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Endophytic fungi from *Oxalis stricta* enhance growth, secondary metabolite production, and photosynthetic efficiency in maize (*Zea mays* L.)

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Abstract

Endophytic fungi play a crucial role in plant growth promotion and stress tolerance by producing bioactive metabolites. In this study, endophytic fungal strains were isolated from the *Oxalis stricta L.* (yellow wood sorrel) belonging to the Oxalidaceae family. The fungal strains were cultured in Czapek medium, and their culture filtrates were analyzed for secondary metabolites, including flavonoids, phenols, sugars, indole-3-acetic acid (IAA), prolein, and ammonia production, using optical density (OD) measurements. The isolated strains exhibited significantly high levels of flavonoids (ranging from 2.5 to 4.1 µg/mL) and phenols (3.2 to 5.6 µg/mL), while the isolates tested positive for ammonia production, indicating their potential role in nitrogen metabolism. Furthermore, greenhouse experiments demonstrated that inoculated maize (*Zea mays L.*) plants exhibited significant improvements in root and shoot length (increase of 34% and 41%, respectively) and biomass accumulation compared to control plants. Seedlings were further grown in water agar medium to assess the impact of fungal inoculation on IAA production and chlorophyll (A and B) content. Treated plants showed a 27% increase in IAA and a 19% increase in total chlorophyll content compared to non-inoculated plants, suggesting enhanced photosynthetic efficiency and auxin-mediated growth promotion. Results showed a marked increase in IAA and chlorophyll levels in treated plants compared to the controls, suggesting enhanced photosynthetic efficiency and auxin-mediated growth promotion. These findings highlight the potential of isolated endophytic fungi as biofertilizers for sustainable agriculture.

Keywords: Biofertilizer, Chlorophyll content, Endophytic fungi, Maize plant, Plant–microbe interaction, Secondary metabolites

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Introduction

Oxalis stricta L, commonly known as sheep weed, sour grass, upright yellow-sorrel, common yellow oxalis, lemon clover, and various other colloquial names, is an herbaceous plant often referred to as the "pickle plant (Swan 2022). It can exist as both an annual and a perennial, particularly in disturbed environments. This plant thrives in areas with full sun or partial shade and is typically considered a weed in lawns, gardens, and fields (Groom et al., 2019). Its leaves are arranged alternately and comprise of three heart-shaped leaflets that can grow up to 2 cm wide. When disturbed, the mature seed capsules of *Oxalis stricta* L explode, scattering seeds up to about 4 meters (or roughly 13 feet) away, a characteristic shared with plants in the genus Impatiens (Dzinyela et al., 2021). The plant's hermaphroditic flowers bloom from July to October. *Oxalis stricta* L is fond of sandy and loamy soil, but it can adapt to both dry and moist, alkaline environments. It can also thrive in soils with poor nutrient content, provided they are well-drained (Sannaiah et al., 2022).

In traditional medicine, various parts of *Oxalis stricta* L have been utilized. Infusions have been made to address issues like nausea, stomach discomfort, and fevers, while poultices made from the plant have been applied to reduce swelling. Boiling the entire plant yields a yellow to orange dye (Vignesh et al., 2025). The extensive use of agrochemicals to enhance crop protection has, in recent decades, led to a decline in crop yield due to the development of disease resistance (Anika et al., 2020). Additionally, these agrochemicals contribute to environmental contamination. Given these environmental concerns, there is a pressing need for transition from agrochemicals to bio fertilizers and bio insecticides (Patil et al., 2021). In this regard, there has been an investigation into the

potential of root endophytic fungi to promote plant growth, aiming to devise effective strategies for enhancing crop development (Jabeen et al., 2023).

Endophytic fungi" is a term used to describe a category of fungi that form a cooperative and mutually beneficial relationship with the plant they inhabit. These fungi dwell inside the plant for either the entirety or a portion of their life cycle, without causing any harm or disease (Syamsia et al., 2021). There has been a specific emphasis on discovering novel sources of unique bioactive compounds. A similar term, "endophytes," has been coined to denote mutualistic fungi associated with various types of plants (Baron et al., 2022). Endophytic strains create secondary metabolites, often serving as antimicrobial agents, which shield their host by inhibiting or suppressing harmful microorganisms (Poveda et al., 2021). Their intricate interactions with the host plant, other organisms, and the external environment led to the production of secondary metabolites with diverse structures and biological activities (Kharkwal et al., 2024). It's noteworthy that once extracted from their host plants, endophytic microbes can be easily cultivated and propagated.

Endophytes produce secondary metabolites which can be influenced by various living and environmental factors. In a controlled laboratory setting, deliberately adjusting cultivation parameters, modifying culture media, and inducing stress through microbial competition can be fascinating methods to generate biological activity, chemical diversity, and potentially discover unique molecules (Khalil et al., 2021). The German botanist Fredrick Link, in 1809, was the first to shed light on endophytes. He coined the term "Endophyte" to describe a distinct group of fungi that reside partially parasitically within plant tissues. Nowadays, endophytes are generally seen as microorganisms, often fungi and bacteria that can inhabit the inner tissues of healthy plants without causing any disease symptoms (Chand et al., 2020). Currently, it is believed that both vascular and nonvascular plants may harbor endophytes within their tissues (Ikram et al., 2023).

