

REVIEW ARTICLE

INDIAN SANDALWOOD (*SANTALUM ALBUM* L.): A COMPREHENSIVE REVIEW OF ITS CULTURAL HERITAGE, TRADITIONAL USES, AND MODERN PERSPECTIVESPadma S¹., Mamatha, N²., Sundararaj, R²., Prashanth, M². and Nalini, U.R.^{2*}¹Indian Institute of Science, Bangalore – 560012, India²*ICFRE- Institute of Wood Science and Technology, Malleswaram Bangalore-560003, IndiaEmail: nalini@icfre.org

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Abstract: Indian Sandalwood (*Santalum album* L.) is one of the most valuable plant species in the world that have been associated with human civilization for more than four millenia. This review aims to document the historical significance of sandalwood, its mythological background, cultural importance, economic value, and its traditional and scientific applications. Indian sandalwood has been widely celebrated and discussed abundantly in old Indian literary works like the Ramayana, Mahabharata, Vedas, and Arthashastra. It is being used extensively in different religious and spiritual practices of Hinduism, Buddhism, Jainism, and Zoroastrianism. Traditional uses of sandalwood are from birth till death, including homa and abhishekam in ceremonies, Ayurvedic medicine preparations like Chyawanprash, skincare products, and handcrafts. The efficacy of various traditional applications of sandalwood has been established by modern science through its pharmacological attributes like antitumor, antibacterial, anti-inflammatory, and neuroprotective effects. Main bioactive constituents are α - and β -santalols. Unfortunately, due to over exploitation for its cultural and economic importance, sandalwood has become a threatened species.

Keywords: *Santalum album*, Cultural heritage, Traditional, Pharmacological, Santalol, Handcraft, Bioactive

INTRODUCTION

From ancient times until now, Indian sandalwood (*Santalum album* L., Family: Santalaceae) has been one of the most highly valued tree species because of its long-lasting fragrance, exquisite carving quality, and high cultural value (Sundararaj *et al.*, 2019a). Unlike many other fragrant plants, sandalwood keeps its aroma for several decades, thus making it an excellent ingredient in perfumes, medicines, and religious artefacts. The English name "Sandal" derived from word Chandana (Srinivasan *et al.*, 1992). In the Indian tradition, the sandalwood tree plays a significant role, being utilized in every sphere of people's life from the cradle to the crematorium, with the help of which spiritual liberation can be reached, tension is relieved, and peace of mind is ensured (Sandeep & Manohara, 2019). This review is designed to gather information about the culture of Sandalwood in order to emphasize its value and the necessity of its conservation.

CULTURAL AND HISTORICAL SIGNIFICANCE

Sandalwood in Ancient Indian Texts

Indian sandalwood has inextricable links with Indian culture and plays a very important role in Indian legends and religious writings. In historical studies, it is stated that Sandalwood has been found in writings like Milinda Panha (200 BC), Patanjali's Mahabhashya (100 BC), Ramayana and Mahabharata. In Ramayana, Valmiki cited the importance of sandalwood as "The breeze that wafts through the sandal trees is very invigorating and cleans the atmosphere with the intoxicating scent of sandal". Rabindranath Tagore described sandalwood as a symbol of selfless virtue and stated "The sandalwood tree is sure to be the sweetest that could even conquer hate, love, and perfumes the cruel axe that lays it low". Kalidasa (4th-5th Century CE) writes about sandalwood trees in his epic poem entitled "Raghuvamsha." His statement goes like this: "The neck halter of elephants breaking their food chains did not slip, being fixed in the marks of coiling serpents round the sandalwood trees". In addition to that, the two mountains named Malaya and Dardura with their peak covered with

*Corresponding Author

sandalwood trees: "Dally over me uplands of Malaya, strew with Tamala leaves, where the trees have cardamom creepers clinging to them and betel trees (Puga) are girt about with betel creepers (Tambula)". Sandalwood is also listed among the objects entered into the treasury in Chapter XI of Kautilya's "Arthashastra," 320 BC to 300 AD). Periplus of the Erythraean Sea or Periplus of the Red Sea is a Greco-Roman periplus. It was written in Greek and describes the navigation and trade prospects from the Roman Egyptian ports such as Berenice along the coast of the Red Sea. During 1st century, the Greco Roman navigational logbook called Periplus had mentioned the traded route of Sandalwood from India. Sandalwood is mentioned in the Tamil -epic poem "Silappathikaram" (2nd -5th century AD) as originated from the Southern Mountain range (Malaya Tenmalai piranda chandanam). Later Ayurvedic texts like Charaka samhita described Chyawanprash formulations, a herbal paste associated with the historic reference of restoration of youth and vitality of sage Chyavana, had components of sandalwood along with amla, cinnamon, and cardamom (Scartezini *et al.*, 2006; Sharma *et al.*, 2019). In Hitopadesa II Verse 163, it was said 'The root is infested by serpents, the blossoms by trees, the branches by the monkeys, and the summit by bears'. Essentially every part of the sandalwood tree is free from impurities but rich in its natural incredible qualities.

Religious and Ritualistic Uses

Indian sandalwood is an integral part of Hindu rituals such as homa/havana (offerings to sacred fire), abhishekam (bathing deities with liquids), and temple constructions (Fig. 1). According to the Brahma Vaivarta Purana, Goddess Lakshmi dwells within the Sandalwood trees, making them symbol of prosperity and fortune. *Vamana Purana*, prescribes the use of sandalwood paste for worshipping Lord Shiva. According to Hindu Mythology, the birth of Lord Ganesha is often associated with the sandalwood and turmeric paste made by Goddess Parvati. As per the Bhagavata Purana, Goddess Parvati bestows her blessings on a dwarf woman called Kubja when she puts sandalwood paste on Lord Krishna's body. The Lord Venkateshwara is often known as 'Chandana Charchita Govinda', which translates to "The Lord with a pure and beautiful form, adorned with sandalwood paste, and possessing a complexion resembling that of a cloud". The Tirumala Tirupati Devasthanam (TTD) uses over half a tonne of sandalwood annually for rituals like *Snapanam* *Thirumanjanam* (Sandeep & Manohara, 2019). Sandalwood is equally important in other religions, in Jainism, the paste of sandalwood mixed with saffron is applied during prayers of the Tirthankaras and in Buddhism, sandalwood incense is offered to Buddha and it is believed that Buddha's remains were burnt upon a pyre made up of sandalwood in 543 BC. Zoroastrians offer Sandalwood twigs to

maintain sacred fires. The people of Greece came to know about this enchanting fragrant oil used in medicine during the period of Alexander, the great and an European Hakeem (physician) of Madarsa Salarno. The ancient Egyptians used sandalwood oil for embalming mummies and treating skin disorders. It is used as perfume or burned as "bukhoor" to fumigate rooms and spaces of worship in the Islamic world.

Economic History and Conservation

Realizing its importance, Tipu Sultan, who ruled over Mysore from 1782-1799, conferred it with the status of being a "Royal tree" and even formed a monopoly for it. The British too monopolised the sandalwood trade in southern India, and overexploited the species as far as the market would allow (Rashkow, 2014). After independence, the states framed state forest laws with provisions of Challenges in the Production and Protection of Indian Sandalwood to maintain the control regime. The state governments of south India held exclusive authority over all the sandalwood resources, even those located on private properties until 2002 which led to rampant smuggling and illicit trade that resulted this species at risk of extinction (Neil, 1986). However, Sundararaj, (2026) concluded that the primary cause of the unfortunate depletion of sandalwood resources in India is undeniably the widespread occurrence of SSD, which is lethal to sandalwood trees and their subsequent extraction. IUCN (2000) has classified it as a vulnerable plant species. The state governments of Karnataka and Tamil Nadu amended the Sandalwood Act in 2001 and 2002, respectively, and made the grower himself an owner of the sandalwood (Dhanya *et al.*, 2010). The amended acts explicitly stipulate that "every land occupant or holder shall have a legal entitlement to the sandalwood tree on their land." These amendments are encouraging the farmers and corporate bodies to take on sandalwood plantations to meet the growing demand (Gowda *et al.*, 2008) and sandalwood cultivation is increasing in areas outside forests (Sundararaj *et al.*, 2019b). The Economic Advisory Council of the Prime Minister and NITI Aayog established "Sandalwood Development Committee" as a guiding force for sustainable production and conservation of Indian sandalwood. Furthermore, the Government of India has released postal stamps (Fig. 2) to promote its conservation (Purohit, 2018).

TRADITIONAL USES

Ceremonial Applications

It is customary in certain communities of Hindus to put a piece of sandalwood in the funeral pyre as a mark of respect to their departed relatives. Sandalwood paste is used in rituals and ceremonies and to mark religious utensils and to decorate the icons of the deities (Fig. 3) and distributed to

devotees, who apply it to their foreheads or the necks and chest. It is also applied on the entire head after shaving. The body of the philosopher saint Ramanujacharya (after 1137 AD) is preserved in Sri Ranganatha Swamy Temple using only sandal wood paste and saffron.

Skin Care and Cosmetics

Conventionally, women in India have applied Sandalwood paste on their skin for beautifying purposes. Sandalwood paste is beneficial for various skins, especially for dry, oily, and acne-prone skins, since it has moisturizing and astringent qualities. It helps relieve irritated skin that is dry or chapped. Moreover, this is applied on stretch marks and scars. Guests in marriages are greeted by putting sandalwood paste on cheeks (for women) or wrists (for men). Furthermore, it is used in making incense or agarbatties and burning incense, and sandalwood mala (Fig. 4) (Dastur, 1962; Farooqui & Sreeramu, 2001). Sandalwood oil is extensively used in the oil skin-care soaps, lotions, perfumes, and creams (Fig. 5).

Handicrafts

Around 5% of the yearly output of sandalwood goes into making beautiful sculptures of deities, boxes to keep jewellery, picture frames, combs, toys and decoratives (Fig. 6). Sandalwood oil has an inherent characteristic of being an insect repellent, thus adding to its utility. Skilled artisans who practice wood sculpture art and who are referred to as "Gudigars" in Karnataka have passed down their tradition from generation to generation. The places famous for this practice include Thirupathi (Andhra Pradesh), Ankola, Bangalore, Honnavar, Kumta, Mysore, Sagar, Sorab, Sirsi Talaguppa (Karnataka), Thiruvananthapuram (Kerala), Surat (Gujarat), Jaipur, Jodhpur, Pali, Mathopur ((Rajasthan) and some places in Tamil Nadu, Uttar Pradesh and West Bengal. "Cauvery" (Karnataka State Arts and Crafts Emporium) and Tamil Nadu's Poompuhar Corporation market promotes these handicrafts of Karnataka and Tamil Nadu respectively.

MEDICINAL USES

Applications in traditional medical systems:

Traditional medical systems like Ayurveda, Homoeopathy, Siddha, Tibetan, Unani, and Western medicine, use sandalwood to treat various illnesses. It was mentioned in 'De Materia Medica' written by Discorides, the father of pharmacology. It principally acts as a demulcent, diuretic, and mild stimulant in Ayurveda (Pande, 1977) and prescribed to treat bronchitis, dysuria, urinary infection, and gonorrhoea (Dikshit & Hussain, 1984). Sandalwood's wood, roots, bark, and leaves are used by native tribal healers to cure liver conditions, including jaundice. In Ayurveda as therapeutic uses, it is recommended for many ailments that include biliousness, fever, bleeding piles, diarrhoea with internal bleeding, eye

infections, haemorrhage, hiccoughs, inflammation of umbilicus poisoning, the initial phase of pox, urticaria, and vomiting (Kirtikar & Basu, 1933; Desai & Hirenath, 1991). Also, applying cooled sandalwood to the forehead helps to calm the mind. In Unani medicine sandalwood is used to neutralise the adverse effects of intense sunlight and fever, quench thirst, and impart a soothing and invigorating sensation. Sandalwood paste is topically applied to treat localized inflammations, boils, and skin illnesses. It is also applied to the forehead to alleviate fever and to the nostrils to alleviate migraines. Decoction of sandalwood is used for genito-urinary abnormalities. Mixture of sandalwood paste and lime juice as a remedy for itching, scabies, eczema, and several other dermatological conditions in Siddha medicine. Sandalwood oil is advocated in Aromatherapy to treat sleeplessness, neurological tension, and stress disorders (Lawless, 1999) because of its santalol content (Holmes, 1989).

Modern Scientific Pharmacological Perspectives

Modern research has validated many traditional claims, revealing diverse bioactivities, primarily attributed to α - and β -santalol, the major constituents of sandalwood oil (Verghese *et al.*, 1990) and its broad pharmacological action renders it extremely significant (Aftab *et al.*, 2023). Sandalwood extracts have shown memory enhancement potential in animal studies (Papaiah *et al.*, 2010) and affect the sleep-wake cycle (Ohmori *et al.*, 2007). Sandalwood oil is demonstrated as a relaxant, calming and soothing nerves, muscles, and blood vessels, effectively relieving spasms and contractions. Due to its calming and relaxing properties, sandalwood oil is used to treat respiratory tract infections (Holmes, 1989; Davis, 1999) and for coughing up blood (hemoptysis) and coughing up pus (purulent expectoration). Furthermore, it is used to treat genitourinary tract infections (Imdorf *et al.*, 1999) and as a cure for malodorous and pus-filled menstrual discharge, excessive menstrual bleeding, abnormal vaginal discharge and abnormal passage of semen. It potentially alleviates dry skin disorders and the symptoms of eczema and psoriasis (Davis, 1999). Applying sandalwood oil during meditation proved to reduce mental distractions and beneficial for promoting healing and self-healing (Rippin, 1997). When sandalwood oil inhaled through the olfactory system, it relaxes the sympathetic system, resulting in decrease in heart rate and blood pressure and an increase in heart rate variability. It has the potential to prevent headaches, insomnia, and nervous tensions (Battaglia, 2007), depressant effects (Ohmori *et al.*, 2007), memory enhancement (Jackson *et al.*, 2009) and treatment of Alzheimer's disease and skincare (Misra & Dey, 2013a). Sandalwood oil is used as a dietary supplement (Burdock & Carabin, 2008), known to have Antihyperglycemic and antihyperlipidemic effects (Kulkarni *et al.*, 2012) and prevents gastric ulcers (Ahmed *et al.*, 2013). Hegde

et al. (2014) reported that the hydroalcoholic extract of its leaves could protect the liver from damage caused by CCl₄ and paracetamol. Water-based sandalwood extract protects the heart muscle by lowering lipid oxidation and the cardiotoxic effects of doxorubicin (Khan *et al.*, 2014) and the saponins present in the leaves of sandalwood have haemolytic effects (Deepak *et al.*, 2014). Experimentally sandalwood oil is proved to have antimicrobial activities that includes antibacterial (Jones *et al.*, 1995, Ochi *et al.*, 2005, Jirovetz *et al.*, 2006; Bakkiyaraj & Pandiyaraj, 2011), antifungal (Chaumont & Bardey, 1989) and antiviral properties (Schnitzler *et al.*, 2007, Koch *et al.*, 2008, Singh & Nulu, 2010, Misra & Dey, 2013b).

The presence of antioxidant principles in the plant by phytochemical and pharmacological tests justified its traditional therapeutic effects (Scartezzini & Speroni, 2000). The extracts of sandalwood demonstrated considerable scavenging effect on Nitric Oxide and DPPH antioxidant activity (Jagetia & Baliga, 2004; Patrick & Timothy, 2002, Pedapati *et al.*, 2012), protect cardiac tissue from oxidative stress-induced damage (Khan *et al.*, 2014) and has anti-hyperglycemic properties (Misra & Dey, 2013c). Ximenynic acid from sandalwood seed oil has the ability to detoxify and strengthens the Extracellular Matrix (ECM) resulting in stronger dermis and more flexible skin (Vasundhara *et al.*, 2015). It also has the potential to treat cellulitis, hair loss, and varicose veins (Hettiarachchi *et al.*, 2013) and has anti-aging activity (Shivatare *et al.*, 2020). The methanolic extracts of heartwood exhibit anti-inflammatory effects (Saneja *et al.*, 2009).

Anticancer effect of sandalwood and its products have been demonstrated during the recent days. J82 human bladder cancer cells were killed by sandalwood oil and stopped the cell cycle (Dozmorov *et al.*, 2014). α -santalol inhibits skin cancer (Dwivedi *et al.*, 2003; Kaur *et al.*, 2005; Zhang & Dwivedi, 2011; Santha & Dwivedi, 2015) and prevents development of skin tumours generated by UVB radiation (Dwivedi *et al.*, 2006; Bommarreddy *et al.*, 2007). It exhibits cytotoxicity against prostate and oral cancer cells (Bommarreddy *et al.*, 2012; Lee *et al.*, 2015). It is also source of novel antitumor sesquiterpenoids. A few new sesquiterpenoids, two aromatic glycosides, and a number of neolignans obtained from heartwood of sandalwood possess a strong blocking effect on EBV-EA activation resulting in strong stopping effect on two-stage carcinogenesis on mouse skin. (Kim *et al.*, 2006). Matsuo and Mimaki (2012) demonstrated tumour-selective cytotoxicity in HL-60 human promyelocytic leukaemia cells and TIG-3 normal human diploid fibroblasts by the derivatives of α -santalol. Two ligands sourced from the

heartwood showed apoptosis induced tumour cell cytotoxicity against HL-60 human promyelocytic leukemia cells and A549 human lung adenocarcinoma cells (Matsuo and Mimaki, 2010). Cytotoxic properties of α - and β -santalol against many head and neck squamous cell carcinoma (HNSCC) were demonstrated by lines by Lee *et al.* (2015).

Other uses

Roy *et al.* (2017) observed the unique properties of sandalwood oil with its potential to use as a pesticide for managing various pests. "Triterpene palmitic ester", a chemo-sterilant compound isolated from the bark of sandalwood showed insect growth regulation property (Shankaranarayana *et al.*, 1980a), that can be used as a third-generation pesticide (Shankaranarayana, *et al.*, 1980b). A triterpenoid isolated from sandalwood bark showed growth inhibition and chemosterilant activities (Chaumont & Bardey, 1989). The sandalwood oil has the potential as termiticide (Kaikini, 1969; Srinivasan *et al.*, 1992) and acaricide (Imdorf *et al.*, 1999). Santalol exhibits protective role against the spider mite *Tetranychus urticae* and prevents it from laying eggs (Roh *et al.*, 2011; Roh *et al.*, 2012). It has been approved as food additives by the United States Food and Drug Administration (FDA), Flavour and Extract Manufacturers Association (FEMA), Council of Europe (COE), and Joint FAO/WHO Expert Committee (Desai & Hiremath, 1991; Kraft *et al.*, 2000). Palmitone, and d-10-hydroxypalmitone, the wax compounds derived from leaves on saponification, are free of paraffin (Sindhu *et al.*, 2010). The fatty oils of sandalwood seeds are suitable for paint production. The tannin present in the bark has good potential in the tanning industry and the leaves make excellent green manure and improving the soil (Kumar *et al.*, 2015).

