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Assessment of Plant growth-promoting activities of *Alternaria* sp.andevaluation of its efficacy on the growth of cash crops (*Vigna radiata* and *Vigna mungo*)

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ABSTRACT

Fungal endophytes have been shown to be frequently found within plant tissues and have the ability to promote plant growth. These endophytes interact with their hosts in various ways, from latent pathogenicity to defensive mutualism, from enhancing stress tolerance to promoting plant growth. Within the study, an endophytic fungus from the *Citrus limon* plant was successfully extracted and evaluated for its plant growth promoting (PGP) traits. The fungal endophyte was specifically identified as *Alternaria* sp. Apart from showcasing notable enzymatic and antimicrobial properties, the endophyte exhibited diverse abilities in phosphate solubilization, amylase synthesis and cellulose degradation. The endophyte also exhibited varying production levels of indole acetic acid (IAA) and ammonia. The assessment of optimizing the growth of *Alternaria* sp. through response surface methodology (RSM) involved investigating the effects of pH, incubation temperature and period on its growth kinetics. This study aimed to determine the optimal conditions for promoting the growth of *Alternaria* sp. by analysing the interaction between these factors. In addition, the potential endophytic isolate was evaluated through a plant bioassay conducted using a soil-pot system. Notably, a notable enhancement was observed in the vegetative growth parameters of both *Vigna radiata* and *Vigna mungo* crops when they were inoculated with the isolate.The results indicate that endophytes derived from terrestrial plants play a significant role in enhancing plant growth and can be utilised as inoculants to establish an eco-friendly approach to crop production.

Keywords: Endophyte,antimicrobial activity, phosphate solubilization, lemon, optimization

1. INTRODUCTION:

Endophytes that promote plant growth reside in plant tissues, and their strong association with one another improves nutrient exchange and enzyme activity(Rana et al., 2020)(Murphy et al., 2014). Endophytic fungi play a crucial role in promoting plant growth through the

distribution of growth-promoting hormones to different parts of the plants(Lin & Xu, 2013).Microbial endophytes penetrate plant tissues without showing symptoms, engaging in competition with other microbial pathogens for the same ecological niches(Matsuoka et al., 2013). Consequently, the established relationship between plants and endophytes improves plant well-being through various mechanisms and could potentially safeguard the host plant against microbial infections (Malhadas et al., 2017). The different bioactive substances produced by endophytes have a wide range of biological functions and can be directly or indirectly referred to as plant growth-promoting agents. Most plants contain endophytes inside their tissues, however, the amount of knowledge about them and their biological activity does not match the prevalence of endophytes. These microorganisms have been isolated from plant species in a wide variety of habitats worldwide, and it is estimated that all terrestrial plants are colonized by one or more species of endophytic fungus.*Citrus limon* is an ecologically and biologically significant therapeutic, terrestrial plant that inhabits a special ecosystem, little is known about the microbial endophytes of this plant.

In the current study, fungal endophyteisolated from the citrus plant was evaluated for its plant growth-promoting potential. More specifically, the enzyme production, antimicrobial activity, IAA and ammonia production, and phosphate solubilization of the microbial isolate was tested to see if they had any effects on the biomass production of *Vigna radiata* and *Vigna mungo*, two significant commercial crops.Therefore, the current research work focuses how endophytic fungus contributes to enhance plant growth and development.

2. METHODOLOGY

To evaluate the plant growth promotion potential of the endophytic fungal isolate, various assays were performed. Further, isolate was identified by microscopic characterizationand DNA sequencing.

2.1.Plant sampling andisolation of microbial endophytes:

Fresh, disease-free plant materials obtained from *Citrus limon* were carefully excised using a sterile scalpel, placed in sterile poly bags, and transported to the laboratory for storage at 4°C. The plant parts were then cut into small pieces (0.5-1.0 cm), thoroughly washed under running tap water, surface sterilized with 70% ethanol for 1 minute, and rinsed three times with sterile distilled water. A culture medium of potato dextrose agar supplemented with streptomycin was utilized to cultivate the fungal isolate, which was then properly incubated at 28 ± 2°C to facilitate its growth cycle(Khan et al., 2015). Subsequently, the fungal growth was assessed for purity, transferred to fresh cultural slants, and preserved at 4°C for further analysis.

2.2.Identification of endophyte:

2.2.1.Morphological and microscopicidentification:

The identification of isolated fungal culture involved assessing different parameters including color, texture, and morphology of conidia and hyphae(Dhayanithy et al., 2019). Utilizing the tease mount method, characteristics such as culture appearance, mycelium formation, and spore production aided in the identification of unknown isolated endophytic fungus(Maurya et al., 2011).

2.2.2.Molecular identification:

Molecular identification was performed using sequence analysis (Thi Minh Le et al., 2019). The DNA fragment that was sequenced has been submitted to GenBank and assigned a specific accession number. The NCBI BLAST tool was utilized to compare the sequences with the GenBank database. Additionally, the sequence was aligned using BLASTn software to conduct a multiple sequence alignment.

