma. **363**, 114170 (2020)

11. C. Buysens, V. César, F. Ferrais, H.D. De Boulois, S. Declerck. Appl. Soil Ecol. **105**, 137–143 (2016)
12. K. Saravanakumar, C.Yu, K. Dou, M. Wang, Y. Li, Y. Chen. J. Biological Control. **94**, 37–46 (2016)
13. M. Ali, A. R. Khan, M. R. Khan, A. A. Khan. J. P. Prot. Res. **61**(2), 131-137 (2021)
14. E.T. Mezadri, K.R. Kuhn, S. Schmaltz, M.V. Tres, G.L. Zabot, R.C. Kuhn, M.A. Mazutti. Biotech. **12**(5):122 (2022)
15. V. Kagot, S. Okoth, M. De Boevre. Toxins. **11**(2), 109 (2019)
16. L. Aziz, M. Hamayun, M. Rauf, A. Iqbal, A. Husssin, S.A. Khan, M. Irshad, I. Lee. J. Plt. Interact. **16**, 104–115 (2021)
17. M. T. Selim, S. S. Salem, A. A. Mohamed, M. S. El-gamal, and M. F. Awad. J. fungi, **7**(3), 193 (2021)
18. S. Ullah, Bano, A. Can. J. Microbiol. **61**, 307–313 (2015)
19. S.R. Niranjana, P. Hariprasad. Springer: New York, NY, USA, pp. 59–108 (2014)
20. N. Katarzyna, Z. Malek, A. de Vos, P. Vellinga. J. Arid Env. **203**. 104775. (2022)
21. S.V.K. Gupta, P.M.C Smith, S.H.A. Natera, U. Roessner. Front Plant Sci. **13**, 908853. (2022)
22. Z. Liu, N. Xu, Q. Pang, R.A.A. Khan, Q. Xu, C. Wu, T. Liu. J Fungi (Basel). **9**(3), 304 (2023)
23. S. Sajid, V.R. de Dios, O.K. Zveushe, F. Nabi, S. Shen, Q. Kang, L. Zhou, L. Ma, W. Zhang, Y. Zhao, Y. Han, F. Dong. J Hazard Mater. **443**(Pt B), 130324 (2023)
24. K. Liu, X. Ding, G. Wang, W. Liu. MPMI. **35(9)** 867–869, (2022)
25. A.Yang, S.S. Akhtar, S. Iqbal, Z. Qi, G. Alandia, M.S. Saddiq, S.E. Jacobsen. J Agron Crop Sci **204**(1):31–39 (2018)
26. A. Hidayah, R.R. Nisak, F.A. Susanto, T. R. Nuringtyas, N. Yamaguchi, Y.A. Purwestri. Bot. Stud. **63**,24 (2022)
27. H. Abdalla, M.H. Adarosy, H.S. Hegazy, R.E. Abdelhameed. BMC Plant Biol. **22**(1), 560 (2022)
28. Sutarman, Tjahjanti, P.H., Prihatiningrum A.E., &Miftahurrohmat, A. Afr. J. Food Agric. Nutr. Dev. **22**(10): 21743-21760 (2022)
29. Z. Rohman, Sutarman. Proceedings of the 1st Seminar Nasional Sains (2021)
30. Sutarman, T. Setiorini, A.S. Li'aini, A. Purnomo, A. Rahmat. Int'l. J. Env. Sci. Dev. **13**(4), 131-137 (2022)
31. Sutarman, A.K. Jalaluddin, A. S. Li'aini, A.E. Prihatiningrum. J. Trop Plant Pests Dis **21**, 8-19 (2020)
32. Sutarman. *IOP Conf. Ser.*: Earth Environ. Sci. **1104**. 012026 (2022)
33. A.A. Farihadina, Sutarman. *IOP Conf. Series*: Earth and Environmental Science 1104 (2022)
34. S. Kumar, G. Stecher, M. Li, C. Knyaz, K. Tamura. Mol. Biol. Evol. **35**(6): 1547–1549 (2018)