CONCLUSION

Sandalwood (*Santalum album* L.), indigenous to India, represents a remarkable natural resource that intertwines culture, tradition, and contemporary practices. It is revered as a sacred plant throughout ancient texts, has significant cultural significance during religious rituals, and is used in skincare products and crafts. Pharmacological studies carried out recently have shown that sandalwood provides a wide range of health effects such as antitumor, antibacterial, anti-inflammatory, and metabolic effects due to santalols. The overexploitation, habitat degradation, illegal harvesting and disease pressures have highly threatened their natural population. In the future, much work needs to be done to sustainably cultivate and trade sandalwood, as well as conduct clinical trials.



Fig. 1. Religious uses of sandalwood (a) Sandalwood, Ghee, Mustard & Myrrh in Homa, (b) Bathing of Shivalinga with sandalwood powder, (c) Abhishekam of Nandi with sandalwood mixed with water.



Fig. 2. Postal stamps from government of India in recognition of the importance of sandalwood (Source: Purohit, 2018)

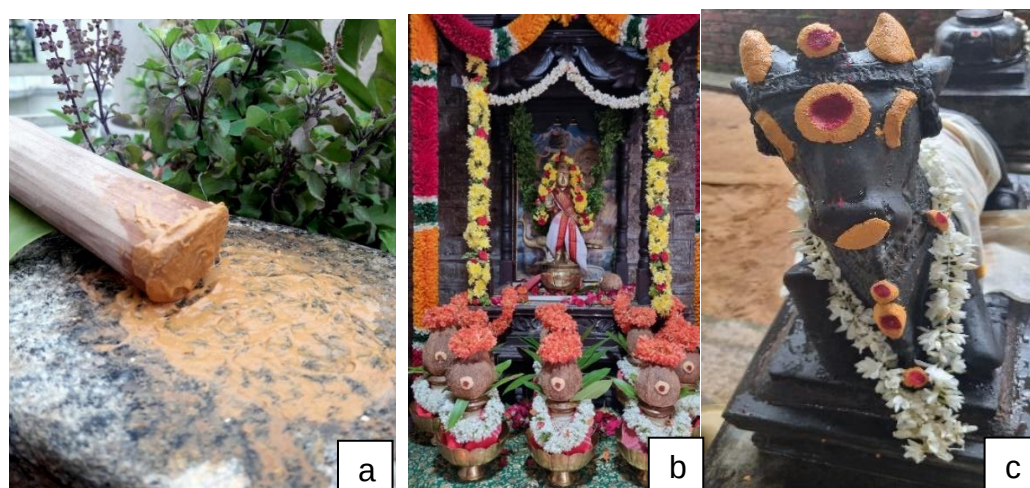


Fig. 3. Traditional use of Sandalwood (a) Preparation of sandalwood paste, (b & c) Decorating the icons of the deities

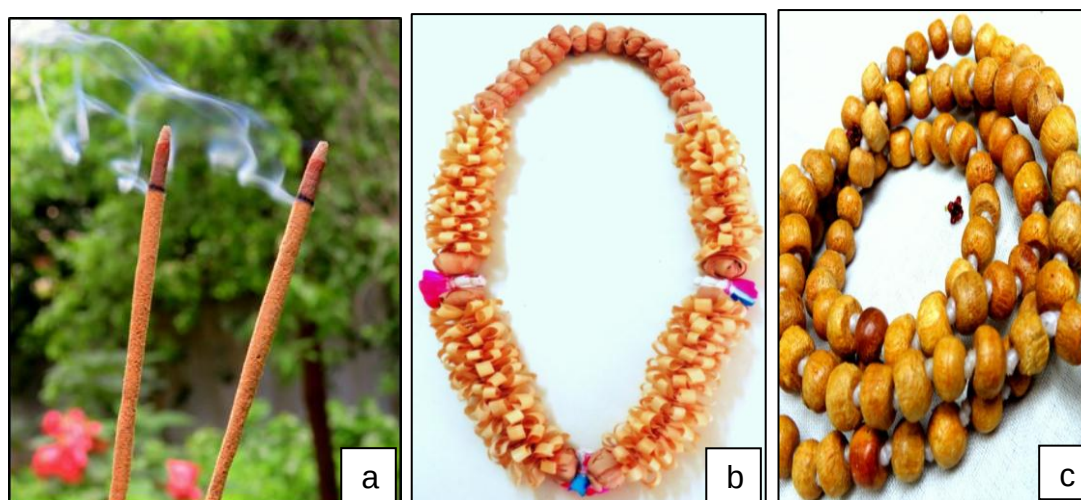


Fig. 4. Spiritual application (a) Incense Sticks, (b) sandalwood garland, (c) Japamala

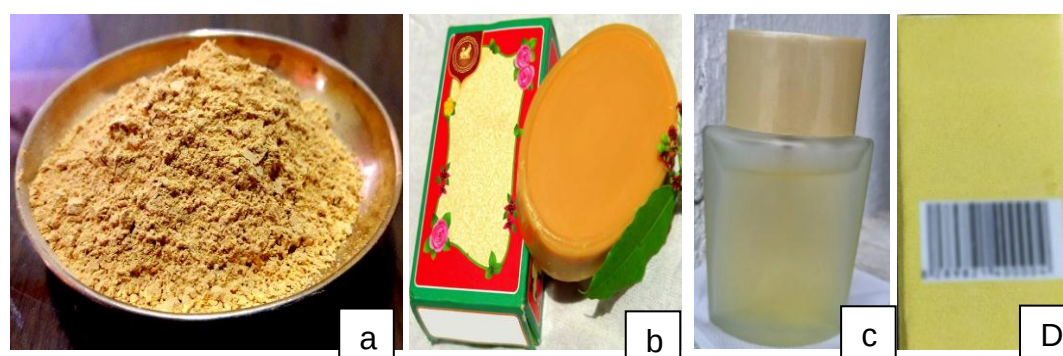


Fig. 5. Cosmetic use of sandalwood, (a) as powder, (b) Soap, (c) sandalwood oil based perfume, (d) sandalwood oil



Fig. 6. Sandalwood based handicrafts (a) toys, (b) God's statues

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RESEARCH ARTICLE

CHANGES IN SELECTED BIOCHEMICAL COMPOUND CONTENTS AND ENZYMATIC ACTIVITIES DURING VIVO-PLANT PRODUCTION IN PLANTAIN (*MUSA AAB*) CV. 'CORNE 1'

Sah Fatoumata Diomandé¹, Kouakou Romaric Kouakou², Yaya Touré^{3*}, Pyminnan Traoré¹, Tchoa Koné¹ and Mongomaké Koné¹

¹Laboratory of Biology and Improvement of Plant Production, Faculty of Natural Sciences, Nangui ABROGUA University, Abidjan, Côte d'Ivoire.

²National Center for Agronomic Research (CNRA), Korhogo Regional Directorate, Ferkessédougou Research Station, Côte d'Ivoire

³Agronomy and Forestry Department, Faculty of Agricultural, Forestry and Environmental Engineering, Université of Man, Man, Côte d'Ivoire.

Email: toureyaya12@yahoo.fr

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Abstract: The limited availability of high-quality planting material is one of the major constraints to increasing plantain production in Côte d'Ivoire. Macropropagation represents an accessible alternative for the rapid production of planting material; however, the biochemical and enzymatic processes associated with bud formation remain poorly documented. This study evaluated the effect of prior sucker dehydration on bud production and characterized changes in selected biochemical compounds and enzymatic activities during macropropagation of the plantain (*Musa AAB*) cv. 'Corne 1'. A total of 90 suckers were collected, and healthy suckers were assigned to two treatments: non-dehydrated suckers (TEM) and suckers dehydrated for two weeks (DESH). Total sugar, total protein, and total phenolic compound contents, as well as phenylalanine ammonia-lyase (PAL) and peroxidase (POX) activities, were determined weekly over a six-week incubation period. The first roots emerged after 7–8 days, whereas the first buds were observed after 14 days. The mean number of buds per explant was significantly higher in DESH (58.28 buds) than in TEM (34.66 buds). In contrast, the mean times to root emergence and bud emergence did not differ significantly between treatments. Total sugar, protein, and phenolic compound contents, as well as PAL and POX activities, showed temporal variations that differed between treatments. These results indicate that prior dehydration for two weeks can enhance bud production and is associated with changes in the biochemical and enzymatic dynamics of suckers during plantain macropropagation.

Keywords: Plantain, *Musa AAB*, Macropropagation, Sucker dehydration, Bud production, Biochemical changes

INTRODUCTION

Plantain is the fourth most important food crop produced in Côte d'Ivoire, after yam, cassava, and rice. With an annual production of approximately 1.6 million tonnes, Côte d'Ivoire is the third-largest supplier of plantain in West Africa. However, this production remains insufficient to meet the continuously increasing demand resulting from rapid population growth and the quantities exported to neighboring countries and Europe. The capacity of production systems to meet this growing demand remains uncertain, particularly due to pest and disease pressure and, above all, the insufficient availability of high-quality planting material.

To overcome these constraints, several propagation approaches have been developed to facilitate the large-scale production of planting material and contribute to improving plantain production. These approaches include in situ methods (Bonte et al.,

1995), macropropagation methods (Koné *et al.*, 2016), and in vitro techniques (Shirani *et al.*, 2009). Among these approaches, macropropagation is particularly attractive because of its relatively low production cost and its potential for implementation by farmers compared with in vitro techniques. Previous studies on plantain macropropagation have mainly focused on production performance as affected by substrate type, sucker size, and the physiological stage of the suckers (Koné et al., 2016), as well as on the conditions required for the acclimatization and hardening of vivo-plants (Koua, 2014). Other studies have investigated the effects of sucker dehydration on the mass propagation of vivo-plants.

Although these agronomic and technical studies have contributed to improving the production of vivo-plants, the physiological processes underlying their formation remain poorly understood. In particular, little information is available on the biochemical and

*Corresponding Author

enzymatic changes occurring during the transition from a sucker or stem fragment to newly formed buds and developing *vivo*-plants. The formation of new organs involves profound physiological changes that require the mobilization and synthesis of structural and reserve compounds, as well as the regulation of enzymatic activities. Understanding these changes could therefore provide useful information for elucidating the physiological basis of plantain *vivo*-plant production and potentially identifying biochemical indicators associated with successful bud initiation and *vivo*-plant development. To our knowledge, no study has specifically characterized the dynamics of biochemical compounds and enzymatic activities during the formation of *vivo*-plants from plantain suckers. The available literature provides only limited information on the biochemical mechanisms occurring within plantain suckers during the processes leading to bud emergence and *vivo*-plant formation. This knowledge gap limits our understanding of the biological mechanisms underlying *vivo*-plant production and may constrain the optimization of production conditions.

The present study therefore aimed to investigate changes in selected biochemical compounds and enzymatic activities during the production of *vivo*-plants from suckers of the plantain ‘Corne 1’ cultivar. The study was based on the hypothesis that bud initiation and subsequent *vivo*-plant development are accompanied by characteristic changes in biochemical compounds and enzymatic activities reflecting the physiological processes involved in *vivo*-plant formation. Specifically, the objectives were to: (i) stimulate bud production from

sucker stem fragments; (ii) quantify selected biochemical compounds at different stages of *vivo*-plant production; and (iii) evaluate changes in enzymatic activities throughout the *vivo*-plant production process.

MATERIALS AND METHODS

Study site

The study was conducted at the Laboratory of Biology and Improvement of Plant Production (LBAPV), Nangui ABROGOUA University, Abidjan, Côte d’Ivoire. The experimental site is located between 5°17’ and 5°31’ N latitude and 3°45’ and 4°22’ W longitude. The study area is characterized by a deep sandy-clay soil and a humid tropical climate, with a mean annual temperature of approximately 27 °C. The suckers used as plant material were collected from the experimental plot of the LBAPV, while the different stages of macropropagation and *vivo*-plant production were conducted in the laboratory’s experimental facilities.

MATERIALS

Plant material

The plant material consisted of suckers of the ‘Corne 1’ cultivar (Figure 1), which were collected from the experimental plot of the Laboratory of Biology and Improvement of Plant Production (LBAPV), Nangui ABROGOUA University. The ‘Corne 1’ cultivar has a production cycle of 10–11 months. The average bunch weight ranges from 9 to 13 kg, and each bunch bears an average of 4–6 hands with large fruits (Boyé *et al.*, 2010).



Figure 1. Suckers of the ‘Corne 1’ cultivar used as plant material in the experiment

Infrastructure and equipment

The infrastructure and equipment used for macropropagation included a plastic film-covered propagation chamber used for establishing the explants in a sawdust-based substrate, a mini-

greenhouse covered with palm-leaf shade, used for the acclimatization and hardening of the *vivo*-plants, and a shade structure used for the subsequent growth and development of the *vivo*-plants.

METHODS

Collection and dehydration of suckers

Suckers measuring 15–50 cm in height, measured from the soil surface, and weighing 500 g to 1 kg were collected using a palm-tree knife. A total of 90 suckers were collected. Following an assessment of their sanitary condition, only healthy suckers were selected for the experiment, whereas suckers showing signs of contamination were discarded. The selected healthy suckers were washed with tap water to remove soil particles adhering to their surfaces. They were then divided into two groups according to the duration before placement in the propagation chamber: a dehydration group and a control group. Suckers assigned to the dehydration group were kept under shelter and exposed to ambient air for two weeks, under conditions that protected them from rainfall and direct sunlight. Suckers in the control group were used on the day of collection, without prior dehydration.

Preparation and establishment of explants in the propagation chamber

The suckers were peeled and trimmed to expose the inner tissues, and were then immersed for 15 min in a solution containing a mixture of Furadan 5G (15 g/L) and mancozeb 880 W (15 g/L). After soaking, the pre-explants, consisting of peeled and trimmed suckers, were removed from the solution and dried under shelter for 48 h. At the end of this period, the surface of the pseudostem was freshly trimmed. Two perpendicular incisions were then made at the center of each explant to induce stress to the apical meristem and promote bud emergence. The prepared explants were placed in a propagation chamber containing sawdust as the substrate. Twenty-four hours after the explants were placed in the propagation chamber, the substrate was thoroughly watered. Thereafter, watering was carried out two to three times per week, depending on the moisture status of the substrate.

The mean time to root emergence, mean time to bud emergence, and mean number of buds produced per explant were determined during the *vivo*-plant production process.

Biochemical and enzymatic assays

The analyses included the determination of total sugar, protein, and phenolic compound contents, as well as the assessment of phenylalanine ammonia-lyase (PAL) and peroxidase (POX) activities, two groups of enzymes notably involved in phenolic compound metabolism. The samples were removed from the freezer and immediately used for the respective assays. The analyses were performed on non-dehydrated suckers (controls) and suckers dehydrated for two weeks.

Extraction and determination of total sugars

A 0.5-g sample of fresh tissue was ground in 10 mL of 80% ethanol. The homogenate was centrifuged at 5,000 rpm for 5 min. The resulting pellet was then

resuspended in 10 mL of 80% ethanol and centrifuged under the same conditions. The supernatants obtained from the two centrifugation steps were combined and adjusted to a final volume of 30 mL with 50% ethanol. The resulting extract was used for the determination of total sugars. Total sugars were extracted and quantified using the phenol–sulfuric acid colorimetric method described by Dubois et al. (1956).

Extraction and determination of proteins

A 0.5-g sample of fresh tissue was ground in 10 mL of 0.1 M sodium phosphate buffer (pH 7.5) in the presence of polyvinylpyrrolidone (PVP). The resulting homogenate was centrifuged at 5,000 rpm for 20 min. The resulting supernatant constituted the protein extract used for the assay. Protein content was determined according to the method described by Bradford (1976).

Extraction and determination of phenolic compounds

Phenolic compounds were determined with modifications and adaptations to the plant material used in this study. The method is based on the reduction, under alkaline conditions, of the phosphomolybdic and phosphotungstic acids in the Folin–Ciocalteu reagent by phenolic compounds, resulting in the development of a measurable color. For the assay, 0.5 mL of extract was mixed with 0.5 mL of 0.5 N Folin–Ciocalteu reagent and 2 mL of 17% sodium carbonate (Na_2CO_3) solution. The remaining reaction conditions and spectrophotometric measurement parameters, including the final reaction volume, incubation time, and wavelength, should be specified according to the protocol actually used.

Enzyme extraction and activity assays

Enzyme extraction

Enzymes were extracted under cold conditions using an extraction buffer appropriate for each enzyme. A 0.5-g sample of fresh tissue was ground in the corresponding extraction buffer. During grinding, polyvinylpyrrolidone (PVP) and polyethylene glycol (PEG) were added to the extraction medium to minimize interactions between phenolic compounds and enzyme proteins. β -Mercaptoethanol was also added to limit oxidation. Sodium thiosulfate and glycerol were added to the extraction medium to contribute to enzyme stabilization and protection.

Preparation of the purified enzyme extract

The enzyme extract was subjected to a purification step using DOWEX 2, an anion-exchange resin. The resin was homogenized with the enzyme extract, and the mixture was then incubated under cold conditions for 30 min. The mixture was subsequently centrifuged at 5,000 rpm for 10 min. The resulting supernatant constituted the purified enzyme extract, which was used for the determination of enzyme activities.

Phenylalanine ammonia-lyase (PAL) activity

Phenylalanine ammonia-lyase (PAL) activity was determined according to the method described by Zucker (1965), with modifications and adaptations to the plant material used in this study. The extraction buffer consisted of 0.1 M sodium phosphate buffer (pH 7.5). Following centrifugation of the homogenate, the resulting supernatant was subjected to purification using DOWEX 2 according to the procedure described above. The supernatant obtained after this purification step constituted the purified enzyme extract used to determine PAL activity.

Peroxidase activity

Peroxidase activity was determined according to the method described by Santimone (1973). Sodium phosphate buffer (0.1 M, pH 7.5) was used as the extraction buffer. Following centrifugation of the homogenate, the resulting supernatant was subjected to purification using DOWEX 2 according to the procedure described above. The supernatant obtained after this purification step constituted the purified enzyme extract used to determine peroxidase activity.