Endophytic fungi play a crucial role in preserving the balance of our biological community. They contribute significantly to preventing ecosystem degradation, biodiversity decline, and the contamination of soil and water caused by highly toxic pesticides, harmful gases, and industrial waste. Endophytes are often harnessed as natural control against diseases and pests, as well as for environmental cleanup efforts (Khan et al., 2015). Numerous reviews have emphasized the significance of endophytes in the process of phytoremediation (Turbat et al., 2020). Fungal endophytes have produced a large number of brand-new secondary compounds. A varied collection of nearly 1.5 million species of endophytic fungus can generate a variety of metabolites, including flavonoids (Shah et al., 2018). Maize (*Zea mays* L.) is a globally important cereal crop, serving as a staple food, livestock feed, and raw material for industrial products (Ahmad & Ahmad, 2018; Rubab et al., 2020; Mehmood et al., 2022; Azam et al., 2023). Enhancing its productivity and resilience is crucial to addressing food security and agricultural sustainability challenges (Zia et al., 2023; Jamilah et al., 2024; Kekere et al., 2024). The present study was undertaken to isolate and characterize endophytic fungal strains from *Oxalis stricta* L. and evaluate their ability to produce bioactive secondary metabolites such as flavonoids, phenols, sugars, indole-3-acetic acid (IAA), proline, and ammonia and to investigate the impact of fungal inoculation on maize (*Zea mays* L.) growth, including root length, shoot length, and biomass accumulation under greenhouse conditions. It also aimed to analyze the influence of fungal inoculation on IAA production and chlorophyll (A and B) content to assess its role in enhancing photosynthetic efficiency and auxin-mediated growth promotion.

Recent research has emphasized the growing relevance of endophytic fungi in modern agriculture due to their capacity to boost plant development and improve the manufacture of key secondary metabolites under stress circumstances. These fungi not only enhance nutrient uptake and phytohormone synthesis, but they also augment the plant's defense systems against biotic and abiotic stressors (Ahmed et al., 2025). For example, endophytic fungal strains derived from medicinal plants were found to dramatically improve antioxidant activity, chlorophyll content, and proline accumulation in host plants under salt stress (Kaur et al., 2025). Furthermore, numerous endophytes have been shown to improve maize growth and production by increasing photosynthetic efficiency and boosting secondary metabolite pathways, offering a sustainable alternative to synthetic agrochemicals (Kumar et al., 2025).

Materials and Methods

Locality and sampling of plant materials and their sterilization

In the current research, *Oxalis stricta* L. plants from the Garden Campus of Abdul Wali Khan University Mardan (AWKUM) were used for endophytic fungus isolation. The plant material was identified, packaged in sterile containers, and transported to (AWKUM), Garden Campus Mardan Plant Microbes Interaction (PMI) facility, where it was kept at 4°C. To ensure that no dust or other debris adhered to the collected plant parts, they were thoroughly rinsed with tap water. Subsequently, the cleaned samples underwent surface sterilization for 30 seconds using a solution of 70% ethanol and 4% sodium hypochlorite to eliminate any adhering bacteria. The plant parts were then placed on sterile filter paper, sectioned into segments measuring 0.5 cm with a sterilized blade, and subsequently rinsed with double-distilled autoclaved water to remove any residual sterilizing agents. Following this, 4 to 5 sterilized segments were carefully placed in a petri dish containing Hagem mineral medium supplemented with the antibiotic streptomycin to inhibit bacterial growth. As per the protocol, the Petri dishes were sealed with parafilm and incubated at 25 °C in darkness for a period of 15 days (Photita, 2004). Using PDA plates, a fungal strain was isolated from the Hagem plates and stored at 4°C for further use (Hallman et al., 2006).

Medium of growth for microbial populations

The first step was to inoculate the fungal spores in a Hagem minimum medium, which contained glucose (0.5%), KH2PO4 (0.05%), MgSO4.7H2O (0.05%), NH4Cl (0.05%), FeCl3 (0.1%), and agar (1.5%). After growing individually on potato dextrose agar (PDA) medium, the fungal cultures were purified and stored at 4°C for further use. PDA medium was prepared by dissolving 19.5g of PDA powder in 500 mL of distilled water containing 50 ppm of streptomycin (Khan and Lee, 2013). To produce secondary metabolites, the fungal strain was inoculated into Czapek broth medium. Czapek medium contained glucose (1%), peptone (1%), KCl (0.05%), MgSO4.7H2O (0.05%), and FeSO4.7H2O (0.001%). The flasks were then placed in a shaking incubator at 28°C, set to 120 rpm, and incubated for 7 days (Arnold, 2007).

