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RESEARCH ARTICLE

HUMBOLDTIA NAIRIANA (FABACEAE-DETARIOIDEAE), A NEW SPECIES FROM KERALA, INDIA

Santhosh Kumar E.S.*, Sabu T. and Shareef S.M.

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

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Abstract: A new species of the genus *Humboldtia* (Fabaceae-Detarioideae), from Kerala (SW India), is described here with photographs. It is closely allied to *Humboldtia ponmudiana*, but differs in possessing warty bark; a creamy-white blaze; angled, glabrous branchlets; distinctly shorter, sparsely hairy, isometric stipules and appendages; relatively long petiolules; entire leaf margins; fewer lateral nerves, obscurely looped near the margins; flowers borne on long, axillary or lateral, erect racemes; larger flowers with comparatively long pedicels; glandular bracts and bracteoles; hairy styles; and elliptic-oblong fruits with a shorter beak.A detailed description, ecology and a key to the species for the identification of the genus is also provided.

Keywords: *Humboldtia*, Fabales, Kerala, Eudicots, South West India, Taxonomy

INTRODUCTION

The genus *Humboldtia* Vahl is a distinctive member of the subfamily Detarioideae within the family Fabaceae, as recognized by the Legume Phylogeny Working Group (LPWG 2017). The genus comprises 11 recognized taxa, in which 10 are strictly confined to the Western Ghats, underscoring this biodiversity hotspot as the primary centre of origin and diversification for the genus. The high degree of endemism exhibited by *Humboldtia* in this region highlights its significant evolutionary and biogeographical importance within the Indian subcontinent.

Among the members of the genus, *Humboldtia laurifolia* Vahl stands apart as the sole species endemic to Sri Lanka. Its restricted distribution outside the Indian mainland further emphasizes the genus's predominantly Western Ghats-centered radiation, while also reflecting historical floristic linkages between the southern Western Ghats and Sri Lanka (Sanjappa 1986, Sasidharan & Sujanapal 2007, Udayan *et al.* 2007, Anoop *et al*. 2016a, 2016b, Kumar *et al*. 2022).

During a floristic exploration conducted in 2010 along the Cheenikkala–Pandimotta forest trail in the Shenduruney Wildlife Sanctuary, an unusual and taxonomically intriguing species of *Humboldtia* was collected. A few healthy seedlings were subsequently introduced and established in the Arboretum of the Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI) for *ex-situ* conservation and detailed observation. The plants adapted well to cultivation and, after a prolonged juvenile phase, commenced flowering and fruiting from 2022 onwards, eventually producing viable seedlings under garden conditions. The availability of flowering and fruiting material enabled comprehensive morphological assessment and critical comparison with existing literature (Sanjappa 1986; Sasidharan & Sujanapal 2007; Udayan *et al.* 2007; Anoop *et al.* 2016; Kumar *et al.* 2022) and type descriptions. Although the specimens exhibit close affinity to *Humboldtia ponmudiana* E.S.S. Kumar et al., they differ consistently in several diagnostic vegetative and reproductive characters. These stable and distinguishable features support the recognition of the taxon as a distinct species, which is herein described as new to science.

MATERIALS AND METHODS

The present study was undertaken as part of an ongoing programme on the ex-situ conservation of imperilled plant species of the Western Ghats. The taxonomic description is based on detailed examination of both living collections maintained under cultivation and herbarium specimens gathered

*Corresponding Author

during field surveys. Morphological characters were critically assessed through comparison with all relevant taxonomic literature, including the protologues, type specimens and authenticated collections of *Humboldtia* housed in the herbaria BM, C, CAL, K, L, MH and TBGT (herbarium acronyms following Thiers 2020+) to ensure accurate evaluation. Specimens preserved at BM, C, K and L were examined through high-resolution digital images accessed via JSTOR Global Plants (https://jstor.org), facilitating direct comparison with historical materials. The formal description was prepared primarily from fresh collections, which were meticulously studied under a Leica Wild M3Z stereo microscope at magnifications ranging from ×6.4 to ×40, enabling precise documentation of critical vegetative and reproductive characters.

Taxonomy

Humboldtia nairianaE.S.S.Kumar, Shareef *et* T.Sabu,**sp.nov. (Figure 1)**

Type:—INDIA, Kerala, Kollam District, Cheenikkala, ±300 m., 15.03.2010, *E.S. Santhosh Kumar & S.M. Shareef85818* (holotype TBGT!, isotype TBGT!, MH!, CAL!).

Diagnosis:—It is closely allied to *Humboldtia ponmudiana*, but differs in possessing warty bark; a creamy-white blaze; angled, glabrous branchlets; distinctly shorter, sparsely hairy, isometric stipules and appendages; relatively long petiolules; entire leaf margins; fewer lateral nerves, obscurely looped near the margins; flowers borne on long, axillary or lateral, erect racemes; larger flowers with comparatively long pedicels; glandular bracts and bracteoles; hairy styles; and elliptic-oblong fruits with a shorter beak.