Parameters evaluated

The parameters evaluated during the vivo-plant production process were the time to root emergence (TAR), time to bud emergence (TAB), and mean number of buds produced (NBP).

The time to root emergence (TAR) was defined as the time required for the first root to emerge after the explant was placed in the propagation chamber. It was calculated as the ratio of the sum of the times required for root emergence to the total number of explants that produced roots.

$$\text{TAR} = \frac{\text{Sum of the times required for root emergence}}{\text{Total number of explants that produced roots}}$$

The time to bud emergence (TAB) was defined as the time required for the first bud to emerge after the explant was placed in the propagation chamber. It was calculated as the ratio of the sum of the times required for bud emergence to the total number of explants that produced buds.

TAB

$$= \frac{\text{Sum of the times required for bud emergence}}{\text{Total number of explants that produced buds}}$$

The mean number of buds produced (NBP) was determined at the end of the observation period. It was calculated as the ratio of the total number of buds produced to the total number of explants that produced buds.

$$\text{NBP} = \frac{\text{Total number of buds produced}}{\text{Total number of explants that produced buds}}$$

Experimental design and statistical analysis

The suckers were arranged in each propagation chamber according to a completely randomized design (CRD). The experiment was conducted with three independent replicates, with 48 suckers per treatment in each replicate, corresponding to a total of 144 suckers per treatment across the three replicates.

All statistical analyses were performed using STATISTICA version 7.1. Analysis of variance (ANOVA) was performed to determine whether significant differences existed among the factors studied at the 5% significance level. When the ANOVA indicated significant differences, means were separated using the Newman-Keuls multiple range test at the 5% significance level.

RESULTS

Bud induction and production from explants in the propagation chamber

Morphological development of explants and bud emergence

After 7–8 days in the propagation chamber, numerous roots of varying lengths emerged at the surface of the substrate (Figures 2A and 2B). The first buds emerged at the substrate surface 14 days after the explants were placed in the propagation chamber. Initially, these buds exhibited lanceolate leaflets and a whitish appearance, and subsequently developed a greenish coloration. They then developed into leafy shoots of varying sizes (Figures 2C and 2D).

No apparent morphological differences were observed between the leafy shoots produced from non-dehydrated control suckers and those produced from suckers dehydrated for two weeks. Bud emergence at the substrate surface occurred progressively throughout the observation period.





Figure 2. Formation of roots, buds, and leafy shoots from suckers placed in the propagation chamber. (A) non-dehydrated control sucker; (B) sucker dehydrated for two weeks; (C) whitish buds; (D) leafy shoots.

Time to root and bud emergence and mean number of buds produced

The mean times to root emergence were 8.12 days for non-dehydrated suckers (T1) and 7.87 days for suckers dehydrated for two weeks (T2), with no significant difference between the two treatments (P

= 0.54) (Table 1). Similarly, the mean time to bud emergence did not differ significantly between treatments (P = 0.65). In contrast, the mean number of buds produced per explant differed significantly between the two treatments (P = 0.041) and was significantly higher in T2 than in T1 (Table 1).

Table 1. Mean number of buds produced and mean times to root and bud emergence according to treatment

Treatment	Mean time to root emergence (days)	Mean time to bud emergence (days)	Mean number of buds per explant
T1	8.12±0.56 ^a	17.01±2.56 ^a	34.66±21.06 ^b
T2	7.87±0.86 ^a	15.56±3.42 ^a	58.28±31.04 ^a
<i>P-value</i>	0.54	0.65	0.041

Dans une même colonne, les moyennes suivies de la même lettre ne diffèrent pas significativement au seuil de 5 % selon le test de Newman-Keuls. Les valeurs sont exprimées en moyenne ± écart-type.

Bud production kinetics

Regardless of the type of sucker used, bud formation was observed from the third week after the explants were placed in the propagation chamber (Figure 3). At the third week (S3), the number of buds produced was approximately 62 buds per explant in the non-dehydrated suckers and 75 buds per explant in the suckers dehydrated for two weeks. This number subsequently decreased to approximately 49 and 60

buds per explant at S4, and then to 38 and 52 buds per explant at S5, respectively, for non-dehydrated and dehydrated suckers. At S6, the corresponding values were approximately 23 and 45 buds per explant, respectively. Thus, throughout the S3–S6 period, the number of buds produced from the dehydrated suckers remained higher than that obtained from the non-dehydrated suckers.

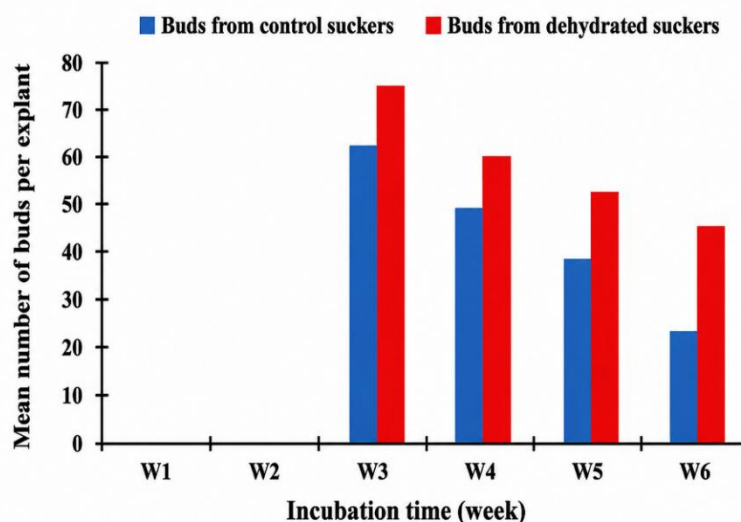


Figure 3. Kinetics of bud production in plantain during incubation of explants in the propagation chamber. W1–W6: 1–6 weeks of incubation;

Changes in biochemical compound contents

During the experiment, total sugar, total protein, and total phenolic compound contents were determined weekly throughout the bud formation process.

Changes in total sugar content

Figure 4 shows the changes in total sugar content of explants derived from non-dehydrated suckers (TEM) and suckers dehydrated for two weeks (DESH). In explants derived from non-dehydrated

suckers, total sugar content decreased during the first week of culture and then progressively increased over the following weeks. In explants derived from dehydrated suckers, total sugar content was higher than that recorded in explants derived from non-dehydrated suckers at the different stages of bud formation. Total sugar content decreased until the third week of culture and then increased following bud emergence.

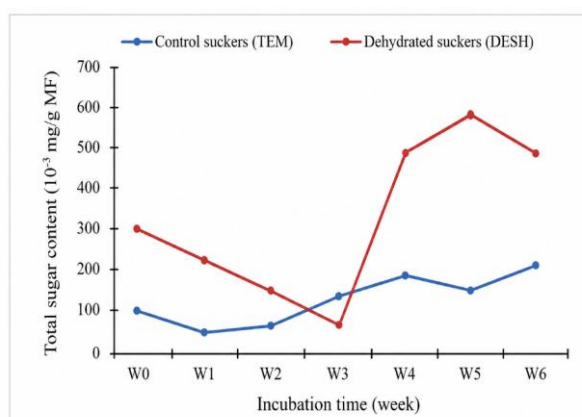


Figure 4. Changes in total sugar content of explants during bud production. W0: initial sampling; W1–W6: 1–6 weeks of incubation; TEM: non-dehydrated control suckers; DESH: suckers dehydrated for two weeks

Changes in total protein content

The changes in total protein content of the dehydrated and non-dehydrated suckers used for bud induction are presented in Figure 5. In non-dehydrated suckers (TEM), total protein content remained relatively stable until the fifth week of incubation, and then increased between the fifth and sixth weeks. In dehydrated suckers (DESH), total protein content was high at the beginning of the experiment and then decreased until the second week. It subsequently increased between the second and fourth weeks before decreasing again until the end of the observation period.

Changes in total phenolic content

Figure 6 presents the changes in total phenolic content of explants derived from non-dehydrated suckers and suckers dehydrated for two weeks. From W0 to W3, both treatments showed a similar pattern in total phenolic content, characterized by an increase from W0 to W1, followed by a decrease until W3. In non-dehydrated suckers (TEM), total phenolic content then increased sharply at W4, decreased at W5, and increased again at W6. In dehydrated suckers (DESH), total phenolic content increased from W3 to W6, reaching approximately 0.95×10^{-3} mg/g FW at W6.

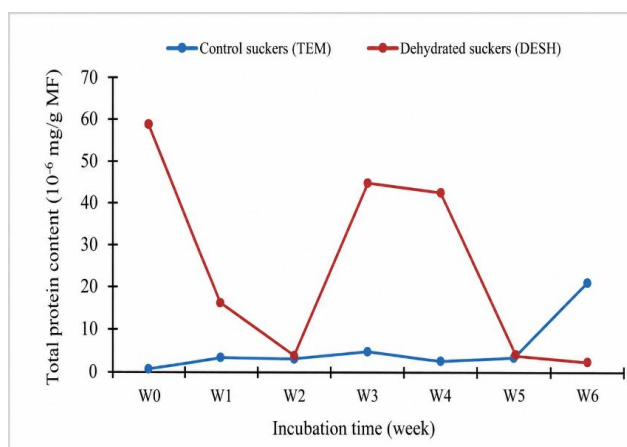


Figure 5. Changes in total protein content of plantain explants during bud production. W0: initial sampling; W1–W6: 1–6 weeks of incubation; TEM: non-dehydrated control suckers; DESH: suckers dehydrated for two weeks

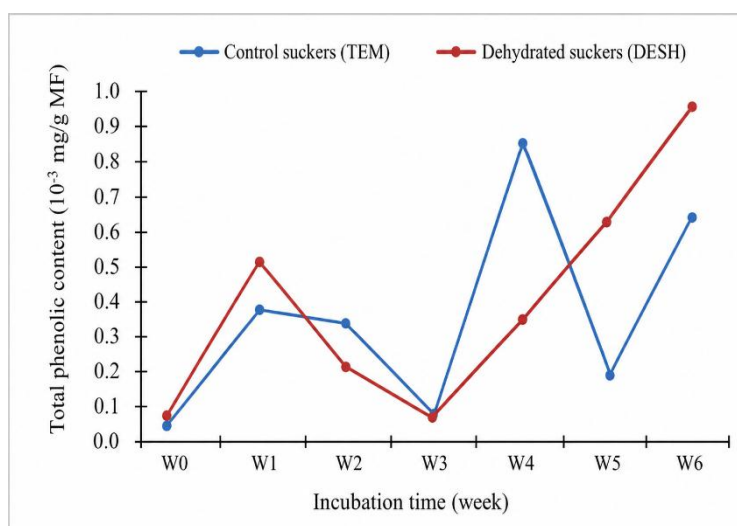


Figure 6. Changes in total phenolic content of plantain explants during bud production. W0: initial sampling; W1–W6: 1–6 weeks of incubation; TEM: non-dehydrated control suckers; DESH: suckers dehydrated for two weeks

Changes in enzymatic activities

The enzymatic activities evaluated during the bud production process included phenylalanine ammonia-lyase (PAL) and peroxidases (POX).

Changes in phenylalanine ammonia-lyase (PAL) activity

Figure 7 presents the changes in phenylalanine ammonia-lyase (PAL) activity during bud production. PAL activity showed different patterns between the two treatments throughout the incubation period. In non-dehydrated suckers (TEM), PAL activity increased sharply from W0 to W1, where it reached its maximum (approximately 7.1×10^{-3} mmol/min/g FW), and then decreased until W3 before increasing again at W4. It subsequently decreased at W5 and W6. In dehydrated suckers (DESH), PAL activity increased slightly from W0 to W1, then increased sharply at W2, where it reached its maximum (approximately 7.0×10^{-3} mmol/min/g FW), before decreasing until W4. It then increased progressively until W6. At W3, PAL activities were similar between the two treatments. At W4 and W5, PAL activity was higher in TEM than in DESH, whereas at W6, it was higher in DESH.

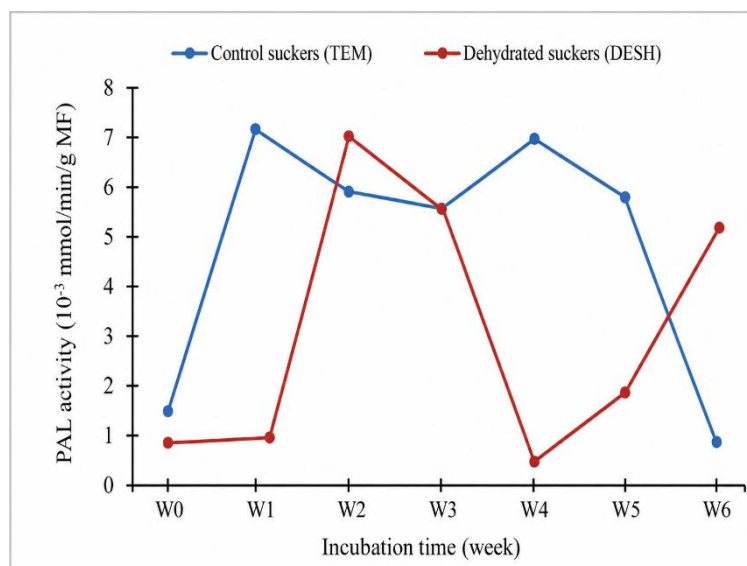


Figure 7. Changes in phenylalanine ammonia-lyase (PAL) activity of plantain explants during bud production. W0: initial sampling; W1–W6: 1–6 weeks of incubation; TEM: non-dehydrated control suckers; DESH: suckers dehydrated for two weeks

Changes in peroxidase (POX) activity

Peroxidase (POX) activity varied differently between non-dehydrated suckers (TEM) and suckers

dehydrated for two weeks (DESH) throughout the incubation period (Figure 8). In non-dehydrated suckers (TEM), POX activity remained relatively

stable between W0 and W1, then increased at W2, reaching its maximum (approximately 2.9×10^{-3} mmol/min/g FW). It then decreased at W3, increased again at W4, and subsequently decreased progressively until W6. In dehydrated suckers (DESH), POX activity increased sharply from W0 to W1 and then decreased at W2. It subsequently increased at W3, reaching approximately 2.3×10^{-3}

mmol/min/g FW, then decreased slightly at W4 and W5 before increasing again at W6.

POX activity was higher in TEM from W0 to W2, whereas it was higher in DESH at W3. The values for the two treatments were similar at W4 and W5. At W6, POX activity was again higher in DESH than in TEM.

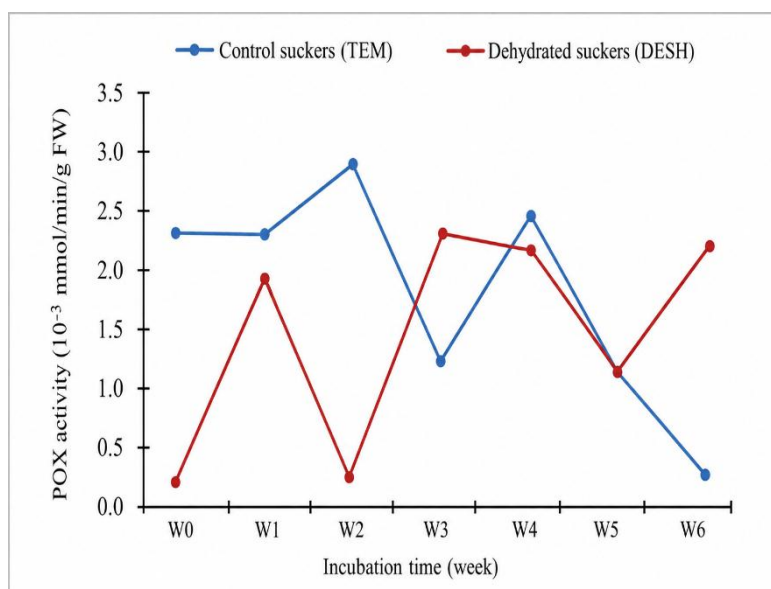


Figure 8. Changes in peroxidase (POX) activity of plantain explants during bud production. W0: initial sampling; W1–W6: 1–6 weeks of incubation; TEM: non-dehydrated control suckers; DESH: suckers dehydrated for two weeks

DISCUSSION

Root emergence was observed 7–8 days after the explants were placed in the propagation chamber, regardless of the treatment applied. Placement in the propagation chamber, followed by tissue rehydration, likely promoted the resumption of metabolic activities required for growth. In dehydrated vegetative organs, rehydration may be accompanied by a resumption of respiratory activity and de novo synthesis of enzymes involved in reserve mobilization (Boyé et al., 2010). In the present study, however, the mean number of buds produced differed significantly between the two treatments, with higher bud production observed in suckers dehydrated for two weeks.

Shoots produced from dehydrated suckers exhibited morphological characteristics comparable to those obtained from non-dehydrated suckers. However, dehydrated suckers produced a significantly higher mean number of buds. This difference suggests that prior dehydration may promote bud induction and/or production under the experimental conditions used. This response could be related to changes in the physiological status of the tissues and the subsequent mobilization of reserve compounds during rehydration. The rhizome of plantain suckers constitutes a reserve organ rich in starch (Turquin,

1998), whose mobilization could contribute to providing the carbon substrates required for renewed growth and the formation of new buds.

The dynamics of total sugar content differed between treatments. In non-dehydrated suckers, sugar content decreased slightly during the first week and then increased during the following weeks. In dehydrated suckers, sugar content decreased until the third week, corresponding to the onset of bud production, and then increased. This initial decrease could be associated with the utilization of available sugars during the resumption of metabolic activity and the formation of new organs. Carbohydrates serve as respiratory substrates and carbon sources required for cell growth (Murphy, 1993). The increase observed after bud emergence could subsequently correspond to progressive sugar accumulation in the tissues. However, this increase cannot be directly attributed to photosynthetic activity, as photosynthesis was not assessed in the present study.

Protein content also showed different patterns depending on the treatment. In dehydrated suckers, a high protein content was observed at the beginning of the experiment, followed by a decrease until the second week and then an increase between the second and fourth weeks. This pattern could reflect changes in protein metabolism during rehydration and new organ formation. Proteins serve as

functional molecules and nitrogen reserves that can be mobilized during the resumption of growth. An increase in protein synthesis is also associated with the release from dormancy in certain vegetative organs (Apelbaum, 1984; Macdonald & Osborne, 1988). In non-dehydrated suckers, protein content remained relatively stable until the fifth week and then increased in the sixth week. These results indicate that protein dynamics depended on both the treatment and the developmental stage of the explants. However, the data obtained do not allow determination of whether these variations resulted primarily from protein synthesis or degradation.

