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Effect of indigenous liquid compatible microbial consortia on seed germination of *Ximenia americana* L. harvested from Amrabad Tiger Reserve Forest of Telangana, India

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Abstract

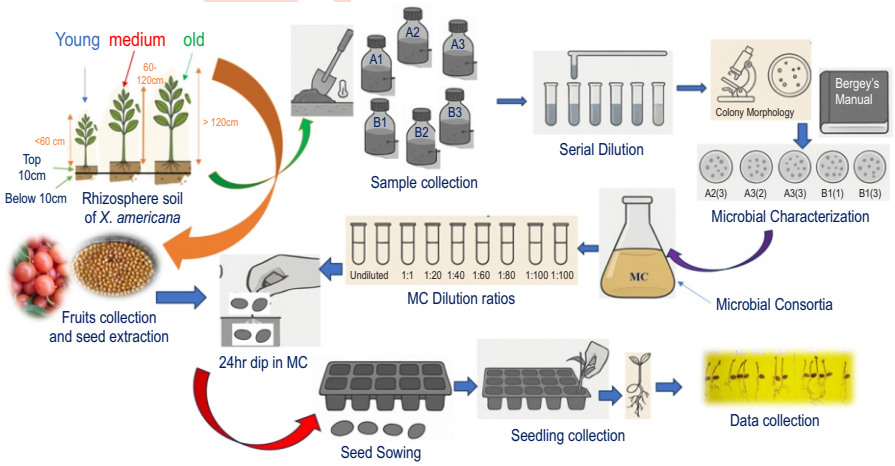
Aim: The present investigation was undertaken to develop a sustainable conservation protocol for the threatened medicinal tree *Ximenia americana* L. by enhancing seed germination and early seedling vigour through biopriming with an indigenous, site-specific liquid microbial consortium isolated from its native rhizosphere in the Amrabad Tiger Reserve.

Methodology: Six morphologically distinct, mutually compatible rhizobacterial strains were isolated from the rhizosphere of *Ximenia americana,* pooled in equal proportion to formulate a stable liquid consortium and applied to surface-sterilized seeds at six serial dilutions (1:1, 1:20, 1:40, 1:60, 1:80, 1:100 v/v) along with a distilled-water control under a completely randomized design with three replications.

Results: Biopriming at the 1:80 dilution (T8) produced the highest germination (83.33%), shortest mean germination time (4.59 days), maximum seedling length (15.90 cm), dry weight (1.65 g) and seedling vigour indices (SVI-I 1244.67; SVI-II 129.13), whereas the undiluted consortium suppressed germination.

Interpretation: An optimally diluted indigenous microbial consortium serves as an eco-friendly, low-cost biotechnological intervention to overcome reproductive bottlenecks and aid *in-situ* and *ex-situ* conservation of *Ximenia americana*.

Key words: Biopriming, Microbial consortia, Rhizospheric soil, *Ximnenia americana* L.



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Introduction

Ximenia americana L. (Olacaceae), commonly known as sour plum or wild plum, is a small thorny shrub or tree widely distributed across the tropical and subtropical regions of Africa, Asia and the Americas. In the Deccan plateau of peninsular India, particularly within the Nallamala forest tract of the Amrabad Tiger Reserve, the species occurs as a characteristic understory component of dry deciduous forests (Dejene *et al.,* 2020). The fruit pulp, kernel oil, bark and leaves possess substantial ethnopharmacological value, with documented antimicrobial, anti-inflammatory, anthelmintic and antioxidant activities, while the kernels yield a high-value edible and cosmetic oil. Despite its medicinal and economic importance, *X. americana* has been classified as ecologically vulnerable owing to chronic over-exploitation, fragmentation of dry-deciduous habitats, low recruitment in nature and persistently poor seed germination (Grover *et al.,* 2021). The reproductive failure of *X. americana* is governed by a combination of physical, chemical and microbiological constraints. The seed consists a thick, lignified and sclerified seed coat that severely restricts imbibition and embryo expansion (Coder and Warnell, 1999).

