

Research Article

Exploration of Endophytic Fungi from *Tecoma Stans* and Their Potential Applications

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Abstract

Endophytic fungi, are symbiotic associations with plants, play a pivotal role in enhancing host resilience, it facilitating nutrient cycling, and synthesizing a diversify bioactive compounds. These microorganisms it gains attention for their potential applications across pharmaceutical, agricultural, and industrial domains. In this study, investigation made to attention on endophytic fungal community associated with *Tecoma stans*, it's a ornamental plant widely cultivated for its ecological adaptability and aesthetic value. The aim of the study was to explore the phytochemical and enzymatic capabilities of the endophytic fungal isolates, with a focus on their relevance to industrial processes. However, two distinct fungal strains were successfully isolated from healthy tissues of *T. stans*. Subsequent screening revealed that presence of bioactive secondary metabolites. Hence, notably anthraquinones and alkaloids, which are known for their antimicrobial, anticancer, and anti-inflammatory properties. In addition to that phytochemical profile of both the strains were demonstrated and pursue significant enzymatic activity. Similarly, Other enzymes like cellulase and amylase enzymes that are crucial in biomass degradation in the textile and food industries. The study concluded that the observation was made to attain the multifaceted potential applications of endophytic fungi are to produce commercially valuable enzymes and pharmacologically active compounds. The ecological significance of these fungi is further highlighted by their contribution to plant health and stress tolerance, suggesting a dual role in both environmental sustainability and commercial exploitation. By tapping into the largely unexplored microbial diversity of *Tecoma stans*, this study opens new avenues for bioprospecting and the development of eco-friendly technologies in sectors such as biopesticides, nutraceuticals, and bio-based manufacturing.

Keywords

Tecoma Stans, Endophytic, Enzymes, Phytochemicals

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1. Introduction

Endophytes are microorganisms that colonies asymptotically intercellular or intracellular part of the plants and play an important role in drug discovery [1]. However, the endophytic fungi are present in almost all types of plants which live as a symbiont [2]. It has been proven to that endophytes have the ability to produce several primary and secondary metabolites such as alkaloids, flavonoids, and terpenoids, which contribute to plant defense against biotic stressors like herbivores and pathogens [3].

Fungi particularly endophytic species, also play a critical role in the degradation of plant materials such as cellulose and lignin. Cellulose, being the most abundant organic molecule on Earth, is highly resistant to degradation due to its crystalline structure and association with lignin. However, certain endophytic fungi are capable of breaking down cellulose more effectively than bacteria due to their production of extracellular enzymes, notably cellulases [4].

The emerging investigation has challenged the scientific community to explore the efficacy of sum medicinal plants and their associated microbial diversity [5]. Even fungal endophytes play vital role in human life because these are source of drug for various deadly disease like bacterial, viral, fungal infections [6]. Present work there is an argent need for bioactive molecules with unique mechanism of action and chemistry to address the issue in correct use of drugs. Natural bioactive chemicals are regarded as critical component in the production of commodities of great values. Their biological activity as enabled usage in the field agricultural, medicinal and nutritional sectors [7].

The major significant challenging scientific under taking are the search for new bio active e molecules and their assessment and ability to have biological impacts [8]. The development of new natural bioactive molecules to solve the problem of seriously harmed by resistant micro-organisms therefore, it is more important than ever to find and create new natural substances to satisfy this pressing and expanding needs.

Utilizing microbial communities, particularly fungus, provides number of benefits that make more effective than alternative tactics due to their number's biotechnological applications as well as their affordability and a easy of use as cultural medium for the development of metabolites from the fungi [9]. In an effort to discover new compounds with a range of functions, recent research as concentrated on bio-prospecting endophytic fungi that reside inside the plant tissues.

Fungi are essential components of ecosystems, and it significantly contributing to nutrient cycling, and forming symbiotic relationships with various organisms. Among these relationships, endophytes microorganisms residing within plant tissues without causing any harm to the host tissue due to their ecofriendly nature. Therefore, Endo-

phytic fungi, are gaining attention for their ability to produce several bioactive compounds with applications such as pharmaceutical, agriculture, and food and textile industries.

