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REVIEW ARTICLE

BRYOPHYTES AS CANDIDATES FOR ANTIBIOTIC DISCOVERY: A REVIEW WITH EMPHASIS ON INDIAN SPECIES

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Abstract: Bryophytes are second-largest group of terrestrial plants, comprising more than 24,000 species. They occupy nearly every terrestrial habitat except the sea. Although they lack the physical barriers of vascular plants, they are rarely damaged by herbivores or pathogens. This resilience is attributed to their production of a wide range of secondary metabolites that also protect them against abiotic stresses. Several extracts and isolated compounds from bryophytes have been reported to inhibit fungi and both Gram-negative and Gram-positive bacteria. Bryophytes have long been used medicinally in China for diseases of bacterial origin; their use elsewhere, however, is uncommon and the chemical and pharmacological profiles of most species remain undisclosed. In India, bryological research has traditionally concentrated on systematics and the antimicrobial research is comparatively recent. This review examines the antimicrobial evidence for bryophytes, with emphasis on studies of Indian species. The review also assesses what is required to move from crude-extract screening to validated leads. In vitro inhibition by crude extracts is an initial screening result, not evidence of therapeutic efficacy and bryophytes are therefore best considered as candidates for further study rather than as established antibiotic sources.

Keywords: Antimicrobial activity, Antibiotic, Bryophytes, Secondary metabolites

INTRODUCTION

Bryophytes are a diverse group of nonvascular plants, which reproduce by spores rather than seeds. They include three clades namely liverworts (Marchantiophyta), hornworts (Anthocerotophyta) and mosses (Bryophyta) and constitute the second largest group of terrestrial plants (Dziwak *et al*., 2022; Benek *et al.*, 2025). Bryophytes collectively comprise more than 24,000 species (Sabovljevic *et al*., 2016; Benek *et al*., 2025). However, the number of taxa continues to change due to synonymization of some species and discovery of new ones (Benek *et al*., 2025). The characteristic feature of the bryophyte life cycle is the alternation of generations with the dominance of the haploid gametophyte. The sporophyte generation is diploid and short lived (Horn *et al*., 2021).

Bryophyte species constitute a significant part of the plant biodiversity. They occur across a wide range of terrestrial ecosystems, with the exception of marine ones. They can be easily found in diverse environments, including tropical forests, Antarctica, deserts and locations that are difficult for many other plant groups to colonize. Tolerance of low-light conditions enables them to grow even in dark environments such as caves (Dziwak *et al*., 2022). Although bryophytes lack well-developed physical defense-barriers unlike higher plants, they are hardly ever damaged by herbivores or pathogens. This is due to their ability to produce a wide range of secondary metabolites which allow them to survive unfavorable abiotic conditions and also protect them against many biotic stresses (Dziwak *et al*., 2022). In fact, the long evolutionary history of bryophytes has given them a wide collection of secondary compounds that serve as chemical defenses. The production of these compounds costs them metabolic energy; some bryophytes are edible and others do not (Glime, 2006).

Secondary metabolites of bryophytes and their antimicrobial potential

Bryophytes are rarely used as food. However, some species have a long history of their medicinal use, particularly in China. Their medicinal use is, however, less common elsewhere (Vollar *et al*., 2018). The ability to tolerate extreme environmental conditions allows bryophytes to produce rich and unique secondary metabolites. More than 400 novel chemical compounds had been isolated and structurally elucidated from bryophytes, despite the fact that only about 3.2% of mosses and 8.8% of liverworts had been chemically investigated (Greeshma *et al*., 2017). This clearly indicates that the group is understudied in this area. Nevertheless, due to their rich secondary metabolite content, bryophytes have attracted scientific attention in recent years (Coteli *et al*., 2026). Studies to date have identified a wide range of phenolic compounds, terpenoids, fatty acid derivatives and alkaloids from

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this group of tiny plants. While, there is extensive terpenoid diversity, the alkaloids occur only rarely in bryophytes (Dziwak *et al*., 2022; Kulshrestha *et al*., 2022).

The available literature indicates that from a phytochemical and pharmacological perspective, vascular plants have been explored far more extensively than bryophytes. On the other hand, the chemical and pharmacological profiles of most bryophyte species remain poorly understood. Bryophytes grow in an unfavorable environment as the earliest land plants and inevitably biosynthesize secondary metabolites to counter biotic and abiotic stresses. These secondary metabolites not only defend them against plant competition, microbial attack and insect or animal predation, but also function in UV protection, drought tolerance and freezing survival (Xie & Lou, 2009). The protective roles of these metabolites are particularly relevant to antibiotic discovery. This is because many of the same compounds that shield bryophytes from stresses may also display antibacterial and antifungal activity in laboratory assays. These compounds and their bioactivity need to be studied in greater depth.

Table 1. Potential biological activities of major secondary-metabolite classes reported in bryophytes. (Adapted from Dziwak *et al*., 2022).

Secondary-metabolite class	Potential activities reported
Benzenoids	Antibacterial and antifungal; insecticidal and molluscicidal; anti-UV
Bibenzyls and bis(bibenzyls)	Antibacterial and antifungal; cytotoxic; insecticidal and molluscicidal; phytotoxic; drought tolerance
Fatty acid derivatives	Antibacterial and antifungal; cold tolerance
Flavonoids	Antibacterial and antifungal; phytotoxic; anti-UV
Phenylpropanoids	Insecticidal and molluscicidal; cold tolerance; anti-UV
Terpenes and terpenoids	Antibacterial and antifungal; cytotoxic; insecticidal and molluscicidal; phytotoxic

Consistent with this, bryophytes appear to be rich sources of antimicrobial compounds. This is evidenced by several studies reporting antibacterial and antifungal activity in extracts of many moss and liverwort species (Vollar *et al*., 2018; Benek *et al*., 2025). For instance, McCleary and co-workers (1960) reported antibacterial effects in *Anomodon rostratus*, *Orthotrichum rupestre*, and *Plagiomnium cuspidatum*. Later in 1979, Banerjee and Sen found that 56% of 52 species inhibited at least one of eight test bacteria. Antimicrobial activity has also been reported in species from the genera *Bazzania*, *Conocephalum*, *Diplophyllum*, *Dumortiera*, *Marchantia*, *Metzgeria*, *Lunularia*, *Pellia*, *Plagiochila*, *Porella*, *Radula* and *Riccardia* (Baek *et al*., 2004; Kim *et al*., 2007). Figueiredo and co-workers (2002) proposed that essential oils contribute to the antimicrobial activity of liverworts, although active compounds have also been identified in non-volatile extracts. For example, sacculatal from *Pellia endiviifolia* was reported to act against *Streptococcus mutans*, and lepidozenolide from *Lepidozia fauriana* showed activity against methicillin-resistant *Staphylococcus aureus*. Marchantins from *Marchantia* species have also shown activity against several pathogenic bacteria (Asakawa, 1990; Shu *et al*., 1994; Scher *et al*., 2002). Herbertane sesquiterpenoids were active against *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Bacillus subtilis* (Harinantenaina & Asakawa, 2004; Komala *et al*., 2010). Chlorinated bibenzyls from *Riccardia marginata* inhibited *Bacillus subtilis*, while diterpenoids from *Jungermannia exertifolia* showed activity against *Mycobacterium tuberculosis* H37Rv (Baek *et al*., 2004; Scher *et al*., 2010).

Olofin (2013) tested extracts from ten bryophytes (*Radula flacida*, *Cyatodium africanum*, *Frullania spongiosa*, *Thuidium gratum*, *Ectropothecium aeruginosum*, *Sematophyllum caespitosum*, *Stereophyllum radiculosum*, *Babula lamberenensis*, *Campylopus aspericuspis* and *Calymperes erosum*) and found that fractions obtained from *Cyatodium africanum* and *Radula flacida* exhibit antimicrobial activity against some pathogenic microorganisms. In the light of this, *Cyatodium africanum* and *Radula flacida* that are effective can be recommended as sources of antimicrobial agents in the treatment of some diseases caused by these etiological agents (Olofin, 2013). Bryophyte extracts and isolated compounds have also shown in vitro cytotoxic activity against cancer cell lines. For example, terpenoids and bibenzyl derivatives are among the compounds that are reported to be most active (Dey & Mukherjee, 2015). Christchellyn and fellow researchers (2020) tested ethanol extracts of a moss *Orthostichella rigida* against strains of Gram-negative (*Escherichia coli* and *Salmonella enteritidis*), Gram-positive bacteria (*Salmonella aureus* and *Listeria monocytogenes*) and yeast fungi (*Candida albicans* and *Candida neoformans*) and reported that all extracts presented antimicrobial activity. The extensive chemical diversity, alongside evidence that bryophyte cultures grown in vitro can produce substances with antibiotic activity, positions bryophytes as promising candidates for extensive antimicrobial screening (Olasoji *et al*., 2019).

Antimicrobial studies on Indian bryophytes

Studies from India also provide several examples of antimicrobial activity in bryophytes. These reported studies are mostly limited to crude extracts and a small number of species. Earlier Indian bryologists mainly focused on the systematics of bryophytes and therefore, no significant work in the past has investigated antimicrobial activity. However, few researchers, namely, Subramoniam & Subhisha (2005), Bodade *et al*., 2008 and Deora & Guhil (2014; 2018) have investigated the antimicrobial activities of mosses. In fact, mosses are the most studied, followed by thalloid and leafy liverworts (Shete *et al*., 2024). In a study conducted by Dhondiyal and co-workers (2013), extracts of the liverwort *Lunularia cruciata* from the Kumaon Himalaya were investigated for antibiotic potential. The results indicated that all the bacterial strains were sensitive to the tested extracts of *Lunularia cruciata* except the aqueous extract. Among the several species tested against various microbes, *Conocephalum conicum* showed the strongest bioactivity. In another investigation, mosses collected in the Eastern Himalaya, particularly *Syrrhopodon subconfertus*, inhibited both Gram-positive and Gram-negative bacteria and also exhibited antioxidative potential (Mukhopadhyay, 2013). Similarly, methanol or dichloromethane extracts of the liverworts *Plagiochasma intermedium*, *Asterella wallichiana* and *Targionia hypophylla* collected from Kolhapur district were reported to inhibit all bacterial growth in agar-well diffusion assays (Sawant & Karadge, 2010).

In another significant study, which also claims to be the first report on the antimicrobial activity of Indian mosses, extract of *Thuidium cymbifolium*, *Thuidium delicatulum*, *Bryum cellulare*, *Bryum argentium* and *Plagiochasma appendiculatum* showed antimicrobial activity against test microorganisms (Bodade *et al*., 2008). The results of this study showed that gram negative microorganisms are more sensitive than Gram positive microorganisms. Conventionally, we know that, antibiotics are generally more active against Gram positive than Gram negative bacteria. However, mosses studied by these workers were found to be active against Gram negative bacteria. This demonstrates the potential of selected bryophytes as antimicrobial agents. Bodade *et al*. (2008) also claim that while most research studies revealed no antifungal activity of Indian bryophytes, their study showed that all the selected fungi were more or less sensitive to all the bryophyte fractions. Many subsequent Indian studies have screened local bryophytes against bacterial and fungal pathogens. For instance, researchers in the Kumaun Himalaya in vitro tested extracts of the liverworts *Reboulia hemisphaerica* and *Marchantia palmata* and the moss *Hydrogonium gracilantum* and the extracts were reported to inhibit pathogens such as *Escherichia coli*, *Bacillus cereus*, *Erwinia chrysanthemi* and *Pseudomonas aeruginosa* (Kandpal *et al*., 2016).

Results from almost all these and several other studies, do not indicate that the extracts or fractions themselves yield a purified antibiotic. In fact, based on the available sources, no bryophyte-derived commercially available antibiotic has yet reached clinical use. However, many potential bioactive candidates derived from several species of bryophytes are undergoing continued preclinical evaluation. For instance, several workers have isolated metabolites such as lunularin and marchantin A and many other (Table 2). Their activity observed in crude extracts or fractions is, however, preliminary. Consequently, their critical evaluation is essential for determining whether bryophyte derived metabolites can contribute to future antimicrobial therapy and eventually antibiotic usage. Future work should therefore focus on bioprospecting of bryophytes through standardized, reproducible screening pipelines that link chemical characterization to antimicrobial activity (Greeshma *et al*., 2017).

Table 2. Antibacterial activity of bryophyte extracts and isolated compounds against selected human pathogenic bacteria (Adapted from Cianciullo *et al*., 2021)

Bacterial species	Antibacterial compound/extract	Compound class	Bryophyte species
Staphylococcus aureus	α-herbertenol	Sesquiterpenoids	*Mastigophora diclados*
	β-herbertenol		
	herbertene-1,2- diol		
	mastigophorene C		
	α-formyl herbertenol		
	Perrotettin A-D	bibenzyl	*Marchantia polymorpha*
	Perrotettin F		*Lunularia cruciata*
	Marchantin A		*Marchantia polymorpha*
	Lepidozenolide	Sesquiterpenoids	*Lepidozia fauriana*

Pseudomonas aeruginosa	Marchantin A	bibenzyl	*Marchantia* spp.
	Bartramiaflavone	Flavonoids	*Bartramia pomiformis*; *Hedwigia ciliata*; *Plagiomnium cuspidatum*; *Plagiomnium affine*; *Dicranum scoparium*
	Lucenin-2		
	Saponarin		
	Apigenin		
	Apigenin-7-O-triglycoside		
	Lunularin	bibenzyl	*Dumortiera hirsute*
	Perrotettin F	bibenzyl	*Lunularia cruciata*
	Bartramiaflavone	Flavonoids	*Bartramia pomiformis*; *Hedwigia ciliata*; *Plagiomnium cuspidatum*; *Plagiomnium affine*
	Lucenin-2		
	Saponarin		
	Apigenin		
Escherichia coli	Apigenin	Flavonoids	*Plagiomnium affine*; *Dicranum scoparium*; *Plagiomnium cuspidatum*; *Hedwigia ciliata*
	Vitexin		
	Saponarin		
	Lucenin-2		
	Marchantin A	bibenzyl	*Marchantia* spp.
Enterobacter spp.	Bartramiaflavone;	Flavonoids	*Bartramia pomiformis*; *Hedwigia ciliata*; *Plagiomnium cuspidatum*; *Plagiomnium affine*; *Dicranum scoparium*
	Luteolin-7-O-neohesperoside;		
	Lucenin-2;		
	Saponarin;		
	Apigenin-7-O-triglycoside;		
	Apigenin		

DISCUSSION

The historical records demonstrate the persistent interest in antimicrobial activity of bryophytes. Indigenous communities around the world have been using bryophytes for antimicrobial, antipyretic, dermatological, hepatic, cardiovascular and wound-healing purposes (Benek *et al*., 2025). They have been used in traditional medicine for centuries and approximately 70 species have established medicinal applications (Motti *et al*., 2023). Extracts obtained from them have demonstrated activity against microbial pathogens of humans and plants, plant-pathogenic fungi and insect pests, as well as allelopathic effects (Kulshrestha *et al*., 2022). Certain preliminary reports appeared in the 1940s and then studies in the 1950s drew attention to the antibacterial effects of *Anomodon rostratus*, *Orthotrichum rupestre* and *Plagiomnium cuspidatum*. A comprehensive study published in 1979 assessed 52 species against eight bacterial strains and found that 56% were active against at least one test bacterium (Vollar *et al*., 2018). A subsequent survey estimated that nearly 200 bryophyte species had been screened, with 63–76% producing positive results (Shete *et al*., 2024). Nevertheless, the phytochemical and pharmacological properties of most species remain unknown (Vollar *et al*., 2018). This indicates that the expansion of screening studies has not been matched by comparable progress in chemical characterization. However, several recent works have identified molecules associated with the observed bioactivity. Some of these are macrocyclic bis(bibenzyls):

marchantin A and perrottetin A–F; the bibenzyl lunularin; the glycosylated flavonoids luteolin-7-*O*-neohesperoside, apigenin-7-*O*-triglycoside and saponarin; the flavonoids apigenin, lucenin-2 and bartramiaflavone; and the sesquiterpenoids α-herbertenol, β-herbertenol, herbertene-1,2-diol, mastigophorene C, α-formyl herbertenol and lepidozenolide. These compounds have shown activity ranging from weak to strong against several human bacterial pathogens (Cianciullo *et al*., 2021). Table 2 brings together extract and compound-level evidence of antibacterial activity in some of the species.

Minimum inhibitory concentration (MIC) is the lowest concentration of a substance that effectively inhibits visible bacterial growth. Vidal *et al*. (2012) noted that an ethanol extract of *Octoblepharum albidum* stopped the growth of *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 33018 at an MIC of 512 µg/mL. When the extract was tested together with aminoglycosides, a family of antibiotics that includes gentamicin and kanamycin, the amount of each antibiotic needed to stop growth decreased. The largest reduction was with the extract plus gentamicin against *Escherichia coli* and the extract plus kanamycin against *Klebsiella pneumoniae*, where the MIC dropped from 128 to only 32 µg/mL. These results are encouraging because both Gram-negative and Gram-positive bacteria are rapidly becoming resistant to nearly all the antibiotics presently available (Cianciullo *et al*., 2021). Some reviews also report that Gram-negative bacteria are more sensitive to bryophyte extracts than to the conventional antibiotics, which usually work better against Gram-positive bacteria. On this basis, bryophyte extracts have been suggested as possible partners for existing antibiotics, a pattern that is rare in higher plants (Dziwak *et al*., 2022). This interaction suggests a valuable area for further investigation.