Phenolic compounds also varied during incubation. From W0 to W3, both treatments showed a similar pattern, characterized by an initial increase followed by a decrease. From W3 onward, the patterns diverged: in dehydrated suckers, phenolic compound content increased progressively until W6, whereas in non-dehydrated suckers, it showed successive fluctuations. Phenolic compounds constitute an important class of plant secondary metabolites, and their accumulation can be affected by various environmental and physiological factors. The increase observed at the beginning of incubation could be associated with the response of tissues to the stresses imposed during explant preparation and placement in the propagation chamber. Subsequent variations could correspond to changes in their synthesis, utilization, or transformation during bud development. Zhao et al. (2004) showed that different signaling pathways can regulate secondary metabolite production in response to external stimuli. The more consistent accumulation of phenolic compounds in dehydrated suckers from W3 onward could therefore reflect a different metabolic response between the two treatments.

Phenylalanine ammonia-lyase (PAL) activity, a key enzyme in the phenylpropanoid pathway, also varied during incubation. In non-dehydrated suckers, PAL activity reached an initial maximum at W1, then decreased until W3 before increasing again at W4. In dehydrated suckers, the maximum was reached at W2, followed by a decrease until W4 and a subsequent increase until W6. These patterns indicate that PAL activity varied according to both treatment and developmental stage. PAL catalyzes the entry of phenylalanine into the phenylpropanoid biosynthetic pathway and may contribute to the regulation of phenolic compound accumulation (Zucker, 1965; Heller & Forkman, 1993). The partial correspondence between changes in PAL activity and those in total phenolic content suggests a relationship between PAL activity and phenolic metabolism during bud production. However, measurement of PAL activity alone does not establish a direct causal relationship with phenolic compound accumulation, which also depends on other enzymes and metabolic pathways.

Peroxidase (POX) activity fluctuated during incubation in both treatments. In non-dehydrated suckers, activity reached its maximum at W2, whereas in dehydrated suckers, the highest level was observed at W3. The patterns also differed between treatments throughout the observation period, with higher activity in non-dehydrated suckers from W0 to W2 and higher activity in dehydrated suckers at W3 and W6. Peroxidases participate in several physiological processes, including oxidation–reduction reactions, responses to stress, and processes associated with plant tissue development (Santimone, 1973). The fluctuations observed may therefore reflect changes in enzymatic activity associated with the different developmental stages of the explants. Nevertheless, POX activity alone does not allow its use as a direct indicator of sucker degradation or preservation.

CONCLUSION

This study showed that prior dehydration of plantain suckers of the ‘Corne 1’ cultivar was associated with increased bud production during macropropagation. Suckers dehydrated for two weeks produced a significantly higher mean number of buds than non-dehydrated suckers, with no apparent morphological differences between the shoots produced. Bud formation was also accompanied by temporal changes in total sugar, protein, and phenolic compound contents, as well as in PAL and POX activities. These patterns differed between dehydrated and non-dehydrated suckers, indicating differences in their biochemical and enzymatic dynamics during bud production. Overall, the findings suggest that prior dehydration could be a useful approach for increasing bud production during plantain macropropagation and provide initial insights into the biochemical and enzymatic changes associated with this process. Further studies involving starch reserves, individual sugars, plant hormones, specific proteins, and additional enzymes involved in reserve mobilization would help clarify the physiological mechanisms underlying the response to dehydration.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest that could have influenced the work reported in this manuscript.

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RESEARCH ARTICLE

EFFECTS OF AQUEOUS LEAF EXTRACTS OF *AGERATUM CONYZOIDES* AND *BIDENS PILOSA* ON GERMINATION AND EARLY GROWTH OF TWO LOCAL RICE GENOTYPES FROM KOHIMA, NAGALAND

Pudumi Rongpipi, Zenwang Konyak* and Keneizoulhou Kesiezie

Department of Botany, Kohima Science College, Jotsoma 797002, India

Email: zenwangnaga@gmail.com

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Abstract: Weeds may affect crop establishment not only through competition for resources but also through chemical interactions. This study evaluated the effects of aqueous leaf extracts of *Ageratum conyzoides* L. and *Bidens pilosa* L. on seed germination and early seedling growth of two locally cultivated rice genotypes, *Thevür* and *Rosolha*. A factorial experiment was conducted in a completely randomized design with three replications. Root length (RL), shoot length (SL), root-to-shoot ratio (R/S), seedling vigor index (SVI), total seedling length (TSL), mean germination time (MGT) and germination percentage (GP) were evaluated. Extract concentration significantly affected SL, RL, R/S, SVI, TSL and MGT ($P < 0.001$), whereas GP was not significantly affected. *Rosolha* showed greater RL, R/S and SVI than *Thevür* ($P < 0.001$). Across weed species, seedlings exposed to *B. pilosa* had greater RL, R/S and SVI than those exposed to *A. conyzoides*, indicating stronger growth inhibition by *A. conyzoides* under the conditions tested. Significant genotype $\times$ concentration interactions were observed for SL ($P < 0.001$), TSL ($P = 0.002$) and MGT ($P = 0.020$), with reductions in growth and greater delays in germination at higher concentrations being more pronounced in *Thevür*. A significant weed species $\times$ concentration interaction was also observed for MGT ($P = 0.042$). Overall, the responses of the two rice genotypes to aqueous leaf extracts depended strongly on extract concentration, with *Thevür* showing greater reductions in early seedling growth at higher concentrations.

Keywords: Angami, Allelopathy, Wet terrace rice, Weeds, Aqueous leaf extract

INTRODUCTION

Weeds compete with crops for light, water, nutrients and space, thereby constraining crop production (Kaur *et al.*, 2018). Weed competition can reduce rice yield and may result in yield losses of about 50-60% in puddled transplanted rice and 70-80% in direct-seeded rice (Dass *et al.*, 2017). Crop losses associated with weeds are not attributable solely to competition for resources but may also involve weed-crop signalling and plant-plant interactions (Horvath *et al.*, 2023). One such form of interaction is allelopathy where secondary compounds produced by plants influence neighbouring plants. These allelochemicals are usually released from plant tissues through the processes such as leaching, volatilization, root exudation and decomposition of plant residues (Weston and Duke, 2003; Singh *et al.*, 2021). The effects of allelochemicals are not uniform and depend on the sensitivity of the plant species and concentration of the allelochemicals (Liu *et al.*, 2025). Depending on these factors, allelochemicals can either inhibit or sometimes at low concentrations stimulate seed germination and seedling growth (An *et al.*, 2005).

Several studies using bioassays and controlled growth experiments have reported the allelopathic potential of *A. conyzoides* L. and its inhibitory effects on the growth and development of several crop plants (Kong *et al.*, 2004; Singh *et al.*, 2003). Likewise, *B. pilosa* L. has been reported to possess allelopathic properties, with bioassays and root-exudate experiments which demonstrated inhibitory effects on other plants (Khanh *et al.*, 2009). Its allelopathic activity has also been investigated in relation to weed management and rice production, where the extracts suppressed the germination, growth and density of paddy weeds (Krumsri *et al.*, 2015). Collectively, these studies indicate that both *A. conyzoides* and *B. pilosa* have the potential to influence the germination and early growth of neighbouring plants through allelopathic interactions. Aqueous weed extracts are commonly used in laboratory bioassays to evaluate the potential phytotoxic effects of donor plants on seed germination and early seedling growth, with previous studies demonstrating that their effects on rice vary depending on weed species, plant parts used, and extract concentrations (Chopra *et al.*, 2017; Lalbiakdika *et al.*, 2022).

Rice is widely cultivated under traditional wet-terraced systems in parts of Kohima district,

*Corresponding Author

Nagaland. A field survey on these fields recorded 22 weed species representing 19 genera and 12 families, demonstrating the diversity of weeds associated with the local rice-growing system (Konyak *et al.*, 2022). Information on the possible allelopathic effects of locally occurring weeds on locally cultivated rice genotypes in this system remains limited. Therefore, this study evaluated the effects of aqueous leaf extracts of *A. conyzoides* and *B. pilosa* at different concentrations on seed germination and early seedling growth of two locally cultivated rice genotypes, *Thevür* and *Rosolha*, under controlled laboratory conditions.

MATERIALS AND METHODS

Fresh leaves of *A. conyzoides* and *B. pilosa*, along with seeds of the two rice genotypes, *Thevür* and *Rosolha*, were collected from Jotsoma Village, Kohima, Nagaland. The weed leaves were thoroughly rinsed with tap water, followed by several washes with sterile distilled water to remove adhering debris. The samples were spread on blotting paper and oven-dried at 48°C until completely dry and then ground into a fine powder. Subsequently, 15 g of powdered leaf material was suspended in 150 mL of distilled water and extracted for 24 h with continuous shaking (Chopra *et al.*, 2017). The extract was then filtered through Whatman No. 1 filter paper. The resulting undiluted extract, corresponding to 100 mg mL⁻¹ of dry leaf material was designated as the stock extract (100% extract). Working concentrations of 25%, 50% and 75% were prepared by diluting the stock extract with distilled water, following Khasabulli *et al.* (2018). The prepared aqueous extracts were stored at 4°C until use (Yusoff and Ismail, 2015) and, in the present experiment, were used within 24 h of preparation.

The rice seeds were soaked overnight and surface-sterilized by immersing them in 5% NaOCl for 20 minutes, followed by several rinses with sterilized distilled water (Wu *et al.*, 2010). The experiment was arranged as a 2 × 2 × 5 factorial in a completely randomized design, comprising two rice genotypes (*Thevür* and *Rosolha*), two weed species (*A. conyzoides* and *B. pilosa*) and five concentration levels (control, 25%, 50%, 75% and 100%). Each Petri dish was considered an independent experimental unit and three Petri dishes were used per genotype × weed × concentration combination. Ten sterilized seeds were placed in each 9 cm Petri dishes lined with sterile filter paper and treated with 5 mL of the respective aqueous extracts. For the control treatment, 5 mL of distilled water was added. The seeds were moistened daily with about 5 mL of the corresponding extract to maintain the moisture. The Petri dishes were then incubated at 25°C for germination (Masum *et al.*, 2016). Seeds were considered germinated when the radicle length

exceeded 2 mm (Haugland & Brandsaeter, 1996). The experiment was terminated after 15 days.

The length of the roots and shoots of all the germinated seeds was measured. Total seedling length (TSL) was calculated as the sum of root length and shoot length. Germination percentage (GP) and mean germination time (MGT) were then calculated using the method described by Ellis and Roberts (1981).

The germination percentage was calculated as:

$$GP = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

Mean germination time (MGT) was calculated using the following formula:

$$MGT = \frac{\sum(Dn)}{\sum n}$$

where *n* represents the number of seeds that germinated on a particular day, and *D* represents the number of days elapsed from the beginning of the germination experiment. Germination was recorded at 24-h intervals throughout the experimental period.

Seedling vigor index (SVI) was calculated according to the method described by Abdul-Baki and Anderson (1973), using the following formula:

$$SVI = \text{Germination percentage} \times \text{total seedling length}$$

Data analysis

The experimental data were analysed using the mixed procedure with *P* ≤ 0.05 in SAS (SAS® Studio, SAS Institute Inc., 2024). Genotype (G), weeds (W), concentration (C) and their interactions were considered fixed effects, whereas replication was treated as random effects. Least-squares means (LS-means) were estimated for the effects and interactions of interest, and pairwise comparisons were performed using the Tukey-Kramer adjustment at *P* ≤ 0.05.

RESULTS AND DISCUSSIONS

Effects of genotype, weed species and extract concentration on seedling growth and germination

The effects of rice genotype, weed species, extract concentration and their interactions on shoot length (SL), root length (RL), root-to-shoot ratio (R/S), seedling vigor index (SVI), total seedling length (TSL), mean germination time (MGT) and germination percentage (GP) are presented in Table 1. Rice genotype significantly affected RL, R/S and SVI (*P* < 0.001), while weed species significantly affected RL (*P* = 0.025), R/S (*P* = 0.018), SVI (*P* = 0.024) and MGT (*P* = 0.005). Extract concentration significantly affected SL, RL, R/S, SVI, TSL and MGT (*P* < 0.001), but not GP (*P* = 0.419). Significant genotype × concentration (G × C) interactions were observed for SL (*P* < 0.001), TSL (*P* = 0.002) and MGT (*P* = 0.020), whereas the weed species ×

concentration ($W \times C$) interaction was significant only for MGT ($P = 0.042$). The other two and three-way interactions were not significant. Germination percentage was not significantly affected by genotype, weed species, extract concentration or their interactions (Table 1).

The significant genotype effect on RL, R/S and SVI indicated differences in early seedling development between the two rice genotypes. *Rosolha* recorded significantly greater RL, R/S and SVI than Thevür, with mean RL of 5.30 cm compared with 4.00 cm, R/S of 0.65 compared with 0.49, and SVI of 537.95 compared with 406.84, respectively (Table 2). The higher R/S value in *Rosolha* indicates relatively greater root development in relation to shoot development. Similar differential responses of root and shoot growth under *B. pilosa* exposure have been reported by Hsueh *et al.* (2020), who observed alterations in shoot-to-root allocation in *Cyperus rotundus* seedlings exposed to *B. pilosa* var. *radiata* residues. However, the present experiment does not provide evidence for the physiological mechanisms underlying the genotype differences.

Weed species also significantly affected RL, R/S and SVI. Seedlings exposed to *B. pilosa* recorded greater RL, R/S and SVI than those exposed to *A. conyzoides*, with RL values of 5.00 and 4.30 cm, R/S values of 0.62 and 0.53, and SVI values of 507.82 and 436.97, respectively (Table 2). These differences correspond to approximately 16-18% higher values under *B. pilosa* across the three traits. Thus, under the conditions of the present experiment, *A. conyzoides* exerted the stronger inhibitory effect on early seedling development. This observation is consistent with Zumbado-Rodríguez *et al.* (2026), who reported that *A. conyzoides* leaf extracts reduced the initial growth of *Oryza sativa*, particularly plant length, although seedling emergence and biomass were not significantly affected. Their findings provide comparative evidence for the inhibitory potential of *A. conyzoides* on early rice growth, while the stronger inhibition relative to *B. pilosa* observed in the present study is specific to the experimental conditions used here.

Extract concentration affected wide range of the measured early seedling-growth traits. Root length decreased from 7.37 cm in the control to 1.41 cm at 100% extract, corresponding to an 80.9% reduction (Table 2). The control and 25% treatments did not differ significantly, whereas significant reductions

were observed at 50%, 75% and 100% concentrations. This concentration-dependent reduction in RL indicates that substantial inhibition of root growth occurred mainly at higher extract concentrations. The observed pattern is consistent with Bhatt *et al.* (2001), who reported increasing phytotoxicity of *A. conyzoides* and *B. pilosa* extracts with increasing concentration, and with Negi *et al.* (2020), who observed concentration-related reductions in rice root length following exposure to *A. conyzoides* extracts.

Root-to-shoot ratio declined from 0.94 in the control to 0.26 at 100% extract, representing a 72.3% reduction. The control differed significantly from the 25% treatment, whereas some adjacent concentration levels did not differ significantly from one another (Table 2). This indicates that the response was not characterized by a statistically significant decline at every successive concentration. Nevertheless, the overall reduction in R/S suggests that increasing extract concentration altered the relative development of roots and shoots. Hsueh *et al.* (2020) similarly reported that *B. pilosa* residues could alter relative root and shoot development, supporting the possibility of differential effects of weed-derived compounds on seedling organs.

Seedling vigor index showed a similar concentration-dependent response, decreasing from 744.81 in the control to 145.97 at 100% extract, representing an 80.4% reduction. The control and 25% treatments were statistically similar, whereas SVI was significantly reduced at 50%, 75% and 100% concentrations (Table 2). Negi *et al.* (2020) also reported concentration-related reductions in rice seedling vigor following exposure to *A. conyzoides* extracts. The consistent decline in RL and SVI at higher concentrations indicates that increasing extract concentration adversely affected early seedling development, although the magnitude of response varied among the rice genotypes and weed species.

Interestingly, germination percentage was not significantly affected by any of the experimental factors, whereas MGT was significantly affected by genotype, weed species and extract concentration, with significant $G \times C$ and $W \times C$ interactions (Table 1). This indicates that the treatments influenced the timing of germination more clearly than the final proportion of seeds germinating.

Table 1. Analysis of variance for the effects of rice genotype, weed species, leaf extract concentrations and their interactions on germination and early seedling growth.

Source of variation	SL (cm)	RL (cm)	R/S	SVI	TSL (cm)	MGT	GP
G	0.028*	<0.001***	<0.001***	<0.001***	<0.001***	0.206 ^{ns}	0.323 ^{ns}
W	0.311 ^{ns}	0.025*	0.018*	0.024*	0.393 ^{ns}	0.005**	0.323 ^{ns}
C	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	0.419 ^{ns}
G × W	0.221 ^{ns}	0.129 ^{ns}	0.200 ^{ns}	0.134 ^{ns}	0.105 ^{ns}	0.058 ^{ns}	0.323 ^{ns}
G × C	<0.001***	0.256 ^{ns}	0.592 ^{ns}	0.254 ^{ns}	0.002**	0.020*	0.419 ^{ns}
W × C	0.311 ^{ns}	0.520 ^{ns}	0.134 ^{ns}	0.537 ^{ns}	0.942 ^{ns}	0.042*	0.419 ^{ns}
G × W × C	0.078 ^{ns}	0.336 ^{ns}	0.716 ^{ns}	0.347 ^{ns}	0.165 ^{ns}	0.563 ^{ns}	0.419 ^{ns}

G = Genotype, W = Weed, C = Concentration, SL = Shoot Length, RL = Root Length, R/S = root-to-shoot ratio, SVI = Seedling Vigor Index, TSL = Total Seedling Length, MGT = Mean Germination Time, GP: germination percentage, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$, ns = non-significant.

Genotype × concentration effects on shoot length and total seedling length

The significant G × C interactions for SL ($P < 0.001$) and TSL ($P = 0.002$) indicated that the two rice genotypes responded differently to increasing extract concentration. In *Rosolha*, SL was highest at 25% extract (9.89 cm) and declined to 7.13 cm at 100%. In *Thevür*, SL increased from 7.79 cm in the control to 10.89 cm at 25%, followed by a decline to 3.71 cm at 100% (Table 3). Thus, the reduction in SL at higher concentrations was more pronounced in *Thevür* than in *Rosolha*.