Above all the aspects indicates that study of endophytic fungi associated with *Tecoma stans* (yellow trumpet bush), a plant known for its medicinal properties and adaptability to tropical and subtropical climates. Further, search and asses the bioactive molecules hence, objectives of the study include isolating fungal endophytes, identifying their phytochemical profiles, and assessing their enzymatic production capabilities, thereby highlighting their potential for industrial and medicinal applications.

2. Materials and Methods

2.1. Collection of Plant Samples

Healthy and mature *Tecoma stans* plants were collected from the Raichur University Botanical Garden. Leaves were gently plucked and placed into pre-sterilized zip-lock plastic bags. The samples were processed in the laboratory within 24 hours after sampling.

2.2. Isolation of Endophytic Fungi from *Tecoma Stans*

All leaves samples were thoroughly cleaned under running tap water to remove surface contaminants. Each leaf cut into small segments. Approximately 1 to 2 cm in length. All procedures were performed in a laminar airflow hood to maintain aseptic conditions. All leaf segments were surface sterilized using 70% ethanol for 1 minute, followed by disinfection with 6% sodium hypochlorite solution for 5 minutes. And repeatedly cleaned at three times with sterile distilled water, the disinfected samples were placed on sterile filter paper to dry leaf segments [10].

To avoid bacterial Contaminant, the sterilized leaf segments were put on Potato Dextrose Agar plate and supplemented with 50 µg/ml of chloramphenicol. To prevent desiccation, the plates were wrapped in parafilm and further kept in to incubation for one to two weeks at 27 °C in the dark place. Every day, fungal growth was observed.

2.3. Phytochemical Analysis

Qualitative tests for phytochemical analysis were conducted to identify primary and secondary metabolites present in the fungal extracts, following the standard procedures as outlined by Harborne, with slight modifications.

2.4. Qualitative Analysis of Secondary Metabolites

- 1) To check for anthraquinones, add a few milliliters of concentrated H_2SO_4 and one milliliter of diluted ammonia to five milliliters of extract. The presence of anthraquinones is indicated by the emergence of a rose-pink tint.
- 2) Quinones were tested by adding alcoholic KOH to 1 milliliter of extract. Quinones are indicated by the presence of a reddish-blue hue.
- 3) The Mayer's Test for Alkaloids: Mayer's reagent was added in two drops to a few milliliters of filtrate. For alkaloids, a creamy or white precipitate signifies a successful test. A few drops of Wagner's reagent were added to around 1 milliliter of extract for the Wagner's test. Alkaloids are present when a reddish-brown precipitate forms. Dragendorff's Test: 1 ml of Dragendorff's reagent and 2 ml of HCl were added to 5 ml of extract. For alkaloids, a positive result is indicated by an orange or crimson precipitate.
- 4) Glycoside Test: Borntrager's Test Three milliliters of chloroform were added to two milliliters of filtrate and shaken. After separating the chloroform layer, a 10% ammonia solution was added. Glycosides are indicated by the appearance of a pink tint. Five milliliters of extract and five milliliters of strong HCl were boiled in a water bath to hydrolyze the glycosides. After cooling, 10% NaOH was added to 2 milliliters of water to create an alcoholic extract. A positive result for glycosides is shown by a yellow tint. In a different test, 0.5 ml of concentrated H_2SO_4 was added after 2 ml of extract and 0.4 ml of glacial acetic acid containing ferric chloride traces were mixed. A blue hue denotes a successful outcome for glycosides.

2.5. Screening of Fungi for Extracellular Enzyme Production

Fungal isolates were screened for the production of extracellular enzymes through qualitative assessment incubated on appropriate media.

- 1) **Cellulase Activity:** The fungal isolates were cultured on Carboxy Methyl Cellulose (CMC) medium based on the method [11]. Fungal isolates were inoculated at the center of Czape-Dox agar plates and incubated at 30 °C for 5 days, followed by 18 hours at 50 °C in the dark. After staining with 1% Congo red for 15 minutes and destaining with 1 M NaCl for 20 minutes, clear zones around the fungal colonies indicated cellulolytic activity.
- 2) **Amylase Activity:** GYP agar medium supplemented with 2% soluble starch was inoculated with a selection of isolates. Cultures were incubated for three to five days before being inundated with 1% iodine in 2% potassium iodide. Amylase activity was revealed by the colonies' surrounding transparent halos.
- 3) **Laccase Activity:** Isolates were grown on GYP agar medium supplemented with 1-naphthol. Laccase activity was indicated by a color change from clear to blue due to the oxidation of 1-naphthol.
- 4) **Lipase Activity:** Lipase production was evaluated on peptone agar media supplemented with 1% Tween 20. Lipase activity was demonstrated by the presence of halos surrounding the colonies.
- 5) **Protease Activity:** The proteolytic activity was examined using GYP agar media supplemented with 0.4% gelatin. After incubation, the plates were flooded with saturated ammonium sulfate solution. The formation of a clear zone around the colony indicated protease activity.