A report of antimicrobial activity in Indian mosses found that *Escherichia coli* and *Staphylococcus aureus* were the most susceptible test organisms, followed by *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Its authors also concluded that Gram-negative microorganisms were more sensitive than Gram-positive ones, in contrast to the reported pattern for conventional antibiotics (Bodade *et al*., 2008). In addition, results from the Kumaun screening study are broadly consistent with this observation when assessed by MIC test. Kandpal *et al*. (2016) reported that the ethanolic extract of *Hydrogonium gracilantum* had MICs of 1.94 µg/mL against *Erwinia chrysanthemi* and 3.91 µg/mL against *E. coli*. On the other hand, ethanolic *Reboulia hemisphaerica* extract had MICs of 3.91 µg/mL against *Pseudomonas aeruginosa* and 7.81 µg/mL against *E. coli*. However, the authors also noted that some of these low MIC values were not fully consistent with the corresponding zone of inhibition results, warranting cautious interpretation of the reported antibacterial potency. Other findings, however, point in a different direction. Chloroform fractions of liverworts were more active against Gram-negative strains, whereas butanol fractions of mosses showed substantial activity against Gram-positive bacteria (Singh *et al*., 2011). Another study on *Platyphylla* and *Dicranum scoparium* likewise reported activity against the Gram-positive bacteria *Bacillus subtilis*, *Staphylococcus aureus* and *Sarcina lutea*, but not against the Gram-negative bacterium *E. coli* (Yayintas & Irkin, 2018). Taken together, these results suggest that susceptibility depends on the bryophyte taxon, extraction solvent and the test organism, rather than on Gram status alone. As shown in Table 2, chloroform extracts from different species of the same organism vary several-fold in potency. In addition, the activity of *Lunularia cruciata* followed the order ethanol > methanol > acetone > chloroform, while its aqueous extract was inactive (Dhondiyal *et al*., 2013). Greater activity against Gram-negative bacteria should therefore be treated as a hypothesis to test for each extract, rather than as a general property of bryophytes. This qualification is also important especially for Indian taxa, which have been investigated only recently and unevenly.

Historically, Indian bryology focused primarily on systematics. Antimicrobial research remains concentrated among a small number of groups, including Subramoniam & Subhisha (2005), Bodade *et al*. (2008) and Deora & Guhil (2014, 2018). Thalloid and leafy liverworts even received far less attention than mosses (Shete *et al*., 2024). Nevertheless, Indian medicinal traditions may provide a relevant ethnopharmacological basis for further work in this field. This is because liverworts have long been used to treat hepatitis and skin disorders (Gahtori & Chaturvedi, 2019). When considered collectively, Indian bryophyte studies establish three main points. First, antimicrobial activity is widespread but not universal. For instance, thirteen of the 16 Marchantiales species tested were inhibitory and the five species of Rebouliaceae showed mainly marked antibiotic activity (Shete *et al*., 2024). Among bryophytes examined in Kolhapur district, *Targionia hypophylla* showed the most promising activity, whereas *Cyathodium cavernarum* was comparatively inactive (Sawant & Karadge, 2010). Second, potency varies substantially. Scientists reported that MIC, MBC and MFC values across 14 species ranged from 3.91 to 500 µg/mL. *Conocephalum conicum* showed the greatest bioactive potential, followed by *Marchantia papillata* and *Anoectangium thomsonii*. Third, very few of these studies have isolated a purified compound, identified or characterized the biomolecules responsible for the observed antimicrobial activity.

These findings are crucial and also point to four priorities. First, research should now move beyond additional crude-extract screening towards bioassay-guided fractionation, so that activity can be attributed to defined compounds. Second, studies should report extraction procedures, yields, chemical fingerprints and reproducibility alongside MIC and MBC values, strain identities, controls and replicate data. Such standardized reporting would enable comparisons among Indian taxa and with bryophytes studied elsewhere. Third, extracts and compounds should be tested against clinically relevant drug-resistant pathogens. Fourth, field-collected plant material should be compared with sterile, microbe-free axenic cultures maintained in the laboratory. Since *in vitro* bryophyte cultures have been shown to synthesize antibiotic substances (Olasoji *et al*., 2019), this comparison will allow researchers to confirm whether the plant itself is creating the antibiotic effect or whether microbes living inside the wild plant are producing it. To further advance these efforts, combination testing should also complement the studies of individual compounds. Bryophyte antimicrobial activity may depend not only on chemical composition, structural configuration or functional groups, but also on synergistic or antagonistic interactions among these compounds (Gahtori & Chaturvedi, 2019). The findings for *O. albidum* illustrate the relevance of such interactions, as its extract modified antibiotic activity rather than relying solely on its intrinsic antibacterial potency (Vidal *et al*., 2012). At the compound level, bioassay-guided fractionation of *Mastigophora diclados* provides a useful model. Among the herbertane sesquiterpenoids, mastigophorene C produced the strongest reported antibacterial effect, with a 17 mm inhibition zone, whereas its monomer, herbertene-1,2-diol, produced a 13 mm inhibition zone (Novakovic *et al*., 2021). These researchers also noticed that crude ether and methanol extracts inhibited *B. subtilis* and *S. aureus* at an MIC of 16 µg/mL and subsequent fractionation yielded compounds that showed moderate activity against *B. subtilis*, with MICs of 2–8 µg/mL. These comparisons may only work if researchers use the same testing methods and pathogenic strains. If the laboratory setups are different, it is impossible to tell whether the results changed because of the unique chemistry of the plant extract or simply because the experiment was run differently.

Overall, the evidence supports a more qualified and useful conclusion than either unreserved optimism or complete dismissal. Bryophytes constitute a therapeutically promising group of plants whose phytochemicals may yield drug leads and *in vitro* culture may facilitate the production and isolation of these compounds at larger scales (Das *et al*., 2022). However, most of the evidence reviewed here identifies candidates rather than establishing sources of antibiotics. This is irrefutably because antibacterial activity of crude extracts observed *in vitro* is an initial screening result, not proof of therapeutic efficacy. In fact, no antibiotic in clinical use has been confirmed to originate from bryophytes (Dziwak *et al*., 2022). To date, *in vivo* studies of bryophyte extracts and compounds have focused on anti-inflammatory, antinociceptive, wound-healing, anticarcinogenic, nanoparticle pharmacokinetic and antilipidemic endpoints (Cianciullo *et al*., 2021). Antibacterial efficacy in an infection model has not been demonstrated or published yet. Further progress will require a staged evaluation which may include purification and identification of active compounds and testing against mammalian cells. Moreover, crude plant extracts may contain toxic constituents and cannot be presumed safe; the selective compound assessment in relevant infection models is crucial for efficacy, mechanism of action, toxicity and pharmacokinetics. In each case, extraction methods, chemical characterization and reproducibility should also be reported alongside MIC values.

CONCLUSION

Bryophytes have been screened for antimicrobial activity since the 1940s, and the results are encouraging enough to keep looking. Since then, researchers around the globe have identified many of the molecules behind this activity. Indian work also fits this picture and has begun contributing to this research. Bryology in India has moved from systematics towards antimicrobial screening under proper controls. In vitro inhibition by a crude extract is a starting point, not proof that a plant can treat an infection. No clinically used antibiotic has yet been confirmed to come from any bryophyte. Several bryophyte extracts have demonstrated appreciable *in vitro* antibacterial activity; their potency, however, varies with species, extract solvent, concentration used and test organism. Some of the evidence reviewed also indicates that when a bryophyte extract is tested along with a low dose of ordinary antibiotics, the antibiotics show substantially greater effectiveness. This evidence of antibiotic potentiating activity in selected bryophytes also warrants further investigations. Such a finding may be the most promising lead so far and it is distinct from finding a new antibiotic. Hence, the way forward is straightforward, if slow. New active compounds need to be extracted, purified and identified. The identified compounds should then be tested for their toxicity against human cells and those showing acceptable selectivity should be further evaluated in suitable infection models.

DECLARATION

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RESEARCH ARTICLE

A MODIFIED CTAB-BASED PROTOCOL FOR EFFICIENT ISOLATION OF HIGH QUALITY RNA FROM SECONDARY METABOLITE RICH RHIZOMES OF *CURCUMA ZEDOARIA* ROSCOE

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Abstract: Presence of high concentration of secondary metabolites in *Curcuma zedoaria* rhizomes hampers the extraction and purification of high quality total RNA, which is pre-requisite for transcriptome and gene expression studies. The main challenge is with its rhizome tissues which are rich in polyphenolics, polysaccharides and tannins, that results in poor RNA yield both in quality and quantity, due to their co-precipitation and enzymatic degradation that further limits their use in molecular processes. Thus, development of a simple, convenient and affordable approach for RNA isolation from *Curcuma zedoaria* rhizomes is necessary to facilitate its molecular studies. In the present study, four different isolation protocols were compared to access the quality and quantity of total RNA isolated from fresh and frozen rhizomes of *Curcuma zedoaria*, where a modified Cetyltrimethylammonium bromide (CTAB) based protocol resulted in distinct and intact 28 S and 18 S rRNA bands. RNA isolated from fresh rhizomes exhibited A260/A280 and A260/A230 ratios of 1.98± 0.11 and 1.94± 0.02 respectively while RNA isolated from frozen rhizomes showed corresponding ratios of 2.08± 0.08 and 2.03± 0.04. The total RNA yield obtained from fresh and frozen rhizomes was 670.1± 0.07 µg/g and 630.8± 0.09 µg/g respectively. The results demonstrated that modified CTAB method minimizes the interference of secondary metabolites and enables the recovery of high quality RNA from *C. zedoaria* rhizomes. To best of our knowledge, this the first report describing a modified CTAB based protocol optimized for RNA isolation from *C. zedoaria* rhizomes for facilitating studies related to gene expression analysis and various molecular processes.

Keywords: *Curcuma zedoaria*, Polyphenols, RNA isolation, Secondary metabolites

INTRODUCTION

Using advanced modern biotechnological tools in functional genomics is highly important for crop improvement. Identifying the underlying molecular factors that affects the plant growth and its productivity under various stress conditions and further evaluating their responses can decipher novel genes/alleles can result in stress-tolerant plants Further, decoding various stress (biotic or abiotic) tolerance mechanisms require isolation of good quality RNA, which is a crucial step in all the molecular studies. High quality RNA is also essential in developing genomic resources so as to gain the genetic information, gene expression and post-translational modifications particularly from less explored/under-utilized plant species.

Curcuma zedoaria Roscoe, also known as white turmeric, belonging to family Zingiberaceae, is native to India, China and Indonesia. Use of *Curcuma* plant rhizomes have been already mentioned in ancient Ayurvedic literature against various ailments like arthritis, asthma, diarrhea, dysentery and skin diseases, due to presence of high content of metabolites like curcumin, B turmenrone, zingiberene etc. (Gharge *et al.*, 2021). Several studies have been reported on analyzing the metabolic pathways for these important medicinal compounds using molecular tools which are primarily based on isolation of high-quality RNA (Tsouka & Masoodi, 2023). Isolation of RNA is difficult from *C. zedoaria* rhizomes due to the presence of abundant polysaccharides, polyphenols and metabolites which co-precipitate with RNA during isolation, resulting in low yield and further cause interference with reverse transcription. Though protocols for RNA isolation using CTAB (Cetyltrimethylammonium bromide), CTAB/LiCl (CTAB/Lithium chloride) SDS (Sodium dodecyl sulfate) and TRIzol method have already been reported from many plant species that are rich in polyphenols and polysaccharides (Singh *et al.*, 2024), however, these methods being expensive and time consuming do not yield good quality intact RNA due to contamination by metabolites. The methods needs to be optimized for specific target tissues, specifically for extracting RNA from under-ground tissues such as rhizomes, tubers, corms and roots which is more challenging due to high content of secondary metabolites that contaminate or degrade RNA, thus making its use inapt in further downstream applications (Mandaliya *et al.*, 2024).

The present study standardized a modified CTAB-

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based RNA isolation protocol through a comparative evaluation of four methods: TRIzol, CTAB, CTAB supplemented with LiCl, and the modified CTAB method. Modified CTAB method was proved to be efficient, reliable and cost-effective method that overcame all the limitations recorded earlier with RNA isolation from *C. zedoaria* rhizomes. Further, the isolated RNA exhibited good quality and quantity, thus making it suitable for downstream applications, including qualitative and quantitative PCR analysis.

MATERIALS AND METHODS

Plant Material and Chemicals

Fresh rhizomes of *Curcuma zedoaria* grown in the experimental fields of School of Biotechnology, University of Jammu (Jammu, India), and frozen rhizomes (*Curcuma zedoaria*) stored at -80 °C (for a minimum period of 6 months) were used for RNA isolation. The rhizomes collected from field were thoroughly washed to remove adhering soil, wrapped in aluminum foil and immediately immersed in liquid nitrogen to inhibit RNase activity and preserve the RNA integrity. Chemicals used for total RNA isolation were of molecular biology grade, thus ensuring high purity and all the required solutions were made with double-distilled water. Plastic ware and pipette tips were treated with 0.1 % DEPC-treated distilled water overnight at room temperature and autoclaved. Glassware and mortar and pestles (0.1% DEPC treated and autoclaved) were baked overnight at 140 °C in the oven.

Solutions and Reagents

RNA extraction buffer used in the study consisted of 100 mM Tris HCl (pH 8.0), 25 mM EDTA (pH 8.0), 2% CTAB (w/v), and 2% beta-mercaptoethanol (V/V). Water-saturated phenol:chloroform:isoamyl alcohol (PCI) 25:24:1 (v/v/v), RNase-free distilled deionized water, and 100% and 75% (v/v) ethanol were also used in the study. Autoclaved double

RNA Isolation

Frozen and fresh *C. zedoaria* rhizomes were sliced into small pieces and ground into a fine powder (using liquid nitrogen), in a sterilized and pre-chilled mortar and pestle. Four different protocols were used to isolate RNA from *C. zedoaria* rhizomes, so as to identify the most efficient method for RNA isolation. The protocols used were TRIzol (Invitrogen, Carlsbad, USA) method, according to the manufacturer's instructions, original CTAB method (as described by White *et al.,* (2008); CTAB+LiCl protocol as proposed by Gasic *et al.,* (2004) and a modified CTAB protocol (method proposed by Gasic *et al.,* (2004) with modifications in few components, incubation temperature and centrifuge time). All the components for various protocols were mixed and prepared as described in table 1.

Protocol for RNA isolation as described by Gasic *et al.,* (2004) was modified (named as modified CTAB method throughout the text). 200 mg of sample (each of fresh and frozen rhizomes) was taken in 2 ml vial and 400ul CTAB buffer (preheated at 65°C) was added to it and mixed well; 500ul of 2.5% Polyvinylpyrrolidone (PVP) and 200ul of 2% of beta-mercaptoethanol were also added to the CTAB buffer and the vials were incubated in water bath at 60°C for 15 min. After incubation, 1 mL of phenol:chloroform:isoamyl alcohol (25:24:1, v/v/v) was added to the vials, and the mixture was thoroughly homogenized by pipetting. The vials were then centrifuged at 13,500 rpm for 12 minutes at 4 °C. The aqueous phase was carefully transferred to a new vial, followed by the addition of an equal volume of chloroform:isoamyl alcohol (24:1, v/v). The mixture was thoroughly mixed on ice by slow pipetting and again centrifuged at 13,500 rpm (12 minutes, 4 °C). Finally, an equal volume of chilled isopropanol was added to the aqueous phase (obtained after centrifugation) to precipitate the RNA, followed by washing the RNA pellet with ethanol (70%) and further air drying the pellet. The collected RNA pellet was dissolved in nuclease free water and stored at -80 °C. This modified protocol also included a pre-freezing step, in which freshly collected rhizomes from field were kept at -80 ^{o}C for one hour, prior to RNA isolation.

