

Role of Seaweed Liquid Fertilizer (SLF) in Controlling Fungal Growth Associated with Okra (*Abelmoschus esculentus* L.) Seeds

Hafsah¹, Amarah¹, Amir Haider² And Laila Shahnaz¹

¹Department of Botany, University of Karachi, Karachi, Pakistan, Postal code:7529

²Chemico Bacteriological Laboratory, Department of Health, Government of Sindh Services Hospital Karachi

Corresponding Author: lailashehnaz@gmail.com

ABSTRACT

Okra (*Abelmoschus esculentus* L.) seeds are easily attacked by a variety of fungi resulting in seed loss. This study was to determine the effect of seaweed liquid fertilizer (SLF) obtained from *Sargassum tenerrimum* on the growth of fungi on okra seeds. Agar Plate, Blotter paper, and Deep Freezing methods were used to study the fungi related to okra seeds. The effects of 0.1, 0.2 and 0.3% concentrations of seaweed liquid fertilizer (SLF) on fungal growth were studied. Results showed that the majority of the seeds appeared to be physically healthy. SLF reduced the attack of fungi on seeds, although certain unidentified bacterial growth was still present on seeds after treatment. The study concludes that SLFs can be used for the protection of okra seeds against fungi, although their specific ratios are yet to be determined.

Keywords *Sargassum*, Okra seeds, *Chaetomium* spp., *Trichoderma* spp.

1. INTRODUCTION

Okra is considered an important vegetable crop in Pakistan, but the seeds of okra are highly susceptible to decaying and rot. Seeds of Okra are easily attacked by different insects, bacteria and fungi. Studies reported that about 75 different species of fungi can attack okra seeds, which include both saprophytic as well as pathogenic genera, and cause multiple diseases in okra seeds (Rahim & Dawar, 2015; Yadav *et al.*, 2020). Therefore, it is necessary to search for precautionary measures against fungi associated with okra seeds. Seaweeds not only act as a soil fertilizer for the growth of okra but are also known to be active against certain fungi including *Fusarium moniliforme* and *Rhizoctonia solani* (Khan *et al.*, 2016). This study is focused on the effect of seaweed liquid fertilizer (SLF) obtained from brown seaweed *S. tenerrimum* on the fungal growth of okra seeds.

2. MATERIALS AND METHODS

2.1 Collection of seeds

Okra is a biannual plant and grows in the spring and rainy seasons. The seeds used in this study were collected from the okra crop which was sown last spring in the fields of interior Sindh, Pakistan. All seeds were stored in dry and cool conditions until tested.

2.2 Seaweed Collection and Extraction

Brown seaweed *S. tenerrimum* was collected from Buleji during low tide. After bringing samples to the laboratory, they were thoroughly washed with tap water to remove epiphytes, and then shade dried. The dried samples were ground into powder form till further use.

The SLF was extracted by boiling powdered seaweeds with distilled water in 1:10 (w/v) ratio. The extract was then centrifuged for 10 minutes at 3000 rpm. The supernatant collected was considered a 100% stock solution. From the stock solution, concentrations of 0.1%, 0.2 and 0.3% were made in distilled water (Parab & Shankhadarwar, 2022).

2.3 Visual Observations for Physically Healthy Seeds of Okra

Around 400 seeds were taken for the test. The seeds were tested through low power binocular microscope for their physical features including smooth and healthy, discoloured, shrunken and shriveled seeds, *etc.* (Sarkar *et al.*, 2015).

2.4 Observations for Fungal Growth on Okra Seeds

The fungi present on seeds were observed by Blotter Paper, Agar Plate and Deep Freezing methods as described by the International Seed Testing Association (ISTA). In the Blotter paper method, Petri plates containing three layers of blotter paper were autoclaved. In each plate, 10 seeds were placed at equal distances. The plates were

incubated by wrapping them completely in paper and then kept in the dark at room temperature for a week. After seven days, the plates were observed for fungal growth.