Description:—Medium sized, evergreen tree, 5–8 m high; bark smooth, reddish brown, creamy-white blaze. Stipules ovate-lanceolate,1.8–3.0× 0.8–1.2 cm, acuminate to cuspidate at apex, depressed glandular, sparsely brown; appendages 2.2–3.0× 1.5–3.0 cm, reniform, glabrescent at maturity. Leaves imparipinnate,4–12 foliolate, rachis 20–45 cm long, obcordately and decurrently winged, wings reticulately veined, glabrous above, sparsely brown tomentose beneath; petiolule 8.0–10 mm long, not covered by rachis wings,sparsely brown tomentose; leaflets 10–30 × 5.0–9.0 cm,ovate-lanceolate,narrowly elliptic or elliptic-lanceolate or elliptic-oblong, subcoriaceous, depressed glandular,broadly acute, rounded to subcordate at base, acuminate to caudate acuminate, margin not undulate, entire; midrib shallowly depressed above, slightly raised beneath. Lateral nerves 8–12 pairs, shallowly depressed above and slightly raised beneath and arching 3.0–9.0 mm away from margin forming an obscurely intramarginal vein. Racemes 9.0–16 cm long, erect, axillary and lateral;peduncle 2.0–3.0 cm long, brown tomentose, many flowered. Flowers c.5 cm across at anthesis, pedicellate, pedicel 1.0–1.8 cm long, silky hairy. Bracts ovate, 1.3×0.8 cm creamy-white, obtuse at apex,silky villous on both surfaces, glands present or absent, fugacious. Bracteoles 2, connate to middle or connate to middle on oneside, hairy on both surfaces, eglandular,1.0–1.5× 1.0 cm, broadly obovate, obtuse at apex.Calyx tube 9.0–10 mm long,cylindrical silky villous;lobes 4, subequal, one larger than the rest,ovate-elliptic or linear-oblong, pink, 1.7–1.9×0.7–0.9 cm, silky villous on both surfaces. Petals obovate to oblanceolate,2.3–2.4× 0.8–0.9 cm, white with pink base, pilose within at the base, clawed, obtuse or acute at apex. Stamens 5, 3.8–4.0 cm long, filaments filiform, pink, sparsely pilose up to the middle; anthers 3.5–4.0× 1.0 mm, pink, obtuse at both ends. Ovary to 6.0–7.0 mm long, obliquely linear, silky villous, stipe 7.0–8.0 mm, silky villous;style 2.0–2.5 cm, pinkish, hairy upto 1/3 from the base; stigma capitate. Fruits 13–20× 4–5 cm, elliptic-oblong, compressed, velvety hairy, reticulate. Seeds 3–4, 3–4 cm across, suborbicular, flattened.

Phenology:—Flowering and fruiting occur during March-June.

Distribution: —It is so far known only from Kerala state (Thiruvananthapuram and Kollam districts).

Habitat, Ecology and Conservation status: —This species is found in the riparian forests of Cheenikkala in ShenduruneyWildlife Sanctuary about 300 m elevation in Agasthyamala Biosphere Reserve (ABR), Kerala. The main associated speciesare *Antidesma montanum* Blume, *Croton malabaricus* Beddome, *Dimocarpuslongan* Loureiro, *Diospyros buxifolia* (Blume) Hiern, *Elaeocarpus tuberculatus* Roxburgh, *Humboldtia decurrens* Bedd. ex Oliv., *Humboldtia vahliana* Wight, *Pandanus thwaitesii* Martelli,*Syzygium stocksii* (Duthie) Gamble and *Vateria indica* L. The present population consistsof less than 10 mature trees and several seedlings of various ages occupying an area of less than 2 km^2. Following the IUCN Red List criteria (IUCN 2024), *H. nairiana* is assessed as Data Deficient (DD).

Etymology: —This new species is named in honour of Prof. (Dr.) G. M. Nair, a distinguished Professor and former Director of the Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI), in recognition of his outstanding contributions to the

field of Botany and his dedicated efforts toward plant conservation. His extensive work in advancing botanical research and promoting the conservation of plant diversity has made a lasting impact on the scientific community.

Additional Specimens Examined: —INDIA, Kerala state, Thiruvananthapuram district, JNTBGRI Arboretum, 18.03.2026, *T Sabu 63235* (TBGT); *ibid*., 25.03.2026, *T. Sabu 63236* (TBGT).

Table 1. Diagnostic morphological characters of *Humboldtia nairiana* and related species