Table 1. Components of four protocols used for RNA isolation from *C. zedoaria* rhizomes

Components	Protocols			
	Original CTAB method	**Modified CTAB method**	**CTAB + LiCl method**	**TRIzol method**
Buffer	2% CTAB	2% CTAB	2% CTAB	TRIzol reagant
Tris-HCl	100 mM	100 mM	100 mM	-
EDTA	25 mM	25 mM	25 mM	-
NaCl	2.0 M	2.5 M	2.0 M	-
PVP	1 %	2.5%	2%	-
β-mercaptoethanol	5%	2%	2%	-
LiCl	4.0 M	-	10.0 M	-
Chloroform	-	-	-	0.2 vol.
Chloroform/Isoamyl alcohol	24:1	24:1	24:1	-

Phenol/Chloroform/Isoamyl alcohol	25:24:1	25:24:1	25:24:1	-
Isopropanol	2/3 vol.	2/3 vol.	0.6 vol.	Equal vol.
Ethanol	70%	70%	70%	75%
Ammonium acetate	-	-	10 mM	-
Sodium acetate	-	-	3.0 M	-

Estimation of Quantity and Quality of Isolated RNA

Purity and the concentration of isolated RNA was determined by measuring absorbance ratios of A260/A280 and A260/A230, using spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific, MA, USA). Further, the integrity of the total RNA was quantified using gel electrophoresis on 1.2% (w/v) agarose gel stained with ethidium bromide and imaged using a Gel Doc system (Bio-RAD).

cDNA Synthesis from isolated RNA and its Confirmation

To remove the co-extracted genomic DNA, total RNA isolated from modified CTAB method was incubated with 2 µL of RNase-free DNase I (Fermentas, Burlington, Canada) for 30 min at 37 °C. DNase I treated total RNA (3µg) was used as a template for the first strand synthesis using Revert Aid First Strand cDNA synthesis kit (Fermentas, Burlington, Canada). The total reaction of 20 µl contained: 3 µg of total isolated RNA, 100 µM of oligo (dT) primer, 5X reaction buffer (250 mM Tris–HCl; pH 8.3; 250 mM KCl; 20 mM MgCl2; 50 mM DTT), 10 mM dNTPs, and 1 µl of RevertAid reverse transcriptase (200 units/µl). The reaction was incubated for 60 min at 42 °C, followed by 5 min at 70 °C to terminate the reaction. Synthesis of first strand cDNA was confirmed with PCR (Denaturation at 95 ^{0}C for 35 sec, Annealing at 50 ^{0}C for 40 sec, Extension at 72 ^{0}C for 30 sec for 35 cycle and final extension at 72 ^{0}C for 10 min) using primer pairs for GAPDH housekeeping gene. The sequence of primer pair used for GAPDH is Forward: 5'-GTCTCCTCTGACTTCAACAGCG-3'; Reverse: 5'-ACCACCCTGTTGCTGTAGCCAA-3'.

RESULTS

Four different approaches viz. TRIzol method, CTAB+LiCl method, original CTAB method and modified CTAB method were used in the present study to isolate RNA from fresh and frozen *C. zedoaria* rhizomes. Using TRIzol method, A260/A280 and A260/A230 ratios of 1.05± 0.19 and 0.51± 0.20 from fresh rhizomes and 1.06± 0.21 and 0.44± 0.24 from frozen rhizomes respectively were observed in the isolated RNA. Compared to Trizol method, RNA isolated using CTAB + LiCl method, A260/A230 and A260/A280 ratios (1.59± 0.11 and 0.78± 0.16 respectively) were higher for fresh rhizomes in comparison to frozen rhizomes which was 1.37± 0.18 and 0.54± 0.13 respectively (Table 2). The low ratios in other approaches were due to co-precipitate of polysaccharides and polyphenols with endogenous RNA, hence restricting nucleotide purification. Also, varying degrees of degradation was observed in RNA samples isolated using TRIzol, original CTAB and CTAB + LiCl methods (Figure 1a,b; Figure 2a,b and Figure 3a,b). However, using a modified CTAB procedure, total RNA isolated from fresh rhizomes of *C. zedoaria* was of high quality as compared to RNA from frozen rhizomes, with distinct and intact 28 S and 18 S rRNA bands (Figure 4,b), with A260/A280 and A260/A230 ratios (1.98± 0.11 and 1.94± 0.02 respectively) for fresh rhizomes, which were observed to be much higher as compared to three other RNA isolation methods used in the present study (Table 2).

Quantity and quality of total RNA isolated from fresh and frozen *C. zedoaria* rhizomes was also compared using original CTAB method given by Gasic *et al.,* (2004) and modified CTAB approach used in the current investigation. Purity and integrity of the isolated RNA (from fresh and frozen rhizomes) was observed in both the methods, though the modified CTAB method gave better results as compared to original CTAB method (Figure 4a,b).

Using various approaches for RNA isolation, variation in yield of total RNA isolated from fresh and frozen *Curcuma* rhizomes was observed (Table 2). The quantity of the RNA extracted from fresh rhizomes using the original CTAB approach was 412.8± 0.18 µg/g tissue whereas it was 670.1± 0.07 µg/g tissue using modified CTAB protocol. For frozen rhizomes the RNA yield was 388± 0.10 µg/g tissue using the original CTAB protocol, whereas with modified CTAB method the yield increased to 630.8± 0.09 µg/g tissue. The quantity of RNA isolated from fresh rhizomes improved with the modified protocol by 1.62 folds. Overall, the modified CTAB protocol resulted in 5.31- and 2.61-folds enhancement in RNA yield as compared to TRIzol and protocol given by Gasic *et al.,* (2004) respectively for fresh rhizomes. Also as compared to frozen rhizomes, the RNA yield obtained with fresh rhizomes was observed to be more in all the four protocols under study (Table 2). Further, the isolated RNA from fresh rhizomes (using modified CTAB method) was subjected to DNase 1 treatment to eliminate any remaining traces of genomic DNA. The first strand of cDNA was synthesized and confirmed on the gel, using GAPDH as housekeeping gene (Figure 5).

Table 2. Quality and yield of total RNA isolated from *C. zedoaria* rhizomes (both fresh and frozen rhizomes)

Optical density	**RNA Quality**		**RNA Yield (µg/g tissue)**	
Original CTAB method (White *et al.*, 2008)	Fresh	Frozen	Fresh	Frozen
A260/A280	1.64± 0.25	1.48± 0.34	412.8± 0.18	388.0± 0.10
A260/A230	1.59± 0.30	1.54± 0.18	----	----
Modified CTAB method	Fresh	Frozen	Fresh	Frozen
A260/A280	1.98± 0.11	2.08± 0.08	670.1± 0.07	630.8± 0.09
A260/A230	1.94± 0.02	2.03± 0.04	----	----
CTAB + LiCl method (Gasic *et al.*, 2004)	Fresh	Frozen	Fresh	Frozen
A260/A280	1.59± 0.11	1.37± 0.18	256.3± 0.41	184.1± 0.38
A260/A230	0.78± 0.16	0.54± 0.13	----	----
TRIzol method	Fresh	Frozen	Fresh	Frozen
A260/A280	1.05± 0.19	1.06± 0.21	126.2± 0.29	118.6± 0.73
A260/A230	0.51± 0.20	0.44± 0.24	----	----

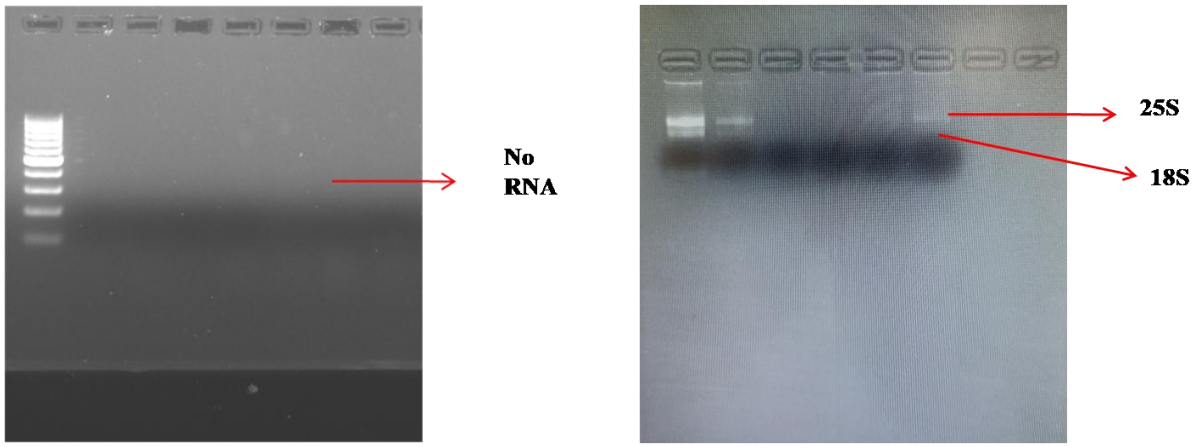


Figure 1. Total RNA isolated from a) fresh and b) frozen rhizomes of *C. zedoaria* using TRIzol method

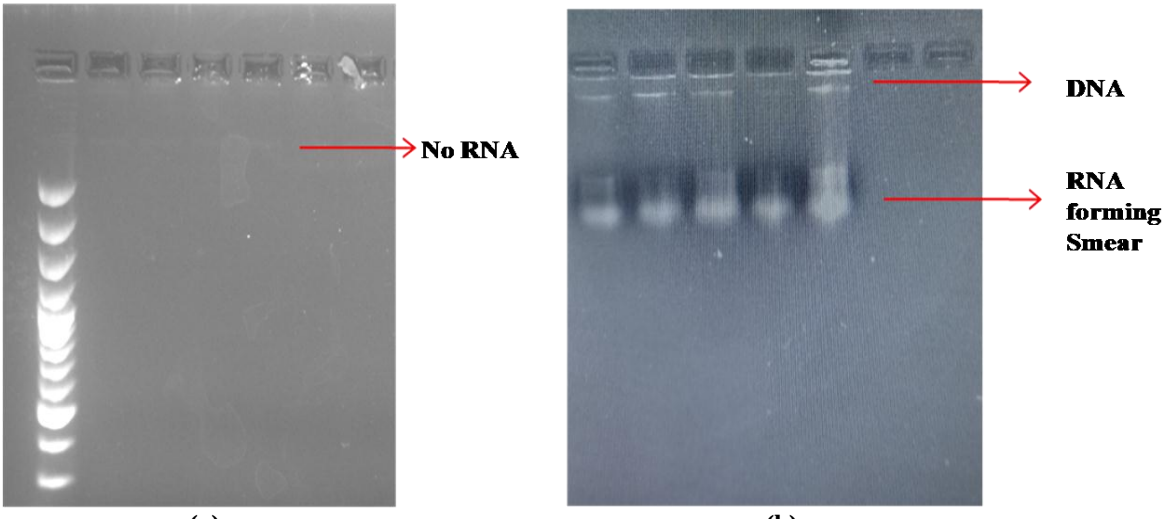
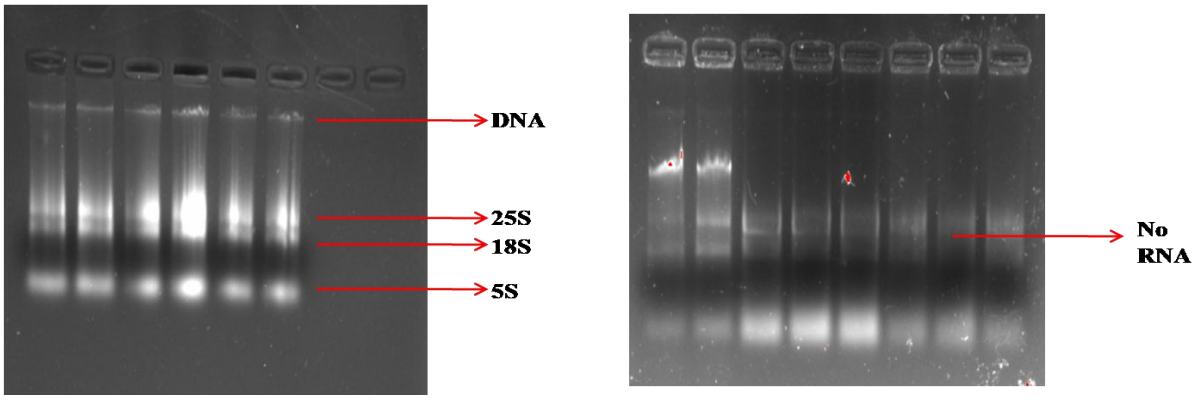
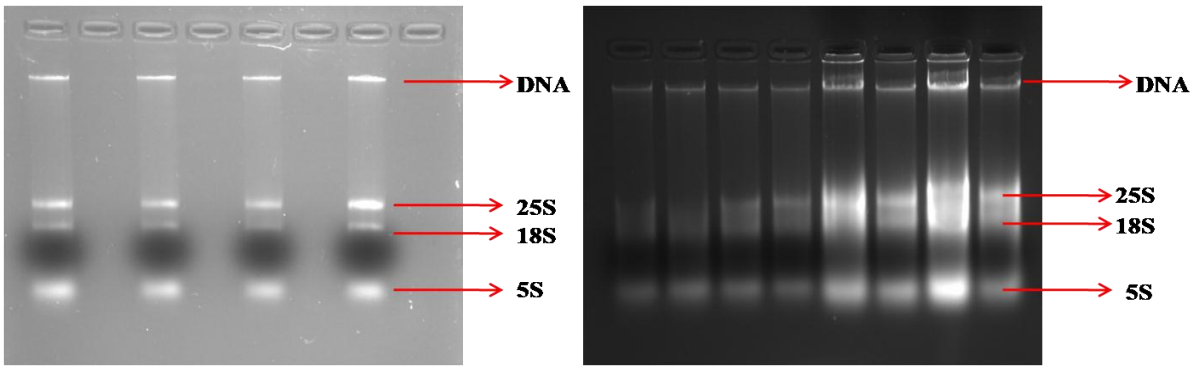


Figure 2. Total RNA isolated from a) fresh and b) frozen rhizomes of *C. zedoaria* using original CTAB method (White *et al.*, 2008)



(a) (b)

Figure 3. Total RNA isolated from a) fresh and b) frozen rhizomes of *C. zedoaria* using CTAB+LiCl method (Gasic *et al*., (2004)



(a) **(b)**

Figure 4. Total RNA isolated from a) fresh rhizomes b) frozen rhizomes of *C. zedoaria* using modified CTAB method

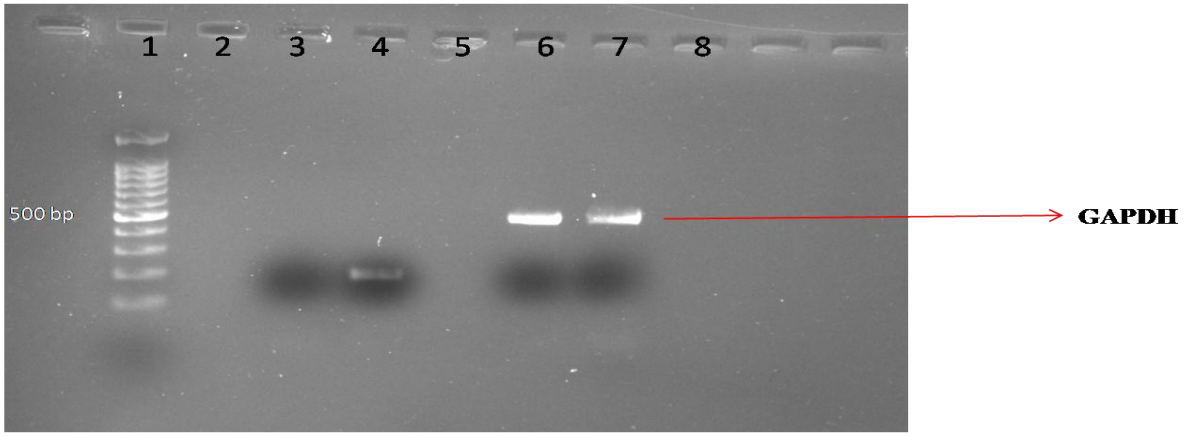


Figure 5. Confirmation of cDNA from RNA isolated from fresh rhizomes using modified CTAB method. Well no. 1: 100bp ladder, Well no. 6&7 (housekeeping GAPDH gene)

DISCUSSION

For molecular studies that involves gene expression and transcriptome analysis, isolating high-quality RNA is a crucial step. However, it is challenging to get RNA in adequate quantity and quality, particularly from those plant species whose high metabolite profile makes RNA extraction difficult and sometimes impossible. Furthermore, due to the diversity of chemical constituents contained in plant cells, there are no guidelines that are relevant for the isolation of RNA from a particular plant species/tissue (Liu *et al.*, 2018).

Presence of polyphenols, polysaccharides and alkaloids in *C. zedoaria* rhizome tissues makes it difficult to isolate good quality RNA. The polyphenolic compounds present in plant tissues undergo rapid oxidation during RNA extraction, resulting in the formation of quinones that bind to RNA and subsequently co-precipitate with it under low-ionic-strength buffer conditions. (Barbier *et al*., 2019; Wang & Stegemann, 2010).

Since the original CTAB-based RNA isolation method was modified to improve the removal of

interfering impurities, a modified CTAB protocol was developed for the efficient isolation of high-quality RNA from *C. zedoaria* rhizomes. A modified CTAB method involved protocol of Gasic *et al.*, (2004), with sight modifications where LiCl was removed from the extraction buffer in modified protocol, while LiCl at a concentration of 10.0M was used in the protocol given by Gasic *et al.*, (2004). Also, in modified protocol an increased salt concentration of 2.5M NaCl instead of 2.0M was used along with subsequent enhanced concentration of phenol:chloroform:isoamyl alcohol (25:24:1) in the extraction buffer. High ionic strength (NaCl) promotes effective partitioning of RNA away from proteins, dissolves polysaccharides and other cellular components during CTAB extraction. Since the rhizomes are rich in polyphenolics, so concentration of PVP was enhanced to 2.5% as compared to 2% used by Gasic *et al.*, (2004) protocol. This is because PVP has a strong affinity for oxidized and unoxidized phenolic compounds that precipitate in various enzymatic reactions, so PVP binds to these compounds and minimizes their interactions during RNA isolation (Wang *et al.*, 2021; Kumar & Shashank, 2025). Also, inclusion of Beta-mercaptoethanol in extraction buffer denatures RNases by breaking down disulfide bridges and disrupting their native conformation, which is necessary for enzyme functioning and further protecting the RNA from degradation.