2.3. Plant growth-promoting activities by endophytic isolate:

The potential of a fungal endophyte to enhance plant growth was investigated through a series of assays. A microbial endophytic isolate was examined for its ability to solubilize

phosphate in Pikovskayas medium (Adnan et al., 2018). Furthermore, the production of ammonia by the endophytic isolate was assessed using Nessler's reagent in a peptone liquid medium(Singh et al., 2014). The synthesis of extracellular enzymes (amylase, cellulase) was evaluated by growing the endophytic isolate on a medium containing 1% soluble starch, cellulose, or carboxy-methylcellulose (Fouda et al., 2015). To determine the endophytic fungus's ability to produce indole acetic acid (IAA), tryptophan and Salkowski's reagent were used. After inoculating the fungal cultures in LB broth, Salkowski's reagent was added, and a positive indication for IAA production was observed based on the change in the color of the media (Glickmann & Dessaux, 1995).

2.4.Evaluation for antimicrobial activity of isolated endophytic strain against cropdiseases causing fungi

In order to assess the antimicrobial properties of a fungal isolate, test organisms such as *Penicillium* sp. and *Aspergillus* sp. were utilized. A mycelial disc measuring 8 mm in diameter was taken from the actively growing margins of a 4-5 day old culture of pathogenic fungi and positioned near the edge of the media in a Petri plate to evaluate the antimicrobial activity of the fungal endophyte(Erfandoust et al., 2020). Following a 24-hour interval, another 8 mm-diameter mycelial disc from a growing culture of the fungal isolate under evaluation for antagonistic properties was placed adjacent to the pathogenic fungus. The examination was conducted to determine any following antagonistic activity present:

1. First, the hyphae of the two fungi grow into one another and eventually cease expanding.
2. Second, co-mingling growth, which occurs when the investigated fungus has slowed its development and is being outcompeted by another colony.
3. Third, there was a little reduction in growth as the fungi got closer together until they were practically touching, leaving a thin demarcation line between the two fungal colonies.
4. A two-millimeter-wide area of mutual inhibition.

2.5.Optimization process:

The media (PDA) were heated to 121 C for 15 minutes to guarantee sterility. After the medium solidified, 15 millilitres were added to the petri plate. For a whole day, the plates holding the hardened material were turned upside down to let the moisture on the surface evaporate. To develop fungal endophyte, 8mm diameter mycelium discs were removed from the edge of a seven-day-old culture and incubated. The selected endophytes were then subjected to tests on several physiological optimisation parameters, including pH (4.5, 5.5, 6.0, 6.5), incubation temperature (15 ˚C, 20 ˚C, 25 ˚C, 30 ˚C, 35 ˚C), and incubation duration (1, 3, 5, 7, and 9 days)(Li et al., 2021).

Response Surface Methodology was used to further improve the culturing conditions, which consisted of three variables: temperature, time, and pH. The Central Composite Design's upper and lower bounds were established using data from the selection from the prior test(Widjajanti et al., 2022). This works well with Stat-Design-Expert Ease's 13.0 for the RSM technique.

2.6.Effect of fungal isolate on the growth of *Vigna radiata* and *Vigna mungo:*

Healthy, disease-free seeds were carefully selected and sterilized prior to the experiment. The spores were collected from lawn cultures of the organism on potato dextrose agar by covering the culture with sterile saline containing 0.01% (v/v) Tween (BDH) and spreading them with a sterile glass spreader (M.K Khatun, M.S Haque, S.Islam, K.M Nasiruddin, 2008). The spore count was determined using a hemocytometer, and spray bottles were then filled with the spore suspension (Jaber, 2018). Two separate sets of agricultural plant seeds were placed in autoclaved Petri plates on sterile filter paper in triplicate. The fungal suspension was sprayed onto the seeds, and the growth conditions were maintained at a 25˚C for a 12-hour

photoperiod. The seeds were watered daily, and the root and shoot growth were regularly monitored.The assay was performed to evaluate seed germination percentage and to depict the effect of inoculant on radicle and plumule lengths of the plantlets, seed vigour index, average dry and fresh weights of seedlings of two different plants (*Vigna radiata* and *Vigna mungo*).

3.RESULTS AND DISCUSSIONS

3.1.Isolation and identification of endophytic fungus:

The endophytic fungus derived from the *Citrus limon* plant (Figure 1) was isolated and examined based on its microscopical and cultural traits. In culture, *Alternaria* sp. was found to grow in elongated chains with dark brown conidiophores. Microscopically, the spores were observed to be larger in size and exhibited a dark appearance.

The 18S rRNA gene sequencing method was utilized to conduct the molecular identification of the endophytic fungus. Following the BLAST analysis, the isolate was verified as *Alternaria* sp., and its sequence (420 base pairs) was submitted to the NCBI with the accession number OP782663. To ascertain the evolutionary relationships among living organisms, a phylogenetic tree was prepared and depicted in Figure 2.

3.2. Screening of plant growth-promoting endophytic strains:

The assessment was conducted to determine the plant growth supporting activities of the fungal endophyte, which could directly or indirectly contribute to plant growth and health. The results revealed that the isolated fungal endophyte exhibited a higher capability in solubilizing inorganic phosphate, as indicated by a phosphate solubilization index of 5.9±0.2cm. Additionally, the fungus synthesized a significant amount of IAA, which was confirmed by a noticeable change in the color of the media. Moreover, the isolated fungal endophyte demonstrated the production of remarkable extracellular enzymes, namely amylase and cellulose. The presence of clear zones in the respective media confirmed the positive tests for the production of amylase and cellulase enzymes by the fungal isolate. Furthermore, the ammonia detection test yielded positive results, as depicted in Figure 3.