This physical barrier is compounded by chemical dormancy imposed by autotoxic allelochemicals released from fruit peels that accumulate beneath the maternal canopy following frugivorous dispersal (Muller, 1969). Furthermore, in semi-arid forest environments such as the Amrabad Tiger Reserve with erratic monsoon patterns, high evapotranspiration and rapid surface drying further reduce the narrow window of viable germination. Seed germination is no longer regarded as a purely physiological event, but as a biologically mediated transition strongly modulated by the rhizosphere microbiome. The plant–soil–microbe interactome assembles before radicle emergence and continues to influence seedling establishment through nutrient mobilization, phytohormone synthesis, allelochemical detoxification and induction of systemic resistance (Carvalhais *et al.,* 2015; Backer *et al.,* 2018). Plant growth-promoting rhizobacteria (PGPR) elaborate indole-3-acetic acid (IAA), gibberellins, cytokinins, ACC-deaminase, siderophores, exopolysaccharides (EPS) and a battery of hydrolytic enzymes that collectively accelerate seed metabolism and seedling vigour (Ansari and Ahmad, 2019; Basu *et al.,* 2021). Seed biopriming, defined as the controlled hydration of seeds in the presence of beneficial microorganisms, has emerged as an inexpensive, eco-friendly alternative to chemical scarification and growth-regulator treatments for breaking dormancy in recalcitrant species (Mahmood *et al.,* 2016).

Despite the proven utility of biopriming, a critical appraisal of the literature published over the last four years (2021–2025) reveals several conspicuous research gaps. First, the overwhelming majority of biopriming studies have been confined to commercially important crop species such as tomato (Singh *et al.,* 2019; Raja *et al.,* 2019b; Rangasamy and Saleh, 2025), blackgram (Raja *et al.,* 2019a), French bean (Kangjam *et al.,* 2023), rice and maize, while wild medicinal tree species remain largely neglected. Second, most reported consortia have been formulated from culture-collection strains or strains isolated from agricultural soils, with limited attention to truly indigenous, site-specific rhizobacteria co-evolved with the target host.

Recent reviews (Adeboye *et al.,* 2025; Shil *et al.,* 2025; Singh *et al.,* 2025) have emphasized that microbial inoculants tailored to the host's native rhizosphere consistently outperform generic formulations, particularly under dryland and semi-arid stress (Timofeeva *et al.,* 2023; Ait-El-Mokhtar *et al.,* 2023). Third, dose–response optimization of liquid microbial consortia for wild tree species remains poorly explored; the few existing reports rarely encompass a graded dilution series capable of distinguishing stimulatory from inhibitory microbial loads. Fourth, to the best of our knowledge, no investigation has been published on the use of native rhizospheric consortia for the conservation propagation of *X. americana* from any Indian biosphere reserve, including the Amrabad Tiger Reserve.

The novelty of the present study lies in the isolation, screening and assembly of a six-strain indigenous liquid compatible microbial consortium derived exclusively from the rhizosphere of mature, medium-aged and juvenile X. americana populations of the Amrabad Tiger Reserve; a systematic serialized dilution dose–response evaluation (1:1 to 1:100 v/v) designed to identify the optimum microbial load for germination enhancement of a hard-coated, allelopathically inhibited wild tree species; and the integration of microbiological intervention with in situ conservation needs of a vulnerable medicinal species of the Deccan plateau. Accordingly, the present investigation was undertaken with the aim to isolate and morphologically characterize culturable rhizobacteria from the native rhizosphere of *X. americana* in the Amrabad Tiger Reserve and formulate a stable, mutually compatible liquid microbial consortium inorder to evaluate the dose-dependent effect of the consortium on seed germination kinetics, seedling morphometrics, biomass accumulation and vigour indices of *X. americana*, and identify the optimum dilution suitable for development of a conservation-oriented biopriming protocol.