IAA quantification using the Salkowski solution

In 50 mL of Czapek medium, endophytic fungal strains were cultivated to produce indole acetic acid (IAA). 1.5 mL of the culture supernatant was collected after seven days of incubation and spun for two minutes at 10,000 rpm in an eppendorf pipette. After that, 1ml of the residue was combined with 2ml of salkowski reagent for 30 minutes at room temperature. The chemical composition of salkowski reagents contains, i.e. 50 mL of per chloric acid (50% concentration) and 1 ml of ferric chloride (0.5 M). The presence of IAA in the endophytic culture supernatant was indicated as pink in appearance. Absorbance was checked at 530 nm using a UV spectrophotometer (Chadha et al., 2014).

Estimation of phenol, flavonoid, sugar and proline

According to the Bhalodia et al. (2011) the Folin-Ciocalteau reagent was used to estimate phenol. Which involve taking 0.5 mL of 70% ethanol and separately mix with 0.5 mL fungal culture filtrate in sterile eppendorf tubes. At 10,000 rpm, the reaction combination was spun for 20 minutes. The supernatant was moved from Eppendorf tubes. With 70% ethanol, the combination was evenly distributed in eppendorf tubes before being spun once more using the same method. In a water bath set to 40C, the supernatant was drained from test containers. Organic liquids were evaporated, and the remainder was then mixed with 3 mL of autoclaved purified water. The mixture was kept in test containers, and 0.5ml of the Folin-Ciocalteau reagent was introduced. 1.5 mL of newly made 20% sodium carbonate (NaCO3) was added to the mixture in test containers after five minutes. A deep blue hue developed after two hours of room temperature incubation, which is a clear sign that phenol is present. At 750 wavelengths, absorbance was measured. Based on the reference plot for Gallic acid, phenol was quantified (Bhalodia 2011). According to description, flavonoid was measured using the aluminum chloride colorimetric technique (2003). Add 0.5 mL of culture supernatant and 4.3 mL of 80% methanol individually. Mix 0.1 mL of 10% potassium acetate (CH3CO2K) with 0.1 mL of 10% aluminum chloride (AlCl3). The absorbance of the reaction combination was measured at 415nm in 30 minutes of incubation at ambient temperature. The existence of flavonoids was strongly suggested by the hue, which appeared milky white. A flavonoid measurement curve using quercetin was created. Add 1 ml of 80% phenol to 0.1 mL of culture supernatant and then incubate for an hour. 2.5 mL of H2SO4 was introduced to each test container and thoroughly mixed after an hour. At 485 nm, the light density was determined using a spectroscope. A mixture of 20 ml distal water and 80 ml phenol. In solid form 8g phenol add to 100ml distal water. The culture filtrate was homogenized in 10 ml of 3% aqueous sulfuric acid. The sample was mixed with 2ml of glacial acetic acid and 2ml. Test tube of acid ninhydrin the reaction mixture was heated in the water bath for an hour at 1000 degrees before being extracted with (ml) of toluene. The absorbance was then measured at 520nm, with toluene serving as a blank (Bagheri et al., 2013).

Ammonia production by Nessler's reagent

By combining 0.25 Nessler reagents with 5 ml of culture supernatant, the generation of ammonia was measured. A favorable response for ammonia production was the appearance of a dark brown or yellow hue by following the protocol of (Chadha et al., 2014).

Screening of fungal culture filtrate for the growth-promoting potential

A bioassay was performed for the ability of endophytic fungal culture filtrates (CFs) to stimulate the growth of mycelium. Each fungal isolate was grown in 50ml of Czapek broth for 5 days at 28C for 7 days in a shaking incubator at 150 rpm. Culture supernatants were therefore gathered by centrifugation at 12,000 rpm for 20 brief periods at 4 °C, drained through 0.45-µm organic compound composed of carbon acetate filters, and lyophilized. The developing lyophilized powder was disintegrated in 1 mL of completely clean water purified by distillation (Khan et al., 2015). The maize surface was completely cleaned by double-distilled water before accompanying 1% sodium hypochlorite for 10 seconds, and then 70% flammable liquid for 30 seconds. After being surface-infected, seedlings were allowed to grow on petri plates using Whatman filter paper and were then submerged in sterile distilled water. Following transplantation into plastic pots with 0.8% agar, seedlings of the same size were allowed to grow in growth chambers. After the application of fungal inoculum on maize plants in the experimental group had their IAA production and chlorophyll content were measured on SPAD chlorophyll meter wherein other agronomic attributes were noted including root length, shoot length, fresh /dry weight were noted as compared to the control plant.

Statistical analysis

The experiments were repeated three times under the same conditions and with the same materials. Analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) was performed using SPSS software (IBM SPSS Statistics 21) to determine the significance level ($p < 0.05$) for inoculated and non-inoculated, and their interaction. Graphs were plotted using Graph Pad Prism (Version 5.03).