Final precipitation with 2/3rd isopropanol also proved to be very effective. Addition of chloroform:isoamyl alcohol is important as they separate nucleic acids into a soluble phase and denatures the protein component. As reported earlier, quinones present in plant tissues bind to RNA and form high molecular-weight complexes that undergo oxidation and establish covalent linkages with polyphenolic compounds. Furthermore, polysaccharides present in plant issues also co-precipitate with RNA in presence of alcohol, which appear as contaminants during the final stages of RNA extraction and thus interfere with various molecular applications (Lee *et al.*, 2021). Additional chloroform washing steps were added in the protocol so as to remove polyphenols, polysaccharides, proteins and other unnecessary secondary metabolites. When phenolic compounds and other proteins (already present in significant amounts in the bark, rhizomes, storage roots and woody parts of plants) are eluted during the DNA/RNA extraction procedures, the quantity and quality of nucleic acid is degraded and altered. Temperature is also a primary factor in determining the integrity of RNA from plant tissues as high temperature results in RNA degradation, thus maintaining low temperature throughout RNA isolation is critical for obtaining high-quality and good yield of RNA. In the present study, all RNA isolation steps were conducted under low temperature conditions (on ice) so as to minimize its degradation and preserve integrity. This is in consistent with the observations reported by Dash, (2013) who observed brown supernatant in RNA isolated at room temperature which was due to reaction of phenolic compounds (present in plants) with the nucleic acids, while RNA isolated at 0 °C resulted in clear supernatant due to less degradation of RNA at low temperature.

CONCLUSION

In comparison to other time consuming and cost-intensive methods, the modified CTAB protocol demonstrated a significant improvement in both yield and purity of RNA isolated from fresh and frozen rhizomes of *Curcuma zedoaria*. The efficacy of this method is isolating high-quality RNA from rhizomes where other commonly used protocols often failed. Moreover, use of low-temperature conditions and optimized reagent composition effectively minimized the interference of secondary metabolites which are typically problematic in isolating high-quality RNA from plants. The reproducibility, simplicity and cost-effectiveness of this modified CTAB protocol make it highly suitable for various molecular applications. Future validation of the protocol across diverse plant species rich in metabolite will increase its applicability. This optimized protocol establishes a strong foundation for molecular techniques, particularly in plants rich in secondary metabolite content.

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CONFLICT OF INTEREST

The authors report no conflicts of interest in this work.

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RESEARCH ARTICLE

CHARACTERIZATION OF WEED FLORA AND EVALUATION OF CHEMICAL WEED CONTROL STRATEGIES FOR IRRIGATED WHEAT IN THE MIDDLE SENEGAL RIVER VALLEY

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Abstract: The objective of this study was to characterize the weed flora of irrigated wheat and to evaluate the efficacy, selectivity, and agronomic performance of chemical weed control strategies in Fanaye, Senegal, in 2022 and 2023. A randomized complete block design comprising five treatments and four replicates was used. The flora inventory identified 35 species belonging to 20 botanical families, including 26 broadleaf weeds, six Poaceae, and three Cyperaceae. Three treatments combining pendimethalin at 1.5 L/ha with different doses of 2,4-D were compared to manual weeding and an untreated control. Treatment T3 provided the best weed control but caused greater phytotoxicity. Treatment T1, with 1 L/ha of 2,4-D, offered the best balance between efficacy, selectivity, and yield, reaching 2,321 and 2,430 kg/ha, respectively.

Keywords : Irrigated wheat, Weed flora, Chemical weed control, Phytotoxicity, Yield

INTRODUCTION

Wheat is an essential grain for human consumption and plays a key role in global food security. In Africa, growing demand and dependence on imports pose major challenges for food systems. In Senegal, wheat imports reached 982,106 metric tons in 2025, up from 904,947 metric tons in 2024 an increase of 8.5% (ANSD, 2026). In this context, improving wheat productivity and addressing the factors that limit its yield are key challenges for the development of national cereal production. Among these factors, weeds represent a major constraint because they compete with the crop for water, light, nutrients, and space.

The intensity of this competition depends, in particular, on the plant community composition, species abundance, the plants' stage of development, and agroecological conditions. Inadequate weed management can thus compromise wheat growth and reduce its yield. In the context of climate change and the intensification of production systems, weed control is an important component of the sustainability of cereal production systems (Benjamin *et al*., 2024 ; Sharma *et al*., 2024).

Understanding the floristic composition of weed communities is an essential step in developing appropriate weed control strategies. Identifying the species present makes it possible, in particular, to better target control measures and to take into account the diversity of botanical groups. In Senegal, Mballo *et al*. (2018) characterized the weed flora of irrigated rice at several sites in the Senegal river valley. However, the weed flora associated with irrigated wheat and the weed control methods suitable for this crop in the middle valley require further documentation.

Chemical weed control is one of the methods used to manage weeds in cereal crops. Pendimethalin, a pre-emergence herbicide, controls certain annual weeds during the early stages of crop development. Its effectiveness, however, depends on the weed community composition, application conditions, and environmental characteristics. Lamani *et al*. (2024) studied the efficacy of pre-emergence herbicides against various weed communities in wheat, highlighting the importance of evaluating weed control strategies under the specific conditions of each production system. Combining a pre-emergence application with a post-emergence treatment can also extend the period of weed control, provided that the products and rates used ensure sufficient selectivity toward the crop.

In the middle Senegal river valley, an evaluation of these strategies is necessary to identify weed control methods that balance herbicide efficacy, wheat tolerance, and yield maintenance. This study was conducted at the Fanaye experimental station of the Senegalese Institute for Agricultural Research (ISRA) during two cold dry-season. Its overall

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objective was to contribute to improving weed management in irrigated wheat. More specifically, it aimed to characterize the weed flora, evaluate the efficacy of three treatment combinations pairing pendimethalin with different doses of 2,4-D, assess their phytotoxicity to wheat, and determine their effects on weed biomass, yield components, and grain yield.

MATERIALS AND METHODS

Overview of the study area

The trial was conducted at the ISRA experimental station in Fanaye during the cold dry seasons of 2022 and 2023. The station is located in the middle valley of the Senegal river, approximately 160 km from Saint-Louis, in the Podor department. The experimental station (16°33 North and 15°46 West) is situated along a river branch known as Ngalanka and covers an area of 117 hectares, 12 of which have been developed and connected to a motor pump.

The Fanaye station has three main soil types: Hollaldés, which are very clayey (50–75% clay); faux Hollaldés, which are clay-loamy (30–50% clay); and Fondés, which have a loamy texture (10–30% clay). The trial was established on Hollaldé-type soil, characterized by a high clay content.

Characterization of weed flora

The study of plant diversity involved conducting inventories of weeds present in the experimental plots every 10 days, from sowing to maturity. Taxa were identified using the flora by Berhaut (1971–1979). The APGIII classification (2009) was used to compile the list of families.

Experimental design

The experiment was conducted using a Fisher-type block design (completely randomized blocks) with 5 treatments and 4 replicates over two years. The basic plot has an area of 17.6 m² (8 m x 2.2 m). The number of elementary plots is 5 × 4 = 20. The distance between elementary plots and between replicates is 30 cm, and the distance between blocks is 1 m. The total area of the experiment is 455 m² (35 m × 13 m). The experimental block is 1 m wide.

Table 1: Treatments studied and administration methods.

Code	Treatment	Application method
T1	Pendimethalin (1.5 L/ha) + 2,4-D (1 L/ha)	Pendimethalin at 2 DAS, followed by 2,4-D at 20 DAS
T2	Pendimethalin (1.5 L/ha) + 2,4-D (1.5 L/ha)	Pendimethalin at 2 DAS, followed by 2,4-D at 20 DAS
T3	Pendimethalin (1.5 L/ha) + 2,4-D (2 L/ha)	Pendimethalin at 2 DAS, followed by 2,4-D at 20 DAS
TP	Manual weeding	At 30 and 60 DAS
TE	Weedy control	No weeding from sowing to harvest

DAS: Days After Sowing

The plant material consisted of soft wheat, Hamat variety, with a maturity cycle of 85 days and an average yield of 3.7 metric tons per hectare. The variety was registered in Senegal in 2020.

Herbicides were applied using conventional spraying with a manual backpack sprayer equipped with a slot nozzle, resulting in a treatment volume of approximately 120 L/ha.

Observations and measurements

The observations made to evaluate the effect of the treatments were as follows:

- ❖ for weeds
- visual assessment of efficacy on a scale from 0 to 100% (CILSS/CSP, 1999), on the 30th, 45th, and 60th days after sowing (DAS);
- collection of weed biomass from 3 squares of 0.25 m² per experimental plot on the 45th DAS.
- ❖ For wheat
- Visual assessment of treatment phytotoxicity on the 30th, 45th, and 60th days after sowing (DAS);
- Number of panicles per square meter (3 quadrats of 0.25 m² each per experimental plot); 1,000-kernel weight; and yield.

Statistical analysis

The data were analyzed using ANOVA to evaluate the effect of the treatments on herbicidal efficacy, weed biomass, and wheat yield parameters. Means were compared using the Newman-Keuls test at the 5% significance level. The analyses were performed using R software, version 3.4.0.

RESULTS AND DISCUSSION

Analysis of the flora

The floristic survey conducted in the irrigated wheat plots in Fanaye identified 35 weed species belonging to 20 botanical families (Table 2). Broadleaf weeds constituted the most diverse group, with 26 species belonging to 17 families, compared with six species of Poaceae and three species of Cyperaceae. Among the broadleaf weeds, the Fabaceae were the most represented, with five species, followed by the Malvaceae with four species and the Amaranthaceae with three species. The Asteraceae, Convolvulaceae, and Euphorbiaceae each had two species, while the other families were represented by only one species.

The Poaceae species identified were *Aristida mutabilis*, *Bothriochloa bladhii*, *Chloris pilosa*, *Echinochloa colona*, *Panicum laetum*, and

Pennisetum pedicellatum. The Cyperaceae included *Cyperus esculentus*, *Cyperus iria*, and *Cyperus rotundus*. The presence of these different species reflects the diversity of botanical groups associated with irrigated wheat cultivation under the agroecological conditions of Fanaye. Among the identified grasses, *Echinochloa colona* deserves special attention due to its significance as a weed in several cropping systems, particularly cereal crops.

The predominance of broadleaf weeds is comparable to the observations of Amare (2014), who studied weed management methods in wheat crops in Ethiopia. In the Senegal river valley, Mballo *et al*. (2018) also highlighted the importance of characterizing weed flora in irrigated agricultural systems. However, differences between the crops studied and the agroecological conditions limit direct comparisons between these studies. This floristic diversity requires adapting weed control strategies to the composition of the flora, the abundance of species, and their sensitivity to herbicides. Galon *et al*. (2021) showed that the efficacy and selectivity of herbicides used on wheat varied depending on the treatment and the target species. Thus, knowledge of the weed community composition is essential for interpreting the performance of the treatments evaluated at Fanaye and for guiding the choice of weed control methods.

Table 2: List of recorded species

Family	Species
POACEAE	
Poaceae	*Aristida mutabulis* Trin. & Rupr.
Poaceae	*Bothriochloa bladhii* (Retz.) S.T.Blake
Poaceae	*Chloris pilosa* Schummach.
Poaceae	*Echinochloa colona* (L.) Link.
Poaceae	*Panicum laetum* Kunth.
Poaceae	*Pennisetum pedicellatum* Trin.
CYPERACEAE	
Cyperaceae	*Cyperus esculentus* L.
Cyperaceae	*Cyperus iria* L.
Cyperaceae	*Cyperus rotundus* L.
BROAD-LEAVED WEEDS	
Aizoaceae	*Trianthema portulacastrum* L.
Amaranthaceae	*Alternanthera nodiflora* R. Br.
Amaranthaceae	*Alternanthera sessilis* (L.) R. Br. ex DC.
Amaranthaceae	*Amaranthus hybridus* (L.) Thell.
Asteraceae	*Blumea aurita* (L. f.) DC.
Asteraceae	*Eclipta alba* (L.) Hassk.
Convolvulaceae	*Ipomoea acanthocarpa* (Ch.) Asch. & Sch.
Convolvulaceae	*Ipomoea coptica* (L.) Roth.
Elatinaceae	*Bergia suffruticosa* (Delile) Fenzl
Euphorbiaceae	*Euphorbia heterophylla* L.
Euphorbiaceae	*Euphorbia hirta* L.
Fabaceae - Caesalpinoideae	*Senna obtusifolia* (L.) Irwin & Barneby
Fabaceae - Faboideae	*Aeschinomene indica* L.
Fabaceae - Faboideae	*Indigefera microcarpa* Desv.
Fabaceae - Faboideae	*Macroptilium lathyroides* (L.) Urb.

Haloragaceae	*Laurembergia tetrandra* auct.
Malvaceae - Malvoideae	*Abutilon pannosum* (G. Forst.) Schltdl.
Malvaceae - Tilioideae	*Corchorus tridens* L.
Malvaceae - Malvoideae	*Sida alba* L.
Malvaceae - Tilioideae	*Corchorus olitorius* L.
Molluginaceae	*Molugo nudicaulis* Lamarck
Phyllanthaceae	*Phyllanthus amarus* Schum. & Thion.
Portulacaceae	*Portulaca oleracea* L.
Scrophulariaceae	*Scoparia dulcis* L.
Zygophyllaceae	*Tribulus terrestris* auct.

Efficacy of treatments against weeds in wheat

In 2022, treatment efficacy ranged from 90 to 92% at 30 days after sowing (DAS). Treatments T2 and T3 performed best, with an efficacy of 92%, compared with 90% for T1 (Figure 1A). At 45 DAS, efficacy decreased for all treatments, with values ranging from 80 to 84%. T2 maintained the best performance (84%), while T1 and T3 showed 80% and 81%, respectively. At 60 days after sowing (DAS), trends varied by treatment: the efficacy of T1 continued to decline, reaching 75% ; that of T2 remained stable at 84% ; while T3 showed a gradual improvement, reaching 90% the highest value at that point.

In 2023, efficacy at 30 DAS ranged from 80% to 92% (Figure 1B). T3 performed best at 92%, followed by T2 at 88%, while T1 had the lowest value at 80%. At 45 DAS, the efficacy of T1 remained stable at 80%, while that of T2 decreased from 88% to 84% and that of T3 from 92% to 84%. At 60 DAS, a further decrease was observed for all three treatments, with efficacy rates of 70%, 78%, and 80% for T1, T2, and T3, respectively. Thus, despite the variations observed between years, T3 maintained the highest final efficacy over the two growing seasons, followed by T2, while T1 showed a more pronounced decline in performance.

The differences in efficacy trends between the two years could be related to variations in weed flora, application conditions, or environmental factors. Ghosh *et al*. (2017) demonstrated that combining pre-emergence pendimethalin with post-emergence 2,4-D reduced the density of dicotyledons and weed biomass in wheat. Hundal and Dhillon (2018) also demonstrated that sequential herbicide applications combining a pre-emergence treatment with a post-emergence treatment improved weed control and wheat yield. These results are consistent with those obtained in Fanaye, where the application of pendimethalin 2 days after sowing, followed by 2,4-D 20 days after sowing, resulted in high efficacy. However, the superior final efficacy of T3 must be interpreted while taking into account its effects on the crop.

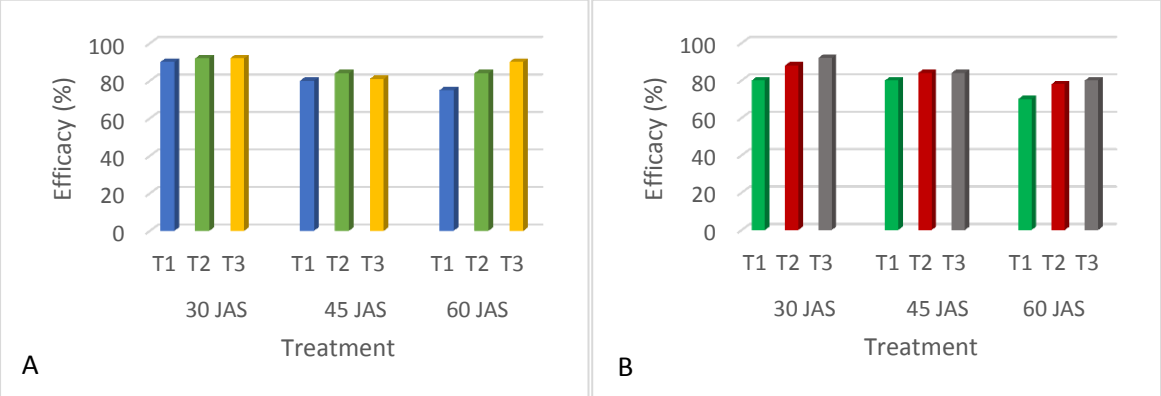

Figure 1 : Evolution of herbicide treatment efficacy across observation periods and years.