Materials and Methods

Study area: The study was conducted using rhizospheric soil and seeds collected from natural populations of *Ximenia americana* L. distributed within the Amrabad Tiger Reserve, Nagarkurnool district, Telangana, India (geographical coordinates 16°00'–16°30' N latitude, 78°30'–79°15' E longitude; elevation 300–900 m above mean sea level). The reserve covers approximately 2611 km² of the Nallamala forest tract and experiences a semi-arid tropical climate with mean annual rainfall of 700–900 mm, mean annual temperature of 25–32 °C, and predominantly red lateritic soils interspersed with rocky outcrops. Subsequent laboratory and nursery experiments were carried out at the Department of Fruit Science, College of Horticulture, Sri Konda Laxman Telangana State Horticultural University, Mojerla, Telangana, India, during 2022–2023.

Rhizospheric soil sampling: To capture the natural variability of root-associated microbiota, samples were collected from three age cohorts of *X. americana* classified on the basis of plant height: young (<60 cm), medium-aged (60–120 cm) and mature (120–240 cm). At each plant, rhizospheric soil firmly adhering to the root system was excavated aseptically from 0–10 cm depth in close proximity to active feeder roots using sterile spatulas. Samples were placed in sterile, airtight polythene bags, transported to the laboratory in an ice box at 4 °C and processed within 24 hr of collection.

Isolation, purification and morphological characterization of rhizobacteria: Bacterial enrichment was carried out on M9 minimal medium and nutrient agar (NA). One gram of each rhizospheric soil sample was serially diluted (10^{-1} to 10^{-7}) in sterile phosphate-buffered saline (PBS, pH 7.2). Aliquots of 100 µl from appropriate dilutions were plated by the streak-plate method and incubated at 25–30°C for 24–72 h. Bacterial growth was monitored by recording the optical density at 600 nm (OD_{600}) at pH 7.0–7.2. Morphologically distinct colonies were repeatedly sub-cultured until pure cultures were obtained. Isolates were characterized for colony attributes (shape, margin, elevation, pigmentation, opacity) and microscopic features (cell shape, arrangement and Gram reaction) using the protocol of Bartholomew and Mittwer (1952).

Compatibility screening and consortium development: From an initial pool of 18 morphologically distinct isolates, six superior strains—designated A2(3), A3(2), A3(3), A3(4), B1(1) and B1(3)-were selected on the basis of in vitro mutual compatibility (absence of inhibition zones during cross-streak assays) and preliminary screening for seed-germination enhancement. The six selected isolates were grown individually in nutrient broth to a uniform population density ($OD_{600} \approx 0.8$) and pooled in equal proportion (v/v) to formulate a stable liquid microbial consortium that mimicked the natural rhizospheric assemblage. The undiluted consortium was further subjected to serial aqueous dilutions to obtain ratios of 1:20, 1:40, 1:60, 1:80 and 1:100 (v/v) using sterile distilled water.

Seed collection, surface sterilization and biopriming: Mature, ripe fruits of *X. americana* were harvested manually from healthy mother trees in the Amrabad Tiger Reserve. Seeds were extracted, depulped, washed thoroughly with running tap water, surface-sterilized with 0.1% (w/v) mercuric chloride for 2 min followed by three rinses with sterile distilled water, and air-dried under aseptic conditions. Biopriming was performed by soaking the sterilized seeds in the respective consortium dilution for 24 h at room temperature. Seeds soaked in sterile double-distilled water (ddH_2O) for 24 h served as the hydropriming control.

Experimental design and sowing: The experiment was laid out in a Completely Randomized Design (CRD) with nine treatments and three replications (10 seeds per replication, 30 seeds per treatment). Treatments were as follows: T_1—Consortium 1:1 (undiluted); T_2—Control (ddH_2O, hydropriming); T_3— Consortium 1:20; T_4 — Consortium 1:40; T_5 — Consortium 1:60 (first set); T_6— Consortium 1:60 (replicate set); T_7— Consortium 1:80 (first set); T_8— Consortium 1:80 (optimized set); T_9— Consortium 1:100. Bioprimed seeds were sown in pro-trays filled with a sterilized growing medium consisting of cocopeat (33%), coarse sand (33%), vermicompost (17%) and red soil (17%) (v/v). Trays were maintained under nursery conditions and irrigated uniformly with sterile water as required for 30 days.