3. Results and Discussion

3.1. Isolation of Endophytic Fungi

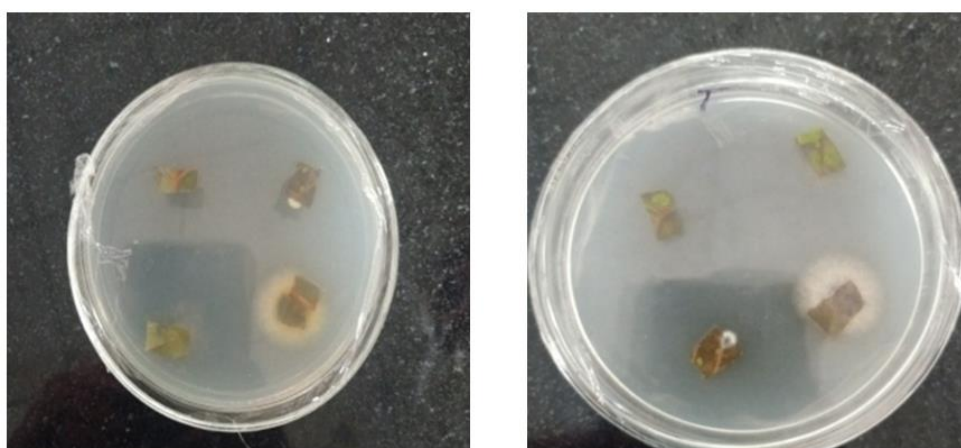


Figure 1. Isolation of Endophytic Fungi From *Tecoma stans* plant leaf.

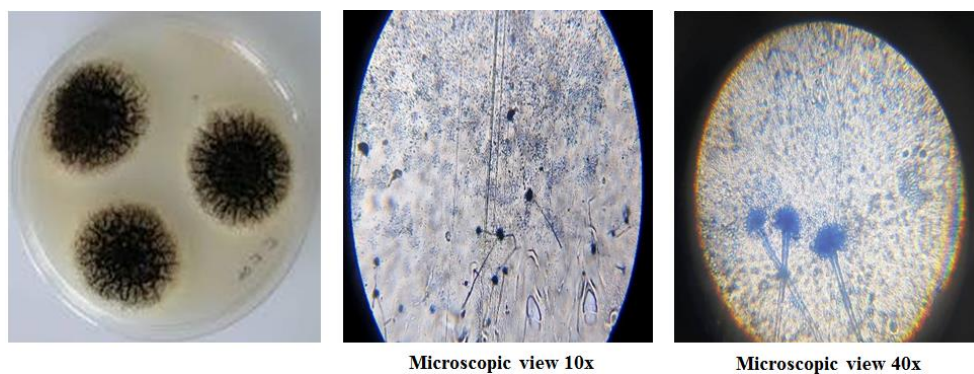


Figure 2. Morphological View of Endophytic Fungi under 10X and 40X Microscopic.

Analysis of the endophytic fungi associated with *Tecoma stans* results reveals that isolation of endophytic fungi were recorded about 26 endophytic fungal isolates were recorded. Among 26 species only two species were recognized on the basis of morphology, physicochemical parameters. By examining morphology of spore pattern under microscopic view

(Figure 2) indicates that the spore patterns distinctive, radiating or columnar pattern consist of foot cell, conidiophore, vesical and sterigmata. similarly, (Figure 3) Microscopic sporulation patterns show spherical or oval spores (Conidia) arises at the tip, brush like appearance in this study. Notable endophytic fungi identified as *Aspergillus* and *Penicillium* species [12].