A similar pattern was observed for TSL. In *Rosolha*, TSL decreased from 16.88 cm at 25% extract to 9.29 cm at 100%, whereas in *Thevür* it decreased from 17.18 cm to 4.36 cm over the same concentration range (Table 3). The greater reduction in *Thevür* indicates that this genotype was more strongly affected by increasing extract concentration with respect to overall seedling growth. The significant G × C interaction confirms that the effect of concentration on SL and TSL depended on rice genotype.

The response across concentrations was non-monotonic. In both genotypes, relatively high SL and TSL values occurred at 25% extract, followed by reductions at higher concentrations. Negi et al. (2020) similarly reported variety-dependent responses of rice to *A. conyzoides* extracts, with the direction and magnitude of response varying with both rice variety and extract concentration. The higher-concentration reductions observed in the present study are also broadly consistent with the findings of Bandeira et al. (2024), who reported reductions in root and hypocotyl length with increasing concentrations of *B. pilosa* leaf extract in lettuce and chicory. Although the species used in those experiments differed from the present study, the results provide supporting evidence for concentration-dependent effects of *B. pilosa* extracts on early seedling development. The relatively high

SL and TSL observed at 25% in the present study therefore indicate a non-monotonic response, while the mechanism responsible for this pattern cannot be determined from the present measurements alone.

Taken together, the significant G × C interactions demonstrate that the response of rice seedlings to extract concentration differed between the two genotypes. The divergence was particularly evident at 75% and 100% extract, where *Thevür* showed substantially lower SL and TSL than *Rosolha*. Thus, genotype-specific responses became more apparent at higher extract concentrations.

Genotype × concentration and weed × concentration effects on mean germination time

Mean germination time was significantly affected by the G × C interaction ($P = 0.020$). In *Rosolha*, MGT increased from 2.17 days in the control to 4.00 days at 100% extract, whereas in *Thevür* it increased from 1.17 to 4.83 days. Significant genotype differences were observed at the control, 50%, 75% and 100% concentrations, with *Thevür* showing greater MGT than *Rosolha* at the higher concentrations (Fig. 1a). These results indicate that increasing extract concentration delayed germination and that the magnitude of this delay differed between the two rice genotypes.

The W × C interaction was also significant for MGT ($P = 0.042$). Under *A. conyzoides*, MGT increased from 1.67 days in the control to 5.33 days at 100% extract. Under *B. pilosa*, MGT increased from 1.67 to 3.50 days. The two weed species differed significantly only at 100%, when MGT was higher under *A. conyzoides* than under *B. pilosa* (5.33 versus 3.50 days) (Fig. 1b). The concentration-related delay in germination is consistent with Akter et al. (2023), who reported an increase in MGT of wheat treated with *A. conyzoides* root exudates. The present results therefore indicate that increasing extract concentration, particularly at 100%, delayed germination, with the magnitude of the response depending on both rice genotype and weed species.

Table 2. Effects of rice genotype, weed species and leaf-extract concentration on root length, root-to-shoot ratio and seedling vigor index of rice.

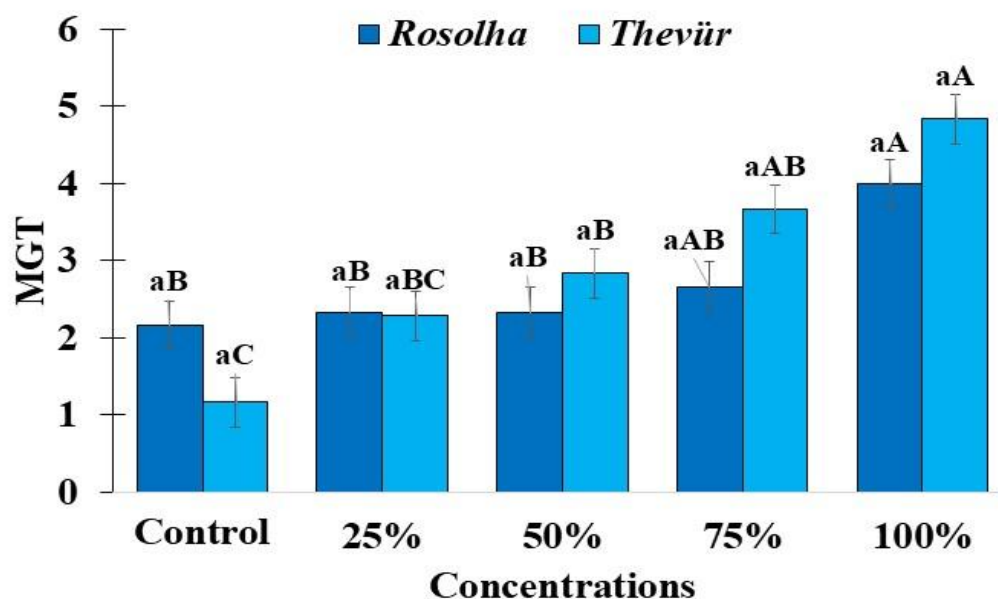
Factor	Level	RL (cm)	R/S	SVI
Genotype	<i>Rosolha</i>	5.30 ^a	0.65 ^a	537.95 ^a
	<i>Thevür</i>	4.00 ^b	0.49 ^b	406.84 ^b
Weed species	<i>Ageratum conyzoides</i>	4.30 ^b	0.53 ^b	436.97 ^b
	<i>Bidens pilosa</i>	5.00 ^a	0.62 ^a	507.82 ^a
Concentrations	Control	7.37 ^a	0.94 ^a	744.81 ^a
	25%	6.64 ^a	0.65 ^b	672.81 ^a
	50%	5.04 ^b	0.63 ^b	512.06 ^b
	75%	2.79 ^c	0.39 ^c	286.32 ^c
	100%	1.41 ^d	0.26 ^c	145.97 ^d

Values are LS-means. Within each factor and column, means followed by different lowercase letters are significantly different according to the Tukey-Kramer adjustment ($P \leq 0.05$). RL = root length; R/S = root-to-shoot ratio; SVI = seedling vigor index.

Table 3. Interactive effects of rice genotype and leaf-extract concentration on shoot length and total seedling length of rice

Genotype	Concentrations	SL (cm)	TSL (cm)
<i>Rosolha</i>	Control	7.93 ^{bcd}	15.98 ^{abc}
	25%	9.89 ^{ab}	16.88 ^{ab}
	50%	7.72 ^{cd}	12.98 ^{cd}
	75%	7.63 ^{cd}	11.66 ^{de}
	100%	7.13 ^{cd}	9.29 ^{ef}
<i>Thevür</i>	Control	7.79 ^{cd}	14.48 ^{abcd}
	25%	10.89 ^a	17.18 ^a
	50%	8.63 ^{bc}	13.45 ^{bcd}
	75%	6.12 ^d	7.68 ^{fg}
	100%	3.71 ^e	4.36 ^g

Values are LS-means. Within each column, means followed by different lowercase letters are significantly different according to the Tukey-Kramer adjustment ($P \leq 0.05$). SL = shoot length; TSL = total seedling length.

(a)

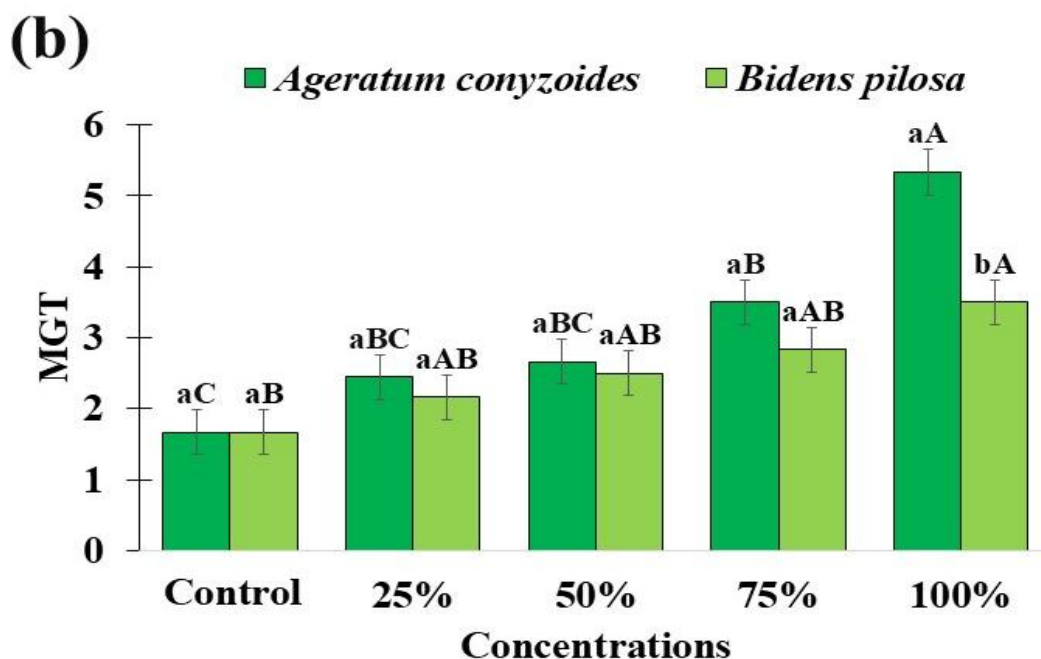


Fig. 1. Interactive effects of leaf-extract concentration with (a) rice genotype and (b) weed species on mean germination time (MGT) of rice. Bars represent LS-means $\pm$ SE. Different lowercase letters indicate significant differences among concentrations within the respective factor, while different uppercase letters indicate differences between genotypes or weed species at each concentration according to the Tukey-Kramer adjustment ($P \leq 0.05$).

CONCLUSIONS

The study demonstrated that aqueous leaf extracts of *A. conyzoides* and *B. pilosa* influenced early growth of the two rice genotypes in a concentration-dependent manner. Extract concentration significantly affected root length, root-to-shoot ratio, seedling vigor index, shoot length, total seedling length and mean germination time, whereas germination percentage was unaffected. *Rosolha* showed greater root development, root-to-shoot ratio and seedling vigor than Thevür. Significant genotype $\times$ concentration interactions for shoot length, total seedling length and mean germination time indicated genotype-dependent responses to increasing extract concentrations. *A. conyzoides* produced greater inhibition of RL, R/S and SVI than *B. pilosa* under the conditions of the experiment. Because the study was conducted using aqueous extracts under controlled laboratory conditions, further experiments using field-relevant exposure levels and plant residues are needed to determine the ecological relevance of these responses.

Credit Statement

Pudumi Rongpipi (PR): Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Validation, Visualization, Writing-original draft

Zenwang Konyak (ZK): Investigation, Data curation, Data analysis, Methodology, Validation, Writing-review & editing

Keneizoulhou Kesiezie (KK): Conceptualization, Supervision, Methodology, Validation, Writing-review & editing.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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RESEARCH ARTICLE

GREEN SYNTHESIS OF SILVER NANOPARTICLES USING *THUJA OCCIDENTALIS* L. LEAF EXTRACT: A COMPREHENSIVE STUDY OF ANTIMICROBIAL POTENTIAL

Rejitha, L.R.* and Gayatri Gireesh

Department of Botany, Mar Ivanios College, Nalanchira, Thiruvananthapuram-695015, India

Email: rejitha.lr@mic.ac.in

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Abstract: The increasing prevalence of antimicrobial resistance has necessitated the exploration of novel therapeutic agents from natural sources. This study investigates the green synthesis of silver nanoparticles (AgNPs) using aqueous leaf extract of *Thuja occidentalis* L. and evaluates their antimicrobial potential against pathogenic bacteria and fungi. The biosynthesis of AgNPs was confirmed by UV-Visible spectroscopy, revealing a characteristic surface plasmon resonance peak at 420 nm, with the rapid formation of stable nanoparticles observed within 20 minutes of reaction. The antibacterial activity, assessed using the agar well diffusion method, demonstrated significant concentration-dependent inhibition against *Pseudomonas aeruginosa*, with maximum zones of inhibition of 16 mm at 60 µg concentration, comparable to the standard antibiotic streptomycin (18 mm). However, *Staphylococcus aureus* exhibited resistance to all tested concentrations, indicating differential susceptibility between Gram-negative and Gram-positive bacteria. Antifungal evaluation revealed selective activity against *Candida albicans* with a zone of inhibition of 16 mm, while *Aspergillus niger* and *Phytophthora capsici* showed resistance. The study successfully demonstrates a simple, rapid, cost-effective, and environmentally friendly approach for synthesizing antimicrobial silver nanoparticles using *T. occidentalis* leaf extract. These findings validate the traditional medicinal use of *Thuja* and suggest its potential as a sustainable source for developing alternative antimicrobial agents. The green nanotechnology approach presented herein offers promising applications in biomedical fields, contributing to the global effort to combat antimicrobial resistance while promoting environmental sustainability.

Keywords: *Thuja occidentalis*, Silver nanoparticles, Green synthesis, Antimicrobial activity, UV-Vis spectroscopy

INTRODUCTION

The escalating global challenge of antimicrobial resistance has intensified the search for novel therapeutic agents from natural sources. Plant-derived bioactive substances have long served as repositories of medicinal compounds, offering significant potential for human health and combating various microbial diseases (Ateş *et al.*, 2003; Kumar *et al.*, 2006; Mathabe *et al.*, 2006). The bioactive constituents of plants, including tannins, flavonoids, saponins (Avato *et al.*, 2006), terpenoids (Funatogawa *et al.*, 2004), and alkaloids exhibit remarkable antimicrobial properties that have garnered increasing scientific attention in recent years. In the context of rising antibiotic resistance, medicinal plants represent promising resources for developing alternative antimicrobial agents capable of effectively controlling infectious diseases (Schulz *et al.*, 2001).

Thuja occidentalis L., commonly known as northern white-cedar and belonging to the family Cupressaceae, has been traditionally utilized for various medicinal applications. Its scaly leaves,

growing from fan-like branches, are rich in vitamin C, and the essential oil derived from the plant has been employed in cleansers, disinfectants, hair preparations, insecticides, liniment, room sprays, and soft soaps (Chambers, 1993). Historically, in the 19th century, *T. occidentalis* was widely used as an externally applied tincture or ointment for treating warts, ringworm, and thrush (David Hoffmann, 2003). The plant is extensively utilized in homeopathy and evidence-based phytotherapy, with its immunopharmacological potential demonstrated through numerous *in vitro* and *in vivo* studies showing immunostimulating and antiviral activities. A critical factor for *Thuja*'s use as a medicinal herb is its content of thujone, which is reported in fresh leaves (David Hoffmann, 2003). From *Thuja* (Esberitox) is used for the treatment of the common cold and as a standard antibiotic treatment for severe bacterial infections.

Nanotechnology has emerged as a transformative field, with silver nanoparticles (AgNPs) gaining particular prominence due to their unique physicochemical properties and broad-spectrum antimicrobial activities (Larue *et al.*, 2014; El-

*Corresponding Author

Chaghaby and Ahmad, 2011). The green synthesis of nanoparticles using plant extracts has become increasingly popular as an environmentally friendly alternative to conventional chemical methods, offering advantages of cost-effectiveness, biocompatibility, and reduced environmental toxicity (Thuesombat *et al.*, 2014; Mittal *et al.*, 2014). The biological synthesis of silver nanoparticles using plant extracts provides a convenient, extracellular, and environmentally safe approach that overcomes the limitations of conventional synthetic antimicrobial compounds (Ahmed *et al.*, 2014). Plants-mediated nanoparticles have demonstrated potential as remedies for various diseases, including malaria, cancer, HIV, hepatitis, and other acute conditions (Logeswari *et al.*, 2012).

The antimicrobial mechanism of silver nanoparticles involves disruption of cell membrane integrity, generation of reactive oxygen species, and interference with protein synthesis, leading to bacterial cell death (Sondi and Salopdek-Sondi, 2004; Kasthuri *et al.*, 2009). AgNPs can effectively disrupt the polymer subunits of cell membranes in pathogenic organisms, with the reciprocal action subsequently breaking the cell membrane and disturbing protein synthesis mechanisms in bacterial systems (Gong *et al.*, 2007; Li *et al.*, 2011). Studies have demonstrated that fungicidal mechanisms of biosynthesized metallic nanoparticles exhibit greater potential than commercial antibiotics such as fluconazole and amphotericin, with plant-derived AgNPs clearly showing membrane damage in *Candida* species and destruction of intercellular components (Logeswari *et al.*, 2012).

Various plant extracts have been explored for the synthesis of silver and gold nanoparticles, including *Azadirachta indica* (Shankar *et al.*, 2004), *Aloe vera* (Chandran *et al.*, 2006), *Bryophyllum* sp., *Cyperus* sp., *Hydrilla* sp., *Chenopodium*, *Cycas* (Jha and Prasad, 2010), *Acalypha indica* (Krishnaraj *et al.*, 2010), *Hibiscus rosa sinensis* (Philip *et al.*, 2010), *Ipomoea aquatica* (Roy and Barik, 2010), *Psidium guajava*, *Garcinia mangostana*, *Ocimum sanctum* (Philip and Unni, 2011), and *Cocos nucifera* (Roopan *et al.*, 2012). The presence of α -pinene, 3-carene, and α -cedrol in *Thuja* oil reveals that *Thuja* possesses potential for use in many medical applications, which is why the plant is also known as "The Tree of Life" (Tsiri *et al.*, 2009).

The present study aims to synthesize and characterize silver nanoparticles from the leaf extract of *Thuja occidentalis* and evaluate their antibacterial and antifungal potential using the agar well diffusion method. The antimicrobial activity is assessed against pathogenic bacteria *Pseudomonas aeruginosa* and *Staphylococcus aureus*, and fungi *Aspergillus niger*, *Candida albicans*, and *Phytophthora capsici* (Tsiri *et al.*, 2009; Mishra *et al.*, 1992). This research contributes to the growing body of knowledge on green nanotechnology and the development of

sustainable, eco-friendly antimicrobial agents from medicinal plant resources.

MATERIALS AND METHODS

Collection and Identification of Plant Material

Fresh leaves of *Thuja occidentalis* were collected, and taxonomic identification was performed by the Department of Botany, University of Kerala, Kariavattom. The plant specimens were authenticated, and voucher specimens were deposited for future reference, following standard herbarium procedures.