Phytotoxicity of treatments on wheat

Observations revealed differences in phytotoxicity among the treatments over the two years of the experiment (Table 3). T1, as well as the manually weeded control (TP) and the weeded control (TE), caused no visible symptoms, with phytotoxicity scores of zero on all three observation dates. In contrast, T2 caused slight and temporary yellowing of the lower leaves. Phytotoxicity scores were 0.5, 0.1, and 0 in 2022, at 30, 45, and 60 DAS, respectively, compared to 0.7, 0.5, and 0.2 in 2023. These results indicate a more pronounced intensity in

the second year, despite a gradual decrease in symptoms.

T3 exhibited the most pronounced symptoms during both growing seasons. In 2022, phytotoxicity scores were 1.5 at 30 DAS, 1.1 at 45 DAS, and 0.5 at 60 DAS. In 2023, they were 1.3, 1.0, and 0.7, respectively. The symptoms observed included leaf necrosis and yellowing, as well as plant death in 2023. Although their intensity decreased over time, these observations indicate that T3 has more limited selectivity toward wheat.

Colombo *et al*. (2022) also showed that herbicides can differ in their selectivity and the damage they cause to wheat. Khogali and Mohamed (2023), in a study conducted in northern Sudan, reported no visible phytotoxic symptoms with the tested doses of pendimethalin, unlike some other herbicides that caused mild symptoms at high doses. These observations are consistent with T1's good tolerance at Fanaye, while taking into account that the treatments evaluated in this study combined pendimethalin with a subsequent application of 2,4-D.

Table 3: Changes in treatment phytotoxicity in wheat across years and observation periods.

Treatment	Year	30 DAS	45 DAS	60 DAS	Visual symptoms observed
T1	2022	0.0	0.0	0.0	No
	2023	0.0	0.0	0.0	No
T2	2022	0.5	0.1	0.0	Mild, transient yellowing of the lower leaves
	2023	0.7	0.5	0.2	Mild, transient yellowing of the lower leaves
T3	2022	1.5	1.1	0.5	Leaf necrosis and yellowing
	2023	1.3	1.0	0.7	Plant death, leaf necrosis and yellowing
TP	2022	0.0	0.0	0.0	No
	2023	0.0	0.0	0.0	No
TE	2022	0.0	0.0	0.0	No
	2023	0.0	0.0	0.0	No

DAS: Days After Sowing

Effects of treatments on weed biomass, yield components, and wheat yield in 2022.

The 2022 results show that weed control strategies influenced weed biomass and wheat yield (Table 4). Weed biomass was particularly high in the weedy control (TE), at 241 ± 58.8 g/m², compared to 11.10 ± 1.19 g/m² for T1, 10.00 ± 1.28 g/m² for T2, and 5.48 ± 0.53 g/m² for T3. Manual weeding (TP) resulted in zero biomass. T3 thus had the lowest residual biomass among the herbicide treatments. However, the observed differences were not statistically significant ($p = 0.448$). These trends are consistent with the results of Ghosh *et al*. (2017), who demonstrated the benefits of sequential applications of pendimethalin and post-emergence herbicides for reducing weed biomass in wheat. Similarly, Pisal and Sagarka (2013) highlighted the effectiveness of integrated strategies combining pendimethalin with complementary measures.

Yield components also varied among treatments. The number of panicles ranged from 342 ± 86.3 panicles/m² in the grassed control to 448 panicles/m² in T1 and TP. T2 and T3 recorded 422 ± 31.9 and 374 ± 68.9 panicles/m², respectively. The thousand-kernel weight ranged from 35 to 36.5 g. However, the differences were not significant for either the number of panicles ($p = 0.279$) or the thousand-kernel weight ($p = 0.353$).

In contrast, grain yield showed highly significant differences among the treatments ($p = 9.51 \times 10^{-6}$). The highest yield was obtained with manual weeding (TP), at 2,343 ± 178 kg/ha, followed by T1 (2,321 ± 300 kg/ha) and T2 (2,306 ± 55.8 kg/ha). T3 recorded a lower yield of 2,050 ± 121 kg/ha, while the weedy control had the lowest yield (1,237 ± 153 kg/ha). These results highlight the importance of weed control for maintaining crop production. They are consistent with Ghosh *et al*. (2017), who reported a yield of 4.3 t/ha in the weeded control, comparable to that obtained with certain sequential herbicide strategies. The differences in yield between the two studies must nevertheless be interpreted in light of the experimental and agroecological conditions.

Despite its low residual weed biomass, T3 yielded less than T1 and T2. This result could be related to the phytotoxic symptoms observed with T3, particularly yellowing and leaf necrosis; however, this relationship remains a hypothesis that has not been statistically demonstrated. Galon *et al*. (2021) thus emphasize the importance of jointly evaluating herbicide efficacy, selectivity, and agronomic performance. Under the trial conditions, T1 appears to offer the best compromise, with a yield close to that of the clean control and no visible symptoms of phytotoxicity.

Table 4: Effects of treatments on weed biomass and wheat yield in 2022.

Treatment	Total weed biomass (g/m^2)	Number of panicles/m^2	Thousand-grain weight (g)	Yield (kg/ha)
T1	11.10 ±1.19	448 ±49.7	35.40 ±0.85	2321 ± 300
T2	10.00 ±1.28	422 ± 31.9	35.40 ±0.25	2306 ±55.8
T3	5.48 ± 0.53	374 ±68.9	35.90 ±0.85	2050 ±121
TE	241 ± 58.8	342 ±86.3	35 ±1.83	1237 ±153
TP	0	448 ±109	36.5 ±0.577	2343 ±178
Mean	53.58	406.65	35.63	2051.37
Cv (%)	49.31	19.29	2.94	9.16
Probability (p-value)	0.448	0.279	0.353	$9.51e^{-06}$ ***

T1, T2 and T3 = herbicide treatments; TP = manual weeding; TE = weedy control; CV = coefficient of variation

Effects of treatments on weed biomass, yield components, and wheat yield in 2023.

In 2023, herbicide treatments significantly reduced weed biomass compared to the weedy control (TE), which had 208 ± 14.4 g/m², versus zero biomass in the clean control (TP) (Table 5). Among the herbicide treatments, T3 had the lowest residual biomass (5.30 ± 0.26 g/m²), followed by T2 (9.88 ± 0.30 g/m²) and T1 (11.80 ± 0.34 g/m²). The differences were highly significant ($p = 2.45 \times 10^{-14}$). These results are consistent with those of Ghosh *et al*. (2017) and Pisal and Sagarka (2013), who demonstrated the benefits of strategies combining pendimethalin with complementary treatments. However, Khogali and Mohamed (2023) noted that herbicide efficacy may vary depending on botanical groups.

The number of panicles ranged from 316 ± 12.5 panicles/m² in the weedy control to 452 ± 10.4 panicles/m² in the clean control. T1 and T2 recorded 450 ± 11.5 and 427 ± 9.63 panicles/m², respectively, compared to 374 ± 7.68 panicles/m² for T3. The differences were highly significant ($p = 2.37 \times 10^{-10}$). In contrast, the thousand-kernel weight, ranging from 35 to 36 g, did not show a significant difference (p = 0.0625). These results are consistent with those of Agostinetto *et al*. (2008), who highlighted the importance of competition between weeds and wheat in determining yield losses.

Grain yield also differed significantly among treatments ($p = 2.38 \times 10^{-15}$). T1 yielded the highest grain yield (2,430 ± 35.4 kg/ha), followed by the clean control (2,412 ± 32.3 kg/ha), T2 (2,316 ± 25.3 kg/ha), and T3 (2,102 ± 24.7 kg/ha). The weeded control recorded the lowest yield, at 1,212 ± 52.0 kg/ha, confirming the importance of weed control for maintaining production. Ghosh *et al*. (2017) also reported a yield of 4.3 t/ha in the weeded control, comparable to that of certain sequential herbicide strategies.

Despite its superior control of weed biomass, T3 yielded less than T1 and T2. This difference may be related to the phytotoxic symptoms observed, including yellowing and leaf necrosis, as well as the presence of dead plants. Under the trial conditions, T1 represents the best agronomic compromise, combining a significant reduction in weed biomass, the absence of visible phytotoxicity symptoms, and the highest yield among the herbicide treatments.

Table 5: Effects of treatments on weed biomass and wheat yield in 2023.

Treatment	Total weed biomass (g/m^2)	Number of panicles/m^2	Thousand-grain weight (g)	Yield (kg/ha)
T1	11.80 ±0.34	450 ±11.50	35.50 ±0.58	2430 ±35.40
T2	9.88 ±0.30	427 ±9.63	35.80 ±0.50	2316 ±25.30
T3	5.3 ±0.26	374 ±7.68	36 ±0.82	2102 ±24.7
TE	208 ±14.4	316 ±12.5	35 ±0.82	1212 ±52.0
TP	0	452 ±10.40	35 ±0.82	2412 ±32.30
Mean	46.91	403.9	35.45	2051.38
Cv (%)	13.84	2.26	1.46	9.16
Probability (p-value)	$2.45e^{-14}$ ***	$2.37e^{-10}$ ***	0.0625	$2.38e^{-15}$ ***

T1, T2 and T3 = herbicide treatments; TP = manual weeding; TE = weedy control; CV = coefficient of variation

CONCLUSION

This study, conducted at the ISRA experimental station in Fanaye in 2022 and 2023, identified 35 weed species belonging to 20 botanical families, with broadleaf weeds predominating. Overall, the herbicide treatments provided good control of these weeds and reduced their biomass compared to the weedy control.

Among the strategies evaluated, T1 (pendimethalin at 1.5 L/ha followed by 2,4-D at 1 L/ha) proved to be the best treatment, thanks to its good balance between herbicidal efficacy, selectivity, and yield. It caused no visible symptoms of phytotoxicity and yielded the highest yields among the herbicide treatments, with 2,321 kg/ha in 2022 and 2,430 kg/ha in 2023. In contrast, despite its superior weed control efficacy, T3 applied with 2 L/ha of 2,4-D resulted in more pronounced symptoms of phytotoxicity and lower yields.

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RESEARCH ARTICLE

CASSIA AURICULATA (L.) BASED HIGH-DENSITY PLANTATION: AN IDEAL APPROACH FOR AUGMENTATION OF NATIVE MEDICINAL PLANT DIVERSITY IN CHAMBAL RAVINES OF RAJASTHAN

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Abstract: One of the key environmental benefits of sustainable resource management strategies in any type of ecosystem is the revival of biodiversity. Due to substantial biotic and abiotic interference from open grazing and falling, the natural vegetation of non-rehabilitated degraded ravine lands was extremely limited in distribution and diversity. Despite this, there is a dearth of information on the variety of medicinal plants found in rehabilitated ravine regions, both treated and untreated. The goal of the current study is to investigate the native flora under the Cassia auriculata-based land-use system in the Chambal ravines in the Kota district (Rajasthan) in terms of plant diversity, distribution, and ethnomedical applications. An account of 67 species observed denoting 38 families of plant kingdom in the rehabilitated study area. The documented indigenous plant species mostly belong to the plant families of Euphorbiaceae, Asteraceae, Apocynaceae, Solanaceae and Caesalpiniaceae, which have high drought tolerance capacity and the ability to grow under harsh environments. The maximum revival of native medicinal understory flora was observed in the *Cassia auriculata*-based rehabilitated lands. Rehabilitation/restoration/ woody shrubs may foster/facilitate revival/reappearance of valuable native medicinal flora through litter addition and residual moisture retention using soil moisture conservation measures in the study sites. Hence, crucial attention to proper woody plant species selection is essential for rehabilitation and enrichment of ravine biodiversity. Further, implementing appropriate conservation measures is highly needed to ensure the augmentation of indigenous medicinal flora under this resource poor region for sustainable ravine land utilization.

Keywords: Ravine, Rehabilitation, Diversity, Ethnomedicinal, Ethnovetetinary, Conservation

INTRODUCTION

Ravine ecosystems are extremely dynamic and drastically altered topography. Indigenous plant species play an important role in environmental rehabilitation in these locations due to their exceptional survival and high level of adaption to local conditions. Ravine lands are among the most degraded dry land ecosystems due to a harsh environment, low soil fertility, low soil moisture, severe temperature change, and high biotic pressure. Several natural and man-made factors have contributed to the creation of ravines. In a number of states, deep gullies have resulted from significant land degradation along some of the country's major river systems. The Chambal ravines form a 10-kilometer-wide band that runs south from the Yamuna confluence for 480 kilometers to the town of Kota in Rajasthan. *Cassia auriculata* (L.), commonly known as *Senna auriculata* (also known as Tanner's Cassia), is a multipurpose perennial legume shrub from the Caesalpinioideae family (Fig.1). It is also known locally as Indian Senna, Avartakgi, Avaram, Tangedu, Anwala, and Awalchawl. The two subfamilies of the family Leguminosae, Fabaceae, Mimoseae, and Papilionaceae, contain a small number of shrubs that grow as pioneer species. According to Banerjee *et al.* (1989), one of these is *Cassia auriculata*, which thrives under low elevated (even <200 msl) arid salt-tolerant plants. Therefore, it is appropriate to plant pioneer shrubs in degraded land afforestation to improve the site so that seral and climax stage species can sprout and flourish. These shrubs frequently act as nurse plants in afforestation. The remarkable concord with which they coexist is one of this shrub's most fascinating features. Native to India and Sri Lanka, *Cassia auriculata* is an Indo-Malaysian species that has been introduced to many regions of Southeast Asia. It can be found naturally from the northern regions of Rajasthan to the arid regions of peninsular India. In northern India, from West Bengal to Haryana, it is also grown for aesthetic purposes in gardens and hedges.

Many review papers indicated that *Cassia auriculata* has a range of phyto-constituents, indicating possible medical applications. Diabetes, liver toxicity, fungal infection, microbial infection, inflammation, pyrexia, and pain alleviation are some of the human illnesses that can be treated with the plant or its constituents.

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The relevance, conservation, and management of *Cassia auriculata* integrated landscapes for the sustainable management of shrub variety in the semi-arid chambal Ravine in Rajasthan, India, have all been attempted to be compiled in this work. However, much more effort remains to be done with *C. auriculata* in order to research and generate a superior genetic resource with a package of processes for production as a commercial plantation for potential use (Kala *et al.,* 2023). In many regions of India, this shrub grows wild and produces flowers and pods all year round. It grows well in wastelands, degraded forests, black soils beside roads, and dry stoney hills. Its blooms and seeds are used to make yellow dye (Chandramouli, 1995).

The research on *Cassia auriculata* L. (Caesalpiniaceae, also known as Tanner's Cassia), a common Asian plant that has been used extensively in traditional medicine to treat diabetes, rheumatism, and conjunctivitis, is described in this work. Additionally, *Cassia auriculata*, the primary ingredient in Kalpa herbal tea and a common ingredient in Ayurvedic medicine as "Avarai Panchaga Choornam," has been the subject of much research because to its antidiabetic properties (Kala *et al.,* 2021). Researchers have been working to find and validate plant-derived compounds for the treatment of a range of illnesses in recent years. Similar to this, it has already been demonstrated that different plant parts-such as leaves, fruits, seeds, etc. Offer chemicals that improve human nutrition and health (Kala *et al.,* 2019). Another Indian plant, *Cassia auriculata* Linn., has several traditional uses against a variety of illnesses.

Therefore, *Cassia auriculata* is not only a culturally significant medicinal plant but also a scientifically proven herb with significant potential for creating new phytopharmaceuticals, especially for the treatment of oxidative stress-related illnesses and diabetes (Kala *et al.,* 2021). Gonorrhea, ulcers, and rheumatism are treated with bark. With demonstrated pharmacological effects and significant ethnopharmacological significance, *Cassia auriculata* is a versatile medicinal herb. In ravine land ecology, a thorough investigation of native plant diversity and their ethnobotanical applications remain unexplored. The study of the entire natural and customary interaction between humans and their domesticated animals and plants is known as ethnobotany. The general perception that "green medicines" or "herbal renaissance" are safer and healthier than synthetic ones has sparked a resurgence of interest in natural medications, particularly those made from plants, in recent decades (Meena, 2015). The market for herbal health goods, such as herbal tea, herbal cosmetics, essential oils, and flavors, is expanding, despite the fact that these items are more expensive than the comparable low-cost synthetic ones (Reddy *et al.*, 2011).