3.3.Antimicrobial activity of isolated endophytic strains against crop diseases causing fungi:

The antimicrobial properties of the endophyte were evident as it successfully suppressed the growth of the plant pathogen *Penicillium* sp. A distinct zone of inhibition was observed on the agar plates after a 6–7-day incubation period. These results highlight the endophyte's ability to hinder the growth of pathogens to some extent by competing for essential resources like nutrients and space (Figure 3).

3.4. Optimization of the various growth parameters for the selected endophytic strains

3.4.1. Effect of pH, incubation temperature and incubation time on the growth of *Alternaria* sp.

Testing a temperature range from 15°C to 35°C, with a 5°C difference, was conducted to determine the optimal temperature range for endophytic fungus. It was noted that the isolate showed minimal growth at 15˚C and 35˚C, which are considered extreme for fungal growth. The results revealed that *Alternaria* sp. displayed the largest growth diameter when grown on PDA medium at 25˚C. Less mycelial growth was observed at 15˚C and 30˚C.Different pH values were introduced to the medium over a nine-day period to observe their effects on different fungus species. When standard temperature of 25°C was utilized to assess the influence of pH and incubation time, it was found that the growth process was significantly influenced by the pH level. The fungal isolate exhibited optimal growth at a pH of 5.5, as shown in Figure 4.

3.4.2. Central Composite Design (Response Surface Methodology):

Based on the outcomes of experiments that focus on a single factor, it can be inferred that for the purpose of screening the important variables influencing the proliferation of endophytic

fungus, Central Composite Design was employed. To find the ideal values of the three chosen variables (pH, incubation temperature, and incubation time), an RSM utilising the CCD was designed and implemented. The responses were subjected to statistical analysis using Stat Ease v13.0 software. At a 95% confidence level, the design indicates that all three factors have the largest impact on the proliferation of endophytes. Twenty sets of tests were conducted using various combinations of the chosen parameters.

3.4.3. Alternaria sp.: Analysis of Variance and Validation of the Model:

The F-test for ANOVA was used to assess the model's statistical significance. According to Table 1, when the F-test was performed at the 95% probability level, the regression sum of squares was statistically significant. Fisher's F-test results showing a very high model F-value (45.66) and a very low p-value ($p < 0.0001$) supported the ANOVA's conclusion that the model was statistically significant. Determination coefficients (R^2) and multiple correlation coefficients (R) can be used to evaluate the quality of a model. Based on the value of the adj-R^2 (0.9549), it was concluded that the independent variables accounted for 95.49 % of the variance in fungal growth, whereas 4.51% of the variance remained unexplained. The model's validity was confirmed by the absence of a statistically significant F-value for the lack-of-fit ($p > 0.05$). The response surfaces were adequately described by the model, and they could be utilised as a predictor in the design space, it was determined (Table1).

3.4.4. Graphical Interpretation of the Response Surface Model

Response surface plots and contour plots can be used to illustrate the relationship between the response and each tested variable, as well as the interactions between any two tested variables. By analyzing 3D response surfaces and 2D contour plots, the interactions between any two variables were swiftly examined, enabling the effective identification of the optimal ranges of the variables to maximize the response. (Figure 5).

3.5.Effect of the endophytic inoculation on the growth of *Vigna radiata* and *Vigna mungo:*

An endophytic suspension was prepared utilizing Tween 20, and the spore concentration was adjusted to 106 ml^{-1} using a hemocytometer. The fungal endophyte was assessed for its ability to colonize two preferred host plant species, specifically Mung bean (*Vigna radiata*) and Urad bean (*Vigna Mungo*) through seed inoculation.

3.5.1. Effect of culture supernatant on the growth of *Vigna radiata*:

A study on seed germination was conducted for 5 days. The experiment was replicated three times. The percentage of seed germination was calculated using the formula Seed Germination Percentage (SGP) = (number of germinated seeds/total number of seeds) x 100. The SGP for the control group was found to be 86%, while it was 93% for the group treated with the isolate (Figure 6).The seedlings' average fresh weight was determined to be 3.612 g for the control group, while the fungal isolate group had an average fresh weight of 7.531 g. In contrast, the average dry weight of the seedlings was 0.215 g for the control group treated with sterile water, and 0.268 g for the fungal-treated seeds. The seed vigor index was calculated as 2080 after treatment, compared to the control group which had a seed vigor index of 1582.05. These results clearly indicate that the endophytic isolate had a significant impact on promoting the growth of the selected plant. When *Vigna radiata* seeds were inoculated with the endophyte, there was a noticeable variation in the lengths of the plumule and radicle compared to the control sample C (Figure 7,8).