Results

IAA and secondary metabolite determination in separated isolates' culture filtrate

The endophytic fungal strain isolated from *Oxalis stricta* L grown in areas of the District Mardan, Pakistan for planned experiments. The strain obtained sustained subculturing onto PDA media plates to achieve purified strain and stored at 4 °C for further experiments (Fig. 1). Endophytic fungus generates a variety of secondary metabolites that are known to be crucial to their interactions with plant roots and their ability to stimulate plant development. Among these significant compounds are flavonoids, phenols, and sugars, which are significant signaling molecules that facilitate relationships between endophytes. Endophytic fungi were grown for seven days in a shaking Czapek broth under the aforementioned circumstances, incubation at 28 °C, continuous shaking at 120 rpm, and maintained in dark conditions to ensure optimal metabolite production. Their culture supernatant was subsequently tested for phenols, flavonoids, and carbohydrates using a spectrophotometer. The straight gradient of quercetin content against OD was plotted, and the standard curve was created. The optical densities of the sample for flavonoids were compared with the stranded curve. A fungal strain produces IAA with maximum reading of 0.176 µg/ml and minimum reading of 0.172 µg/ml (Fig. 2). The analysis of culture filtrate showed the flavonoid was produced by a fungal strain (Fig. 3). With maximum reading as 0.256 µg/ml and the minimum reading as 0.168 µg/ml. The present study showed the fungal strain produces phenol (Fig. 4). Maximum reading was 0.284 µg/ml and minimum 0.281 µg/ml. That fungal strain produces a sugar in which maximum reading is 2.2 µg/ml and minimum reading is 2.0 µg/ml (Fig. 5). We take the optical density of the proline as optimum value of 0.636 µg/ml and minimum value secured 0.618 µg/ml respectively (Fig. 6).

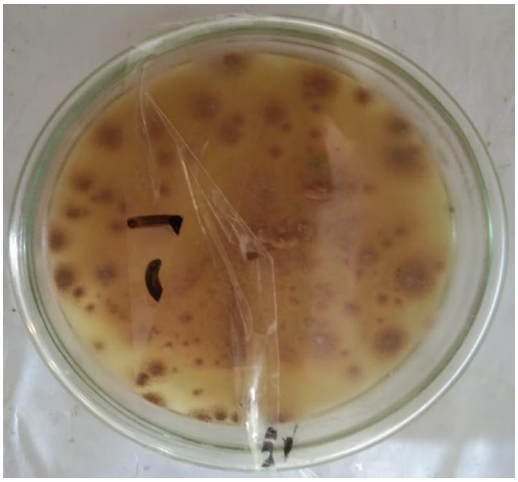

Fig. 1 Endophytic fungal strain isolated from *Oxalis stricta* L grown on potato dextrose agar (PDA) medium

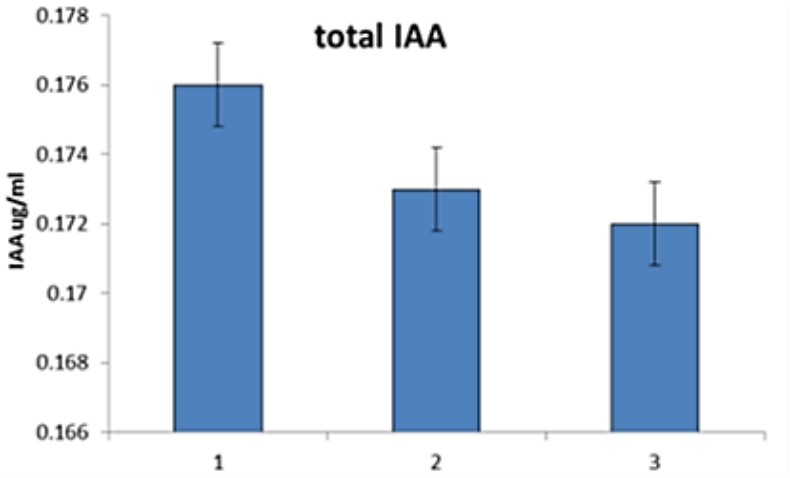

Fig. 2 Represent the total IAA content from culture filtrate. Column represent mean values of triplicates with error bars represent standard deviation, significant difference among treatments at $p \leq 0.05$

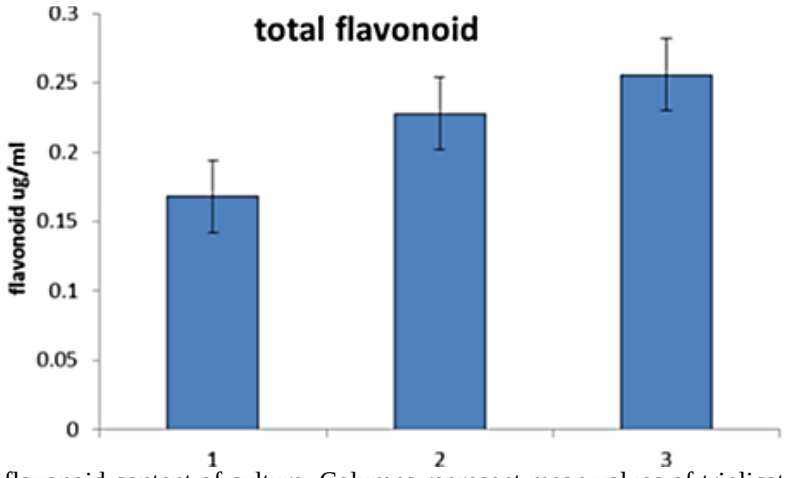

Fig. 3 Represent the total flavonoid content of culture. Columns represent mean values of triplicates with error bars represent standard deviation, significant difference among treatments at $p \leq 0.05$