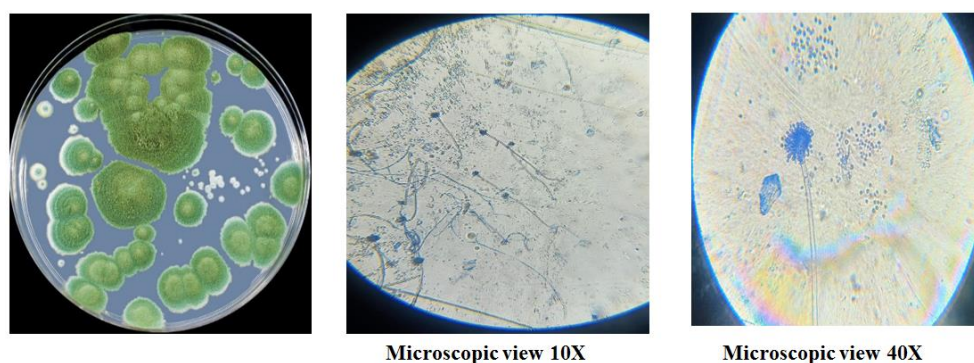


Figure 3. Morphological View of Endophytic Fungi under 10X and 40X Microscopic.

3.2. Phytochemical Analysis

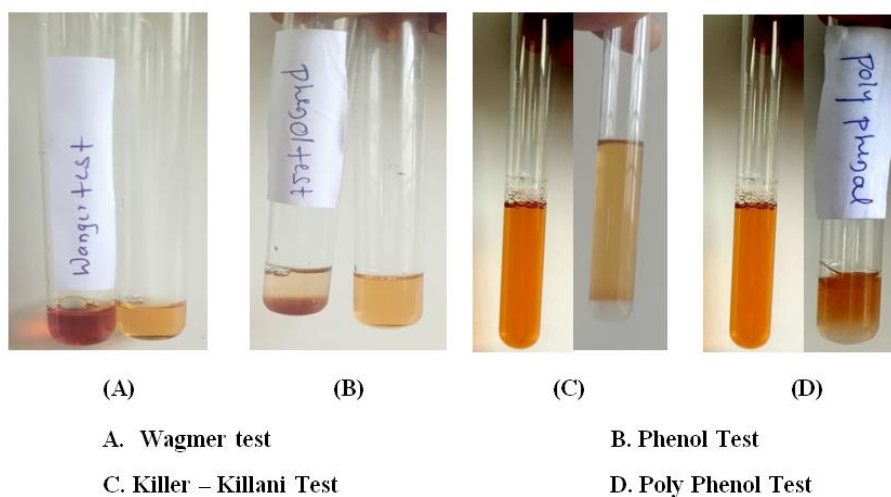


Figure 4. Qualitative tests for Endophytic (*Penicillium* species) Fungi from *Tecoma stans*.

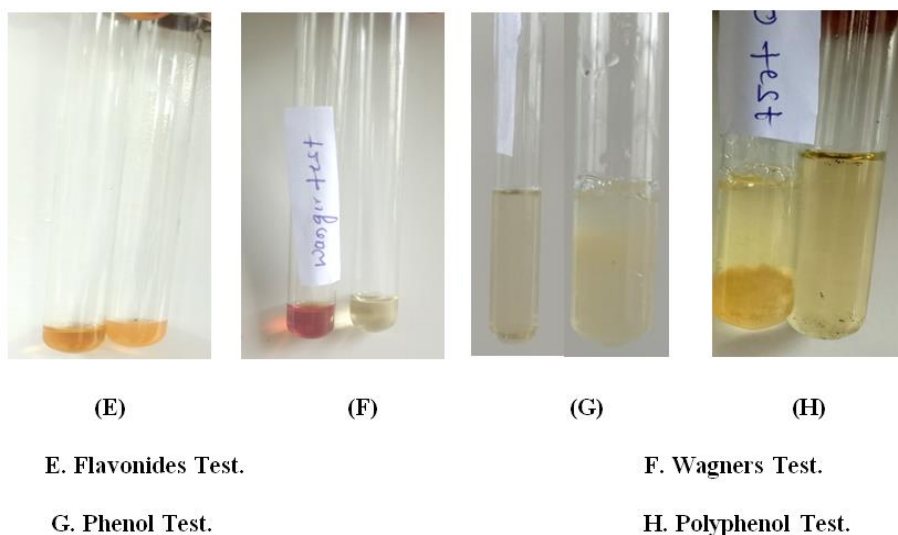


Figure 5. Qualitative tests of Endophytic (*Aspergillus* species) Fungi from *Tecoma stans*.