3.5.2. Effect of culture supernatant on the growth of *Vigna mungo* Plants:

Upon the application of a fungal elicitor during seed inoculation, it was observed that the endophytic isolate significantly enhanced plant growth by stimulating both root and shoot elongation, which was clearly visible in Figure 7 and 8. The SGP for the control group was determined to be 83%, whereas it was 90% for the group treated with the isolate (Figure 6). The average fresh weight of the seedlings was determined to be 3.883 g for the control group

and 6.148 g for the group treated with the fungal isolate. Similarly, the average dry weight of the seedlings was found to be 0.198 g for the control group and 0.281 g for the fungal isolate-treated group. Furthermore, the seed vigour index was calculated to be 1732.5 for the control group and 2113.64 for the group treated with the fungal isolate (*Vigna mungo*).

4. CONCLUSION:

In this investigation, based on our study, the isolated fungal strain *Alternaria* sp. has not been previously documented in the host plant *Citrus limon* (family *Rutaceae*). The microscopic and molecular identification of the strain was verified as *Alternaria* sp. (accession no. OP782663), a deuteromycetes fungus. Additionally, the well-characterized strain was evaluated for its plant growth-promoting characteristics, revealing significant levels of IAA synthesis, ammonia production, and phosphate solubilization abilities in vitro. Moreover, the strain exhibited a notable production of extracellular enzymes (amylase, cellulase). The plant growth-promoting activities of fungal endophytes isolated from the host plant were assessed to determine their impact on root and shoot growth in two economically important crops, *Vigna radiata* and *Vigna mungo*. The fungal endophyte formulation was applied to the seeds of the selected crops, resulting in enhanced plant growth. Furthermore, the efficiency of the strain was confirmed through antimicrobial assays against two crop pathogens (*Aspergillus* sp., *Penicillium* sp.), demonstrating suppression of pathogen growth to a certain extent. The endophytic strain significantly enhanced plant growth compared to the control group. This study contributes novel insights to the field, shedding light on how fungal endophytes influence plant growth. However, due to the complexity of these effects, further investigations using diverse methods are necessary, especially if field applications are to be considered.

CONFLICT OF INTEREST: None

REFERENCES :

Adnan, M., Alshammari, E., Ashraf, S. A., Patel, K., Lad, K., & Patel, M. (2018). Physiological and Molecular Characterization of Biosurfactant Producing Endophytic Fungi *Xylaria regalis* from the Cones of *Thuja plicata* as a Potent Plant Growth Promoter with Its Potential Application. *BioMed Research International*, *2018*, 1–11. https://doi.org/10.1155/2018/7362148

Dhayanithy, G., Subban, K., & Chelliah, J. (2019). Diversity and biological activities of endophytic fungi associated with Catharanthus roseus. *BMC Microbiology*, *19*(1), 22. https://doi.org/10.1186/s12866-019-1386-x

Erfandoust, R., Habibipour, R., & Soltani, J. (2020). Antifungal activity of endophytic fungi from Cupressaceae against human pathogenic Aspergillus fumigatus and Aspergillus niger. *Journal de Mycologie Médicale*, *30*(3), 100987. https://doi.org/10.1016/j.mycmed.2020.100987

Fouda, A. H., Hassan, S. E.-D., Eid, A. M., & Ewais, E. E.-D. (2015). Biotechnological applications of fungal endophytes associated with medicinal plant Asclepias sinaica (Bioss.). *Annals of Agricultural Sciences*, *60*(1), 95–104. https://doi.org/10.1016/j.aoas.2015.04.001

Glickmann, E., & Dessaux, Y. (1995). A critical examination of the specificity of the salkowski reagent for indolic compounds produced by phytopathogenic bacteria. *Applied and Environmental Microbiology*, *61*(2), 793–796. https://doi.org/10.1128/aem.61.2.793-796.1995

Jaber, L. R. (2018). Seed inoculation with endophytic fungal entomopathogens promotes plant growth and reduces crown and root rot (CRR) caused by Fusarium culmorum in wheat. *Planta*, *248*(6), 1525–1535. https://doi.org/10.1007/s00425-018-2991-x

Khan, A. R., Ullah, I., Waqas, M., Shahzad, R., Hong, S.-J., Park, G.-S., Jung, B. K., Lee, I.-J., & Shin, J.-H. (2015). Plant growth-promoting potential of endophytic fungi isolated from

Solanum nigrum leaves. *World Journal of Microbiology and Biotechnology*, *31*(9), 1461–1466. https://doi.org/10.1007/s11274-015-1888-0

Li, H., Dou, M., Wang, X., Guo, N., Kou, P., Jiao, J., & Fu, Y. (2021). Optimization of Cellulase Production by a Novel Endophytic Fungus Penicillium oxalicum R4 Isolated from Taxus cuspidata. *Sustainability*, *13*(11), 6006. https://doi.org/10.3390/su13116006

Lin, L., & Xu, X. (2013). Indole-3-Acetic Acid Production by Endophytic Streptomyces sp. En-1 Isolated from Medicinal Plants. *Current Microbiology*, *67*(2), 209–217. https://doi.org/10.1007/s00284-013-0348-z

Malhadas, C., Malheiro, R., Pereira, J. A., de Pinho, P. G., & Baptista, P. (2017). Antimicrobial activity of endophytic fungi from olive tree leaves. *World Journal of Microbiology and Biotechnology*, *33*(3), 46. https://doi.org/10.1007/s11274-017-2216-7