Characters	***H.decurrens***	***H. bourdillonii***	***H.vahliana***	***H.ponmudiana***	***H.nairiana***
Bark	Rolled up, bluish green or brown; blaze pale pink	Smooth, dark brown mottled with white; blaze pale pink	Smooth, dark brown mottled with white; blaze pink	Warty, black, lenticellate; blaze light crimson	Warty, pale brown, lenticellate; blaze creamy-white
Branchlets	Pubescent when young, glabrous at maturity	Glabrous	Glabrous	Densely brown tomentose, glabrescent at maturity	Glabrous
Stipules	5–8× 1–2.5 cm, ovate to narrowly ovate, falcate	2–3.5 × 1–1.5 cm, ovate, acute, prominently veined, glabrous	1.5–3 × 2.2 cm, ovate-cordate, parallel veined, glabrous	5.5–6.0 × 1.5–2.0 cm, ovate–lanceolate, slightly falcate, densely brown tomentose when young and glabrescent at maturity	1.8–3 x 0.8–1.2 cm, ovate-lanceolate, depressed glandular, sparsely brown
Appendage	2–4 × 1–2.5 cm, reniform, brown tomentose, depressed glandular	0.8 × 0.6 cm, falcate-reniform, rounded, veined, slightly hairy	1 × 2.2 cm, reniform, divergently veined, glandular, glabrous	1.0–1.7 × 1.6–2.3 cm, reniform, obtuse or rarely acuminate on one side, densely tomentose when young, glabrescent at maturity	2.2–3 × 1.5–3 cm, reniform, glabrescent at maturity
Leaves	10–12 foliolate	6–8 foliolate	6–8 foliolate	8–12 foliolate	6–12 foliolate
Leaflets	15–38 × 4–12 cm, narrowly ovate or elliptic, sometimes linear	7.5–12 × 1.5–4 cm, linear to narrowly ovate	12–25 × 3–5 cm, ovate to narrowly ovate or elliptic to narrowly elliptic	10.5–37 × 3.0–7.0 cm, lanceolate or ovate-lanceolate	10–30 ×5–9 cm, ovate-lanceolate, narrowly elliptic or elliptic-lanceolate or elliptic-oblong
Leaf base	Obtuse or rounded	Obtuse	Obtuse	Subcordate or rounded	Broadly acute, rounded to subcordate
Leaf apex	Obtuse to acuminate	Obtusely acuminate	Obtusely acuminate	Acuminate to caudate-acuminate	Acuminate to caudate acuminate
Leaf margin	Undulate	Slightly undulate	Entire	Slightly undulate	Entire
Lateral nerves	15–18 pairs, deeply impressed above, prominently looped near margin	7–10 pairs, obscurely looped near margin	8–14 pairs, obscure above, slightly visible beneath, obscurely looped near margin	10–19 pairs, shallowly depressed above, prominently raised beneath and looped near margin	8–12 pairs, shallowly depressed above, slightly raised beneath, obscurely looped near margin
Petiolules	4–5 mm long, covered by rachis wings, densely brown tomentose	3–4 mm long, covered by rachis wings, stout, glabrous	7–10 mm long, not covered by rachis wings, glabrous	2–4 mm long, not covered by rachis wings, densely brown tomentose	8–10 mm long, not covered by rachis wings, sparsely brown tomentose
Inflorescence	Racemes 5–8.5 cm long,	Corymbs to 5 cm long on	Racemes 10–15 cm long, axillary,	Racemes 5.0–9.0 cm long, axillary	Racemes 9–16 cm long, axillary and

	axillary or cauliflorous, tawny villous	tubercles on main trunk and old branches; tawny velvety	racemes sometimes 2–3 per axil, brown velvety	or cauliflorous, brown tomentose	lateral
Flowers	Pink or white	Pinkish	White	White	White with pink base
Pedicels	1–1.2 cm long, brown villous	1.4–1.7 cm long, brown velvety	5–6 mm long, brown tomentose	Sessile or rarely up to 0.2 cm long, brown villous	1–1.8 cm long, silky hairy
Bracts	Broadly ovate, 7–8×5 mm, obtuse to acute at apex, brown villous without, glandular, fugacious	Ovate, 4 × 1.8 mm, acute, densely brown tomentose on both surfaces, glandular, fugacious	Lanceolate, 8–10×4 mm, acute, brown villous on both surfaces, eglandular, persistent	Broadly ovate, 1.0–1.2 × 0.7 cm, greenish white, acuminate at apex, brown villous and eglandular without, glabrous within, fugacious	Ovate, 1.3×0.8 cm, creamy-white, obtuse at apex, silky villous on both surfaces, glands present or absent, fugacious
Bracteoles	Connate at base, 7–13 × 4–5 mm, ovate, glandular, obtuse, brown villous on both surfaces	Connate almost throughout when young, splitting down to 1/3 at maturity, 8× 1.5 mm, ovate-oblong, obtuse or rounded at apex, densely brown tomentose on both surfaces, glandular	Connate at base, 8–10 × 8 mm, obovate, rounded at apex, brown villous on both surfaces	Connate to middle in cauliflorus flowers or connate to middle on one side and free up to the base on the other side in axillary flowers, 1.0–1.3 × 0.7 cm, ovate, obtuse atapex, greenish-white, silky villous and glandular without, glabrous within	Connate to middle or connate to middle on one side, hairy on both surfaces, eglandular,1–1.5×1cm, broadly obovate, obtuse at apex
Sepals	Oblong or linear-oblong, rounded at apex, brown villous without, equal in size	Oblong, rounded at apex, densely brown tomentose on both surfaces, equal in size	Obovate-oblong, rounded at apex, slightly concave, brown villous without, equal in size	Elliptic-oblong or linear-oblong, subequal, one sepal larger than the rest, rounded at apex, silky villous on both surfaces	Ovate-ellitic or linerar-oblong, crimson,subequal, one larger than the rest, silky villous on both surfaces
Petals	Pink or white, 2–2.5 × 0.5 cm,obovate or oblanceolate, clawed, obtuse to rounded at apex, pilose within at base (in cauliflorous flowers the petals to 2 × 0.5 cm, lanceolate, distinctly clawed, abruptly acute at apex, sparsely pilose at base within)	White with pink veins, 3 longer and 2 shorter, 16–20 × 6 mm, shortly clawed, obovate, obtuse to rounded at apex, sparsely pilose within, glabrous without, sometimes a brown gland present in the middle within, fugacious	White, 10–12 × 4 mm, obovate, shortly clawed, broadly rounded at apex, glabrous	White, 1.6–2.3 × 0.6–1.0 cm, obovate to oblanceolate, clawed at base, acute or slightly acuminate at apex, glabrous or minutely silky pilose along the midrib abaxially	White with pink base, 2.3–2.4× 0.8–0.9 cm, obovate to oblanceolate,pilose within at the base, clawed, obtuse or acute at apex.
Ovary	4.0–6.0 mm long, obliquely linear, brown villous, 3–6-	8.0–10 mm long, obliquely oblong, brown	4.0–5.0 mm long; obliquely linear, brown villous, 3–4ovuled	0.8–1.0 cm long, stipe 1.0–1.5mm long, obliquely linear, silky	6.0–7.0 mm long, obliquely linear, silky villous, 3–4 ovuled