Preparation of Leaf Extract

Fresh leaves of *Thuja occidentalis* (10 g) were thoroughly washed with distilled water to remove surface contaminants and finely chopped to increase surface area for extraction. The chopped leaf material was placed in a 250 mL beaker containing 100 mL of distilled water and boiled at 80°C for 20 minutes. The resulting extract was filtered through Whatman No. 1 filter paper to remove particulate matter, and the filtrate was stored at 4°C for subsequent experiments (Mittal *et al.*, 2014; Krishnaraj *et al.*, 2010).

Synthesis of Silver Nanoparticles

Silver nitrate (AgNO_3) was obtained from Sigma-Aldrich and used without further purification. Double distilled water was employed throughout all reactions. All glassware was thoroughly washed with aqua regia ($\text{HCl}:\text{HNO}_3$, 3:1), rinsed with double distilled water, and dried in a hot air oven.

For the synthesis of silver nanoparticles, 100 mg of AgNO_3 was dissolved in 300 mL of double distilled water with continuous stirring until complete dissolution. Subsequently, 10 mL of the filtered plant extract was treated with the aqueous AgNO_3 solution in an Erlenmeyer flask and boiled for 10 minutes (Ahmed *et al.*, 2014; Roopan *et al.*, 2012). The formation of silver nanoparticles was indicated by a color change from pale yellow to brown-yellow, suggesting the reduction of aqueous silver ions by the plant extract constituents (Gilaki, 2010). The biosynthesized nanoparticles were isolated by repeated centrifugation at 5000 rpm for 5-10 cycles, and the resulting pellets were dissolved in acetone and air-dried for further characterization (Kasthuri *et al.*, 2009).

Characterization of Silver Nanoparticles

UV-Visible spectroscopy was employed to monitor the formation and stability of silver nanoparticles. Approximately 2 mL of the nanoparticle solution was placed in a quartz cuvette, and UV-Vis spectra were recorded at 400-600 nm using a UV-Vis spectrophotometer. The absorbance of the leaf extract alone was measured to compare the spectral differences and confirm nanoparticle formation (Krishnaraj *et al.*, 2010; Philip and Unni, 2011).

Microbial Cultures

Pure cultures of *Pseudomonas aeruginosa* and *Staphylococcus aureus* were obtained for antibacterial activity assessment. For antifungal studies, *Aspergillus niger*, *Candida albicans*, and the plant pathogen *Phytophthora capsici* were employed. All microbial cultures were maintained and subcultured according to standard microbiological protocols (Mishra *et al.*, 1992; Tsiri *et al.*, 2009). Antimicrobial studies were conducted at the Department of Biotechnology, University of Kerala, and the Department of Biotechnology, Mar Ivanios College.

Antibacterial Activity Assessment

The antibacterial activity of synthesized silver nanoparticles was evaluated using the agar well diffusion method (Alzoreky and Nakahara, 2003; Trombetta *et al.*, 2005). Mueller-Hinton Agar (MHA) medium was prepared by dissolving 38 g of commercially available nutrient medium (Hi Media) in 1000 mL of distilled water, followed by sterilization at 121°C for 15 minutes at 15 lbs pressure. The medium was dispensed into sterile petri plates and allowed to solidify.

Bacterial cultures were adjusted to 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL) and swabbed uniformly onto the surface of MHA plates. Wells of approximately 10 mm diameter were bored using a sterile cork borer, and varying concentrations (20-60 μ L) of silver nanoparticles dissolved in dimethylsulfoxide (DMSO) were added to each well. Streptomycin (1 mg/mL) served as the positive control, while DMSO alone was used as the negative control (Nisha *et al.*, 2017; Tsiri *et al.*, 2009). The plates were incubated at 37°C for 24 hours, and the zones of inhibition were measured in millimeters. All experiments were performed in triplicate.

Antifungal Activity Assessment

The antifungal activity of silver nanoparticles was evaluated using the agar well diffusion method against *Aspergillus niger*, *Candida albicans*, and

Phytophthora capsici (Mishra *et al.*, 1992). Potato Dextrose Agar (PDA) was prepared and sterilized according to standard protocols. Fungal cultures were inoculated onto PDA plates, and wells of 10 mm diameter were bored. Silver nanoparticle suspension (100 μ L) was added to each well, and mancozeb (ethylene-bis-dithiocarbamate) was used as the positive control. Plates were incubated at 28°C for 48-72 hours, and zones of inhibition were measured (Tsiri *et al.*, 2009).

RESULTS

Biosynthesis and Characterization of Silver Nanoparticles

The green synthesis of silver nanoparticles using *Thuja occidentalis* leaf extract was confirmed by the characteristic color change from pale yellow to dark brown upon addition of silver nitrate solution. This color transformation, observed within 20 minutes of reaction, resulted from the excitation of surface plasmon resonance vibrations in the silver nanoparticles. The biosynthesized nanoparticles were successfully recovered by centrifugation and stored for further characterization and antimicrobial studies. UV-Visible spectroscopic analysis revealed a characteristic absorbance peak at 420 nm with an absorbance value of 1.061, confirming the formation of silver nanoparticles (Fig 1). The control solution containing silver nitrate without plant extract showed no color change and exhibited an absorbance maximum at 340 nm, specific for silver nitrate solution. The spectral difference between the leaf extract alone and the extract with silver nanoparticles was noted as 0.859, confirming the bioreduction of silver ions. The rapid reduction of silver ions and formation of stable nanoparticles occurred within 20 minutes, with no evidence of aggregation, indicating effective stabilization by phytochemicals present in the leaf extract.

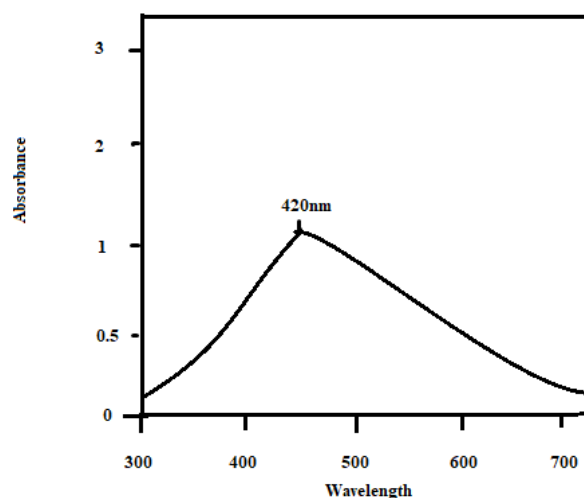


Fig 1: Absorbance peak at 420nm

Microscopic examination of the synthesized nanoparticles revealed the presence of discrete particles, confirming successful biosynthesis. The stability and morphological characteristics of the nanoparticles suggest potential applications in various biomedical fields.

Antibacterial Activity

The antibacterial activity of biosynthesized silver nanoparticles was evaluated against *Pseudomonas aeruginosa* and *Staphylococcus aureus* using the agar well diffusion method. The results demonstrated concentration-dependent antibacterial activity, with increasing zones of inhibition corresponding to higher concentrations of silver nanoparticles (Table 1, Fig 2)

Table 1: Antibacterial Activity of Silver Nanoparticles from *Thuja occidentalis*

Concentration (μL)	<i>Pseudomonas aeruginosa</i> (Zone of Inhibition, mm)	<i>Staphylococcus aureus</i> (Zone of Inhibition, mm)
20	11	Resistant
40	13	Resistant
50	14	Resistant
60	16	Resistant
Streptomycin (1 mg/mL)	18	20

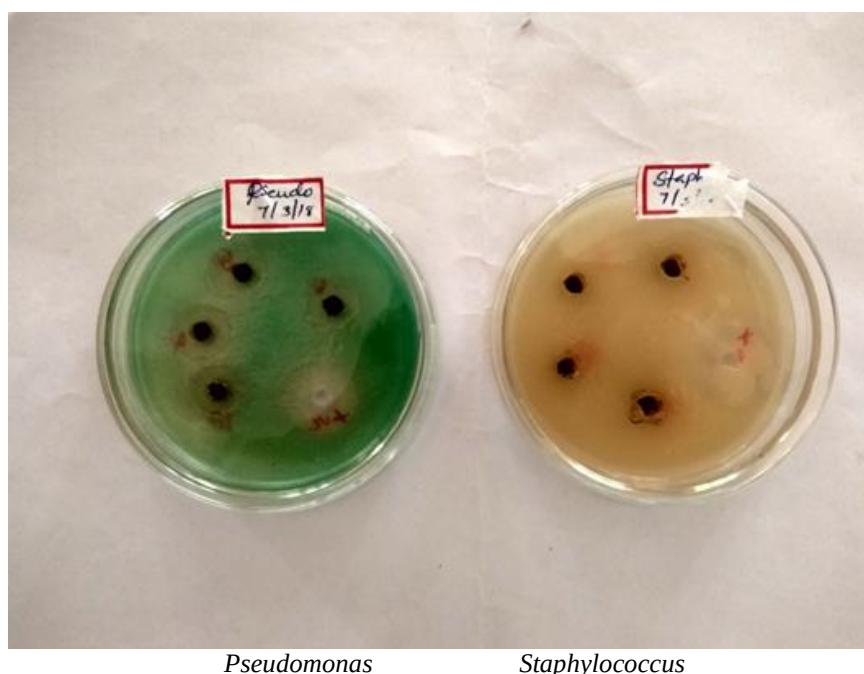


Fig 2: Diameter of zone of inhibition against the different test bacteria

Against *Pseudomonas aeruginosa*, the synthesized silver nanoparticles exhibited significant antibacterial activity with inhibition zones of 11 mm at 20 μL , 13 mm at 40 μL , 14 mm at 50 μL , and 16 mm at 60 μL concentration. The maximum zone of inhibition (16 mm) was observed at the highest tested concentration (60 μL). This activity was comparable to the positive control streptomycin, which produced an inhibition zone of 18 mm against *P. aeruginosa*. The results indicate that *P. aeruginosa* was susceptible to the synthesized silver nanoparticles, with activity approaching that of the standard antibiotic.

In contrast, *Staphylococcus aureus* demonstrated resistance to all tested concentrations of silver nanoparticles, showing no inhibition zones even at

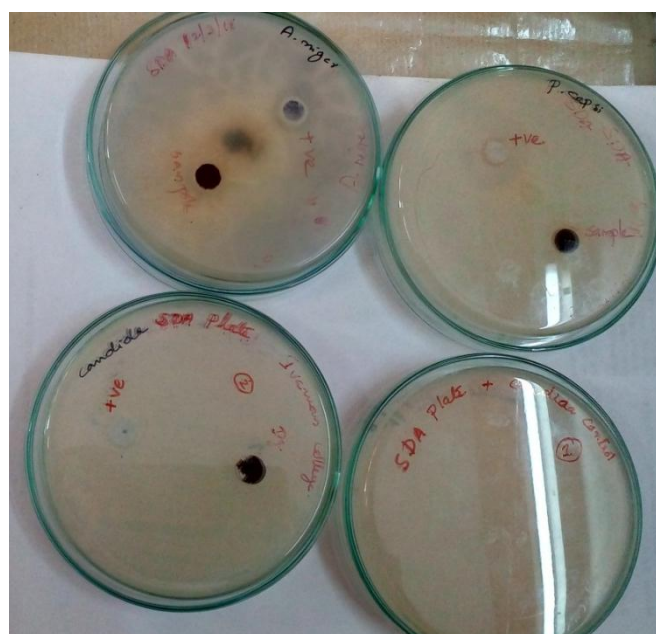
the highest concentration (60 μL). The positive control streptomycin exhibited a zone of inhibition of 20 mm against *S. aureus*, confirming the validity of the assay. This differential susceptibility suggests that *S. aureus* may possess inherent resistance mechanisms or cell wall characteristics that limit the penetration or efficacy of the biosynthesized silver nanoparticles.

Antifungal Activity

The antifungal activity of *T. occidentalis* silver nanoparticles was evaluated against *Aspergillus niger*, *Candida albicans*, and *Phytophthora capsici*. The results demonstrated selective antifungal activity, with significant inhibition observed against *Candida albicans* only (Table 2, Fig 3)

Table 2: Antifungal Activity of *Thuja occidentalis* Silver Nanoparticles

Extract/Concentration	<i>Aspergillus niger</i>	<i>Candida albicans</i>	<i>Phytophthora capsici</i>
AgNPs (100 μ L)	Resistant	16 mm	Resistant
Mancozeb (Positive Control)	Resistant	20 mm	20 mm

**Fig 3:** Diameter of zone of inhibition against the different test fungi

The biosynthesized silver nanoparticles exhibited a zone of inhibition of 16 mm against *Candida albicans*, while mancozeb (positive control) showed 20 mm inhibition against *C. albicans*. The nanoparticles showed no antifungal activity against *Aspergillus niger* or *Phytophthora capsici*, indicating selective antifungal efficacy. This differential activity may be attributed to variations in cell wall composition and susceptibility mechanisms among fungal species.

DISCUSSION

The present study successfully demonstrated the green synthesis of silver nanoparticles using aqueous leaf extract of *Thuja occidentalis* and evaluated their antimicrobial potential. The biosynthesis of metal nanoparticles using biological systems represents an expanding research area with significant implications for nanomedicine and antimicrobial therapy (Ahmed *et al.*, 2014; Mittal *et al.*, 2014). The green synthesis approach offers distinct advantages over conventional chemical methods, including environmental safety, cost-effectiveness, biocompatibility, and absence of toxic byproducts (Thuesombat *et al.*, 2014; El-Chaghaby and Ahmad, 2011).

The formation of silver nanoparticles was confirmed by the characteristic color change from pale yellow to dark brown upon addition of silver nitrate solution, attributed to the excitation of surface plasmon resonance vibrations (Savithramma *et al.*, 2011;

Gilaki, 2010). The absorption spectra showed a maximum peak at 420 nm, consistent with previous reports for silver nanoparticles synthesized using plant extracts (Krishnaraj *et al.*, 2010; Chandran *et al.*, 2006; Philip and Unni, 2011). The rapid reduction of silver ions within 20 minutes, without evidence of aggregation, suggests that the phytochemicals present in *T. occidentalis* leaf extract act as effective reducing and capping agents, providing stability to the nanoparticles (Ahmed *et al.*, 2014; Roopan *et al.*, 2012). This is consistent with earlier studies that have reported the presence of various phytochemicals including phenolics, flavonoids, terpenoids, and alkaloids in *Thuja* species, which can facilitate metal ion reduction and nanoparticle stabilization (David Hoffmann, 2003; Tsiri *et al.*, 2009).

The antibacterial activity of biosynthesized silver nanoparticles demonstrated concentration-dependent inhibition against *Pseudomonas aeruginosa*, with the maximum zone of inhibition of 16 mm at 60 μ g concentration. This activity was comparable to streptomycin (18 mm), indicating the potential of *T. occidentalis* AgNPs as effective antibacterial agents (Nisha *et al.*, 2017; Tsiri *et al.*, 2009). The antibacterial efficacy of green-synthesized AgNPs against *P. aeruginosa* has been previously reported for various plant extracts (Krishnaraj *et al.*, 2010; Shankar *et al.*, 2004; Roy and Barik, 2010). The mechanism of action likely involves the attachment of AgNPs to the bacterial cell membrane, penetration into the bacterial cell, and subsequent interaction

with phosphorous and sulfur-containing compounds such as DNA and proteins, leading to bacterial cell death (Sondi and Salopdek-Sondi, 2004; Gong *et al.*, 2007; Li *et al.*, 2011). Furthermore, AgNPs can induce the generation of reactive oxygen species, resulting in oxidative stress and cell damage (Kasthuri *et al.*, 2009).

Pseudomonas aeruginosa, a Gram-negative bacterium, is among the main agents in nosocomial infections, contributing to longer hospital stays and mortality. Among Gram-negative bacteria, *P. aeruginosa* is highly susceptible to genetic modifications leading to resistance to antimicrobials and consequent complications in impaired or immunocompromised patients. Due to its ability to survive in harsh environments, *P. aeruginosa* is known as one of the most important agents in hospital infections. The significant activity observed against *P. aeruginosa* in this study is particularly noteworthy and supports the potential application of *T. occidentalis* AgNPs in combating hospital-acquired infections.

Interestingly, *Staphylococcus aureus* showed resistance to all tested concentrations of the synthesized nanoparticles. This differential susceptibility between Gram-negative *P. aeruginosa* and Gram-positive *S. aureus* may be attributed to differences in cell wall structure and composition (Gong *et al.*, 2007; Li *et al.*, 2011). Gram-positive bacteria possess a thick peptidoglycan layer that may act as a barrier to nanoparticle penetration, whereas Gram-negative bacteria have an outer membrane that may be more susceptible to nanoparticle-induced damage (Sondi and Salopdek-Sondi, 2004). However, previous studies have reported variable susceptibility of *S. aureus* to AgNPs, suggesting that the efficacy may depend on nanoparticle size, shape, concentration, and the specific phytochemical composition of the plant extract used for synthesis (Krishnaraj *et al.*, 2010; Kasthuri *et al.*, 2009). It was reported that when streptomycin was supplemented with AgNPs, its antibacterial activity was increased up to 57.98%, suggesting potential synergistic effects (Gong *et al.*, 2007).

The antifungal activity assessment revealed that *T. occidentalis* AgNPs exhibited selective activity against *Candida albicans* with a zone of inhibition of 16 mm, while mancozeb showed 20 mm inhibition. *Candida albicans* is responsible for a number of major diseases as well as recent cases of resistance to the main antifungals (Logeswari *et al.*, 2012). Therefore, new substances should be researched as an alternative to combat such resistance. Ezzat (2001) studied the inhibition of *Candida albicans* in the plant extract of *Thuja* leaves and male cones and showed considerable inhibition for *C. albicans*, supporting our findings. Mishra *et al.* (1992) studied antifungal activity of aqueous leaf extract of *T. orientalis* and reported similar results. The fungicidal mechanism of biosynthesized metallic nanoparticles

has been shown to have more potential than commercial antibiotics such as fluconazole and amphotericin (Logeswari *et al.*, 2012). The plant-derived AgNPs have clearly shown membrane damage in *Candida* sp. and damage in fungal intercellular components, finally destroying cell functions (Logeswari *et al.*, 2012).

The resistance observed against *Aspergillus niger* and *Phytophthora capsici* in this study is consistent with previous reports. Studies of *Thuja* oil in control of biocontamination in libraries and archives storage areas showed that this oil had little inhibitory effect on the fungal combination (Tsiri *et al.*, 2009). However, Tsiri *et al.* (2009) tested the essential oils of four varieties of *Thuja*, including *T. occidentalis* 'globosa' and *T. occidentalis* 'aurea' against six Gram-positive and Gram-negative bacteria and three pathogenic fungi. They demonstrated significant antimicrobial activity, with this being especially powerful in the isolates of the mixture of α - and β -thujone and beierene. The presence of α -pinene, 3-carene, and α -cedrol in *Thuja* oil reveals that *Thuja* possesses potential for use in many medical applications (Tsiri *et al.*, 2009).