Matsuoka, H., Akiyama, M., Kobayashi, K., & Yamaji, K. (2013). Fe and P Solubilization Under Limiting Conditions by Bacteria Isolated from Carex kobomugi Roots at the Hasaki Coast. *Current Microbiology*, *66*(3), 314–321. https://doi.org/10.1007/s00284-012-0276-3

Maurya, S. P., Prakash, P. Y., & Bairy, I. (2011). A simplified touch tape preparation from tube cultures for microscopic examination of filamentous fungi. *Journal of Microbiological Methods*, *86*(1), 128–129. https://doi.org/10.1016/j.mimet.2011.04.015

M.K Khatun, M.S Haque, S.Islam, K.M Nasiruddin. (2008). *In vitro REGENERATION OF MUNGBEAN (Vigna radiata L.) FROM DIFFERENT EXPLANTS*. *19*(2), 13–19.

Murphy, B. R., Doohan, F. M., & Hodkinson, T. R. (2014). Yield increase induced by the fungal root endophyte Piriformospora indica in barley grown at low temperature is nutrient limited. *Symbiosis*, *62*(1), 29–39. https://doi.org/10.1007/s13199-014-0268-0

Rana, K. L., Kour, D., Kaur, T., Devi, R., Yadav, A. N., Yadav, N., Dhaliwal, H. S., & Saxena, A. K. (2020). Endophytic microbes: Biodiversity, plant growth-promoting mechanisms and potential applications for agricultural sustainability. *Antonie van Leeuwenhoek*, *113*(8), 1075–1107. https://doi.org/10.1007/s10482-020-01429-y

Singh, P., Kumar, V., & Agrawal, S. (2014). Evaluation of Phytase Producing Bacteria for Their Plant Growth Promoting Activities. *International Journal of Microbiology*, *2014*, 1–7. https://doi.org/10.1155/2014/426483

Thi Minh Le, T., Thi Hong Hoang, A., Thi Bich Le, T., Thi Bich Vo, T., Van Quyen, D., & Hoang Chu, H. (2019). Isolation of endophytic fungi and screening of Huperzine A–producing fungus from Huperzia serrata in Vietnam. *Scientific Reports*, *9*(1), 16152. https://doi.org/10.1038/s41598-019-52481-2

Widjajanti, H., Nurnawati, E., Muharni, M., & Zahwa, E. D. (2022). Optimization of Antibacterial Production of Endophytic Fungi with Various Sources of C, N, and pH using The Response Surface Methodology. *Science and Technology Indonesia*, *7*(2), 149–157. https://doi.org/10.26554/sti.2022.7.2.149-157

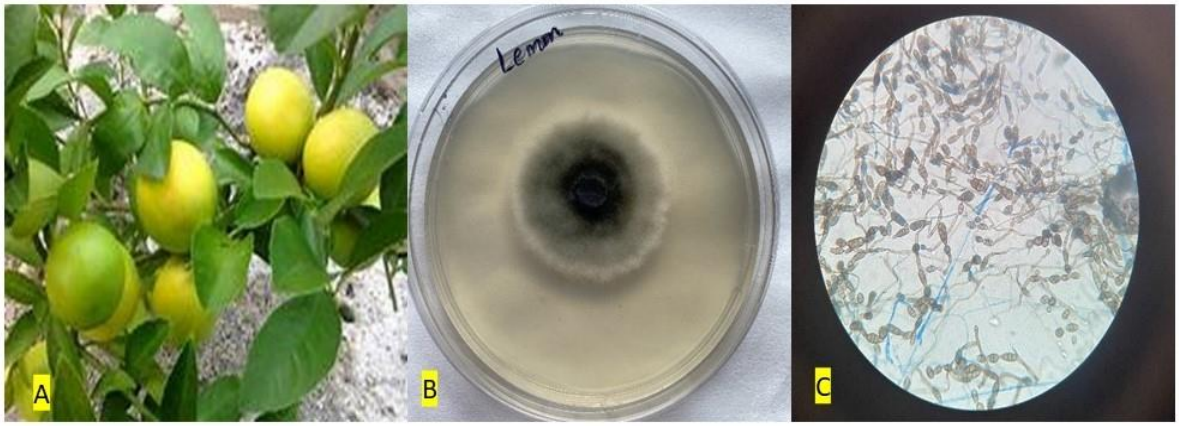

Figure 1. Isolation of the fungal endophyte: *Citrus limon*(A); Fungal growth or colony appearance on PDA media (B); Microscopic view of the isolate (C)