Germination and growth parameters: Daily germination counts were recorded over a 30-day observation period. The following parameters were computed (ISTA, 1995): Germination percent, Germination Rate Index Esechie (1994), Mean germination rate (Ellis and Roberts, 1981) Speed of Germination (Maguire, 1962). Mean daily germination (Czabator., 1962). Peak value of germination and Germination value (GV) Germination value was determined using the formula given by Czabator (1962). At 30 days after sowing, the seedlings were carefully uprooted, washed and the following morphometric parameters were recorded: root length (cm; root tip to hypocotyl), shoot length (cm; collar to apex), root:shoot ratio and total seedling length (cm). Fresh weight (g) was recorded immediately on an electronic balance; dry weight (g) was determined after oven-drying at 82 ± 2 °C for 24 hr.

Seedling Vigour Indices

Shoot length: Shoot length was measured in centimetre (cm) from collar to apex after 30 days of sowing.

Root length: To measure root lengths, two seedlings at the two-leaf stage were selected from each treatment and the primary root length was measured from its tip to the hypocotyl and expressed in centimetre.

Seedling length: The length of seedlings from each treatment were randomly selected and measured forlength in centimetre (cm), as described by Ghosh *et al.* (2003).

Root: shoot ratio: By calculating the mean value of length of roots and shoot of seedlings, the root-shoot ratio was determined.

Fresh weight: Three germinated seedlings were to measure for their fresh weight (g).

Dry weight: Biomass (dry weight) of phenotypically normal seedlings was estimated by wrapping them inan aluminium foil and subsequentdrying in a hot air oven at 82±2°C for 24 hours. The dried seedlings were then weighed. The dry weight was expressed in gram (Yao *et al.,* 2012).

Seedling Vigor Index I: The seedling vigour index I was calculated by taking the average seedling length (root, shoot length) of randomly selected normal seedlings (Abdul - Baki and Anderson, 1973).

Seedling vigor index II: The seedling vigour index II (SVIII) values were determined in relation to vigour index. The average

Table 1: Morphological characteristics of bacterial colonies and cells from rhizosphere of *Ximenia americana*

Isolates	Shape	Margin	Elevation	Color	Opacity	Gram's nature	Shape & Arrangement
A2(3)	Punctiform	Smooth	Flat	Whitish	Opaque	Gram positive	Rod
A3(2)	Irregular	Wavy	Flat	Pale yellow	Transparent	Gram positive	Cocci
A3(3)	Circular	Entire	Convex	Yellow	Transparent	Gram positive	Cocci
A3(4)	Circular	Smooth	Raised	Whitish	Opaque	Gram positive	Diplococcus
B1(1)	Circular	Entire	Flat	Creamy white	Opaque	Gram negative	Diplobacilli
B1(3)	Circular	Smooth	Slightly raised	Whitish	Transparent	Gram positive	Streptobacilli

seedling dry weight of selected seedlings were used to determine normal seedlings number (Abdul - Baki and Anderson, 1973).

Statistical analysis: All data were subjected to One-way ANOVA using the SAS statistical package (v6.12). Treatment means were separated by Duncan's New Multiple Range Test (DMRT) at $p \leq 0.05$.

Results and Discussion

The present study constitutes the first global report on the isolation and morphological characterization of native rhizospheric bacteria associated with *Ximenia americana* L., thereby establishing a foundational baseline for future microbiome-based conservation studies of this species. Six pure rhizobacterial strains-A2(3), A3(2), A3(3), A3(4), B1(1) and B1(3)-were successfully isolated from the rhizosphere of *X. americana* within the Amrabad Tiger Reserve. The isolates exhibited considerable phenotypic diversity in colony morphology (punctiform, irregular and circular shapes; smooth, wavy and entire margins; flat, convex, raised and slightly raised elevations; pigmentation ranging from whitish, creamy white and pale yellow to yellow) and microscopic features (rods, cocci, diplococci, diplobacilli and streptobacilli). Five of the six isolates were Gram-positive, while B1(1) was the sole Gram-negative diplobacillus (Table 1). The morphological heterogeneity observed in the present study reflects a functionally diverse rhizospheric assemblage capable of complementary biochemical activities. Such phenotypic and structural complementarity is widely regarded as a prerequisite for the formulation of effective multi-strain consortia (Backer *et al.,* 2018; Basu *et al.,* 2021).