The methanol extracts of the endophytic fungi underwent phytochemical screening, reveals that presence of several metabolites such as saponins, phenolic compounds, anthraquinones, flavonoids, steroids, cardiac glycosides, and tannins. All different fungal species produce various bioactive molecules exhibiting a wide range of biological activities. Fungal isolates like *Aspergillus* and *Penicillium* species demon-

strated a complete profile of phytochemical compounds, whereas other isolates were missing tannins. Notably, the presence of anthraquinones was restricted to three endophytes (*Penicillium* and *Aspergillus* species). This diverse array of compounds underscores the medicinal potential of these endophytes, suggesting they may be valuable sources of pharmacologically active agents.

Table 1. Qualitative analysis of secondary metabolites from *Tecoma stans*.

Sl. No	Name of the Test	Observation	Penicillium Spores	Penicillium Mat	Aspergillus Spores	Aspergillus Mat
01	Anthraquinones test	—	—	—	—	—
02	Quinones test	—	—	—	—	—
03	Alkaloids test	White ppt	—	—	—	—
	Mayer's test	—	—	—	—	—
	Dragendroff's test	Red to brown precipitate	+	+	+	+
04	Glycosides test Borntrager's test	Pink color	—	—	—	—
05	Cardiac glycosides test	Brown ring	+	+	—	+
	Keller – Killani test	—	—	—	—	—
	Phenol test	White ppt	+	+	+	+
06	Gelatin test	—	—	—	—	—
07	Lead acetate test	Bulky white precipitate	+	—	—	—
	Polyphenols test	Yellow precipitate	+	—	+	+
	Tannins test	Dark green color	—	—	—	—
09	Flavonoids test	Yellow precipitate	+	+	—	+
10	Phytosterols test	Bluish green color	—	—	—	—
11	Phlobatannins test	Reddish precipitate	—	—	—	—

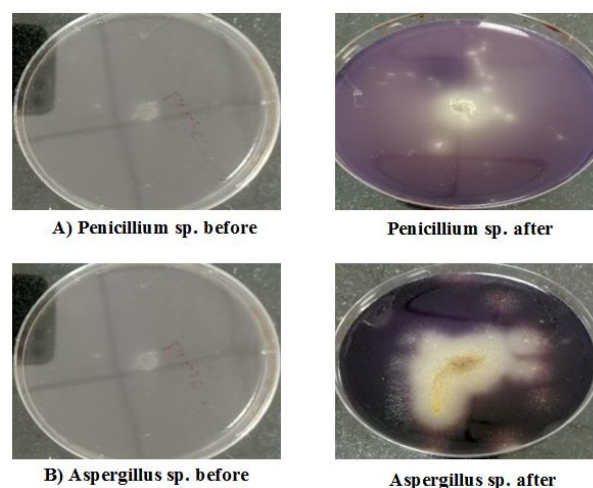
Sl. No	Name of the Test	Observation	Pencillium Spores	Pencillium Mat	Aspergillus Spores	Aspergillus Mat
12	Saponins test	Formation of Frothing	—	—	—	—
13	Steroids test	Red color and yellow green	—	—	—	—
14	Xanthoproteins test	Reddish brown precipitate	—	—	—	—
15	Chalcones test	Red color	—	—	—	—
16	Test for Terpenoids Salkowski test	Reddish brown color	—	—	—	—
17	Anthocyanins test	Pink-red turns to Blue-violet	—	—	—	—
18	Leucanthocyanins test	Red color	—	—	—	—
19	Coumarins test	Yellow color	—	—	—	—
20	Test for Emodins	Red color	—	—	—	—

3.3. Enzyme Assay

In our screening for extracellular enzymes, two endophytic fungal isolates were examined for the presence of amylase, cellulase, laccase, lipase, and protease, which were grown on previously discussed specific media. Interestingly, *Tecoma stans* served as a starch source, available for degradation by endophytes post-mortem. Our investigation yielded promising results, where eight endophytic fungi exhibited amylase activity. Conversely, only two isolates, *Penicillium* and *Aspergillus*, showed cellulase and laccase activity. Previous studies indicate that fungal amylases tend to be more stable than their bacterial counterparts [11], adding significance to our findings.