	ovuled	villous, 3–5 ovuled		villous, 1–4 ovuled	
Style	2 cm long, glabrous	2 cm long, pilose	1.0–1.5 cm long, pilose at base	2.7–3.0 cm long, glabrous, rarely coiled	2–2.5 cm, pinkish, hairy up to 1/3 from the base
Fruits	7–12 × 4 cm, oblong or dolabriform, 2–3-seeded, brown, veined, rugulose and dark brown tomentose when young; sutures thick	10–11 × 2.5 cm, oblong or dolabri-form, velvety brown pubescent, bright red or crimson, 3–5-seeded; sutures thick, valves prominently veins	15–20 × 4–6 cm, elliptic, compressed;sutures thick (especially upper), valves prominently veined, brown villous, 3–4-seeded	13–15 × 3–4 cm, oblong, silky tomentose; beak 2–2.5 cm long	16-18 × 4–5 cm, elliptic- oblong, compressed, minutely densely hairy when young, obscurely reticulate

Key to the species of *Humboldtia* Vahl

1. Stipules appendiculate at base 2

1. Stipules not appendiculate at base 9

2. Leaflets 4 3

2. Leaflets 6–12 4

3. Flowers crimson-red; petals up to 8 mm long; pods 1–2 seeded ..... *H.brunonis* var. *raktapushapa*

3. Flowers white suffused with pink; petals 1.2–1.5 cm long; Pods 3–4 seeded ..... *H.brunonis* var. *brunonis*

4. Rachis of leaves terete ..... *H. laurifolia*

4. Rachis of leaves flattened and winged 5

5. Stipules 5–8 cm long 6

5. Stipules 2–3 cm long 7

6. Flowers with 1–1.2 cm long pedicels ..... *H.decurrense*

6. Flowers sessile or rarely up to 0.2 cm long pedicels ..... *H.ponmudiana*

7. Inflorescence corymbose, pods falcate, velvety ..... *H. bourdillonii*

7. Inflorescence racemose, pods straight, tomentose *8*

8. Bracts and bracteoles eglandular, sepals equal in size ..... *H. vahliana*

8. Bracts and bracteoles glandular, one sepal larger than the rest ..... *H. nairiana*

9. Flowers crimson, staminal filaments glabrous 10

9. Flowers white, staminal filaments pilose at base ..... *H.sanjappae*

10. Leaflets 2 ..... *H.unijuga* var. *unijuga*

10. Leaflets (4–) 6 (–8) ..... *H.unijuga* var. *trijuga*

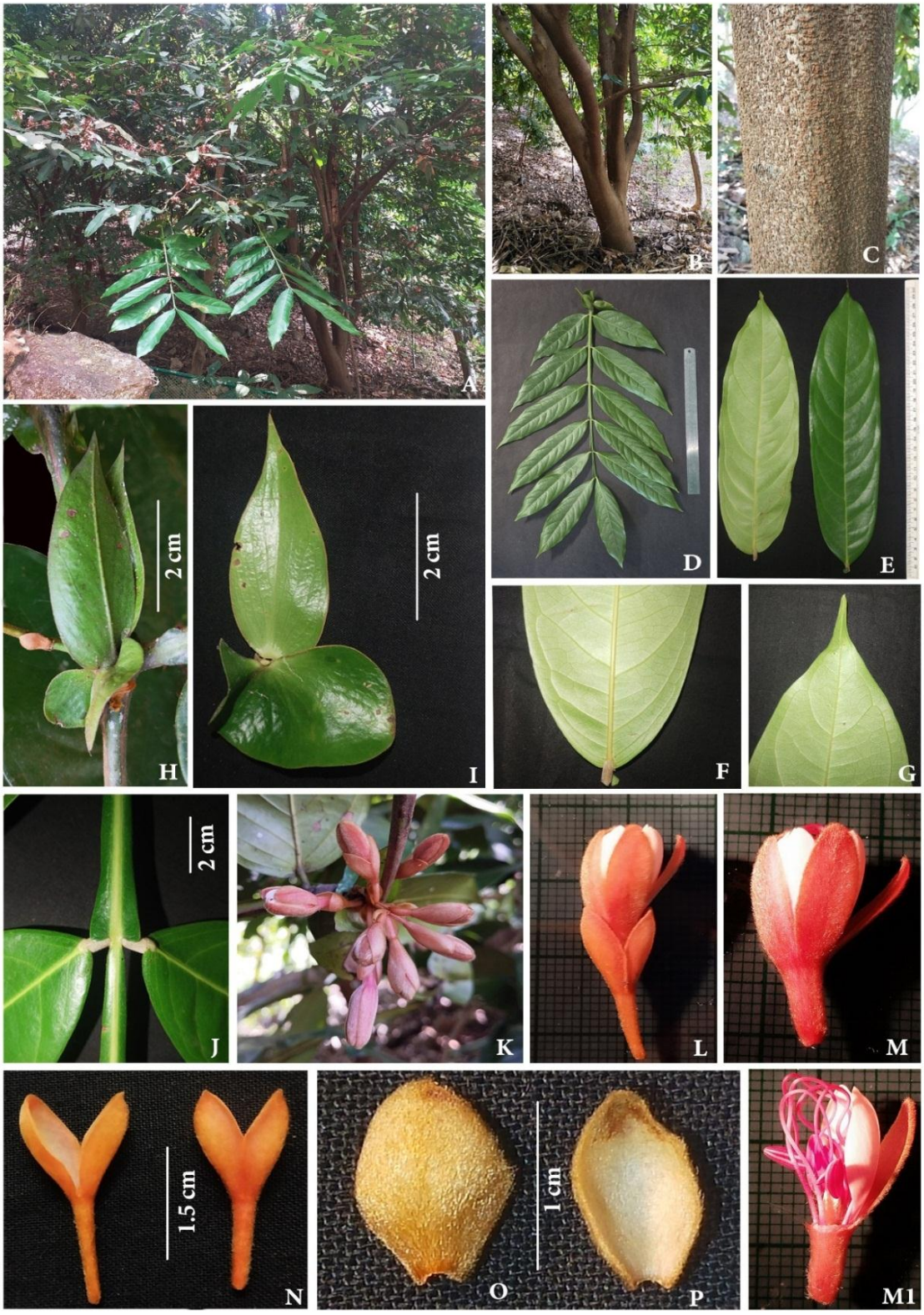

Figure 1. ***Humboldtia nairiana****sp.nov*. A. Habit, B. Basal stem region, C. Bark, D.Leaves, E.Leaflets, F.Leaflet-base, G. Leaflet-apex, H. & I. Stipules, J. Rachis showing petiolules, K. Flower buds, L. A Flower bud with bracteole, M. A flower bud removed bracteole, M1. A flower bud showing floral parts, N. Bracteoles with pedicels, O. & P. Bracts- dorsal and ventral view.

Figure 2.*Humboldtia nairiana**sp.nov.* A. Inflorescences, B. A flower at anthesis, C. Calyx with ovary, D. A petal, E. A flower-(petals removed), F. A pistil, G. Ovary, H. L.S. of ovary showing ovules, I. Fruits, J. A seed.