The co-existence of Gram-positive and Gram-negative members further enhances ecological resilience under fluctuating soil moisture and temperature regimes typical of semi-arid forest ecosystems (Ait-El-Mokhtar *et al.,* 2023). Comparable assemblages of indigenous PGPR have been reported from the rhizosphere of stressed perennial species and have been associated with enhanced plant growth promotion (Grover *et al.,* 2021; Timofeeva *et al.,* 2023). Biopriming with the indigenous liquid consortium produced a clear and statistically significant dose-dependent response in all germination parameters (Table 2). The highest germination percentage (83.33%) was recorded in T_8 (1:80 dilution), followed by T9 (73.33%; 1:100). The undiluted consortium (T_1, 1:1) recorded the poorest germination (20.00%), which was lower than the hydropriming control (T_2, 26.67%). The mean germination time was sharply reduced from 8.38 days (T_2) and 7.61 days (T_1) to 4.59 days in T8, indicating a marked acceleration.

The observed inverted U-shaped dose–response curve provides strong mechanistic evidence that microbial inoculation enhances germination only within a critical population window. At intermediate dilutions, particularly 1:80, the microbial load is sufficient to colonize the seed coat and elaborate IAA, gibberellins, organic acids and hydrolytic enzymes that soften the lignified testa, neutralize autotoxic allelochemicals and accelerate imbibition (Mahmood *et al.,* 2016; Ansari and Ahmad, 2019). The pronounced reduction in MGT closely parallels the findings of Singh *et al.* (2019) in tomato and Raja *et al.* (2019a, b) in blackgram and tomato, where indigenous liquid consortia advanced germination by 2–4 days. In contrast, the inhibitory effect at high microbial densities (T_1) is attributable to competition for oxygen at the seed–microbe interface, accumulation of microbial metabolites, and possible quorum-mediated antagonism among co-inoculated strains (Nayaka *et al.,* 2010; Rangasamy and Saleh, 2025). A similar suppression at undiluted inoculation levels was reported in maize by Rudolph *et al.* (2015) and reviewed by Adeboye *et al.* (2025), reinforcing the present interpretation that microbial dose must be optimized for each host–consortium combination. Treatment T_8 consistently produced the most vigorous seedlings (Table 3), recording the maximum root length (11.57 ± 0.97 cm), shoot length (5.90 ± 0.19 cm), seedling length (15.90 ± 0.62 cm) and root:shoot ratio (1.96 ± 0.18). The corresponding values for T_1 were 5.27 ± 0.50 cm, 3.51 ± 0.30 cm, 8.25 ± 0.97 cm and 1.50 ± 0.04, respectively. Intermediate dilutions (T_4, T_6) also significantly out-performed both the undiluted treatment and the control.

The pronounced enhancement of root architecture under T_8 is of particular ecological significance for *X. americana* growing in shallow, drought-prone soils of the Amrabad Tiger Reserve. PGPR-elaborated IAA stimulates lateral and adventitious root proliferation, while ACC-deaminase activity lowers stress-induced ethylene and prolongs root elongation (Backer *et al.,* 2018; Singh *et al.,* 2025). Phosphate-solubilizing and siderophore-producing rhizobacteria additionally mobilize otherwise unavailable nutrients in the immediate seed micro-environment (Duarah *et al.,* 2011; Ghosh *et al.,* 2003). The higher

Table 2: Effect of indigenous liquid compatible microbial consortia on Germination (%), Germination Rate Index, Mean Germination Time, Mean Daily Germination, Peak Value, Germination Value, Speed of Germination, Seedling Vigor Index I and II of *Ximenia americana*