The findings of this study have significant implications for the development of alternative antimicrobial agents. The biosynthesis of silver nanoparticles using *T. occidentalis* leaf extract provides a simple, rapid, and economical route for nanoparticle synthesis (Ahmed *et al.*, 2014; Mittal *et al.*, 2014). The environmental sustainability of this approach, avoiding the use of hazardous and toxic solvents and waste, makes it particularly attractive for large-scale production and biomedical applications (Thuesombat *et al.*, 2014; El-Chaghaby and Ahmad, 2011). The antimicrobial potential demonstrated in this study supports the traditional use of *Thuja* as "The Tree of Life" and provides scientific validation for its therapeutic applications (David Hoffmann, 2003).

CONCLUSION

The present study successfully demonstrated the synthesis of silver nanoparticles using aqueous leaf extract of *Thuja occidentalis* through a green, cost-effective, and environmentally friendly approach. The formation of AgNPs was confirmed by UV-Vis spectroscopy, showing a characteristic absorption peak at 420 nm, and the nanoparticles exhibited excellent stability without aggregation. The biosynthesized silver nanoparticles demonstrated significant concentration-dependent antibacterial activity against *Pseudomonas aeruginosa*, with a maximum zone of inhibition of 16 mm at 60 μ g concentration, comparable to the standard antibiotic streptomycin. However, *Staphylococcus aureus* showed resistance to all tested concentrations, indicating differential susceptibility between Gram-negative and Gram-positive bacteria. In antifungal

studies, the AgNPs exhibited selective activity against *Candida albicans* (16 mm zone of inhibition), while showing no activity against *Aspergillus niger* or *Phytophthora capsici*. This research validates the traditional medicinal use of *Thuja occidentalis* and demonstrates its potential as a source for green synthesis of antimicrobial silver nanoparticles. The eco-friendly synthesis approach, utilizing readily available plant material, presents a sustainable alternative to conventional chemical methods for nanoparticle production. Future studies should focus on characterizing the phytochemical constituents responsible for nanoparticle synthesis, evaluating the cytotoxicity and biocompatibility of the synthesized nanoparticles, exploring synergistic effects with conventional antibiotics, and investigating the molecular mechanisms of antimicrobial action. The development of such plant-based nanomedicines could contribute significantly to addressing the global challenge of antimicrobial resistance while promoting sustainable and environmentally conscious approaches in nanotechnology.

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RESEARCH ARTICLE

SEASONAL OCCURRENCE OF MAJOR SUCKING INSECT-PESTS AND THEIR NATURAL ENEMIES ON OKRA (*ABELMOSCHUS ESCULENTUS* L. MOENCH) DURING SUMMERFanendra Kumar¹, G.P. Painkra*², K.L. Painkra³, P.K. Bhagat³, S.K. Sinha⁴ and Neelam Chouksey⁵¹Department of Entomology, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Raj Mohini Devi College of Agriculture and Research Station, Ambikapur (C.G.) India²Department of Entomology, Indira Gandhi Krishi Vishwavidyalaya, Raipur, College of Agriculture and Research Station, Shankargarh, Distt- Balrampur -Ramanujganj (C.G.) India³Department of Entomology, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Raj Mohini Devi College of Agriculture and Research Station, Ambikapur (C.G.) India⁴Department of Genetics and Plant Breeding, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Raj Mohini Devi College of Agriculture and Research Station, Ambikapur (C.G.) India⁵Department of Agricultural Statistics, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Raj Mohini Devi College of Agriculture and Research Station, Ambikapur (C.G.) IndiaEmail: fanendrasahu2000@gmail.com

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Abstract: The present study investigated the seasonal occurrence of major sucking insect pests of okra (*Abelmoschus esculentus* L. Moench) and their natural enemies during the summer. Field observations were recorded from seedling stage to crop maturity on leafhopper, whitefly, and aphid populations, along with associated natural enemies, and were correlated with weather parameters. Pest populations appeared soon after crop establishment and peaked during the mid-season, with leafhopper being the most predominant pest. Natural enemy activity closely followed pest population trends, while temperature and relative humidity significantly influenced population fluctuations. The findings highlight the importance of understanding seasonal pest dynamics for developing effective and eco-friendly Integrated Pest Management strategies.

Keywords: Aphid, Coccinellids Okra, Leafhopper, Whitefly

INTRODUCTION

Okra (*Abelmoschus esculentus* L. Moench), commonly known as bhindi or lady's finger due to the characteristic shape of its pods, is an important vegetable crop widely cultivated in tropical and subtropical regions of the world. In India, okra is grown extensively during the kharif and summer seasons. It is cultivated throughout the country, with major contributions from states such as Andhra Pradesh, West Bengal, Bihar, Gujarat, Odisha, Jharkhand, Maharashtra, Madhya Pradesh, Chhattisgarh, Assam, Uttar Pradesh, and Haryana. The crop occupies an area of about 532.66 thousand hectares with an annual production of approximately 6,513 thousand metric tonnes. In Maharashtra alone, okra is cultivated over an area of 8.91 thousand hectares, producing nearly 139.28 thousand tonnes annually (Anonymous, 2021).

Okra is attacked by several insect pests that cause significant yield losses. The major pests include shoot

and fruit borers, *Earias insulana* (Boisd.) and *Earias vittella* (Boisd.); leafhopper, *Amrasca biguttula biguttula* (Ishida); leaf roller, *Sylepta derogata* (Fab.); whitefly, *Bemisia tabaci* (Genn.); aphid, *Aphis gossypii* (Glover); and red spider mite, *Tetranychus cinnabarinus* (Boisd.). Among these, sucking pests such as leafhoppers, whiteflies, and aphids pose a major threat to okra production. The leafhopper (*A. biguttula biguttula*) is considered one of the most destructive pests, as it feeds on the sap from the undersurface of leaves, leading to leaf margin curling, chlorosis, and ultimately substantial reduction in yield.

Effective management of insect pests at the appropriate stage of crop growth is essential to ensure higher yield and better-quality produce. Although the use of chemical insecticides has contributed significantly to increased agricultural productivity, their indiscriminate and excessive use has resulted in several adverse effects, including the development of insecticide resistance, pest resurgence, secondary pest

*Corresponding Author

outbreaks, and accumulation of pesticide residues in the environment. Insecticide resistance has emerged as a major challenge in modern pest management, often leading to unexpected and severe crop losses.

Under the present scenario of climate change, insect pest dynamics are undergoing significant alterations due to variations in abiotic factors. Therefore, understanding the relationship between insect pest populations, their natural enemies, and prevailing weather parameters is crucial for predicting pest outbreaks and developing effective management strategies. Despite their importance, natural enemies have received relatively limited attention as biological control agents, highlighting the need for further research in this area.

MATERIALS AND METHODS

The experiment was conducted during summer at research-cum-Instructional farm of Raj Mohini Devi College Agriculture and Research Station, Ajirma, Ambikapur, District, Surguja (C.G.). The observations of leafhopper, aphids, and whiteflies were recorded on a per-plot basis. For each observation, five plants were randomly selected from each plot, and three leaves (one each from the bottom, middle, and top) of each plant were examined visually to assess pest infestation levels.

The seasonal occurrence of sucking insect-pests population was recorded weekly on five randomly selected plants per plot from their appearance until harvest. Populations of sucking pests (leafhopper, whitefly, and aphids) were counted. The population of natural enemies were also recorded from the same plants. Weekly pooled meteorological data (temperature, rainfall, morning and evening relative humidity) from RMD CARS, Ambikapur (C.G.) were correlated with pest incidence.

RESULTS AND DISCUSSION

Seasonal occurrence of aphids

Okra crop was sown during the March and recorded aphid populations ranging from 4.42 to 18.51 aphids per three leaves from five selected plants throughout the summer season. The initial presence of aphids was observed in the 12th Standard Meteorological Week (SMW), approximately 15 days after sowing, with a population of 4.42 aphids per three leaves per plant. The population gradually increased, reaching a peak of 18.91 aphids per three leaves per plant in the 17th SMW, corresponding to the last week of April. Interestingly, the aphid population remained relatively stable at 18.51 aphids per three leaves per plants until the end of the cropping season.

The present findings are consistent with those of Parasai and Shastri (2009), who observed the highest incidence of aphid populations during the first week of September, corresponding to the 37th SMW. Similarly, the results align with the observations of

Thara *et al.* (2019), who reported a gradual increase in aphid population, reaching its peak during the second week of October.

Seasonal occurrence of leafhopper

During the summer season, leafhopper infestation on okra began in the 12th SMW with a population of 7.56 leafhoppers per five leaves per plant. The population gradually increased, reaching 18.10 leafhoppers per five leaves per plant in the 17th SMW, followed by a temporary decline. However, it rose again and peaked in the 26th SMW, corresponding to the last week of June, with 21.45 leafhoppers per five leaves per plant. Throughout the season, the leafhopper population ranged from 7.56 to 21.45 leafhoppers per five leaves. Similar observations were reported by Mahmood *et al.* (1990), who noted that leafhoppers began emerging in June and remained active until the end of the cropping season. These findings are also supported by Hegde *et al.* (2004), who recorded peak populations during August to September. The present results are further in agreement with those of Potai and Chandrakar (2018), who reported that the major activity period of leafhoppers occurred between August and October, with a distinct peak during the 38th SMW. Likewise, Thara *et al.* (2019) observed that leafhopper incidence began in the second week after sowing and peaked in the fourth week of September, corresponding to the 39th SMW.

Seasonal occurrence of whitefly

During the summer, whitefly infestation on the okra crop was observed throughout the growing season. The initial infestation appeared in the second week of March, corresponding to the 11th SMW, with a mean population of 5.52 whiteflies per five leaves per plant. The population ranged from 5.52 to 10.67 whiteflies per five leaves per plant. The highest infestation was recorded in the 18th SMW, with a peak population of 10.67 whiteflies per five leaves per plant. Thereafter, a gradual decline in the population was observed.

The present findings are broadly in agreement with those of earlier researchers. Yadav and Singh (2013) and Aarwe *et al.* (2016) reported that the whitefly population peaked in August, during the 34th SMW. These results are also supported by Potai and Chandrakar (2018), who observed the initial appearance of the pest in the second week of August, with peak population levels recorded in the third week of September, corresponding to the 38th SMW.

Seasonal occurrence of coccinellids

During the summer season, predatory coccinellids were observed throughout the cropping period, co-existing with various pest species. The initial population was recorded in the 13th SMW (last week of March) at 1.82 coccinellids per five leaves per plant. Over the season, the population fluctuated between 1.82 and 4.51 coccinellids per five leaves per plant. The highest activity was recorded in the 22nd SMW, with a peak population of 4.51 coccinellids per five leaves.

The findings of Purohit *et al.* (2006) and Singh *et al.* (2013) also support the present results, as they recorded peak coccinellid activity during the first week of September and the second week of October, respectively. Similarly, the present observations are in

agree with those of Gaikwad *et al.* (2020), who reported the initial appearance of coccinellids during the 31st SMW, followed by a gradual increase in population in the subsequent weeks.

Table 1. Seasonal occurrence of sucking insect-pests and natural enemies on okra during summer.

SMW	Weather parameters					Population of sucking pests/plant			Population of natural enemies/plant
	Max. T. (°C)	Min. T. (°C)	RH-I (%)	RH-II (%)	Rainfall (mm)	Aphids	Leafhoppers	Whitefly	Coccinellids (Grubs/ Adults)
10	31.1	11.00	79.7	47.9	0.00	0.00	0.00	0.00	0.00
11	34.3	14.3	50.3	34.0	0.00	0.00	0.00	5.52	0.00
12	31.4	15.8	98.3	94.1	0.00	4.42	7.56	5.30	0.00
13	35.9	15.4	69.1	43.9	0.00	8.50	15.10	5.87	1.82
14	38.6	23.4	72.0	41.6	0.00	9.30	13.50	4.50	2.56
15	36.3	22.1	72.1	43.3	5.10	8.93	18.30	5.83	2.79
16	27.4	22.3	95	89.3	0.00	15.63	16.3	5.23	4.23
17	37.2	22.3	79.1	33.7	0.00	18.91	20.11	7.62	4.50
18	34.1	23.6	77.0	45.4	4.10	16.24	17.36	8.33	3.14
19	36.8	27.2	71.7	38.0	1.30	13.65	15.85	7.50	3.99
20	39.4	27.9	77.7	36.4	0.20	10.17	14.67	9.30	3.90
21	32.9	26.3	80.6	59.1	11.4	11.42	14.21	2.30	3.64
22	34.4	21.2	67.2	64.2	4.70	14.61	17.93	8.89	3.49
23	36.8	26.3	84.1	75.3	3.40	17.39	19.6	5.45	4.51
24	36.2	28.1	81.0	64.4	0.10	15.47	18.61	7.82	3.45
25	26.0	22.0	94.7	75.6	16.4	11.50	18.46	6.30	3.98
26	28.1	20.1	93.6	84.6	0.00	18.51	21.45	8.00	2.37

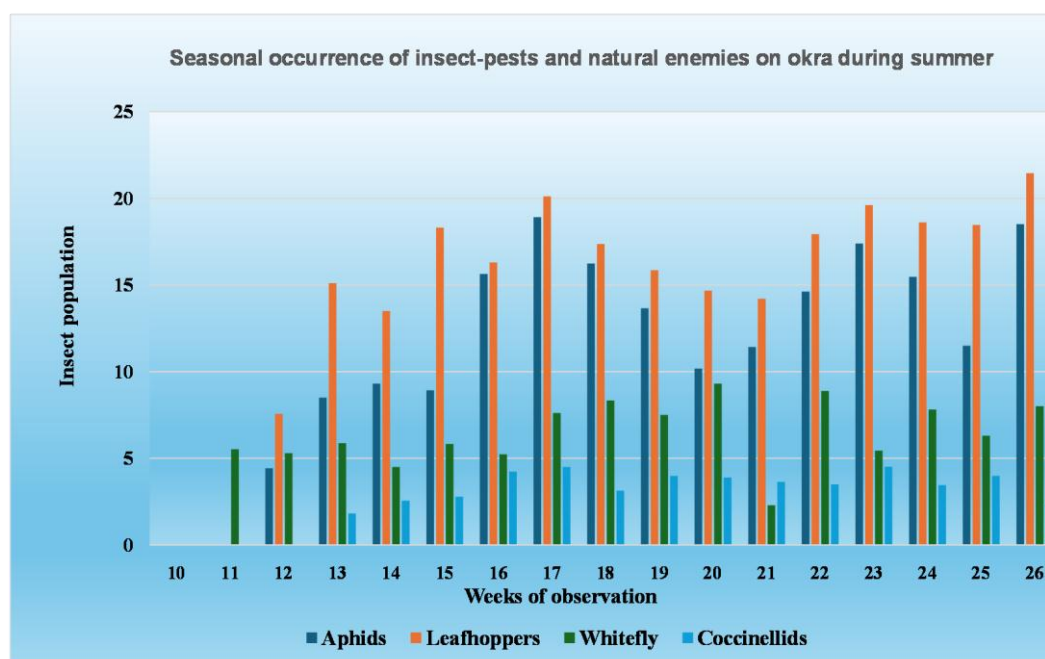


Fig. 1 Correlation of insect-pest and natural enemies with weather parameters during summer.

Correlation of insect-pests and natural enemies with weather parameters during summer.

Correlation coefficients between various weather parameters with the population of sucking pests viz., aphids, leafhoppers and whitefly and their natural enemies i.e., Coccinellids presented in Table 2.

Table 2. Correlation of insect-pests and natural enemies with weather parameters during summer.

Weather parameters	Correlation coefficient value			
	Aphid	Leafhopper	Whitefly	Coccinellid
Max. Temperature	0.425*	0.265	0.456*	0.160
Min. Temperature	-0.586**	-0.548*	-0.276	-0.209
Morning RH	-0.028	0.339	0.165	0.212
Evening RH	-0.575**	-0.213	-0.182	-0.026
Rain fall	-0.104	-0.056	0.114	-0.338

*5% level of significance $t_{15}=0.412$, **1% level of significance $t_{15}=0.558$

Correlation study investigated the relationship between maximum temperature and various insect populations. The positive correlation was observed between maximum temperature, leafhopper and Coccinellid. However, these correlations were not statistically significant, with correlation coefficients of 0.265 and 0.160 respectively. In contrast, a significant positive correlation (coefficient 0.425* and 0.456*) was observed between maximum temperature, aphid and whitefly populations.

Minimum temperature showed negative significant correlation with the aphid (-0.586**), leafhopper (-0.548*) population. In contrast, a non-significant and negative correlation was observed between min. temperature and whitefly (-0.276) and Coccinellid (-0.209) population.

The aphid population correlated negatively and non-significant with the morning relative humidity with correlation coefficient value -0.003. In contrast a positively non-significant correlation was observed between morning RH and leafhopper (0.035), whitefly (0.235) and coccinellid (0.075) population.

The negative correlation was recorded between evening relative humidity the Aphid and leafhopper which was negatively significant with correlation coefficient value (-0.609**) and (-0.472*) respectively. And negatively non-significant correlation observed between evening RH and whitefly (-0.286) and coccinellid (-0.185) population.

As regards rainfall positive non-significant correlation showed with the whitefly with correlation coefficient value (0.114). Whereas aphid, leafhopper and coccinellid with correlation coefficient value (-0.105), (-0.005) and (-0.109) respectively showed negative non-significant correlation with rainfall.

The correlation studies between pests, natural enemies and major weather parameters during summer season revealed that there was a negative correlation between aphid and minimum temperature, morning relative humidity, evening relative humidity and rainfall, while positive correlation with maximum temperature. Investigations by Dhandge *et al.* (2018) revealed that aphid population showed positive correlation with maximum temperature while, negative correlation

with morning and evening relative humidity. Potai and Chandrakar (2018) reported negative correlation between aphid and minimum temperature in confirmation with the present findings.

There was a positive correlation between leafhopper and maximum temperature. Whereas leafhopper showed negative correlation with minimum temperature, morning and evening RH and rainfall. The present findings are in close agreement with Ratanpara *et al.*, (1994) reported that minimum temperature showed negative correlation with leafhoppers. Another findings by Dhandge *et al.* (2018) revealed that pest population showed positive correlation with maximum temperature while, negative correlation with evening relative humidity.