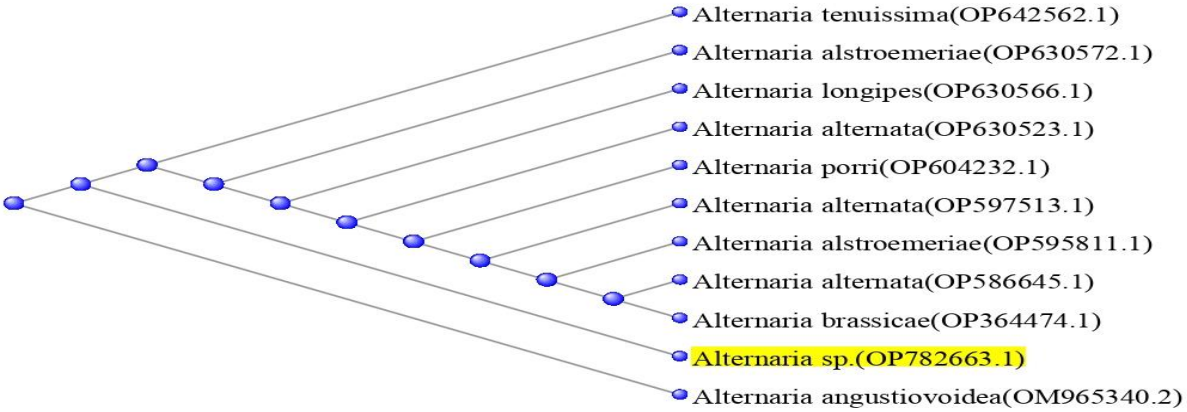

Figure 2. Phylogenetic analysis of endophytic isolate *Alternaria* sp.

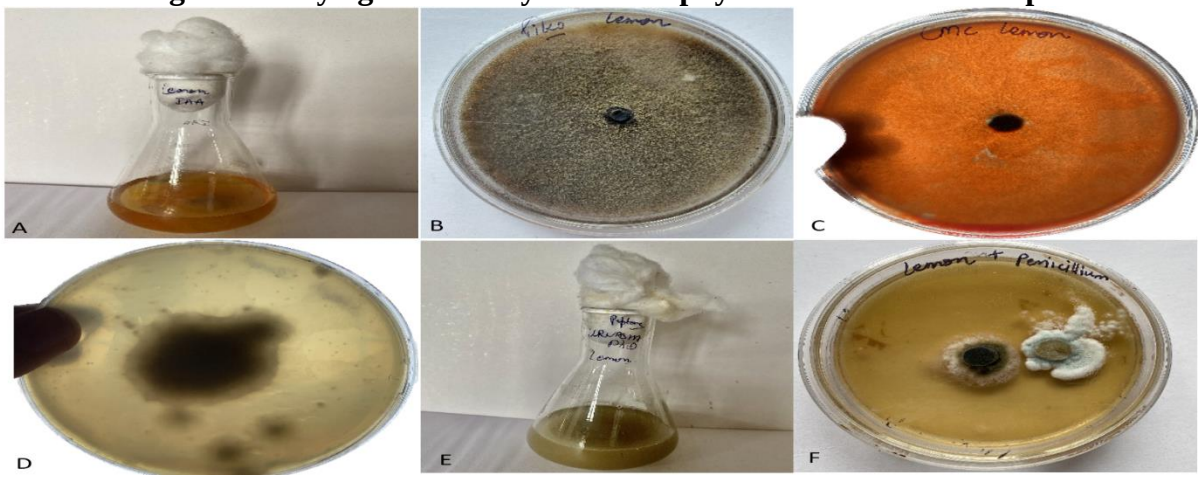

Figure 3. Plant Growth Promotion assays: IAA production (A), Phosphate solubilisation (B),Cellulytic Activity (C),Starch production (D),Ammonia Production(E), Antimicrobial activity (F)

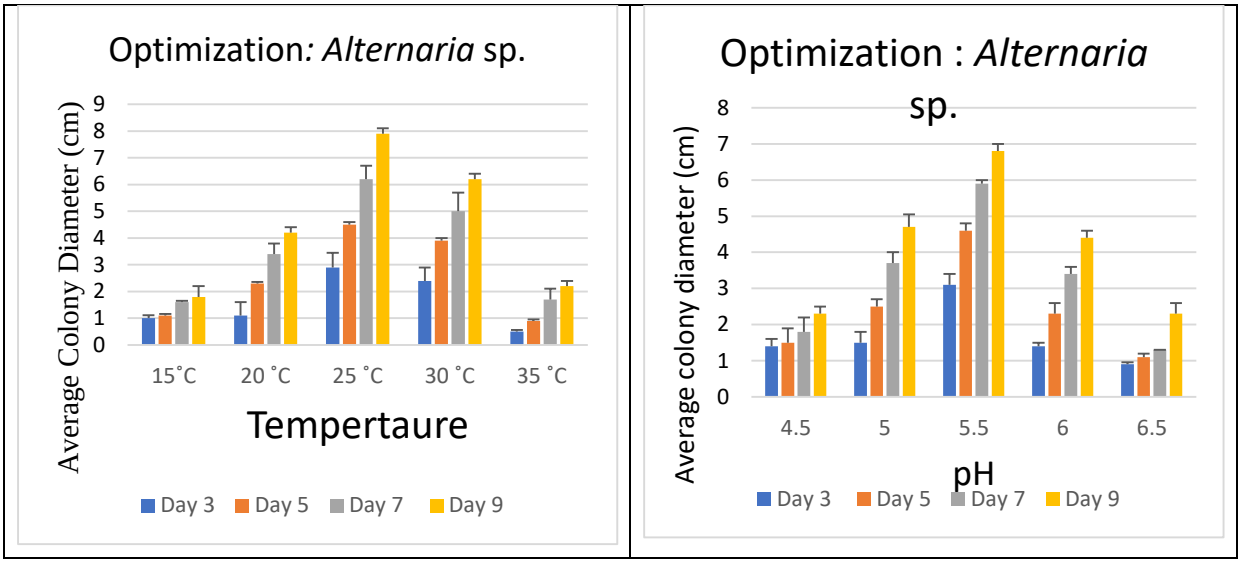

Figure 4 Effect of temperature and pH on the growth of *Alternaria* sp.