In addition to endangering biological resources, deforestation, urbanization, industrialization, transmigration, colonialism, and other developmental activities have also put traditional culture and ethnobotanical knowledge at risk. India is already well-known for being a treasure trove of valuable medicinal plants that are utilized in both Indian medical systems and regional folk health customs. Numerous high-value medicinal plant species from various parts of India have seen population declines over time due to the ongoing illegal exploitation of these species and the significant destruction of their habitats (Kala *et al.,* 2023). Several additional variables, such as habitat specificity, limited range of distribution, land use disturbances, introduction of non-native species, habitat alteration, climatic changes, heavy livestock grazing, population explosion, fragmentation and degradation, population bottleneck, and genetic drift, can all contribute to medicinal plant species' rarity. Natural enemies such as diseases, herbivores, and seed predators can also drastically diminish the number of unusual medicinal plant species in a given area (Bevill *et al.,* 1999). There are 4,000 to 10,000 medicinal plant species that could become extinct locally, nationally, regionally, or globally, wreaking havoc on livelihoods, economies, and health-care systems (Dhyani and Kala 2005).

An estimate states that throughout the past two fiscal years, the amount of Ayurvedic goods exported from India has tripled. Indian medicinal herbs are becoming more and more in demand on the global market. An important part of a country's natural resources are medicinal plants. They have a major role in providing the rural population with basic healthcare services (Gowda *et al.,* 2022). Several types of degraded lands in the nation could be used to meet the demand for indigenous medicinal plants on the international market. This practice will help prevent the overuse of cultivable crop land, and various studies explain that the cultivation of different medicinal plants helps to reclaim the degraded lands. Despite their significance, not much is known about the medicinal plants found in ravine flora in particular. There is a scarcity of information about the type and distribution of medicinal plants in the current study area, as well as publications on traditional knowledge of how residents use indigenous resources.



Fig. 1. *Cassia auriculata* – An indigenous multipurpose shrub species

The study's goals were to: 1. record the resurgence of native medicinal plants in Chambal ravines' untreated and treated ravine areas; and 2. compile possible ethnomedical knowledge and applications of medicinal plants through a phytosociological survey in Chambal ravines. These goals were chosen in order to inform future conservation and regeneration strategies for efficient use of ravines using potential medicinal plant species in India. In light of this, exploration research has been carried out in the restored Chambal Ravines in Kota district (Rajasthan) to record the richness, use, and management of indigenous medicinal plants.

MATERIALS AND METHODS

Due to overuse and inadequate management, this large area of existing ravine lands in northwest India may pose a threat to neighboring productive areas (Fig.2). With an average annual soil loss of around 17 tons per hectare, the ravines are growing at a rate of 8 to 9 meters per year. Both natural and man-made land degradation are prevalent in the ravine lands. This area's vegetation faces a number of unfavorable conditions, including biotic interference, moisture stress, and nutrient deficiencies. Below is a description of the study region and survey details.

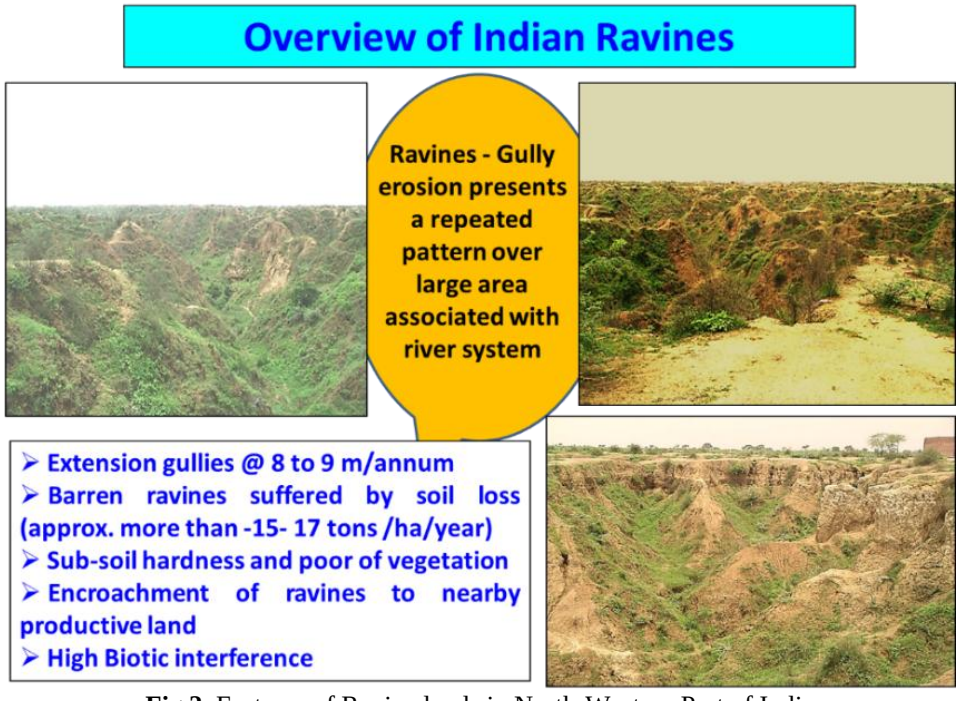

Fig.2. Features of Ravine lands in North-Western Part of India

Study Area:

The research region is located in the Kota district in southeast Rajasthan (longitude: 75°52′ to 75°52′ E; latitude: 25°13′to 25°14′N). The study farm consists of two distinct landscapes: agricultural tablelands (30 ha) and rehabilitated ravine lands (34 ha) along the Chambal River boundary (Fig.3). The soils at the research farm belong to the Kota series. These deep to exceptionally deep soils occur on flat, moderately sloping terrain with a slope of less than 2%. Typically, the $CaCO_3$ layer is found below 100 cm. The soils in the hyperthermic family of Typic Chromusterts are mostly fine-textured (>35% clay). Approximately 80% of the average annual rainfall (748 mm) falls between June and September, with the remaining 20% occurring in the winter. The temperature is normally moderate. In the winter, frost is common. In the assessment year 2024-2026, the average monthly temperature ranged from 4.7°C (lowest in January) to 43.10°C (highest in May). The rehabilitation of ravine lands and the enhancement of land productivity and biodiversity in wheezing regions were made possible by the implementation of situation-specific, economically viable technologies using agroforestry or other perennial plantation systems. In agreement with this, during the years 2023-2025, a phyto-sociological and ethnobotanical survey was conducted in the high-density plantation of *Cassia auriculata* raised with staggered contour trenches in the Chambal ravines through frequent field visits. In order to determine and comprehend the ecological indication and distribution of medicinal plant diversity in protected and rehabilitated regions of the Chambal ravines region, a transect study and in-person observations were implemented. Repeated field surveys were conducted in randomly selected sample plots (20 m x 20 m) to document the floral variety. The quadrats were physically demarcated using stakes and colored nylon ropes to ensure boundary clarity. All opposing species were photographed and identified in situ or in the field lab using standard floral keys. A field guide for recognizing native arborescent species found in Chambal ravines was one of the authoritative regional floras used to verify species identifications.

In the field notebooks, observations were recorded regarding the ecosystems and morphological characteristics of each species of medicinal plant. The plant species were gathered and identified down to the family or species level. The questionable species were verified by a botanist or online resources. Herbs, shrubs, and trees were the categories into which all the data pertaining to the sample plots recognized plant species were divided. Tables 1 and 2 list medicinal plants along with their uses in the following order: common name, botanical name, family, habit, economic aspect, and ethno-medical usage. Primary data were collected through field surveys in representative ravine villages nearby research site, targeting informants /healers verified with two age -groups viz., 41–60 years, and above 60 years. Structured questionnaires and oral interviews were employed to gather quantitative and qualitative data on knowledge of identity of medicinal plants, usage, mode application methods, interest in practicing own use/services and awareness of its cultural and environmental significance. The study was conducted with the consent of participants, with careful attention given to respecting cultural perspectives and authentically representing traditional knowledge. This knowledge was cross-checked using pertinent literature from the center's library and online sources.

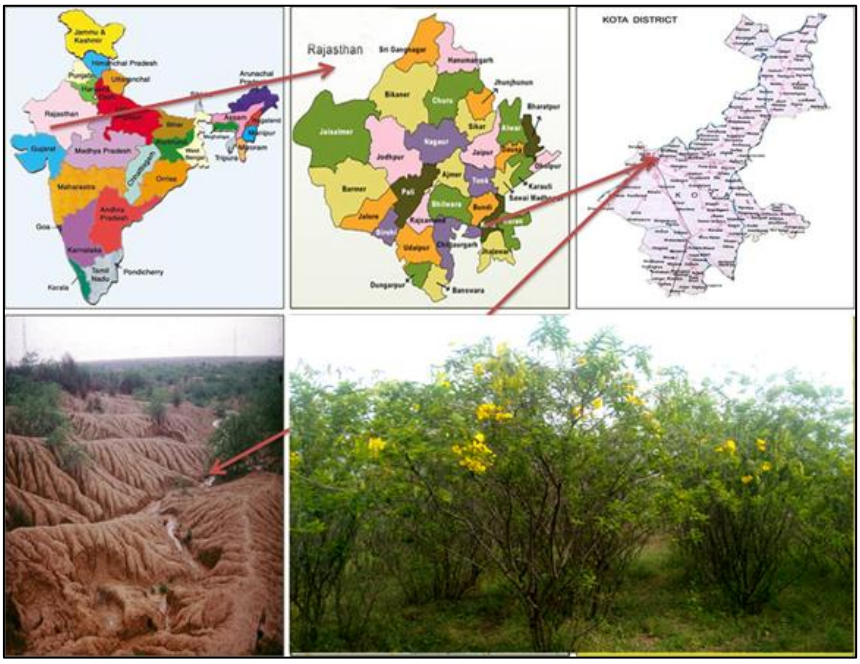

Fig.3. Survey areas of Chambal ravine in Rajasthan (India)

RESULTS AND DISCUSSION

Multipurpose medicinal plants are a significant component of the understory flora and are crucial to the revitalization of the ravine from an ecological and socioeconomic standpoint. This study found 67 medicinal plants from 38 families that local and common people in this area utilize as folk medicine to cure a variety of illnesses or diseases in humans and domestic animals. Trees, shrubs, climbers, and herbs made up the majority of the recognized therapeutic plants. The table .1 & Fig .3. indicate that habit wise distribution of medicinal plants under Cassia rehabilitated Chambal Ravines. In the study site, herbs make up the majority (42), followed by shrubs (15), climbers (9), and grass (1). According to the current study, the groups Euphorbiaceae, Asteraceae, Apocynaceae, Solanaceae, and Caesalpiniaceae comprised the bulk of the species. The identified and recorded native plant species are very drought-tolerant and may thrive in challenging conditions. Through forest cover-induced climatic adjustment, sustainable management of restored Chambal ravines with adequate protection undoubtedly improves species richness and conserving biodiversity. However, compared to unprotected open ravine lands, Cassia-based rehabilitated ravine lands have a significantly greater variety of medicinal plants (Kala *et al.*, 2023). These results are consistent with research by a number of authors showing that understory plant species are more diverse and abundant in rehabilitated or protected or undisturbed ravine than in degraded ravine open forest areas (Uthappa *et al.*, 2018).

Table 1. Listed of Medicinal Plants list in the Cassia based rehabilitated Chambal Ravine in Kota -Rajasthan

Sl. No	Common Name	Scientific Name	Plant Family	Habit Type
1.	Choti-ber	*Ziziphus jujuba*	Rhamnaceae	Shrub
2.	Bichhu-butti	*Xanthium indicum*	Asteraceae	Shrub
3.	Nahtoota	*Tridax procumbens*	Asteraceae	Herb
4.	Lutanki	*Trianthema triquetra*	Aizoaceae	Herb
5.	Mundi	*Sphaeranthus indicus*	Asteraceae	Herb
6.	Kalijibi	*Sonchus asper*	Asteraceae	Herb
7.	Ankhali	*Sonchus ankhali*	Asteraceae	Herb
8.	Pasarghatali	*Solnaum suranttense*	Solanaceae	Herb
9.	Makoy	*Solanum nigrum*	Solanaceae	Herb
10.	Desikharenti	*Sida ovata*	Malvaceae	Herb
11.	Kharenti	*Sida cordifolia*	Malvaceae	Shrub
12.	Lunkia	*Portulaca oleracea*	Portulacaceae	Herb
13.	Lalbuti	*Polygonum plebeium*	Polygonaceae	Herb
14.	Chirpotan	*Physalis minima*	Solanaceae	Herb
15.	Cap berry	*Physalis angulata*	Solanaceae	Herb
16.	Bhui anola	*Phyllanthus niruri*	Euphorbiaceae	Shrub
17.	Milk weed	*Pergularia daemia*	Apocynaceae	Climber
18.	Dakhni	*Pedalium murex*	Pedaliaceae	Herb
19.	Aaera	*Oxalis corniculata*	Oxalidaceae	Herb
20.	Tulsi	*Ocimum sanctum*	Lamiaceae	Herb
21.	Van tulsi	*Ocimum gratissium*	Lamiaceae	Herb
22.	Bapchi	*Ocimum americanum*	Laminaceae	Herb
23.	Kaner	*Nerium oleander*	Apocynaceae	Shrub
24.	Kirmich	*Mucuna pruriens*	Fabaceae	Climber
25.	Khakoda	*Momordia foetida*	Cucurbitaceae	Climber
26.	Chirio ghas	*Mollugo cerviana*	Molluginaceae	Herb
27.	Laajivanthi	*Mimosa pudica*	Mimosaceae	Herb
28.	Alai	*Mimosa hamata*	Mimosaceae	Shrub
29.	Alfa-alfa	*Medicago sativa*	Fabaceae	Herb
30.	Bichtukando	*Martynia annua*	Martyniaceae	Shurb
31.	Darkan	*Leucas urticaefolia*	Lamiaceae	Herb
32.	Timadia	*Impatiens balasamina*	Balsaminaceae	Herb
33.	Gurmar	*Hemidesmus indicus*	Asclepediaceae	Climber
34.	Gangeran Phalsa	*Grewia tenax*	Tiliaceae	Shrub

35.	Gandhi-buti	*Glinus lotoides*	Molluginaceae	Herb
36.	Dudhi	*Euphorbia prostrata*	Euphorbiaceae	Herb
37.	Danda -thor	*Euphorbia nerifolia*	Euphorbiaceae	Shrub
38.	Choloharnach	*Dicoma tomentosa*	Asteraceae	Herb
39.	Dhatura	*Datura metal*	Solanaceae	Herb
40.	Doob grass	*Cynodan dactylon*	Poaceae	Grass
41.	Patharchatti	*Corbichonia decumbens*	Molluginaceae	Herb
42.	Bajar - bel	*Cocculus hirsutus*	Menispermaceae	Climber
43.	Aparajita	*Clitoria ternatea*	Papilionaceae	Climber
44.	Singali	*Cleome viscosa*	Cleomaceae	Herb
45.	Karalia	*Cleome gynandra*	Cleomaceae	Herb
46.	Gar-tumba	*Citrullus colocynthis*	Cucurbitaceae	Climber
47.	Chilva	*Chenopodium album*	Chenopodiaceae	Herb
48.	Karonda	*Carrisa carandas*	Apocynaceae	Shrub
49.	Karil	*Capparis decidua*	Capparaceae	Shrub
50.	Aakda	*Calotropis procera*	Asclepediaceae	Shrub
51.	Santhi	*Boerhavia procumbens*	Nyctaginaceae	Herb
52.	Ankh-Phorniki	*Bergia suffruticosa*	Elatinaceae	Herb
53.	Bajrandanti	*Bareria prionitis*	Acanthaceae	Shrub
54.	Wild onion	*Asphodelus tenuifolius*	Xanthorrhoeaceae	Herb
55.	Satawari	*Asparangus racemosus*	Asparagaceae	Climber
56.	Prickly poppy	*Argemone mexicana*	Papaveraceae	Herb
57.	Kalmegh	*Andrograpis paniculata*	Acanthaceae	Herb
58.	Jal bhangro	*Ammannia baccifera*	Lythraceae	Herb
59.	Chauli	*Amaranthus caudatus*	Amaranthaceae	Herb
60.	Pigweed	*Amaranthus blitoides*	Amaranthaceae	Herb
61.	Gwar Patha	*Aloe vera*	Liliaceae	Herb
62.	Kali-Bui	*Aerva lanata*	Amaranthaceae	Herb
63.	Ardusa	*Adhatoda vasica*	Acanthaceae	Shrub
64.	Senna	*Cassia auriculata*	Caesalpiniaceae	Shrub
65.	Chauriya	*Acalypa indica*	Euphorbiaceae	Herb
66.	Kanghil	*Abutilon indicum*	Malvaceae	Herb
67.	Chirmi	*Abrus precatorius*	Fabaceae	Climber

The study result indicated that grasses (2.70%), herbs (56.76%), climbers (18.91%), and shrubs (21.62%) were discovered in the study areas. The reported medicinal plants were classified based on habit and distribution (Fig. 4). In contrast to the slope and upper portion of the ravine lands, the valley beds of the ravines are covered in lush vegetation. This could be because during the monsoon and post-monsoon seasons, there is more soil moisture available in the valley beds. Herbaceous families like Asteraceae, Apocynaceae, Amaranthaceae, and Acanthaceae make up the majority of the herbaceous stratum of the rehabilitated cassia-based landuse system. Beyond nutrient acquisition, arbuscular mycorrhizal fungi (AMF) promote soil aggregation through the production of glomalin-related soil proteins, which improve aggregate stability and contribute to greater nutrient and water retention and enhanced soil fertility indicates that plant–microbe interactions likely play an important role in sustaining nutrient cycling in these nutrient-deficient sandy soils (Zaman *et al.,* 2025).These families play crucial ecological roles in soil stabilization, moisture retention, and pollinator support networks (Swarupanandan *et al.,* 2001). Legume plant species in the Caesalpiniaceae family, which are frequently or highly occupy in open or disturbed areas, are especially good ground cover forming vegetation at reducing erosion and encouraging the return of vegetation after disturbance events. Alsaveri *et al* (2026) findings suggested that *S. italica* enhances soil fertility through integrated plant–soil–microbe interactions involving biological nitrogen fixation, rhizosphere-mediated nutrient mobilisation, and mycorrhizal associations in arid soils of Jeddah - Saudi Arabia. *Senna italica* as a potential ecosystem engineer with promising applications in ecological restoration, soil rehabilitation, and sustainable management of degraded sandy soils in arid and semi-arid regions.