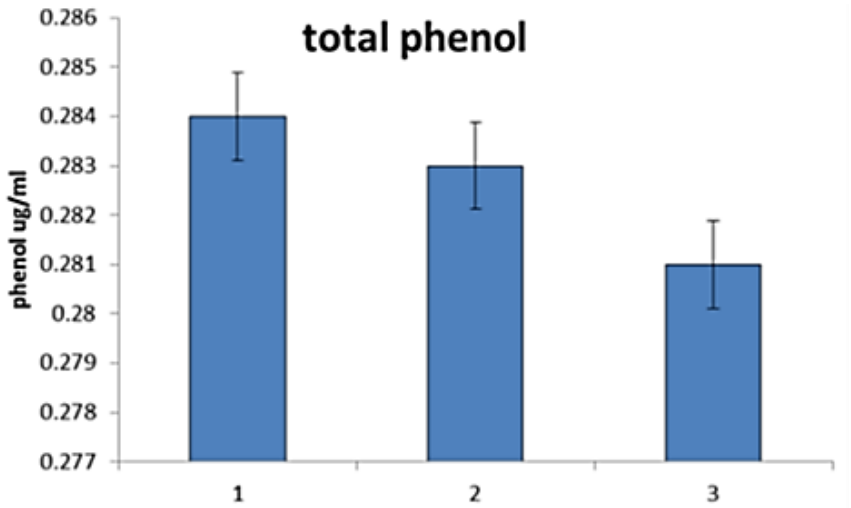

Fig. 4 Represent the total phenolic content from culture filtrate. Columns represent mean values of triplicates with error bars represent standard deviation, significant difference among treatments at $p \leq 0.05$

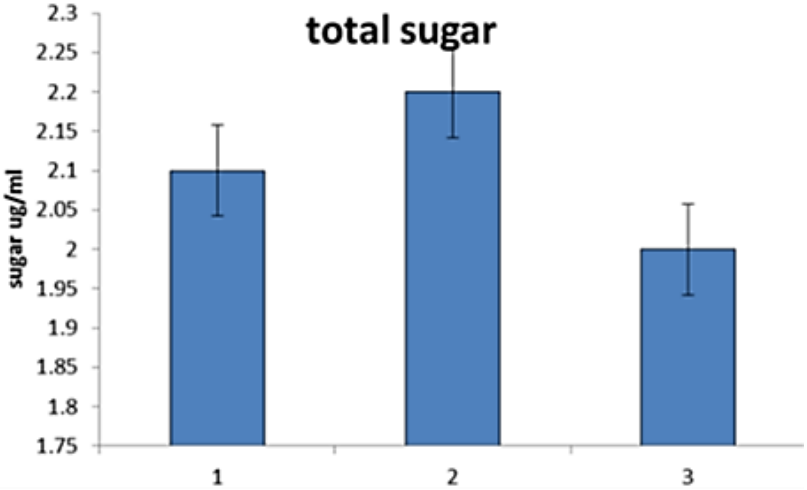

Fig. 5 Represent the total sugar content from culture filtrate. Columns represent mean values of triplicates with error bars represent standard deviation, significant difference among treatments at $p \leq 0.05$

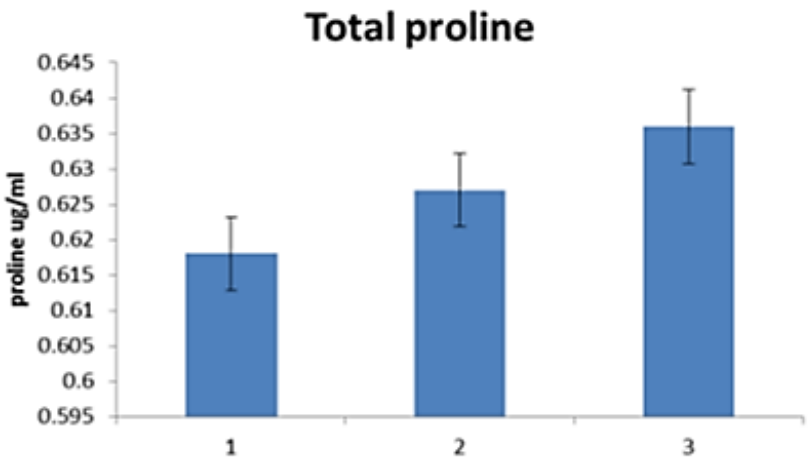

Fig. 6 Represent the total proline content from culture filtrate. Columns represent mean values of triplicates with error bars represent standard deviation, significant difference among treatments at p≤0.05

Endophytic fungi as plant growth promoter determines the IAA and chlorophyll content