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RESEARCH ARTICLE

MORPHOLOGY AND CULTURAL CHARACTERISTICS OF *CURVULARIA LUNATA* (WAKKER) BOEDIJN OF COTTON

Payal Kodavala[1]*, Prashant B. Sandipan[2], R.K. Patel, Satish Kumar Sain, Kishor Sharma, Navya Sharma, Pushpa Ruwali and Vedansh Sharma

[1]Department of Plant Pathology, N. M. College of Agriculture, Navsari Agricultural University, (NAU), Navsari, Gujarat, India.
[2]Main Cotton Research Station (MCRS), Navsari Agricultural University (NAU), Surat 395 007 (Gujarat), India
Email: payalkodavla1309@gmail.com

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Abstract: Cotton (*Gossypium hirsutum* L.) is one of the most important fiber crop playing a key role in the economic and social scenario of the globe. Curvularia leaf spot appear initially as small circular brown to brownish black spot surrounding with yellow, later it become dark yellow to brown hallow surrounding to brownish black spots and in severe cases, the leaves turned yellow colour and detached easily from the branch resulting in the defoliation. There was a good deal of variation in cultural and morphological characters of the pathogen under the different temperature. In morphological characteristics, the maximum dry mycelium weight (194.70mg) with abundant (16.43 millions/ml) sporulation on potato dextrose broth medium was observed at 25°C and at 15°C there was no growth and sporulation observed after 15days of inoculation. In cultural characteristics, the maximum colony diameter (87.76mm) and abundant (++++) sporulation was recorded at 25°C on potato dextrose agar medium after 10 days of incubation.

Keywords: Cotton, Morphology, Cultural, Sporulation, *Curvularia*

INTRODUCTION

Cotton is the world's most widely grown fibre crop, which belongs to the genus *Gossypium* in the family *Malvaceae* (Anonymous, 2017). It has delicate, white, soft and fluffy fibre that is made of about 87 to 90 per cent of cellulose. Cotton has been cultivated as over thousands of years for both the food and fibre. It is versatile crop, its fibre is used as raw material in textile, pulp and paper industries and oil extracted from the cotton seed is used in food, cosmetics, chemicals and pharmaceuticals (Proto *et al*., 2000).

The symptoms of Curvularia leaf spot appear initially as small circular brown to brownish black spot surrounding with yellow hallow, later it become dark yellow to brown hallow surrounding to brownish black spots (Joshi *et al*., 2023).