Treatments	Germination (%)	Germination Rate Index (%/day)	Mean Germination Time (day)	Speed of Germination	Mean Daily Germination	Peak Value	Germination Value	Seedling Vigor indexI	Seedling Vigor indexII
T_1	20 ± 0^{f}	8.79 ± 0.21^{e}	7.61 ± 0.19^{de}	0.09 ± 0^{e}	0.08 ± 0^{f}	0.08 ± 0^{f}	0.01 ± 0^{f}	153.99 ± 20.5^{g}	21.95 ± 2.64^{d}
T_2	26.67 ± 5.77^{ef}	12.24 ± 2.03^{e}	8.38 ± 2.19^{e}	0.12 ± 0.02^{e}	0.09 ± 0.0^{f}	0.11 ± 0.02^{ef}	0.01 ± 0.01^{f}	206.63 ± 103.16^{g}	28.67 ± 13.78^{d}
T_3	33.33 ± 5.77^{e}	15.58 ± 3.17^{e}	7.19 ± 0.26^{cde}	0.14 ± 0.01^{e}	0.16 ± 0.05^{e}	0.14 ± 0.02^{e}	0.02 ± 0.01^{ef}	339.83 ± 63.04^{f}	41.17 ± 7.66^{d}
T_4	63.33 ± 5.77^{bc}	37.67 ± 2.79^{c}	5.75 ± 0.7^{abc}	0.38 ± 0.03^{ac}	0.25 ± 0.02^{bc}	0.29 ± 0.02^{c}	0.07 ± 0.01^{c}	778.67 ± 90.69^{d}	95.8 ± 4.85^{b}
T_5	50 ± 10^{d}	25.92 ± 4.91^{d}	6.58 ± 1.44^{bcde}	0.26 ± 0.05^{d}	0.2 ± 0.04^{de}	0.21 ± 0.03^{d}	0.04 ± 0.01^{de}	506.7 ± 119.22^{e}	63.77 ± 17.74^{c}
T_6	53.33 ± 5.77^{cd}	30.43 ± 3.24^{cd}	6.02 ± 0.74^{bcd}	0.3 ± 0.03^{cd}	0.21 ± 0.02^{cde}	0.24 ± 0.01^{cd}	0.05 ± 0.01^{d}	910.3 ± 49.54^{c}	92.71 ± 10.61^{b}
T_7	56.67 ± 5.77^{cd}	29.58 ± 4.04^{d}	6.53 ± 0.68^{bcde}	0.3 ± 0.04^{d}	0.23 ± 0.02^{cd}	0.24 ± 0.03^{d}	0.06 ± 0.01^{cd}	537.53 ± 75.23^{e}	79.17 ± 16.35^{bc}
T_8	83.33 ± 5.77^{a}	63.78 ± 6.33^{a}	4.59 ± 0.3^{a}	0.64 ± 0.06^{a}	0.33 ± 0.02^{a}	0.47 ± 0.03^{a}	0.16 ± 0.02^{da}	1244.67 ± 38.18^{a}	129.13 ± 0.42^{a}
T_9	73.33 ± 5.77^{ab}	49.39 ± 7.65^{b}	5.15 ± 0.25^{ab}	0.49 ± 0.08^{b}	0.29 ± 0.02^{ab}	0.37 ± 0.03^{b}	0.11 ± 0.02^{b}	1092.83 ± 70.43^{b}	96.75 ± 14.05^{b}
Mean	51.111	30.38	6.42	0.303	0.206	0.240	0.059	641.24	72.12
C.V.	11.907	14.37	15.22	14.317	14.838	10.547	21.043	11.85	15.82
F Prob.	0.000	0.00	0.00	0.000	0.000	0.000	0.000	0.000	0.000
S.E.M.	3.514	2.52	0.56	0.025	0.018	0.015	0.007	43.89	6.59
C.D. 5%	10.440	7.49	1.68	0.074	0.053	0.043	0.021	130.40	19.57
C.D. 1%	14.303	10.26	2.30	0.102	0.072	0.059	0.029	178.65	26.81

Means within a column followed by different superscript letters differ significantly (DMRT, $p \leq 0.05$).