In the second experiment, maize seedlings grown in pots filled with sterilized soil were treated with different fungal strains. After 5 days, found that all of the fungal strains increased the growth of the maize seedlings compared to the control group. The seedlings that were treated with the strain had longer shoots and roots, as well as more fresh and dry weight than the control seedlings as shown in Fig. 7 and 8. In this experiment we took the leaf part form the fungal treated plant and control plant and ensured difference between them. The culture filtrate provided more IAA concentration as compared to control plant. The fungal treated plant showed maximum value of 0.464 µg/ml and minimum 0.462 µg/ml, while control plant showed the maximum value of 0.434 µg/ml and minimum as 0.432 µg/ml. After measuring IAA levels, we conducted a chlorophyll content test in plants treated with the culture filtrate and control plants. The fresh and dry weights of the culture filtrate-treated plants were 2.03 mg and 1.22 mg, respectively, while those of the control plants were 1.41 mg and 0.94 mg. The shoot and root lengths of the fungus-treated plants were 11.4 cm and 7.8 cm, respectively, compared to 7.9 cm and 5.7 cm in the control plants (as shown in Fig. 8). This strain produced more IAA and had higher chlorophyll concentrations than the control group. IAA is a plant growth hormone, and chlorophyll is the green pigment in plants that help them to photosynthesize. These results suggest that the fungal strain could be used to develop a new biofertilizer that can help farmers produce more crops as shown in Fig. 9, 10 and 11.

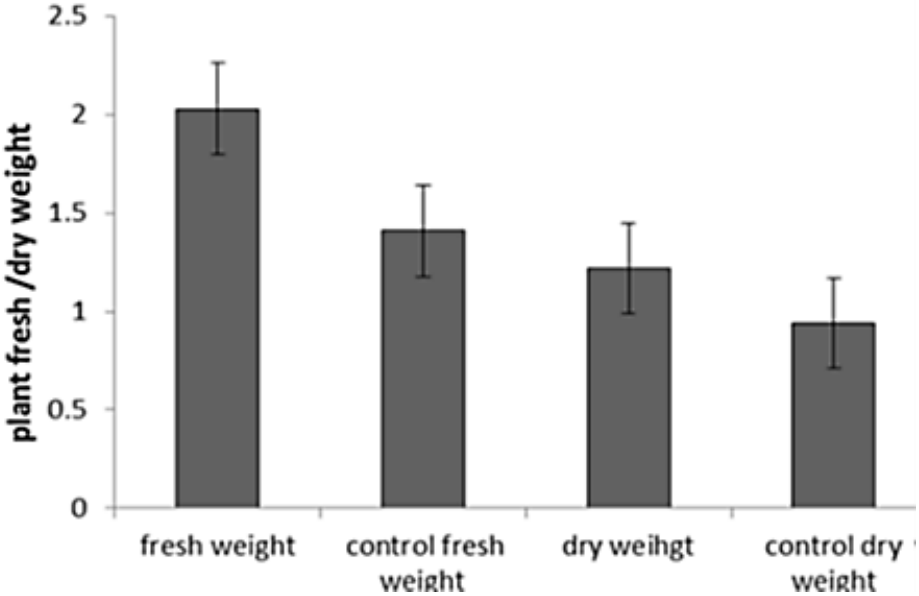

Fig. 7 Effect of association of the endophytic fungal strain on fresh and dry weight, seedlings grown in control condition. Columns represent mean value of triplicates with error bars represent standard deviation, significant difference among treatments at p≤0.05

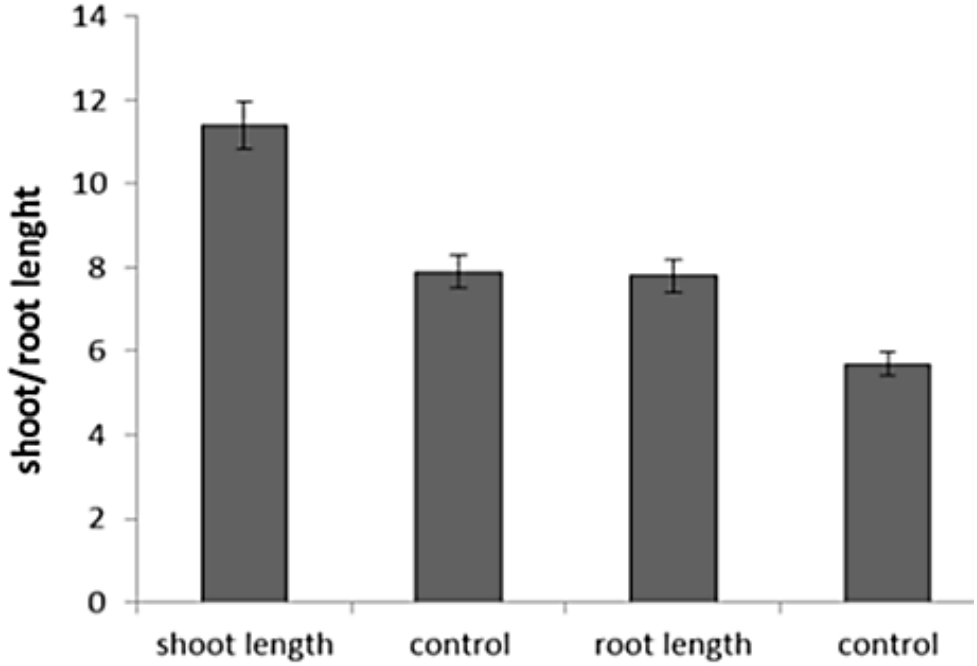

Fig. 8 Effect of association of the endophytic fungal strain on Shoot and Root length, seedlings grown in control condition. Columns represent mean value of triplicates with error bars represent standard deviation, significant difference among treatments at $p \leq 0.05$