Curvularia is a wide spread air borne facultative weak pathogen, which mostly survives as a saprophyte in tropical and sub-tropical areas. It is a dematiaceous, filamentous fungus. *Curvularia* spp. are darkly pigmented fungi with spores (conidia) efficiently adapted for most aerial dissemination. *Curvularia* is characterized by septate mycelium, dark brown hypha 1.5-4.5µ in diameter, conidiophores are erect, swollen at the base, lower part straight, upper part flexuous, geniculate, septate, smooth, brown in colour, 30-268µ long, 3-6µ thick bearing conidia in clusters of 3-8. Conidia are straight or slightly curved 3 septate, 18-30×7-15µ in size (Bhatt and Kumar, 2018). The Curvularia leaf spot injures or kills the leaf tissues and thereby reduces the area of chlorophyll which involved in photosynthesis. If considerable leaf area is killed, then vigour and yields are reduced drastically. The genus *Curvularia* belongs to *Pleosporaceae*, *Pleosporales*, *Ascomycota* (Nelson and Haasis, 1964). *Curvularia* contains about 133 species including saprobes, endophytes and pathogens (Manamgoda *et al*., 2015). Thus, the present study has been taken up with the specific objective.

MATERIALS AND METHODS

Morphological Variation
Design: Completely Randomized Design
Treatments: 7 (Different temperature)
Repetitions: 3
Location: Department of Plant Pathology, Post Graduate Laboratory, N. M. C. A., N. A. U., Navsari,

*Corresponding Author

Gujarat

In this method, the isolate was cultured in liquid media in 100ml flask containing 20ml potato dextrose broth in different temperature as 15, 20, 25, 30, 35, 40 and 45ºC for fifteen days. After incubation, average measurements were taken by the micrometry method.

The morphological characters like dry mycelium weight, sporulation, size (length and width) of conidia and conidiophore were recorded. The observations were recorded in three repetitions of isolate in different temperature. The study was carried out using ocular and stage micrometer after mounting them on the slides containing sterile distilled water at magnification of 40X.

Data was analyzed statistically using complete randomized design.

The following morphological characters were recorded under different temperatures on PDB medium after 15 days of incubation.

- Dry mycelium weight (mg)
- Sporulation (million/ml)
- Size (µm) of conidia and conidiophore

Cultural Variation

Design: Completely Randomized Design

Treatments: 7 (Different temperature)

Repetitions: 3

Location: Department of Plant Pathology, Post Graduate Laboratory, N. M. C. A., N. A. U., Navsari, Gujarat

The isolate was separately cultured on potato dextrose agar medium in different temperature as 15, 20, 25, 30, 35, 40 and 45ºC for ten days. The 5mm disc of *Curvularia lunata* isolate was inoculated on PDA Petri plate and incubates at different temperature.

After ten days of incubation period, colony diameter, sporulation, cultural characteristics including colony character, colour of mycelium and substrate was recorded. The results were tabulated and three repetitions were made for the isolate. Data was analyzed statistically using complete randomized design.

The following cultural characters were recorded under different temperatures on PDA medium after 10 days of incubation.

- Colony diameter (mm)
- Colony characters
- Sporulation category: - Absent, + Scanty, ++ Moderate, +++ Good, ++++ Abundant

RESULTS AND DISCUSSION

Morphological characteristics

Morphological studies were carried out under different temperature on potato dextrose broth medium revealed variation in growth and sporulation, size of conidia and conidiophore of *Curvularia lunata*. The results are presented in Table: 1, Photo 4.5.

Growth and sporulation

The maximum dry mycelium weight (194.70mg) with sporulation (16.43 million/ml) was observed at 25°C, while at 15°C there was no growth and sporulation observed (Table: 1).

Conidia

Conidia were obovoidal to broadly clavate, curved at the sub terminal cell and have 0-3 septa. The size of conidia was maximum at 25°C (27.14×9.10µm) followed by 30°C (26.94×9.00µm), 40°C (26.35×7.57µm), 20°C (26.30×7.10µm), 45°C (25.50 ×9.40µm) and 35°C (25.35×8.10µm). At 15°C temperature no conidia were produced (Table: 1, Photo 4.4).

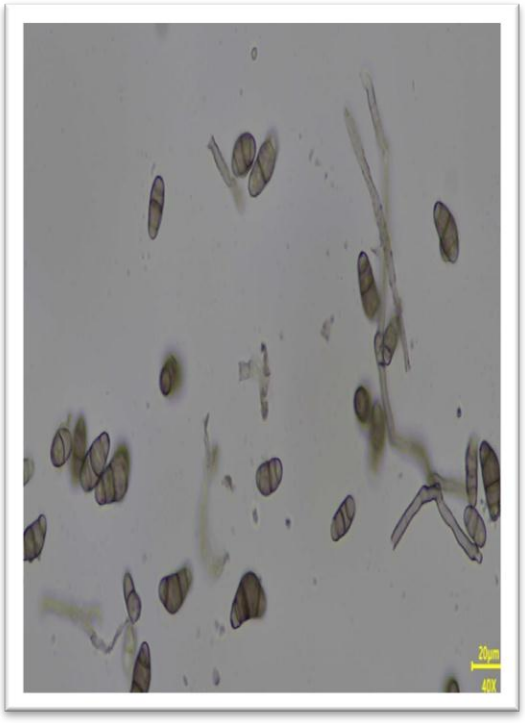
Conidiophore

Conidiophore was straight to flexuous, slightly geniculate towards apex, uniformly sub hyaline to pale brown, smooth and septate. The size of conidiophore was maximum at 30°C (150.80×5.10µm) and minimum at 45°C (107.90x4.020µm). At 15°C conidiophore were not produced (Table: 1).