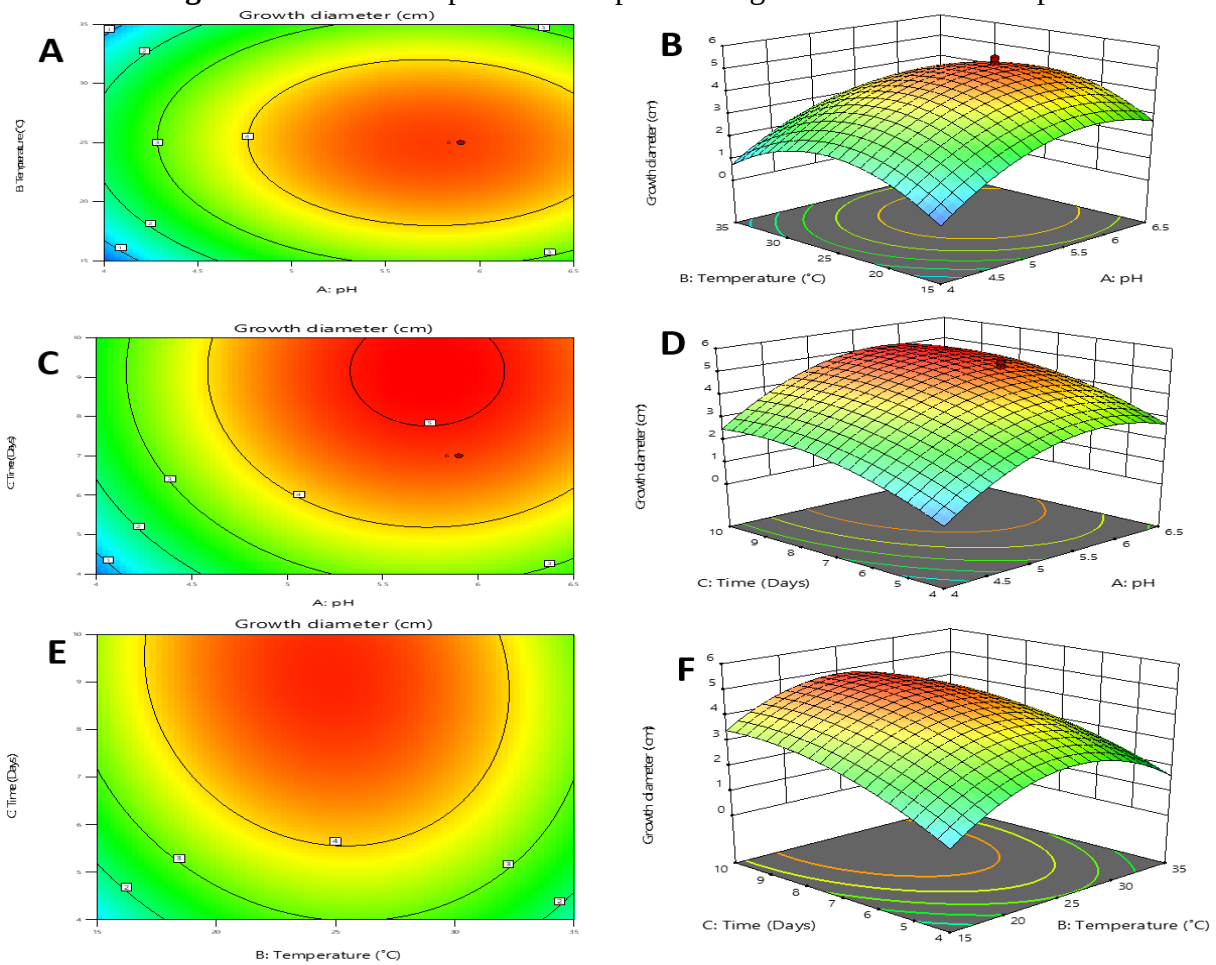

Figure 5 Contour plots (**A**, **C** and **E**) and three-dimensional response surfaces (**B**, **D** and **F**) showing the effects of temperature, pH, and incubation time on fungal growth diameter of *Alternaria* sp.