The exploration into 4000 known secondary metabolites across fungal species highlighted compounds derived predominantly from *Penicillium*, *Aspergillus* and *Acremonium*. The cellulases identified among terrestrial fungi have notable applications in the paper industry [13], while laccases, produced by various marine fungi, play essential roles in lignin degradation [14]. Moreover, increased lipase activity observed in isolates like *Curvularia brachyspora*, *C. vermiformis*, *Drechslera hawaiiensis*, *Colletotrichum falcatum*, and *Phyllosticta* species. confirms their potential to utilize cholesterol as an energy source. Previous research reported Tween as an effective substrate for lipase enzyme assays [13], further validating our enzyme selection criteria. It is important to note that some endophytes displayed a lack of certain active enzymes, potentially as a mechanism to protect host plants from damage. Enzymes from fungal sources generally exhibit higher stability compared to those derived from plants and animals, indicating their viability in industrial applications spanning food processing, beverage production, and textiles [15].

Cellulase Enzyme Screening



Amylase Enzyme Screening

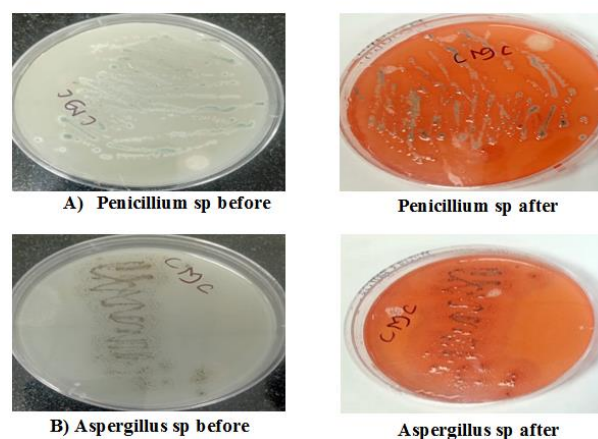


Figure 6. Qualitative screening of Secondary Metabolites (Amylase and Cellulase Enzymes) From *Tecoma* stance.

4. Summaries

- 1) *Overview of Fungi and Endophytes*: Fungi play a vital role in ecosystems, particularly through nutrient cycling and forming symbiotic relationships. Endophytes reside within plants without causing harm, often producing bioactive compounds useful in medicine and agriculture. This study emphasizes fungal endophytes, which are classified into bacterial and fungal categories.
- 2) *Role of Endophytes*: Endophytes exhibit relationships ranging from mutualistic to pathogenic, often benefiting the host plant by enhancing defense mechanisms and productivity. Secondary metabolites like alkaloids and terpenoids produced by endophytes contribute to plant resistance against pathogens.
- 3) *Cellulose Breakdown*: Certain endophytic fungi efficiently degrade cellulose, the most abundant organic molecule, using extracellular enzymes like cellulases. This highlights their industrial relevance, particularly in biofuel production and waste management.
- 4) *Isolation and Identification*: Healthy *Tecoma stans* samples were collected, surface sterilized, and cultured on PDA medium. Two fungal isolates were identified based on morphological and molecular analyses, including *Aspergillus* and *Penicillium* species.
- 5) *Phytochemical Analysis*: The methanol extracts of the fungi revealed bioactive compounds like anthraquinones, alkaloids, flavonoids, and tannins. These compounds suggest significant medicinal potential, particularly as anticancer and antimicrobial agents.
- 6) *Enzyme Production*: Screening of endophytes demonstrated their ability to produce extracellular enzymes such as cellulase, amylase, and laccase. These enzymes have applications in various industries, including textiles, food processing, and environmental remediation.

5. Conclusion

This study underscores the industrial and ecological significance of endophytic fungi associated with *Tecoma stans*. The isolated fungi demonstrated diverse phytochemical profiles and enzymatic capabilities, making them valuable resources for biotechnological applications. Future research should focus on optimizing the production of bioactive compounds and enzymes, as well as exploring their potential in sustainable agriculture and medicine. The findings contribute to the growing understanding of endophytes as vital components in developing eco-friendly technologies and improving quality of life.

Abbreviations

Sp Species

+ Present
- Absent

Conflicts of Interest

The authors declare no conflicts of interest.

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