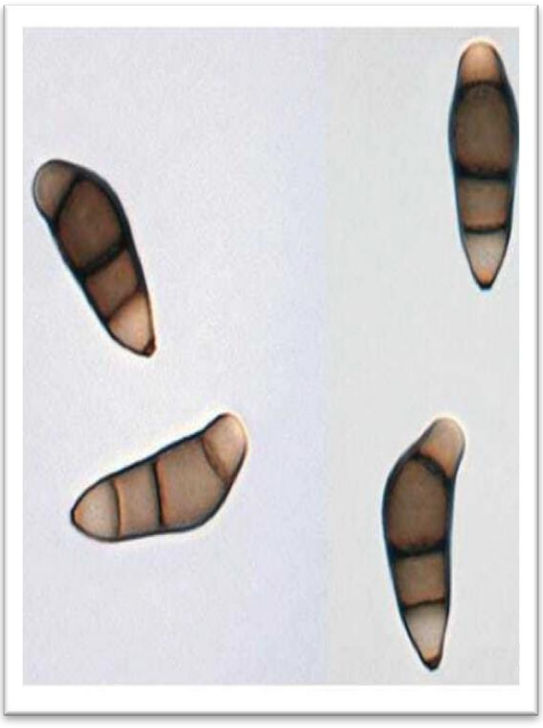
These results are in agreement with the results obtained by various research workers. Ming *et al*. (2005) studied the leaf blight of Cynodon hybrid caused by *Curvularia lunata* based on morphological characteristics. The pathogen grew well at a temperature ranged from 20 to 35°C in which the optimal temperature is 25°C. Yashwant *et al*. (2010) studied the effect of temperature on the growth and sporulation of *Cochliobolus lunata* caused leaf spot of okra crop. The effect of nine different temperatures (between 10 to 40°C) was studied to determine their effect on fungal growth. Maximum mycelial dry weight of the pathogen (830.00mg) was obtained at 28°C. The minimum fungal dry weight (144.66mg) was recorded at 15°C. Excellent sporulation of the pathogen was observed at 28 and 30°C, good at 25°C, fair at 20 and 32°C and poor growth at 15 and 40°C.

Mycelium (10 X)

Conidiophore (40 X)



Conidia (20 X)



Conidia (40 X)

Photo 4.4. Microphotographs showing Mycelium, Conidiophore, Conidia (20 X) & Conidia (40 X) of Curvularia *lunata*

Table 1. Dry mycelium weight, sporulation and size of conidia and conidiophores of *Curvularia lunata* in different temperature

Temp°C	#Dry mycelium weight (mg)	#Sporulation (million/ml)	Conidia		Conidiophore
			Size (µm)	No. of septa	Size (µm)
15	00.00	00.00	-	-	-

20	150.70	15.70	26.30× 7.10	1-2	128.67 × 4.15
25	194.70	16.43	27.14 × 9.10	2-3	140.98 × 4.00
30	173.37	13.53	26.94 × 9.00	1-2	150.80 × 5.10
35	90.43	12.20	25.35 × 8.10	0-1	145.12 × 4.19
40	41.47	10.63	26.35 × 7.57	0-1	129.55 × 5.00
45	10.02	07.81	25.50 × 9.40	0	107.90 × 4.02
SEm ±	1.25	0.07	-		
CD at 5%	3.83	0.24	-		
CV %	2.29	1.26	-		

On PDB (Average of three repetitions)

Photo 4.5. Mycelial growth of *Curvularia lunata* under different temperature on PDB

Mehi *et al*. (2014) studied to know the effect of different temperatures (8, 15, 20, 25, 28, 30, 35 and 37°C) on *Curvularia lunata* causing Curvularia leaf spot on black gram crop. They observed that the fungus can grow up to the temperature ranged of 15°C to 37°C.Whereas, 28°C (830.00mg) was the optimum for the growth of fungus followed by 30°C (734.66mg). Abdel *et al*. (2015) observed *Curvularia lunata* causing disease in rice crop and found that conidia were smooth-walled, olivaceous brown, end cells somewhat paler, conidia obovoidal to broadly clavate, curved at the subterminal cell, 25-30×10-14µm.Conidiophores were erect, septate, unbranched and lexuose in the apical part with lat, dark brown scars, 3 septate. Joshi *et al.* (2023) studied the morphological characters of *C. lunata* in cotton crop by using the microscope. They found that the spores were slightly curved or straight, mycelium was septate with brown to black in colour and conidia was found 25-27×8-10µm in size.

Cultural characteristics

The cultural studies of *Curvularia lunata* was made by growing single spore culture on potato dextrose agar medium under the different temperature and colony diameter (mm), sporulation, cultural characteristics as colony characters and colony colour as mycelium and substrate were recorded (Table: 2, Photo: 4.5).

The maximum colony diameter (87.76mm) was recorded at 25°C after ten days of incubation followed by 78.69mm at 30°C, 59.42mm at 20°C, 45.36mm at 35°C, 28.00mm at 40°C, 9.00mm at 45°C and no colony growth was recorded at 15°C temperature.

Curvularia lunata was differed in colony characters at different temperature. At 20°C produced profuse fluffy aerial growth with irregular margin, brownish grey mycelium, at 25°C moderate fluffy aerial

growth with regular margin with dark blackish mycelium, at 30°C produced flat mycelial growth with regular margin, brownish black mycelium, at 35°C moderate fluffy aerial mycelial growth, cottony raised growth, grayish black mycelium, at 40°C flat growth with irregular cottony margin, light brown and white mycelium, at 45°C submerged mycelial growth with regular margin with whitish brown mycelium was observed.