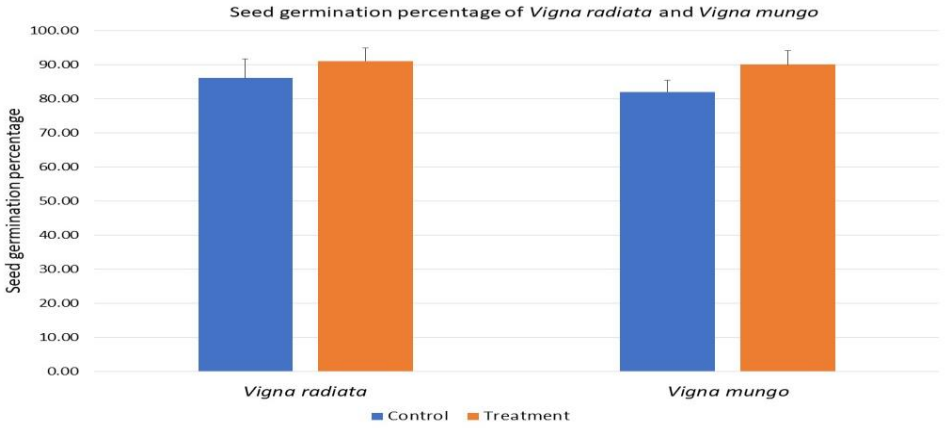

Figure 6. Seed germination percentage assay of *Vigna radiata* and *Vigna mungo*

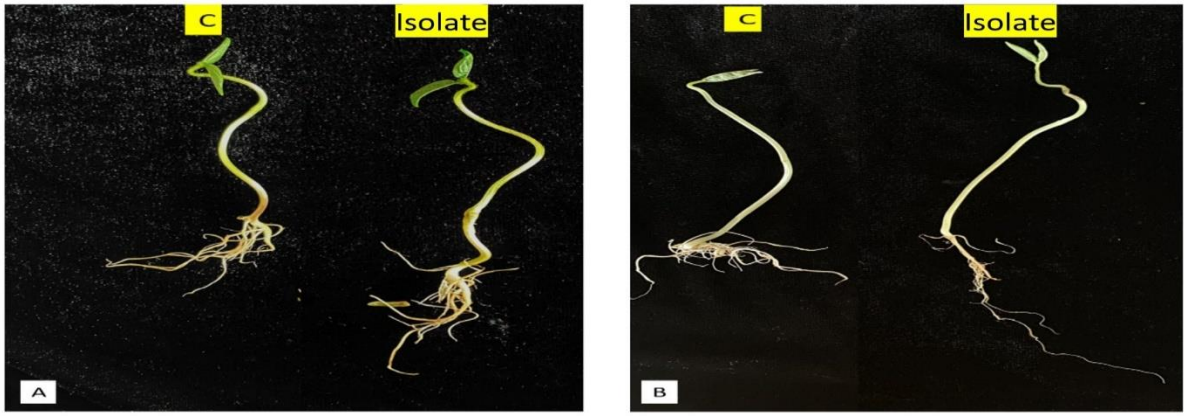

Figure 7. Comparison of growth shown by crop beans A (*Vigna radiata*), B (*Vigna mungo*) when inoculated with control (C) and endophytic fungus isolate.

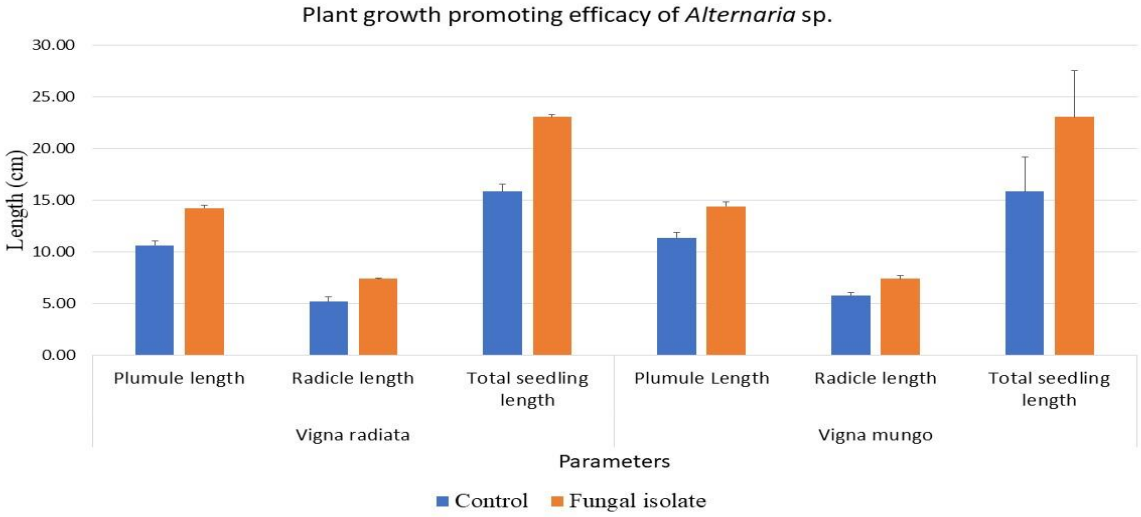

Figure 8. Comparison of fungal formulation on root and shoot lengths of selected crops

Table 1. Fit Statistics for optimization of fungal growth

Std. Dev.	0.4312		**R^2**	0.9762
Mean	2.37		**Adjusted R^2**	0.9549
C.V. %	18.19		**Predicted R^2**	0.8664
			Adeq Precision	16.1204