Table 3: Effect of indigenous liquid compatible microbial consortia on root length, shoot length, root:shoot ratio, seedling length, fresh weight and dry weight of *Ximenia americana* L. seedlings at 30 days after sowing.

Treatments	Root length (cm)	Shoot length (cm)	Root: Shoot ratio	Seedling length (cm)	Fresh weight (g)	Dry weight (g)
T_1	5.27 ± 0.5^{f}	3.51 ± 0.3^{e}	1.5 ± 0.04^{cd}	8.25 ± 0.97^{e}	1.46 ± 0.05^{e}	1.17 ± 0.07^{c}
T_2	5.89 ± 0.38^{ef}	4.31 ± 0.49^{bcd}	1.38 ± 0.18^{d}	9.61 ± 0.25^{d}	1.52 ± 0.05^{de}	1.25 ± 0.11^{c}
T_3	6.64 ± 0.36^{de}	4.3 ± 0.19^{cd}	1.54 ± 0.1^{bcd}	10.18 ± 0.29^{d}	1.56 ± 0.05^{cde}	1.23 ± 0.02^{c}
T_4	8.94 ± 0.56^{bc}	4.89 ± 0.22^{b}	1.83 ± 0.08^{a}	12.3 ± 1.05^{c}	1.73 ± 0.1^{bc}	1.52 ± 0.15^{ab}
T_5	7.27 ± 0.54^{d}	4.17 ± 0.28^{d}	1.75 ± 0.24^{abc}	9.52 ± 0.47^{d}	1.52 ± 0.09^{de}	1.19 ± 0.06^{c}
T_6	8.32 ± 0.2^{c}	4.61 ± 0.36^{bcd}	1.81 ± 0.19^{ab}	12.27 ± 0.85^{c}	1.58 ± 0.15^{cde}	1.25 ± 0.11^{c}
T_7	7.15 ± 0.41^{d}	4.78 ± 0.59^{bc}	1.51 ± 0.15^{cd}	9.47 ± 0.55^{d}	1.8 ± 0.11^{ab}	1.4 ± 0.26^{bc}
T_8	11.57 ± 0.97^{a}	5.9 ± 0.19^{a}	1.96 ± 0.18^{a}	15.9 ± 0.62^{a}	1.96 ± 0.06^{a}	1.65 ± 0.06^{a}
T_9	9.55 ± 0.85^{b}	4.89 ± 0.24^{b}	1.95 ± 0.16^{a}	13.67 ± 0.57^{b}	1.67 ± 0.17^{bcd}	1.21 ± 0.2^{c}
Mean	7.844	4.597	1.694	11.240	1.645	1.319
C.V.	7.335	7.488	9.354	6.052	6.125	10.204
F Prob.	0	0	0.001	0	0	0.003
S.E.M.	0.332	0.19	0.091	0.393	0.058	0.078
C.D. 5%	0.987	0.590	0.272	1.167	0.173	0.231
C.D. 1%	1.352	0.809	0.372	1.599	0.237	0.316

Table 4: Effect of indigenous liquid compatible microbial consortia on Seedling Vigour Indices (SVI-I and SVI-II) of *Ximenia americana* L.

Treatment	Dilution	SVI I	SVI II
T_1	1:1	153.99	21.95
T_2	Control	256.30	33.34
T_3	1:20	339.30	41.00
T_4	1:40	778.96	96.27
T_5	1:60	476.00	59.50
T_6	1:60	654.36	66.66
T_7	1:80	536.66	79.33
T_8	1:80	1244.67	129.13
T_9	1:100	1002.43	88.74

root:shoot ratio observed in T_8 indicates a shift in resource allocation favouring below-ground exploration-an adaptive trait essential for seedling establishment in semi-arid environments (Janmohammadi *et al.,* 2008; Shil *et al.,* 2025).