In agar plate and deep freezing methods, 10 seeds of okra were placed at equal distances on Petri plates containing Potato Dextrose Agar (PDA). For the Agar Plate method, the Petri plates containing okra seeds were wrapped and incubated at room temperature in the dark for seven days. For deep-freezing technique, the plates along with the seeds were wrapped and then placed at -2 °C for four days, and then at room temperature, 28 °C for three days. After a week all petri plates were observed, and the fungi grown were recorded (Anonymous, 1993). Both methods were carried out in triplicates, while the data was recorded as mean values. The fungal growth on okra seeds was calculated by using the equation (Surovy *et al.*, 2020)

$$\text{Infection \%} = \frac{\text{Number of seeds on which fungus attacked}}{\text{Total number of seeds}} \times 100$$

2.5 Seeds Treated with SLF

Three layers of blotter paper were placed on Petri plates, and in each plate, 10 equal-sized seeds of okra were placed at equal distances. The Petri plates were labeled as control, 0.1%, 0.2% and 0.3% SLF. In each plate, 10 ml of respective concentrations of 0.1, 0.2 and 0.3% of seaweed liquid fertilizer (SLF), obtained from seaweed *S. tenerrimum* were poured. In control, instead of SLF, 10 ml of distilled water was poured. The plates were left in dark conditions for a week, after which the growth of fungi was observed. The experiment was carried out in triplicate (Ali, *et al.*, 2022).

3. RESULTS & DISCUSSION

This study was conducted to observe the effects of seaweed liquid fertilizer (SLF) on fungi that usually grow on okra seeds. The okra seeds were incubated through the blotter paper method, agar-plate method and deep freezing method as well as placed in three different concentrations of SLF (0.1, 0.2 & 0.3%). Fungal growth in each method was recorded, and the difference in infection rate between seeds treated with and without SLF was observed. This whole experiment was carried out in triplicate. The seeds were also tested visually for physical abnormalities including discoloured and wrinkled seeds.

3.1 Visual Observation of Healthy Seeds

When seeds were graded through visual observation, it was observed that 50% of seeds were completely healthy, 26% of seeds were healthy yet discoloured, 16% of seeds had a wrinkled surface, and 8% of seeds were either shrunken or broken (Fig 1). Only broken and shrunken seeds were discarded, while the rest of the seeds were used for fungal study.

3.2 Fungi Associated With Okra Seeds

It was observed that when seeds were tested using Blotter Paper, Agar-Plate method and Freeze-Drying method, the infection rate was 100%, as all seeds were covered with fungi (Fig 2). Moreover, more than one type of fungi was detected on all seeds. In the case of the Blotter Paper test, *Aspergillus flavus* was the most dominating fungal species. In the case of agar plate & deep freezing methods, *A.niger* & *A. flavus* were present as the most dominant fungi in all seeds. The other fungi present in different replicates included *Rhizopus* spp., *Trichoderma* spp., *Fusarium* spp., and *Dreschlera* spp. (Table 1). Overall, *Rhizopus* spp., (Fig. 2f), dominated over all other fungi in terms of infection rate, which was 90%, followed by *A. flavus* (Fig. 2e), which caused 82.85% infection, *Dreschlera* spp. causing 60% infection, *A. niger* (Fig. 2g) causing 48.88%, *Trichoderma* spp. causing 40%, and *Fusarium* spp. causing only 30% infection rate (Fig 2d). It was also observed that in replicates containing growth of *Trichoderma* spp., other fungi did not grow (Fig 2c). In some seeds, the white-coloured colony of bacteria was also observed.

3.3 Effect of SLF on Fungal Growth

When treated with three different concentrations of SLF, the seeds did not show fungal growth, although some bacteria were grown on some replicates. In control, two different types of fungi; *Chaetomium* spp. & *Stachybotrys* spp. were present, but their infection rate was 20%, which was lower as compared to the seeds of the agar plate and deep freezing methods (Fig 2a & 2b).