Investigations revealed the positive correlation between whitefly and maximum temperature, minimum temperature, morning RH, evening RH and rainfall. Reports of Purohit *et al.* (2006) stated positive correlation of whitefly with all the abiotic factors. These results are in confirmation with the findings of Yadav and Singh (2013) who revealed positive correlation between whitefly and maximum temperature.

The present study revealed that there was a positive correlation between coccinellid and maximum temperature, minimum temperature, morning RH, evening RH and rainfall. Reports of Purohit *et al.* (2006) stated correlation between coccinellid and minimum temperature and rainfall in confirmation with the present findings. These findings are similar with reports by Dhaka and Pareek (2007) who revealed correlation between Coccinellid and evening relative humidity. Potai and Chandrakar (2018) reported positive correlation between aphid and morning relative humidity in confirmation with the present findings and Kumar *et al.* (2025) on seasonal occurrence of pests and natural enemies of okra.

CONCLUSION

During the summer of 2025, major sucking insect pests of okra, namely aphids, leafhoppers, and whiteflies and natural enemy were observed

throughout the crop growth period. Aphids appeared in the third week of March and peaked in the last week of April, while leafhoppers persisted throughout the season with maximum abundance in late June. Whiteflies showed moderate fluctuations, attaining peak activity in mid-May. Coccinellids, the predominant natural predators, appeared from late March and reached their highest population in mid-June. Correlation analysis indicated that aphid and leafhopper populations were positively influenced by maximum temperature but negatively affected by minimum temperature, relative humidity, and rainfall. Whitefly incidence was also positively correlated with maximum temperature. In contrast, coccinellid

populations showed positive associations with temperature, morning relative humidity, and rainfall, but a negative correlation with evening relative humidity.

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Fig.1. *Amrasca biguttula biguttula*



Fig. 2. *Bemisia tabaci*



Fig.3. *Aphis gossypii*



Fig. 4. *Coccinella septempunctata*

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SHORT COMMUNICATION

RELOCATION, *EX-SITU* CONSERVATION AND VEGETATIVE PROPAGATION OF *ANDROGRAPHIS CHENDURUNII* E.S.S. KUMAR, A.E.S. KHAN & S.G. GOPAL (ACANTHACEAE): A STENO-ENDEMIC SPECIES OF THE SOUTHERN WESTERN GHATS

Anusha, P. P., Rejitha Joseph, Shareef, S. M. and Santhosh Kumar E. S.*

Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI), Palode,
Karimancode P.O., Thiruvananthapuram-695562, Kerala, India
Email: santhoshkumares@gmail.com

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Abstract: *Andrographis chendurunii* E.S.S. Kumar, A.E.S. Khan & S.G. Gopal, a steno-endemic species of the southern Western Ghats in Kerala, was successfully relocated to the Shendurney Wildlife Sanctuary after a lapse of three decades. Notes on its ecology, habitat, ex-situ conservation and propagation techniques are provided as complementary measures towards the long-term recovery and conservation of this narrowly distributed endemic species.

Keywords: *Andrographis chendurunii*, Rediscovery, Steno-endemic, Ex-situ conservation, Vegetative propagation

INTRODUCTION

The genus *Andrographis* Wall. ex Nees (Acanthaceae) comprises 27 accepted species (Table-1) distributed mainly in tropical and subtropical Asia, with peninsular India, particularly the Western Ghats, representing an important centre of diversity and endemism (Mabberley, 2008; Tomar, 2024; POWO, 2026). Kerala supports 18 species of *Andrographis*, among which *A. chendurunii* is unique as the only species strictly endemic to the state. The species has an extremely restricted distribution in the southern Western Ghats, particularly the Shendurney Wildlife Sanctuary and

adjoining areas, making it a species of high phytogeographical and conservation significance. *Andrographis chendurunii* was first collected from Pandimotta, Shendurney Wildlife Sanctuary, in 1994 and described as a new species by Kumar *et al.* (2002). Since the original description was based only a once collected herbarium materials, the other information on its ecology, population status and reproductive biology remained limited. Despite repeated botanical surveys, the species was not relocated for nearly three decades, and habitat degradation at the type locality, including landslides, raised concerns about its possible local extinction.

Table 1. World checklist of the genus *Andrographis* Wall. ex Nees with distribution

Sl No	Species Name	Distribution
1	<i>Andrographis affinis</i> Nees	India
2	<i>Andrographis alata</i> (Vahl) Nees	S. India, Sri Lanka
3	<i>Andrographis atropurpurea</i> (Dennst.) Alston	India
4	<i>Andrographis beddomei</i> C.B.Clarke	India (Andhra Pradesh)
5	<i>Andrographis chendurunii</i> E.S.S.Kumar, A.E.S.Khan & S.G.Gopal	India (Kerala)
6	<i>Andrographis echioides</i> (L.) Nees	Indian Subcontinent to Myanmar
7	<i>Andrographis elongata</i> (Vahl) T.Anderson	S. India
8	<i>Andrographis explicata</i> (C.B.Clarke) Gamble	India
9	<i>Andrographis glandulosa</i> (Roth) Nees	S. India
10	<i>Andrographis gracilis</i> Nees	SW. India
11	<i>Andrographis lawsonii</i> Gamble	S. India
12	<i>Andrographis lineata</i> Nees	S. India
13	<i>Andrographis lobelioides</i> Wight	S. India
14	<i>Andrographis longipedunculata</i> (Sreem.) L.H.Cramer ex Karthik. & Moorthy	Indian Subcontinent to Myanmar

*Corresponding Author

15	<i>Andrographis macrobotrys</i> Nees	S. India, Sri Lanka
16	<i>Andrographis megamalayana</i> Gnanasek., Karupp. & G.V.S.Murthy	SW. India
17	<i>Andrographis neesiana</i> Wight	India
18	<i>Andrographis paniculata</i> (Burm.f.) Wall. ex Nees	Indian Subcontinent
19	<i>Andrographis producta</i> (C.B.Clarke) Gamble	India
20	<i>Andrographis rothii</i> C.B.Clarke	S. India
21	<i>Andrographis rotundifolia</i> (Sreem.) Sreem.	India (Tamil Nadu)
22	<i>Andrographis saxatilis</i> Raja Kullayisw. & Sarojin.	India (Andhra Pradesh)
23	<i>Andrographis serpyllifolia</i> (Rottler ex Vahl) Wight	S. India
24	<i>Andrographis stellulata</i> C.B.Clarke	S. India
25	<i>Andrographis stenophylla</i> C.B.Clarke	India
26	<i>Andrographis theniensis</i> Karupp. & Bharath	India (Tamil Nadu)
27	<i>Andrographis viscosula</i> Nees	S. India

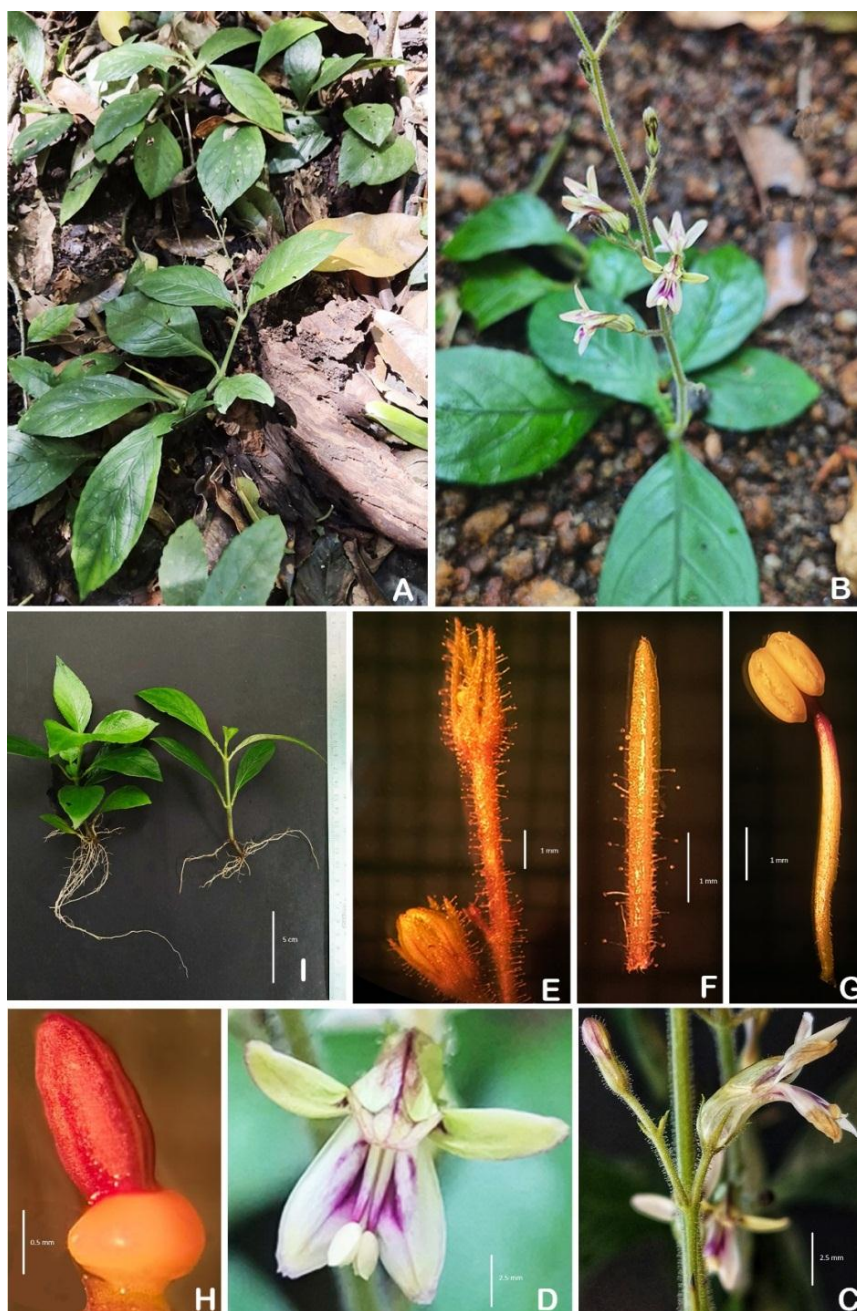


Figure 1. *Andrographis chendurunii*. A.&B. Habit. C Closeup view of a flower and bud. D. Flower-front view. E. Calyx. F. Bracteole. G. Stamen. H. Pistil. I. Rooted stem cuttings.

Relocation of *Andrographis chendurunii*

The Shendurney Wildlife Sanctuary is one of the most important centers of plant endemism in the southern Western Ghats, supporting 951 species of flowering plants belonging to 118 angiosperm families (Sasidharan, 1997). Continued botanical exploration has revealed several narrowly endemic taxa, including *Ardisia stonei* Sasidh. & Sivar., *Monoon shendurunii* (Basha & Sasidh.) B.Xue & R.M.K.Saunders [= *Polyalthia shendurunii* Basha & Sasidh.], *Ophiorrhiza shendurunii* A.E.S.Khan, E.S.S.Kumar & Pushp., *Humboldtia nairiana* E.S.S.Kumar, Shareef et Sabu, and several other species known only from this protected area or its immediate surroundings. *Andrographis chendurunii* is a narrowly endemic species confined to the southern Western Ghats of Kerala, was first collected from Pandimotta in the Shendurney Wildlife Sanctuary in 1994 and described as a new species by Kumar *et al.* (2002). The original description was based solely on herbarium specimens, and little was known about its ecology, population size, reproductive biology, or conservation status. Despite repeated botanical surveys over the following three decades, the species was not relocated, and extensive habitat degradation at the type locality due to landslides raised concerns about its possible local extinction.

Extensive floristic surveys conducted in previously unexplored evergreen forests of the sanctuary led to the finding of two healthy populations of *A. chendurunii* along the Palaruvi–Rosemala route and at Aryankavu. More than 50 mature individuals were recorded in both localities, growing in moist shaded stream banks beneath the evergreen forest canopy, indicating a strong preference for humid, low-light microhabitats. The main associated species are *Diospyros montana*, *Elatostemma lineolatum*, *Gomphostemma eriocarpon*, *Lea indica*, *Justicia* spp., *Pellionia heyneana*, etc. The rediscovery facilitated detailed ecological observations, the preparation of an emended morphological description, documentation of its phenology, and an assessment of its conservation status. A limited number of stem cuttings were collected with minimal disturbance to the natural populations and were successfully established in the Threatened Species Park of the Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI), where they are being maintained for ex situ conservation and vegetative propagation studies. The rediscovery of *A. chendurunii* further emphasizes the importance of sustained botanical exploration, long-term population monitoring, habitat protection, and integrated in situ and ex situ conservation measures to safeguard the unique and highly localized flora of this globally significant biodiversity hotspot.

Taxonomic description

Andrographis chendurunii E.S.S.Kumar, A.E.S.Khan & S.G.Gopal, Nordic J. Bot. 22: 683.

2002; Nayar *et al.*, Flow. Pl. Kerala 10.2006; Nayar *et al.*, Flow. Pl. Western Ghats 12. 2014. (Figure 1) Perennial herbs or subshrubs. Stems creeping, rooting at the lower nodes, quadrangular, glandular-pubescent along the furrows. Leaves 6–15 × 3.5–5.5 cm, broadly ovate-obovate to elliptic-oblancheolate; base acute to cuneate; apex acute to shortly acuminate; margin entire; lamina glabrous above, minutely pubescent along the nerves beneath; lateral nerves 5–7 pairs, arching; petiole 0.5–2 cm long. Inflorescence a profusely branched terminal panicle, 20–45 cm long; peduncle stout, minutely hairy; bracts and bracteoles subulate, up to 4 mm long; pedicels slender, 4–5 mm long, glandular-hairy. Calyx deeply 5-lobed almost to the base; lobes 2.5–3 mm long, glandular-hairy outside and glabrous within. Corolla bilabiate, greenish-white; tube up to 6.5 mm long, slightly curved, glabrous externally; lower lip of limb up to 6.5 × 4.5 mm; upper lip up to 7 × 8 mm; lobes ovate-oblong, up to 5 × 3.5 mm, obtuse to rounded at the apex, middle lobe larger than the laterals, with reddish-brown spots at the base. Stamens 2, exserted; filaments up to 7 mm long, not dilated at the base, minutely puberulous towards the base; anthers 2-celled, glabrous. Ovary ovate-oblong, 1.2–1.5 mm long; style slender, up to 7 mm long, sparsely hairy; stigma inconspicuously bifid at the apex. Capsule linear-obovate, up to 1.5 × 0.3 cm, dorsally compressed, acuminate, glabrous. Seeds 4–6, brown, to 1.8 × 1 mm, subquadrate, rugose, glabrous; retinacula acute.

Ex-situ Conservation and Propagation

Ex-situ conservation is widely recognized as an essential component of integrated biodiversity conservation strategies, particularly for rare and threatened species with restricted distributions (Heywood, 2017). Considering its unique morphology, rarity and highly restricted distribution, an ex-situ population of *Andrographis chendurunii* was established at the Threatened Species Park, JNTBGRI. The primary objective was to develop an insurance population to safeguard the species against potential extinction in the wild and to provide living material for long-term monitoring, reproductive studies, propagation research and future restoration programmes.

A limited number of stem cuttings were collected from healthy plants with minimal disturbance to natural populations and successfully established under ex-situ conditions. The establishment of healthy plants at JNTBGRI provides an important safeguard for the species and reduces dependence on wild populations for future research and conservation activities. Field observations indicated poor seed production and limited natural recruitment, suggesting that seed-based propagation may not be adequate for rapid multiplication. Therefore, stem-tip cuttings were evaluated as an alternative propagation method.

Healthy, mature shoots bearing two to three nodes were collected and planted in polybags containing a mixture of forest soil and well-decomposed cow dung. The cuttings were maintained under controlled mist-chamber conditions to promote rooting and subsequent establishment. The propagation trials resulted in 80–100% rooting, with healthy plantlets developing within 45–60 days. The high rooting percentage demonstrates that stem-tip cuttings are an efficient and reliable method for the rapid multiplication of *A. chendurunii*. This protocol can facilitate the establishment of genetically representative ex-situ collections and provide planting material for population reinforcement, habitat restoration and future reintroduction programmes.

DISCUSSION

The rediscovery of *A. chendurunii* after more than three decades demonstrates the continuing importance of systematic botanical exploration in the southern Western Ghats. Although the species had been described from a single collection made in 1994, the absence of subsequent records had raised concerns about its possible extinction. The discovery of viable populations within Shendurney Wildlife Sanctuary confirms the persistence of remnant populations in suitable microhabitats and highlights the role of protected areas as refugia for narrowly endemic species.

Fresh material obtained during the rediscovery substantially improved knowledge of the species' morphology, habitat and ecology. Its strong association with moist, shaded stream banks under evergreen forest canopies suggests a high degree of habitat specialisation, which may partly explain both its rarity and the difficulty in relocating it during earlier surveys.

The disappearance of the species from its type locality, together with its survival at other localities, emphasises the vulnerability of steno-endemic plants to habitat modification, floods, landslides and other stochastic events. Climate change and the increasing frequency of extreme climatic disturbances may further increase extinction risks for species with highly restricted distributions.

A major outcome of the study is the successful establishment of an ex-situ population and the development of an efficient vegetative propagation method. The 80–100% rooting success obtained from stem cuttings demonstrates that vegetative propagation can overcome the limitations associated with poor seed production and low natural recruitment. Propagated plants can serve as an insurance population and provide material for future population reinforcement, habitat restoration and reintroduction.

The occurrence of *A. chendurunii* together with other narrowly endemic species, including *Ardisia stonei*,

Monoon shendurunii, *Ophiorrhiza shendurunii* and *Humboldtia nairiana*, further demonstrates the importance of Shendurney Wildlife Sanctuary as a centre of plant endemism in the southern Western Ghats. Continued floristic surveys, ecological monitoring and integrated conservation planning are therefore essential.

CONCLUSION

The rediscovery of *Andrographis chendurunii* after more than three decades, together with the establishment of healthy ex-situ collections and successful vegetative propagation, represents an important advance in the conservation of this steno-endemic species of the southern Western Ghats. Stem-tip cuttings showed 80–100% rooting within 45–60 days, providing a practical method for rapid multiplication and production of planting material for conservation programmes. Long-term survival of the species will depend on integrating protection of its natural habitat with ex-situ conservation, population monitoring, propagation, genetic assessment and carefully planned recovery interventions.

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