Fresh and dry weights of 30-day-old seedlings followed the same general trend as morphometric parameters (Table 3). Maximum fresh weight (1.96 ± 0.06 g) and dry weight (1.65 ± 0.06 g) were recorded in T_8, representing increases of 34.2% and 41.0%, respectively, over the undiluted T_1. Treatment T_4 (1:40) ranked second in biomass accumulation (fresh 1.73 g; dry 1.52 g). The enhanced biomass accrual under optimal biopriming reflects the cumulative effect of accelerated germination, prolonged photosynthetically active period and improved nutrient acquisition. Exopolysaccharides produced by PGPR retain rhizospheric moisture and protect the seedling from osmotic stress, while microbial mobilization of phosphorus, nitrogen and micronutrients supports rapid tissue construction (Lynch, 1985; Basu *et al.,* 2021). Comparable biomass gains following biopriming with indigenous consortia have been reported for French bean (Kangjam *et al.,* 2023), canola (Ghosh *et al.,* 2003) and tomato (Rangasamy and Saleh, 2025), corroborating the present results. The dose-dependent advantage of T_8 was most pronounced in the integrated vigour indices (Table 4). SVI-I peaked at 1244.67 in T_8 against 153.99 in T_1, an 8.1-fold increase, while SVI-II rose from 21.95 (T_1) to 129.13 (T_8). The hydropriming control (T_2) yielded intermediate values, confirming that the observed gains in T_8 were attributable specifically to microbial intervention rather than to hydration alone. Because both indices integrate germination performance with subsequent seedling growth or biomass, they provide a robust composite measure of physiological seed quality (Abdul-Baki and Anderson, 1973). The magnitude of vigour enhancement recorded here exceeds values commonly reported for agricultural seeds primed with single-strain inoculants (Basra *et al.,* 2005; Joodi and Sharif, 2006) and is comparable to multi-strain consortium studies in horticultural crops (Singh *et al.,* 2019; Raja *et al.,* 2019b), demonstrating that wild medicinal tree species respond equally-if not more-favourably to well-formulated indigenous consortia.

The consistent superiority of T_8 across all eighteen parameters measured points to an optimal microbial load that maximizes beneficial functions while avoiding antagonistic interactions. At 1:80, the diluted consortium retains a viable population (~106–107 CFU ml^{-1}) sufficient to colonize the seed coat surface, secrete phytohormones and hydrolytic enzymes, and neutralize autotoxic allelochemicals released from residual fruit-peel tissues (Muller, 1969; Coder and Warnell, 1999). Simultaneously, the lower microbial biomass minimizes competition for oxygen during imbibition and prevents the build-up of inhibitory metabolites observed at higher inoculum loads (Yao *et al.,* 2012; Rangasamy and Saleh, 2025).

The aqueous matrix of diluted consortium also ensures uniform hydration of hard-coated *X. americana* seed, addressing the physical component of dormancy. These convergent mechanisms collectively explain why T_8 simultaneously achieved

the highest germination, shortest MGT, greatest seedling biomass and maximum vigour indices, and they identify 1:80 as the operationally optimum dilution for conservation-scale biopriming of *X. americana*. The present investigation conclusively demonstrates that biopriming with an indigenous, site-specific liquid microbial consortium isolated from the native rhizosphere of *Ximenia americana* of the Amrabad Tiger Reserve is a highly effective, eco-friendly and low-cost biotechnological intervention for overcoming the chronic germination bottleneck of this vulnerable medicinal tree species. Among the dilutions tested, the 1:80 ratio (T_8) emerged as the operationally optimum dose, simultaneously maximizing germination percentage, accelerating germination time, enhancing root–shoot development, increasing biomass and elevating seedling vigour indices.

The undiluted consortium proved inhibitory, underscoring the necessity of dose optimization in multi-strain liquid formulations. The protocol developed herein offers a readily deployable, chemical-free conservation tool for the propagating *X. americana* and provides a replicable template for the bioprospecting of native rhizobacteria for the restoration of other ecologically vulnerable tree species of the Deccan Plateau. To the best of our knowledge, this is the first study in the world to explore, isolate and apply native rhizospheric microorganisms of *Ximenia americana* L. for enhancing seed germination and seedling vigour, opening new avenues for site-specific microbial conservation biotechnology of vulnerable medicinal tree species.

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