Okra is rich in carbohydrates, minerals, fibers, proteins, and vitamins A & C, therefore it is considered a high-nutrition vegetable crop. Okra is used worldwide in foods, pickles, and confectionary industries (Elkhalifa *et al.*, 2021; Ofori *et al.*, 2022). The seeds of okra in particular have higher levels of amino acids as compared to other plants. But okra seeds are highly susceptible to pathogenic attacks, which yearly cause yield loss (Begum *et al.*, 2005). Seaweeds are known to act as antifungal agents against root damaging fungi (Sultana *et al.*, 2005), but work on seed protection against fungi using SLF is quite low. As seed treatment with biological agents such as seed coating by using powder of Neem tree reduces the risk of attack by pathogens and insects and enhances

seed quality (Dawar *et al.*, 2008; Sultana *et al.*, 2015), this study is focused on the use of seaweed liquid fertilizer (SLF) obtained from *S. tenerrimum* as a possible treatment for protection of okra seeds against fungi.

All of the fungi found in this research have been reported previously (Rahim & Dawar., 2015; Sohi & Puttoo., 1973). In this study, *A. niger* appeared as the most dominating fungi on the agar plate and deep freezing methods. Previously, *Aspergillus* species have been reported as the dominating fungus in two varieties of okra (Al-Kassim & Monawar., 2000; Sitara *et al.*, 2018). Overall, in this study, *Rhizopus* spp. caused the highest infection rate. The high infection rate of *Rhizopus* is associated with the high moisture content of the infected plant as well as high humidity in the environment (Iwuagwu, *et al.*, 2014). It was also observed during this study that in seeds infected with *Trichoderma* spp., other fungi were not recorded. The non-appearance of other fungi in such seeds is because *Trichoderma* is known as an antagonist, and inhibits the growth of other saprophytic and pathogenic fungi (Mahmood & Al-Abedy., 2018).

When seeds were treated with 0.1, 0.2 and 0.3% SLF, no fungal growth was observed. However in seeds treated with 0.1 and 0.2% SLF, some unidentified bacterial growth was observed. Previous studies have reported similar results in which 0.15, 0.25 & 0.5% concentrations of oils obtained from black cumin (*Nigella sativa*) and asafoetida (*Ferula asafoetida*) showed high antifungal activity against fungi attacking okra seeds (Sitara *et al.*, 2008) and essential oils obtained from herbs is effective against multiple fungi including *Trichoderma* (Tullio *et al.*, 2007). It is also reported that marine green seaweed *Chaetomorpha antennina* shows antifungal activity against multiple fungi, including *A. niger*, *A. flavus* and *Rhizopus* spp., brown seaweeds *Padina gymnospora* and *Sargassum liebmannii* showed antifungal activity against *Alternaria solanii* in tomato plants, while the activity of *Sargassum dentifolium*, *Gracilaria compressa* and *Ulva lactuca* against *Fusarium* attacking tomato plants has also been previously reported (Shahnaz & Shameel., 2009; Esserti *et al.*, 2017; Mostafa *et al.*, 2022).

Table 1. Infection rate caused by different fungi in Okra seeds.

Sr. No	Fungi Name	Infection Rate (%)
1	<i>Aspergillus niger</i>	48.88
2	<i>A. flavus</i>	82.85
3	<i>Trichoderma</i> spp.	40
4	<i>Rhizopus</i> spp.	90
5	<i>Chaetomium</i> spp.	20
6	<i>Stachybotrys</i> spp.	20
7	<i>Dreschlera</i> spp.	60
8	<i>Fusarium</i> spp.	30