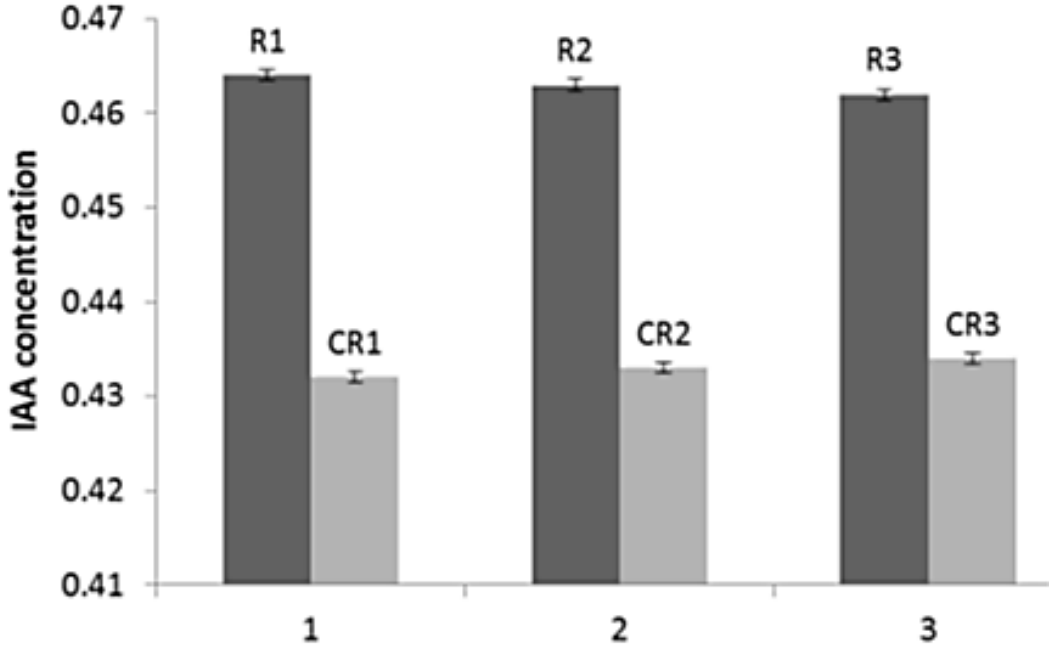

Fig. 9 Effect of association of the endophytic fungal strain on IAA concentration, seedlings grown in control condition. Columns represent mean value of triplicates with error bars represent standard deviation, significant difference among treatments at $p \leq 0.05$

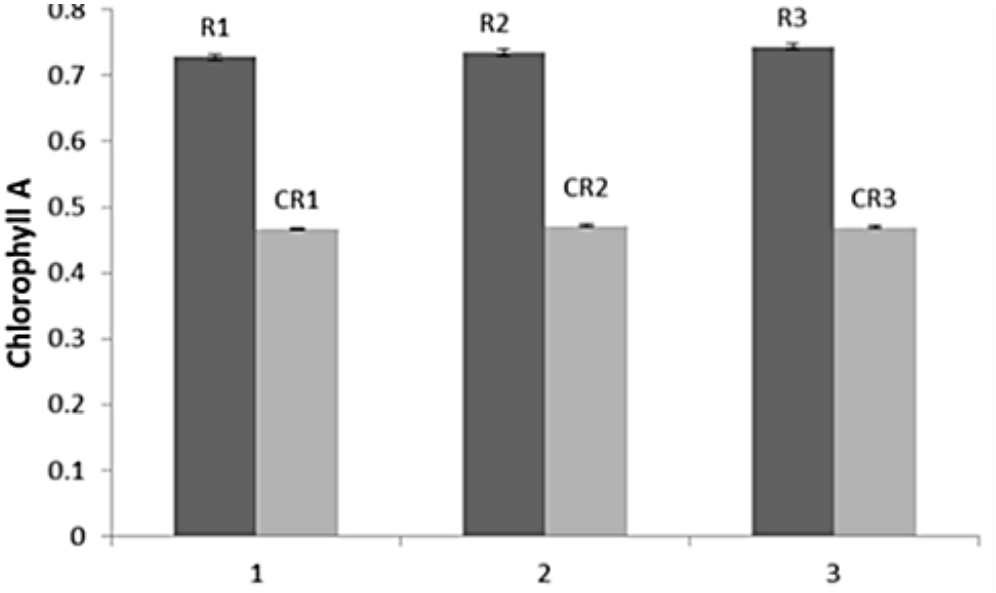

Fig. 10 Effect of association of the endophytic fungal strain on Chlorophyll A content, seedlings grown in control condition. Columns represent mean value of triplicates with error bars represent standard deviation, significant difference among treatments at $p \leq 0.05$