According to Archana *et al.* (2007), Banaras Hindu University in Varanasi, Uttar Pradesh, has a wide diversity of medicinal plant resources and their traditional usage. Similar trends of highlighting medicinal diversity and ethnomedical usage were found in another research carried out in Uttar Pradesh's Chandauli district (Singh and Singh, 2009). A properly restored ravine forest area has a much greater variety of medicinal plants as understory vegetation due to microclimate modification. This is

also consistent with a few other authors' findings that the regrowth of forest vegetation modifies soil fertility, light, temperature, and moisture, all of which have an impact on ground-level competition dynamics (Barbier *et al.* 2008; Carnevale and Montagnini 2002; Shankar *et al.* 1998, Kala *et al.*, 2023). In our study, greater tree diversity under forest regrowth was correlated with higher densities and diversities of medicinal plants.

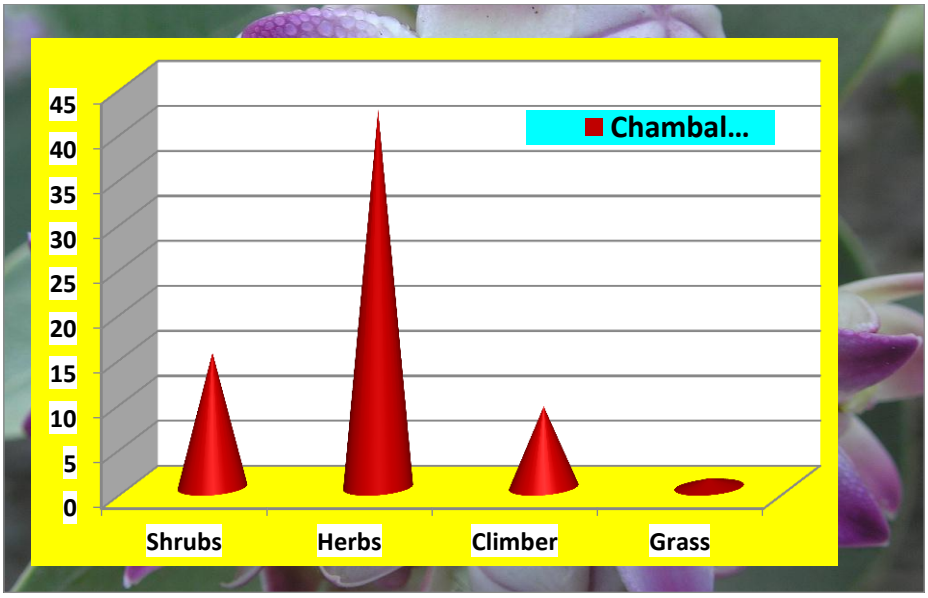

Fig.4. Habit wise distribution of medicinal plants under Cassia based rehabilitated Chambal Ravines

This demonstrates the variety and distribution pattern of medicinal plants in places that have been restored via successful conservation initiatives. The majority of the recognized medicinal plants have ecological responsibilities by preventing soil erosion by lowering runoff water velocity and the erosive effects of raindrops. Traditional healers and local residents (elderly people) in the sample region were also consulted for ethno-medical uses and knowledge. Each plant has a different component that is utilized to make medications. The most popular methods for preparing herbs were crushing, homogenizing with additives, and chewing. The most common application methods were drinking, inhaling smoke, and using a strap and hold-on.

A few therapeutic plants, including Keir, Ber, and Aloe vera, were found to be valuable in local marketplaces. Their market price was still unappealing, though. Although they were offered to the market in many forms, leaves, roots, seeds, and fruits were the most often marketed portions. Leaves, roots, bark, fruit, flowers, and seeds are used to make medicinal remedies. Plants applied as a paste, powder, decoction, extracts, and juices, as well as others applied as oil, smoke, and chew, are examples of the mode or formulation of intake. According to this survey, the most commonly used plant part was the leaf (28%), followed by the root (20%), fruit (14%), seed (11%), entire plant (9%), flower (6%), bark (8%), and gum (4%) (Fig. 5) in Chambal ravine region people. To create effective management strategies, indigenous knowledge and its applications must be examined. To guarantee equitable benefit sharing and greater involvement in conservation, the indigenous botanical knowledge of the local/traditional herbal healers must be respected.

Ethnoveterinary medicine deals with traditional animal health care, which includes methods, practices, beliefs, knowledge, and abilities. Therefore, it would be extremely important to describe ethnoveterinary medicine from areas with a rich cultural and biological environment. There is virtually little information known about ethnoveterinary medicinal plants in the ravine region. Compared to allelopathic medications, locally accessible and readily available medicinal plants offer a less expensive option for treating a variety of illnesses and diseases in livestock. With the goal to acquire data concerning the use of ethnoveterinary medicinal plants available in the area for treating various ailments and diseases of livestock, old, educated farmers and seasoned traditional healers who have traditional knowledge of these ethnoveterinary medicinal plants in the villages of the surveyed district were directly interviewed. Authours received consent of traditional healers and farmers were interviewed and acknowledge properly. The most significant native ravine ethnoveterinary medicinal plants may be crucial to the administration of animal health care, according to Table 2 and Fig.6. To record, preserve, and assess the effectiveness of these priceless medications, botanists, ethnobotanists, and ethnopharmacologists must work together urgently.

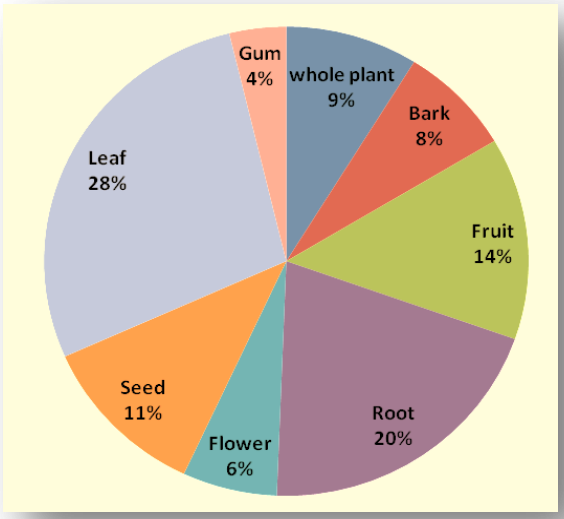

Fig 5. Plant parts wise number of medicinal plants used as a medicine.

These woody and herbaceous understory communities are particularly vulnerable to anthropogenic stressors and climate variability due to their ecological, functional, and economic significance. The resilience of the Chambal ravine forests and the stability of the ecosystem depend on their conservation. By enhancing canopy development, continuous litterfall, nutrient cycling, improving soil properties, and improving moisture regime, *Senna italica* may facilitate the revival of ground cover/ vegetation and contribute to long-term ecosystem resilience. Therefore, this native woody legume shows significant potential as a keystone plant species for degraded land mitigation, ecological restoration, soil rehabilitation, and sustainable land productivity in degraded arid landscapes (Alsaveri *et al.*,2026). Therefore, in order to maintain the biological integrity of these frequently disregarded but crucial understory components of ravine land for improved resource conservation, targeted management tactics with native shrubs and habitat-specific soil-moisture conservation interventions are necessary. Campaigns to raise awareness are therefore urgently needed to increase the local community's understanding of the significance and proper use of medicinal plants. Many people now believe that in situ conservation is the only practical and economical way to let a species continue on its natural evolutionary path while interacting with its environment and with itself. Even though the present data on the nation's medicinal plant variety is unclear and scattered among multiple research institutes, a thorough program to document the diversity and current state of medicinal plants in the country has to be started.

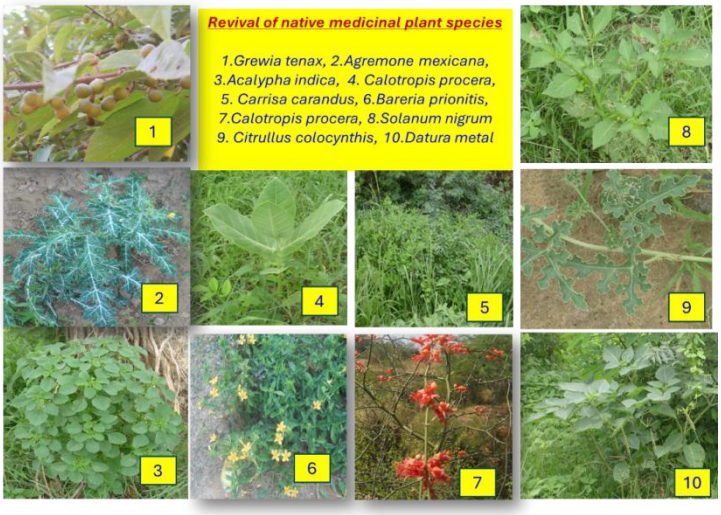

Fig.6. Revival of medicinal plants under *Cassia auriculata* based rehabilitated land use in Chambal ravine of Rajasthan

Table 2: Key plant species used as an ethno-veterinary medicine in Chambal ravines

Sl.No	Common Name	Plant Species	Ethno-veterinary medicinal uses	Part and Utility form
1.	Ardusa	*Adhatoda vasica*	Cure diarrhea	Leaf and bark
2.	Gwar Patha	*Aloe vera*	Ecto parasites	Leaf pulp
3.	Prickly poppy	*Argemone mexicana*	rheumatism	Leaf juice decoction
4.	Satawari	*Asparagus racemosus*	Cure arthritis and foot infection	Root powder and leaf juice
5.	Aakda	*Calotropis procera*	Snake bite	Latex
6.	Doob grass	*Cynodan dactylon*	Increasing lactation	Whole plant
7.	Datura	*Datura metal*	Wound healing	seed paste
8.	Bajrandanti	*Bareria prionitis*	Cure Fever and redness in body	Leaf juice and flower paste
9.	Kainth	*Grewia tenax*	Cure ulcers	Leaf +Flower paste
10.	Van Tulsi	*Ocimum grantissimum*	Cure cold and cough	Leaf decoction
11.	Tulsi	*Ocimum sanctum*	Removal stomach parasites	Leaf decoction
12.	Gokshura	*Tribulus terrestris*	Cure colic cough	Leaf juice
13.	Keir	*Capparis decidua*	Diarrhea	Leaf fodder
14.	Ber	*Ziziphus jujuba*	Skin burn	Leaf paste + Neem oil

CONCLUSION

According to the study, the groups Euphorbiaceae, Asteraceae, Apocynaceae, Solanaceae, and Caesalpiniaceae comprised the bulk of the species. Because they can withstand drought and thrive in challenging conditions, the identified and recorded indigenous plant species exhibit high intensity natural regeneration in ravine environments. In spite of its unpalatable nature, it can also be used as a hedge plant. It is used as a shelter and windbreak shrub in Rajasthan's desert to control wind erosion and enhance the productivity of degraded soils. Through cassia green cover-induced microclimatic conditions, enhance revival native plant species diversity in restored Chambal ravines, which undoubtedly facilitates / improves species richness and conserving biodiversity. Compared to other tree-based land-use systems, Tanner's Cassia-based rehabilitated ravine lands have rich biodiversity support future than according to the current study. Hence, crucial attention with proper species selection is essential towards enrichment of ravine biodiversity. Therefore, it is immediately required to preserve these significant plant species for long-term, sustainable uses. In the ravine areas, initiatives for sustainable farming and harvesting should be started. Furthermore, effective conservation measures, followed by appropriate documentation, conservation, sustainable utilization, and awareness building, are critical to ensuring the future expansion of indigenous medicinal flora in this resource-poor region for sustainable ravine land use.

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RESEARCH ARTICLE

NEMATODE COMMUNITIES OF MANGO AND GUAVA PLANTATIONS OF KASHIPUR, UTTARAKHAND: MORPHOLOGY AND MULTILOCUS MOLECULAR ANALYSIS

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Abstract: The nematode community is an essential part of the soil ecosystem, playing several trophic roles and taking part in plant health, decay processes, nutrient recycling and biocontrol. Nevertheless, there is no data concerning diversity and host specificity of nematodes occurring in the perennial fruit orchards of the Terai belt of Uttarakhand. In the present paper, the diversity, abundance, host specificity and taxonomic composition of nematodes occurring in mango (*Mangifera indica* L.) and guava (*Psidium guajava* L.) orchards of Kashipur, Udham Singh Nagar, Uttarakhand, India have been studied. Rhizosphere soil samples were collected from various locations, and nematodes were extracted by adequate techniques. Characterisation of representative nematodes was done by morphological and morphometric traits, such as body length, width, stylet, oesophagus, reproductive system, tail structure, etc. Special attention was paid to the analysis of plant-parasitic nematodes, especially root-knot nematodes, along with free-living and entomopathogenic nematodes. To ensure accurate species determination, representative nematode specimens were characterised at the molecular level by the analysis of ribosomal and mitochondrial gene loci (ITS, D2-D3 domain of 28S rRNA, 18S rRNA, COI). The present study presents an overall view of nematode communities in relation to two economically important fruits and provides the baseline information on nematode diversity, host preference and molecular affinities in the region of Kashipur. The results of the study will be helpful in the identification of important plant parasitic nematodes and also serve as the basis for future studies related to nematode ecology and management.

Keywords: Fruit orchards, Guava, Mango, Morphometrics, Phytonematodes

INTRODUCTION

Nematodes are one of the most abundant metazoans in terrestrial ecosystems and are found in multiple trophic levels in soil food webs. Plant-parasitic nematodes can lead to loss of root function and susceptibility to secondary pathogens in agricultural soils, while free-living nematodes and entomopathogenic nematodes play a role in decomposition, nutrient cycling and biological control of insects. Perennial orchards have relatively stable soil root systems and microhabitats that support complex nematode communities, the composition of which depends on the identity of the host, soil conditions and management.

India is an economically important tropical-subtropical country where mango and guava are important fruit crops. Root-knot nematodes (especially *Meloidogyne enterolobii*) have been associated with guava decline and wilt complexes on several occasions and may be present in the rhizosphere of mango trees as a combination of plant-parasitic and free-living species. In spite of the significant progress in molecular systematics, morphological identification remains essential, as diagnostic structures are stylet, oesophagus, reproductive system, tail, spicules, gubernaculum and female perineal pattern, which provide taxonomic information directly. However, species-level identifications can be challenging due to phenotypic plasticity, overlapping morphometric ranges and cryptic diversity.

Therefore, molecular markers are of value as an additional layer. The internal transcribed spacer (ITS) region and part of the D2-D3 expansion domains of the 28S rDNA region are commonly used for nematode species delimitation and phylogenetic reconstruction; 18S rDNA is useful for deeper relationships, and mitochondrial COI can enhance discrimination of closely related nematodes. In regional surveys where morphologically similar taxa

*Corresponding Author

can co-occur, an integrative approach is of great value.

Kashipur is part of the Terai–Bhabar transition of Uttarakhand, where intensive horticulture is carried out in a warm and humid subtropical climate. Although this landscape is of great agricultural significance, there are limited integrated data available on mango and guava nematodes. Objective of the current study was (a) to record the nematode communities found in association with M. indica and P. guajava, (b) measure the variations in nematode population density and diversity in the host plants, (c) morphological identification of the nematodes and (d) relate their morphological structure to molecular evidence.

MATERIALS AND METHODS

Study area and sampling design

Mango and guava orchards were taken from the study area, and fine-root and rhizosphere soil samples were collected in and around Kashipur, Udham Singh Nagar district, Uttarakhand. A host-stratified design with 60 samples (30 of *M. indica* and 30 of *P. guajava*). Within the active root zone of each tree, subsamples were taken from four locations around the tree at a depth of ~15–30 cm and pooled. Samples were taken in labelled polyethene bags and processed in cool laboratory conditions (Bhat *et al.,* 2021).