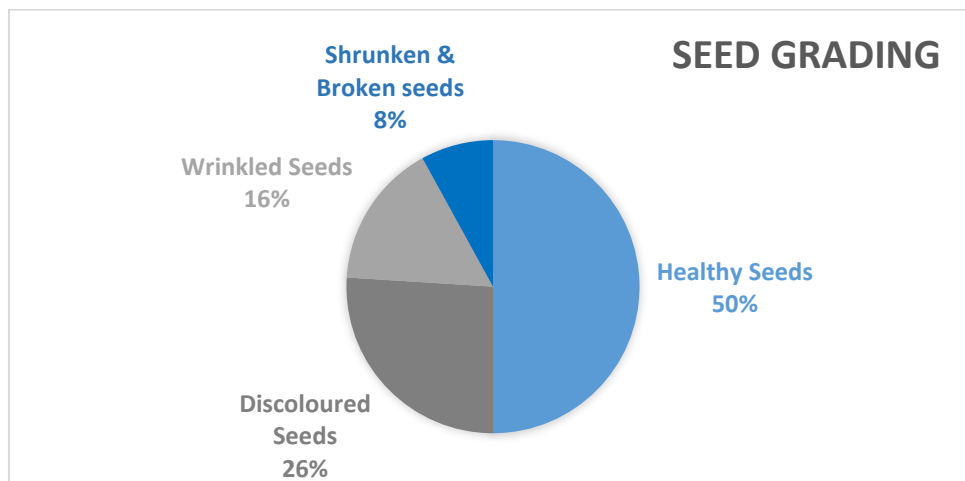


Figure 1: Seed grading through visual observation

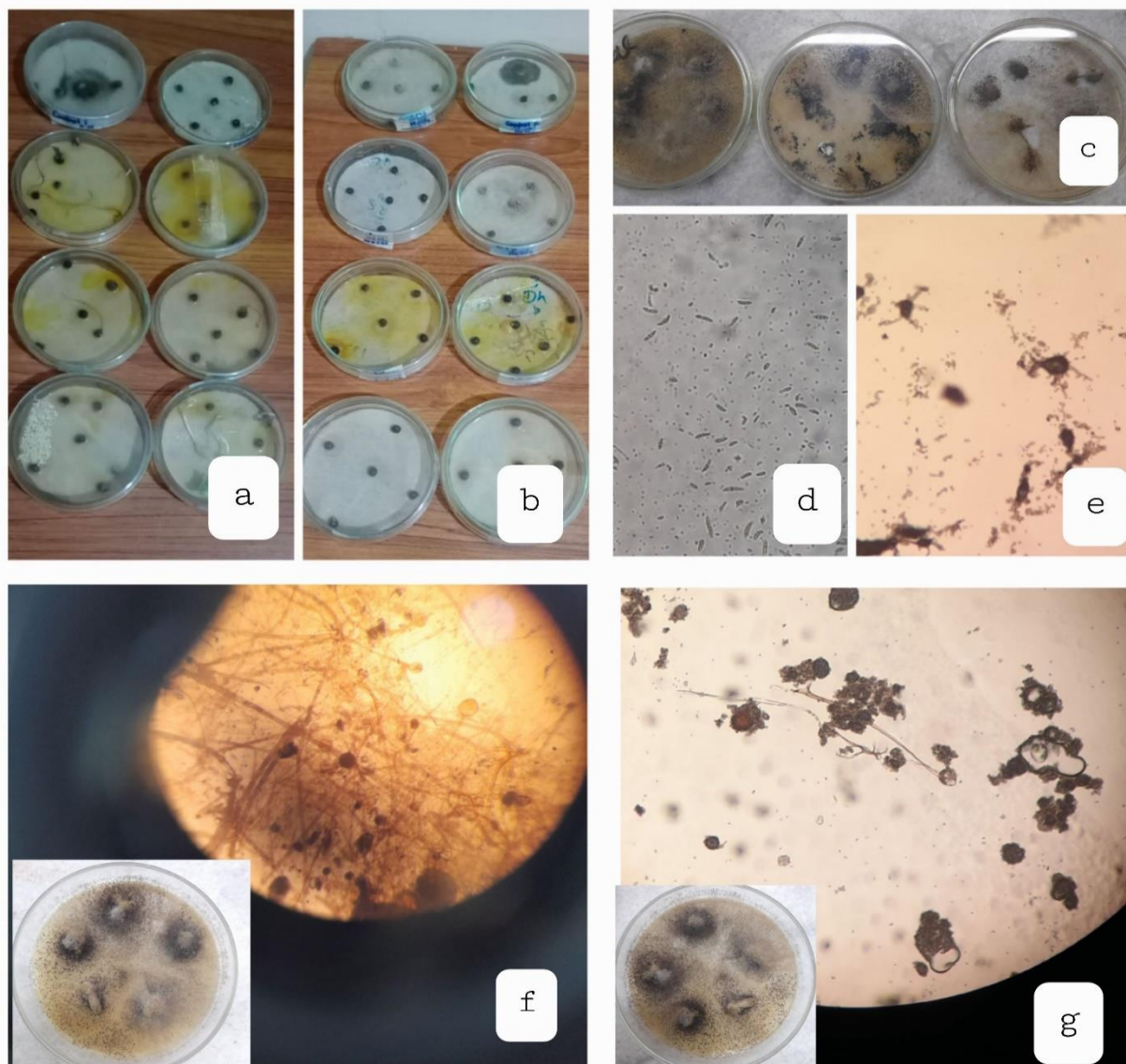


Figure 2: Okra seeds treated with SLF (a-b), seeds incubated through deep freezing method (c), Microscopic view of *Fusarium* spp. (d), *Aspergillus flavus* (e), *Rhizopus* spp. (f) and *A. niger* (g)

4. CONCLUSION

Okra seeds are highly susceptible to a variety of fungal and bacterial attacks. This study concludes that the use of seaweed liquid fertilizer (SLF) inhibits fungal growth in okra seeds when used in small quantities. Still, further studies are required to determine such concentrations of SLF, which can act both as antifungal as well as antibacterial agents in okra seeds.

5. ACKNOWLEDGMENT

We are grateful to Prof. Dr. Saleem Shahzad, Dr. Asma Hanif and Mrs. Noureen Rehan for their guidance in fungal identification.

6. REFERENCES

- Ali, J., Jan, I., Ullah, H., Ahmed, N., Alam, M., Ullah, R., El-Sharnouby M, Kesba H, Shukry M, Sayed S, & Nawaz, T. (2022). Influence of *Ascophyllum nodosum* extract foliar spray on the physiological and biochemical attributes of okra under drought stress. *Plants*, 11(6), 790.
- Al-Kassim, M. Y., & Monawar, M. N. (2000). Seed-borne fungi of some vegetable seeds in Gazan province and their chemical control. *Saudi Journal of Biological Sciences*, 7(2), 179-184.
- Anonymous. 1993. International Rules for Seed Health Testing. *Seed Science & Technology*., 21, 1-288.