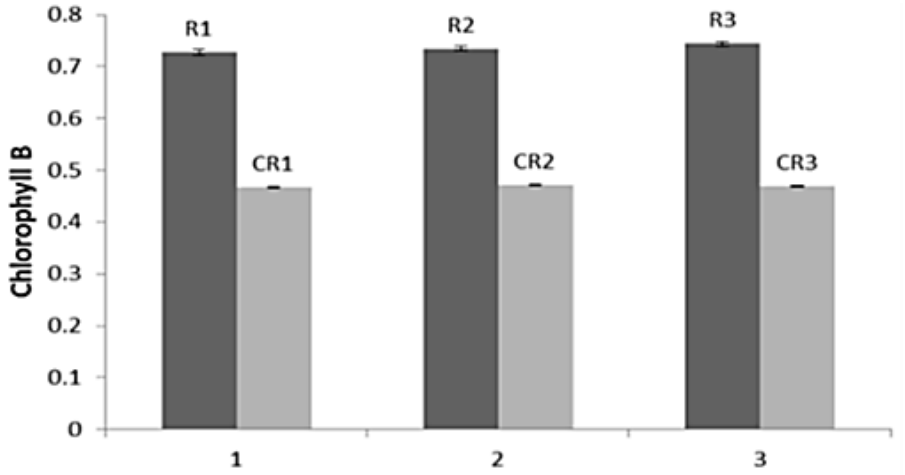

Fig. 11 Effect of association of the endophytic fungal strain on Chlorophyll B content, seedlings grown in control condition. Columns represent mean value of triplicates with error bars represent standard deviation, significant difference among treatments at $p \leq 0.05$

Discussion

The present study highlights the significant role of endophytic fungi isolated from *Oxalis stricta* L. in promoting plant growth and improving physiological characteristics in maize (*Zea mays* L.). The ability of these fungal isolates to produce secondary metabolites, including flavonoids, phenols, sugars, indole-3-acetic acid (IAA), proline, and ammonia, suggests their potential as plant growth-promoting endophytes (PGPEs). Among these, the production of IAA is overall plant vigor. Similar to our findings, endophytic *Fusarium oxysporum* KB-3 was reported to enhance seed germination and vegetative growth in *Brassica napus* through IAA production (Cheng et al., 2022). Endophytic fungi can produce a wide range of beneficial secondary metabolites (Bano et al., 2016). These metabolites can resemble those produced by their associated host plants 2011; (Kusari et al., 2012). These secondary metabolites can help host plants overcome adversity and various environmental stresses (Porras-Alfaro et al., 2014). In addition to promoting growth, the isolated fungal strains exhibited ammonia production, an important trait for nitrogen metabolism. Nitrogen availability is a key factor in plant productivity, and ammonia-producing fungi have been shown to improve nitrogen use efficiency in several crop species (Liu et al., 2021).

We applied the endophyte fungal strain on maize seedlings to check their effects on the studied traits as compared to control like agronomic parameters, IAA production, chlorophyll contents. Our results showing an increase in root and shoot length in maize plants inoculated with endophytic fungi underscores the positive influence of these isolates on

plant development. The greenhouse experiments revealed a 34% increase in root length and a 41% increase in shoot length, highlighting the potential application of these fungi as biofertilizers. Similar to our findings, improvement can be attributed to enhanced nitrogen availability due to ammonia production, which facilitates better nutrient assimilation by plants (Xu et al., 2012).

The results of fungal inoculation significantly boosted chlorophyll (A and B) content in maize plants, indicating an improvement in photosynthetic efficiency. The 19% increase in chlorophyll content in treated plants suggests that these fungi may enhance the synthesis of photosynthetic pigments, leading to greater light absorption and energy conversion. Similar effects have been reported in other plant-fungal associations, where endophytes enhance chlorophyll biosynthesis and delay leaf senescence under stress conditions (Sena et al., 2024).

In the current study, IAA level was enhanced in the treated group as compared to control. Our findings align with previous studies demonstrating that fungal-derived IAA enhances plant growth by modulating root system architecture and increasing nutrient uptake efficiency (Zhao et al., 2025). In another study, the characterization of fungal isolates for specific traits, such as IAA production, was reported, which could potentially enhance agricultural plant yield (Kleopper et al., 1992). Research suggests that there are over a million species of endophytic fungi that live in symbiotic association with plants. These fungi produce a diverse range of bioactive secondary metabolites, including flavonoids, phenols, phytochemicals, and anticancer agents, which benefit the host plant (Aly et al., 2010)

Conclusion

This study highlights the potential of endophytic fungi isolated from *Oxalis stricta* L. as effective plant growth promoters. The fungal strains produced significant levels of secondary metabolites, including flavonoids, phenols, sugars, IAA, proline, and ammonia, contributing to enhanced plant development. The positive effects on maize (*Zea mays* L.) growth, including increased root and shoot length, as well as elevated chlorophyll A and B content, suggest their role in improving photosynthetic efficiency and auxin-mediated growth. The results underscore the potential application of these fungal isolates as biofertilizers, offering an eco-friendly approach to sustainable agriculture and crop productivity enhancement. Future research should focus on field-scale validation and the molecular mechanisms underlying their growth-promoting properties.

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