Nematode extraction and preservation

Soil samples were collected as 200 g soil aliquots and extracted using the Cobb-type decanting and sieving followed by migration using a Baermann funnel (Baermann, 1917). Any root-associated stages were gently washed and processed separately from the root material. Specimens selected for permanent mounts were killed by gentle heat, fixed in TAF and transferred gradually to glycerol following a Seinhorst-type procedure (Seinhorst, 1959). Live specimens for DNA extraction were moved to sterile water and then placed in appropriate storage prior to lysis (Soni *et al.,* 2026).

Morphological and morphometric characterisation

The temporary and permanent preparations were studied under compound light microscope. The total body length (L) and width of the body at its broadest point, the length of the stylet for Leucocytozoon, the length of the oesophagus, the length of the tail, anal/cloacal body diameter and reproductive characters were measured. These ratios include a = L/maximum body width; b = L/oesophageal length; c = L/tail length and c’ = tail length/body diameter at anus or cloaca. Records of female V% were obtained for appropriate taxa. Published characters were compared to the diagnostic characters (Bhat *et al.,* 2023).

Extraction of DNA, PCR and sequence analysis

For the genomic DNA extraction, representative individual nematodes were subjected to either a silica-column or single-nematode lysis protocol. ITS1-5.8S-ITS2, D2-D3 expansion segments of 28S rDNA, 18S SSU rDNA and mitochondrial COI were chosen depending on the taxonomic and diagnostic need. The PCR products were analysed using an agarose gel, purified and sequenced in both directions. The consensus sequences were searched for nucleotide similarity against curated reference sequences. Multiple sequence alignment was performed with the algorithm, and the relationships among species were assessed by a model-based maximum likelihood framework with bootstrap resampling (Lakshman *et al.,* 2026).

Statistical analysis

The abundance of nematodes was standardised as the number of individuals per 200 g soil. The prevalence was determined as the percentage of the samples in which a taxon was present. Morphometric and abundance variables were presented as means, with ranges and standard deviations, and presented as coefficients of variation. Distributional assumptions were assessed and independent-samples tests used to evaluate host-wise differences with non-normal variables, for which non-parametric tests were considered. Shannon diversity (H′), Simpson diversity (1–D) and Pielou evenness (J′) were used to summarize community structure. As appropriate, Pearson or Spearman correlations were used, and principal-component interpretation to summarise the multivariate morphometric patterns. The level of statistical significance was set at $\alpha = 0.05$ (Lakshman *et al.,* 2026).

RESULTS

Occurrence and abundance

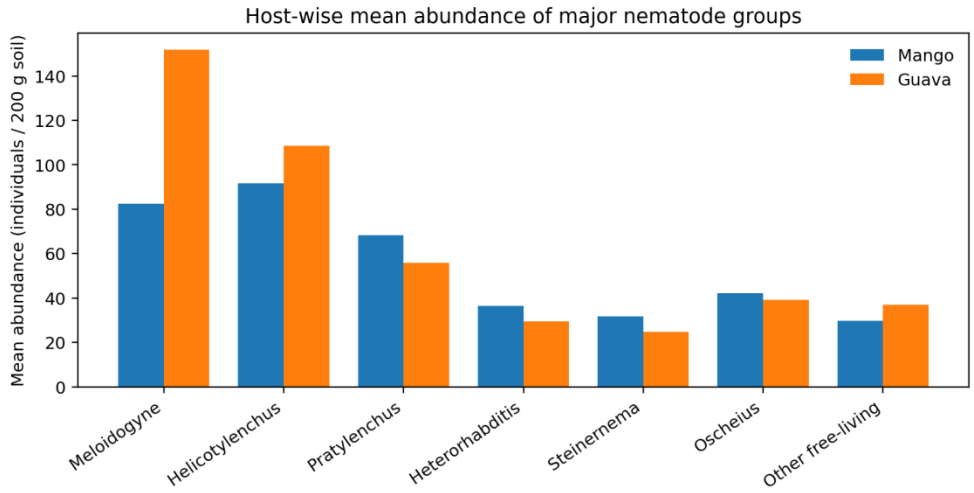

Figure 1. Host-wise mean abundance of major nematode groups recovered from mango and guava rhizosphere samples.

Taxon	Mango mean ± SD	Guava mean ± SD	Prevalence % Mango/Guava	p value
Meloidogyne spp.	82.4 ± 24.6	151.8 ± 39.7	73.3 / 93.3	<0.001
Helicotylenchus spp.	91.7 ± 26.1	108.5 ± 29.4	80.0 / 86.7	0.023
Pratylenchus spp.	68.3 ± 21.8	55.9 ± 18.6	66.7 / 60.0	0.021
Heterorhabditis spp.	36.5 ± 13.2	29.6 ± 11.4	43.3 / 36.7	0.035
Steinernema spp.	31.8 ± 11.6	24.7 ± 9.8	40.0 / 30.0	0.014
Oscheius spp.	42.1 ± 14.5	39.3 ± 13.8	50.0 / 46.7	0.447
Other free-living taxa	29.8 ± 10.9	37.0 ± 12.1	46.7 / 53.3	0.019
Total	382.6 ± 71.3	446.8 ± 82.4	100 / 100	0.002

The total nematode population was much higher in guava than in mango. The difference was primarily attributed to *Meloidogyne*, with its mean abundance being about 84% higher in guava than in mango. *Pratylenchus*, *Heterorhabditis* and *Steinernema* had higher mean abundance in mango compared to the others. There was no significant difference in abundance of *Oscheius* between hosts (Lalramnghaki *et al.*, 2018).

Community diversity

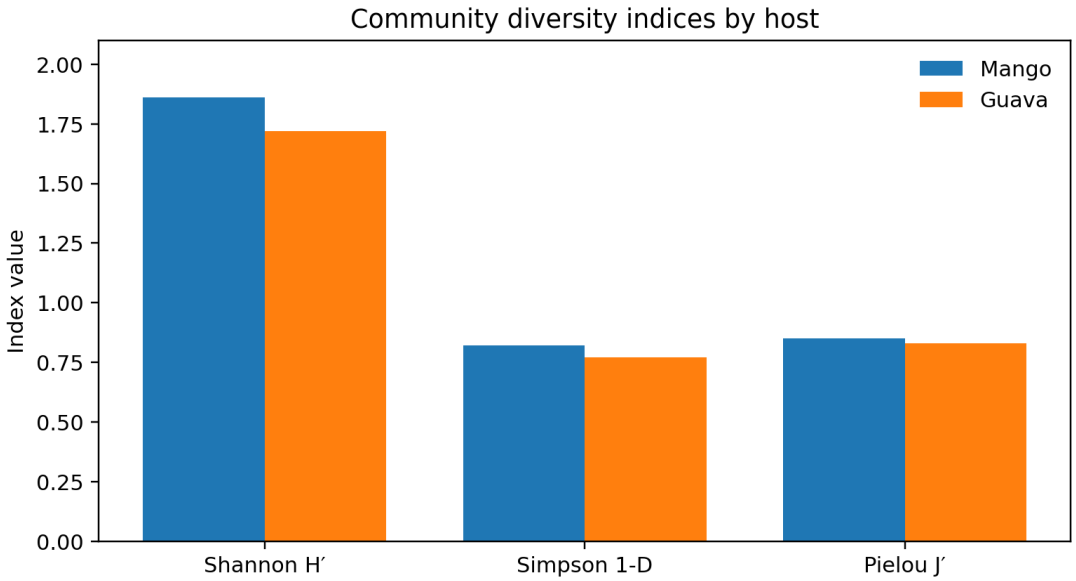

Figure 2. Comparison of Shannon diversity, Simpson diversity and Pielou evenness between mango- and guava-associated

Index	Mango	Guava	Interpretation
Taxon richness (S)	9	8	Slightly greater richness in mango
Shannon diversity (H′)	1.86	1.72	Greater diversity in mango
Simpson diversity (1–D)	0.82	0.77	Greater dominance in guava
Pielou evenness (J′)	0.85	0.83	Comparable evenness
Plant-parasitic: free-living ratio	1.73	2.41	Stronger plant-parasitic dominance in guava

The overall abundance was higher in guava, whereas mango had more richness and diversity. Guava showed numerical dominance of root-knot nematodes for the lower Shannon and Simpson values. The plant-parasitic/free-living ratio was also significantly higher in guava (Chandana *et al.*, 2026).

Representative morphometrics

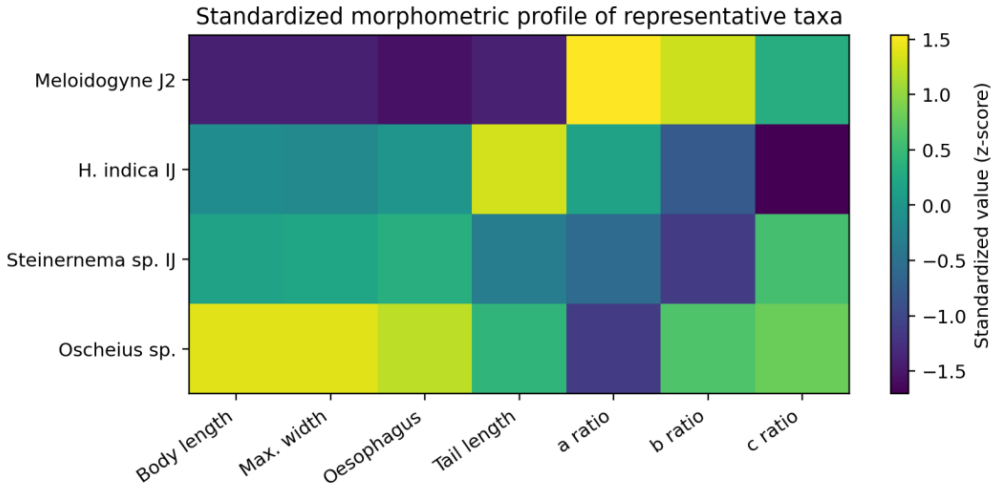

Figure 4. Standardised morphometric profiles of representative nematode taxa. Values are expressed as within-character z-scores to permit comparison across measurements with different units

Character	*Meloidogyne* J2 (n=20)	*H. indica* IJ (n=20)	*Steinernema* sp. IJ (n=20)	*Oscheius* sp. (n=20)
L (µm)	421.6 ± 18.7	566.2 ± 29.4	598.5 ± 34.1	735.8 ± 41.6
Max. width (µm)	15.8 ± 1.2	22.7 ± 1.8	24.9 ± 2.1	31.5 ± 2.8
Stylet (µm)	15.3 ± 0.7	—	—	—
Oesophagus (µm)	92.4 ± 5.8	132.6 ± 8.4	141.8 ± 9.2	164.7 ± 10.3
Tail (µm)	51.7 ± 3.6	99.8 ± 7.5	70.6 ± 5.4	83.9 ± 6.8
a ratio	26.7 ± 2.1	25.0 ± 2.4	24.1 ± 2.2	23.4 ± 2.5
b ratio	4.56 ± 0.31	4.27 ± 0.35	4.22 ± 0.37	4.47 ± 0.39
c ratio	8.16 ± 0.62	5.68 ± 0.51	8.48 ± 0.73	8.77 ± 0.81

The root-knot juvenile population was characterised by a slender vermiform body, a distinct stylet and a gradually tapering tail. The *Heterorhabditis* infective juveniles were comparatively slender with an elongated tail, while the *Steinernema* population had a moderately longer body and shorter proportionate tail. The oesophageal organisation was *rhabditid*, and *Oscheius* specimens were larger (Subbotin *et al.*, 2006).

Molecular characterisation

Representative taxon	Marker	Amplicon/consensus length (bp)	Closest reference-level identity	Interpretive support
Meloidogyne cf. enterolobii	ITS rDNA	~760	99.4–99.8%	Strong
Meloidogyne cf. enterolobii	D2–D3 28S	~780	99.5–99.9%	Strong
Heterorhabditis cf. indica	ITS rDNA	~786	99.3–99.8%	Strong
Steinernema sp.	ITS rDNA	~820	98.8–99.5%	Moderate–strong
Oscheius sp.	18S rDNA	~900	99.0–99.6%	Strong at genus/lineage level
Selected isolates	COI	~650	97.9–99.2%	Supporting

Concordance between the marker comparisons and the principal morphological assignments was obtained. *Meloidogyne* was most similar to *M. enterolobii* reference sequences in both ribosomal markers, with greatest similarity in the IGS. The IGS had the greatest similarity to *M. enterolobii* reference sequences for *Meloidogyne*, and *M. enterolobii* had the greatest similarity in the IGS. The *Heterorhabditis* population grouped with *H. indica*-like reference sequences, which is in agreement with the morphology of the infective juveniles. The additional discrimination provided by COI was due to the relatedness of the isolates (Vrain *et al.,* 1992).

Host-wise statistical relationships

Relationship/test	Statistic	p value	Inference
Host vs total abundance	t = 3.22	0.002	Significant
Host vs *Meloidogyne* abundance	t = 8.13	<0.001	Highly significant
Host vs Shannon diversity	t = 2.18	0.033	Significant
***Meloidogyne* abundance vs root gall index**	r = 0.78	<0.001	Strong positive association
Soil moisture vs total nematode abundance	r = 0.46	<0.001	Moderate positive association
pH vs plant-parasitic abundance	r = −0.29	0.025	Weak negative association

Root galling was most closely correlated with *Meloidogyne* abundance. The number of nematodes did not show a strong increase with soil moisture, and a weak negative correlation with plant-parasitic abundance increased with pH. The two-axis principal component interpretation explained 67.4% morphometric variation, with PC1 being largely associated with body length, oesophageal length and body width, while PC2 was associated mainly with tail length and c/c′-related shape variables (Kumar *et al.,* 2024).

DISCUSSION

The measured host-associated contrast is biologically feasible for perennial orchard systems. Total nematode abundance and the size of the *Meloidogyne* component were higher in guava, while the taxonomic diversity was slightly higher in mango. The occurrence of root-knot nematode follows the general trend seen in Indian literature, with *M. arenaria* and *H. annosum* being identified as important constraints in guava; in recent community-level investigations, high dominance of *Meloidogyne* and *Helicotylenchus* was observed in guava orchards. The morphology of the root-knot population and high similarity of the ribosomal marker genes confirmed similarities with *M. enterolobii*. The need to make careful morphometric measurements of Indian guava populations, as the earlier descriptions may be inconsistent. The juvenile and female morphology, together with the D2–D3 28S, COI and SCAR evidence, confirmed *M. enterolobii* in Kerala (Anes *et al*., 2023). Aligning with this integrative diagnostic approach, this work was followed in 2026 by using morphology, ITS and COI (Lakshman *et al.,* 2026). This is the same principle in the present analytical framework, where sequence evidence should support, and not replace, morphology.

From an ecological and applied standpoint, the recovery of *Heterorhabditis*-like populations is interesting in three respects. Relationships in *Heterorhabditis* species have been established through the use of ITS and 28S (Bhat *et al.,* 2021), and most recently, *Heterorhabditis* indica VLEPN01 has been described from the Mid-Indian Himalayas

(Singh *et al.*, 2025). IENs could be a beneficial part of the orchard soil microbial community and could be used in orchard-based biological-control programs following pathogenicity and field persistence testing.

The variability within the recovered populations was generally low, although a few characters were common to related taxa. This overlap exemplifies that measurements can be inadequate for species delimitation. This uncertainty can be minimised by multilocus analysis, especially in conjunction with ITS and 28S that provide evidence for ribosomal lineages, and COI that provides mitochondrial differentiation. On the other hand, if a diagnostic morphology is in disagreement to high sequence identity at a conserved marker, it should not be considered definitive species identity.

Community indices showed that there is no need for numerical abundance, and ecological diversity must change concurrently. The nematode assemblage at Guava was large, with a low H′ and 1-D value due to the dominance of a small number of plant-parasitic taxa. In the case of mango, there was a more balanced distribution between parasitic, entomopathogenic and other free-living groups. These data reinforce the need to report density and diversity in orchard nematology.

The correlation between *Meloidogyne* population and root galling is strong, giving a quantitative relationship between soil populations and root damage. However, a correlation does not necessarily imply a complete disease complex. Nematodes, fungi, soil and plant age and management may interact in guava decline. Experimental work in the future should, therefore, be integrated by using pathogenicity tests, fungal diagnostics, and seasonal sampling.

The study provides practical application of a diagnostic workflow for regional nematode surveys: standardised sampling, extraction, morphologic screening, morphometric quantification, targeted marker amplification and sequence comparison and phylogenetic verification. This can help differentiate dominant plant parasites from beneficial and neutral soil nematodes and may be useful in prioritising taxa to manage or conserve.

CONCLUSION

The integrative assessment revealed that the mango and guava rhizospheres had different nematode assemblages in the Kashipur region. *Meloidogyne* appeared to be the dominant species in guava, and mango had slightly higher diversity in the community, but more abundance of some entomopathogenic/free-living groups. The major taxonomic assignments were confirmed by the morphometric patterns in conjunction with multilocus marker comparison. The findings highlight the importance of taking simultaneously into account the morphology, quantitative population structure and molecular evidence to define the nematode. This information may help with orchard health monitoring, early detection of damaging populations of root-knot and future assessments of native entomopathogenic nematodes for sustainable root-knot pest control.

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