- Begum, M., Lokesh, S., Ravishankar, R.V., Shailaja, M.D., Kumar, T.V., & Shetty, H.S. (2005). Evaluation of certain storage conditions for okra (*Abelmoschus esculentus* (L.) Moench) seeds against potential fungal pathogens. *International Journal of Agriculture and Biology*, 4, 550-554.
- Dawar, S., Hayat, S., Anis, M., & Zaki, M.J. (2008). Effect of seed coating material in the efficacy of microbial antagonists for the control of root rot fungi on okra and sunflower. *Pakistan Journal of Botany*, 40(3), 1269-1278.
- Esserti, S., Smaili, A., Rifai, L.A., Koussa, T., Makroum, K., Belfaiza, M., Kabil E.M, Faize L, Burgos L, Albuquerque N, & Faize, M. (2017). Protective effect of three brown seaweed extracts against fungal and bacterial diseases of tomato. *Journal of Applied Phycology*, 29, 1081-1093.
- Elkhalifa, A.E.O., Alshammari, E., Adnan, M., Alcantara, J.C., Awadelkareem, A.M., Eltoun, N.E., Mehmood K, Panda B.P, & Ashraf, S.A. (2021). Okra (*Abelmoschus esculentus*) as a potential dietary medicine with nutraceutical importance for sustainable health applications. *Molecules*, 26(3), 696-718.
- Iwuagwu, C.C., Mbah, B.N., & Nwogboga, A.C. (2014). Identification and control mycopathogens associated with storage of five horticultural produce in evaporative coolant-vegetable basket. *European Journal of Biotechnology and Bioscience*, 2(2), 24-27.
- Khan, A. M., Naz, S., & Abid, M. (2016). Evaluation of marine red alga *Melanothamnus afaqhusainii* against *Meloidogyne incognita*, fungus and as fertilizing potential on okra. *Pakistan Journal of Nematology*, 34(1), 91-100.
- Mahmood, A.M., & AL-Abedy, A.N. (2021). Effect of some species of *Trichoderma* spp. and the chemical fungicide Topsin m in control of the disease of seed rot and seedling damping off of okra caused by *Fusarium culmorum*. *Journal of Agricultural and Statistical Sciences*, 3899, 17-1701.
- Mostafa, Y.S., Alamri, S.A., Alrumman, S.A., Hashem, M., Taher, M.A., & Baka, Z.A. (2022). In vitro and in vivo biocontrol of tomato *Fusarium* wilt by extracts from brown, red, and green macroalgae. *Agriculture*, 12(3), 345-362.
- Ofori, J., Tortoe, C., & Agbenorhevi, J. K. (2020). Physicochemical and functional properties of dried okra (*Abelmoschus esculentus* L.) seed flour. *Food Science & Nutrition*, 8(8), 4291-4296.
- Parab, A., & Shankhadarwar, S. (2022). Growth enhancement of agricultural crops using seaweed liquid fertilizer. *Plant Science Today*, 9(2), 322-330.
- Rahim, S., & Dawar, S. (2015). Seed borne mycoflora associated with okra *Abelmoschus esculentus* (L.) Moench. *Pakistan Journal of Botany*, 47(2), 747-751.
- Sarkar, D. D., Chowdhury, M. S. M., Akhtar, N., Bhuiyan, M. Z. R., & Nisha, H. A. C. (2015). Health status of okra (*Abelmoschus esculentus*) seeds collected from different locations of Bangladesh. *World Journal of Agricultural Sciences*, 11(6), 371-379.
- Shahnaz, L., & Shameel, M. (2009). Chemical composition and bioactivity of some benthic algae from Karachi coast of Pakistan. *International Journal on Algae*, 11(4), 377-393.
- Sitara, U., Akbar, A., Abid, M., & Ahmad, A. (2018). Essential oils show antifungal activity against seed-borne mycoflora associated with okra seeds. *International Journal of Biotechnology and Molecular Biology Research*, 15, 855-863.
- Sitara, U., Niaz, I., Naseem, J., & Sultana, N. (2008). Antifungal effect of essential oils on in vitro growth of pathogenic fungi. *Pakistan Journal of Botany*, 40(1), 409-414.
- Sohi, H.S., & Puttoo, B.L. (1973). Studies on the Fungal Flora of Okra [*Abelmoschus Esculentus* (L.) Moench.] Seeds. *Indian Journal of Horticulture*, 30(1and2), 428-431.
- Sultana, R., Chowdhury, M.S.M., Islam, M.R., Mohsin, S.M. and Ahmmed, A.N.F. (2015). Effect of seed treatments and storage periods on the incidence of seed borne disease and seed quality of okra seed (*Abelmoschus esculentus*). *International Journal of Sustainable Agricultural Technology*, 11(9): 28-35.
- Sultana, V., Ehteshamul-Haque, S., Ara, J., & Athar, M. (2005). Comparative efficacy of brown, green and red seaweeds in the control of root infecting fungi and okra. *International Journal of Environmental Science & Technology*, 2, 129-132.
- Surovy, M.Z., Mahmud, N.U., Bhattacharjee, P., Hossain, M.S., Mehebab, M.S., Rahman, M., Majumdr, B.C., Gupta, R.D., & Islam, T. (2020). Modulation of nutritional and biochemical properties of wheat grains

infected by blast fungus *Magnaporthe oryzae* *Triticum* Pathotype. *Frontier in Microbiology*, 11:1174. doi: 10.3389/fmicb.2020.01174.

Tullio, V., Nostro, A., Mandras, N., Dugo, P., Banche, G., Cannatelli, M.A., Cuffini A.M, Alonzo V, & Carlone, N.A. (2007). Antifungal activity of essential oils against filamentous fungi determined by broth microdilution and vapour contact methods. *Journal of Applied Microbiology*, 102(6), 1544-1550.

Yadav, R.K., Ghasolia, R.P., & Yadav, R.K. (2020). Management of *Rhizoctonia solani* of okra (*Abelmoschus esculentus* L. Moench) through plant extracts and fungicides in vitro and field conditions. *International Journal of Chemical Sciences*, 8(2), 2982-2987.