35. T. Alshaal, H. El-Ramady, A.H. Al-Saeedi, T. Shalaby, T. Elsakhawy, A.E.D Omara, A. Gad, E. Hamad, A. El-Ghamry, A. Mosa, M. Amer, N. Abdalla. In Essential Plant Nutrients: Uptake, Use Efficiency, and Management. pp. 275-308 (2017)
36. A. Syed , A.M. Elgorban, A.H. Bahkali, R. Eswaramoorthy, R.K. Iqbal, S. Danish. Sci Rep. **13**(1):4471 (2023)

37. G. Bizos, E.M. Papatheodorou, T. Chatzistathis, N. Ntalli, V.G. Aschonitis, N. Monokrousos. Plants. **9**(6), 743 (2020)
38. A. Sharma, A. Shukla, M. Gupta. Sci Rep. **13**(1), 6052 (2023)
39. S. Boamah, S. Zhang, B. Xu, T. Li, A. Calderón-Urrea, R.J. Tiika. Peer J. **10**, 12923 (2022)
40. R. Gangaraj, A. Kundu, V.S. Rana, A. Das, G. Chawla, G. Prakash, R. Debbarma, A. Nagaraja, N.K. Bainsla, N.C. Gupta, D. Kamil. Front. Microbiol. **14**,1142144. (2023)
41. K. Darshan, R. Aggarwal, B.M. Bashyal, J. Singh, A. Kundu, S. Yadav. Indian J. Agric. Sci. **91**, 776–782 (2021)
42. H. El-Ramady, T. Alshaal, N. Elhawat, A. Ghazi, T. Elsakhawy, A.E.D Omara, S. El-Nahrawy, M. Elmahrouk, N. Abdalla, E. Domokos-Szabolcsy, E. Schnug. Springer: Singapore, pp. 297–324 (2018)
43. Z. Ding, A.M. Kheir, O.A. Ali, E.M. Hafez, E.A. El Shamey, Z. Zhou, B. Wang, X. Lin, Y. Ge, A.E. Fahmy, et al. J. Environ. Manag. **277**, 111388 (2020)
44. E.M. Hafez, A.M.S. Kheir, S.A. Badawy, E. Rashwan, M. Farig, H.S. Osman. Plants **9**, 1346 (2020)
45. A.S. Shabbir, M. Zaman, L. Heng. Guideline for Salinity Assessment, Mitigation and Adaptation Using Nuclear and Related Techniques. p. 1-42 (2018)
46. G.L.F. José, S. Elisa. Orth. Env. Poll. **330**,121802 (2023)
47. R. Sarfraz, A. Hussain, A. Sabir, I. B. Fekih, A. Ditta, S. Xing. Environ. Monit. Assess. (2019)
48. M. Guo, W. Song, W. Tian, J. Front. Environ. Sci. **8**, 183 (2020)
49. Y. Zhang, C. Tian, J. Xiao, L. Wei, Y. Tian, Z. Liang. AMB Express, **10**(1) (2020)
50. R. Cen, W. Feng, F. Yang, W. Wu, H. Liao, Z. Qu. J. Environ. Manag. **286**, 112198 (2021)
51. N. Ullah, A. Ditta, A. Khalid, S. Mehmood, M.S. Rizwan, M. Ashraf, F. Mubeen, M. Imtiaz, M.M. Iqbal. J. Soil Sci. Plant Nutr. **20**, 346–356 (2019)
52. Singh UB, Malviya D, Singh S, Singh P, Ghatak A, Imran M, Rai JP, Singh RK, Manna MC, Sharma AK, Saxena AK. Int J Environ Res Public Health., **18**(18):9936 (2021)
53. S.V.K. Gupta, P.M.C. Smith, S.H.A. Natera, U. Roessner. Front Plant Sci. **13**:908853 (2022)