Findings of the cultural variations such as mycelial growth, colour and sporulation are in conformity with the findings obtained by Olufolaji (1983) conducted an experiment on the growth and sporulation of *Curvularia pallescens* of maize on different solid media at different temperatures. The maximum sporulation and growth were observed at 24°C on malt extract agar and potato dextrose agar. Sumangala and Patil (2010) conducted cultural and physiological studies on *Curvularia lunata*, a causal agent of grain discoloration in rice. The maximum growth of *Curvularia lunata* was observed at the temperature of 25°C.

Table 2. Colony diameter, sporulation and cultural characteristics of *Curvularia lunata* under different temperature on PDA

Temp (°C)	Colony diameter (mm)#	Sporulation category##	Cultural characteristics		
			Colony characters	Colour	
				Mycelium	Substrate
15	00.00	-	No growth	-	-
20	59.42	+++	Profuse fluffy aerial growth with irregular margin	Brownish grey	Slight brown
25	87.76	++++	Moderate fluffy aerial growth with regular margin	Dark blackish	Brown to light black
30	78.69	++++	Flat mycelial growth with regular margin	Brownish black	Dark brown
35	45.36	++	Moderate fluffy aerial mycelial growth, cottony raised	Greyish black	Grey
40	28.00	+	Flat growth with irregular cottony margin	Light brown and white	No color
45	9.00	+	Submerged mycelial growth with regular margin	Whitish brown	Light black
SEm±	0.911	-			
CD at 5%	2.790				
CV %	3.584				

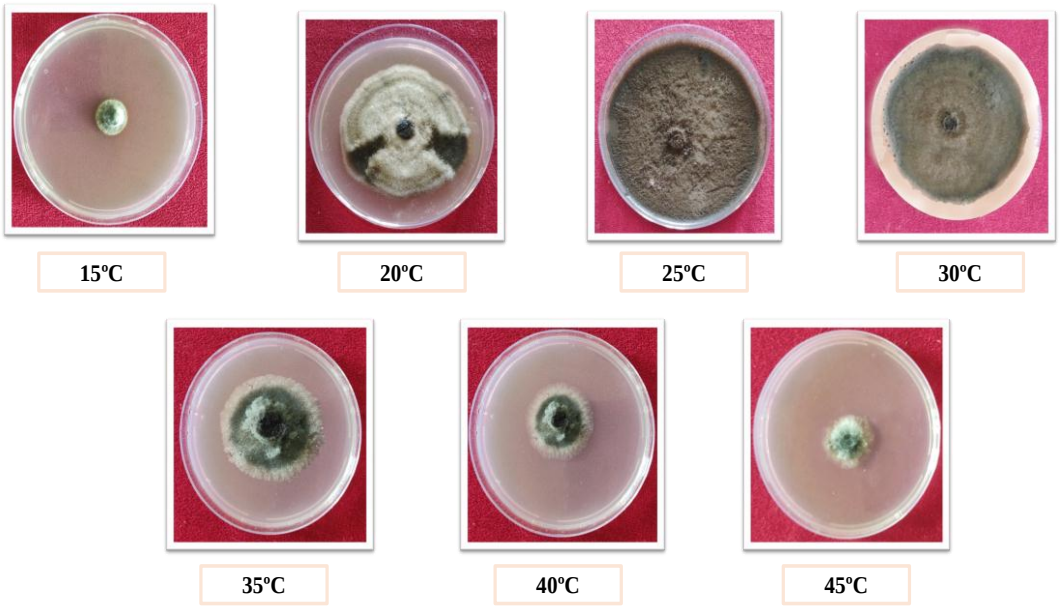


Photo 4.6. Mycelium growth of *Curvularia lunata* on different temperature on PDA

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RESEARCH ARTICLE

STUDIES ON FLORAL BIOLOGY AND INTER-POPULATION PHENOLOGICAL VARIABILITY IN THREATENED *ALLIUM STRACHEYI* BAKER FROM WESTERN HIMALAYA

Pooja Devi, Hrishi Manchanda and Geeta Sharma*

Department of Botany, University of Jammu, Jammu
Email: geetaji@yahoo.com

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Abstract: *Allium stracheyi* Baker, an important species used for seasoning dishes, is endemic to Himalaya. The identified germplasm of this species from Pir-Panjal comprised fifteen populations growing between 2224–3080 masl. The plants of this bear tunicated bulbs, flat leaves and a single long scape bearing umbel inflorescence having trimerous flowers. In just opened flowers, anthers appear at two levels with respect to stigma, with maximum flowers having 3 long and 3 short filaments and minor ones having long and short filaments in proportion of 4:2, 2:4, 1:3median:2. Another interesting condition revealed by this species is distylous, with some flowers having long style and others having short style at the same stage of development. The studied plants differ slightly in flower colour, with more plants of higher reaches bearing inflorescences having light-pink flowers, with plants of lower altitudes bearing outnumbering inflorescence with pale-yellow flowers. The studied *A. stracheyi* plants of varying altitudes also show variation in the duration of different phenological events. The plants of higher reaches (2600-3100 m) emerge in April ending and depict vegetative growth till July, flower from 1st to 3rd week of August, develop fruits from mid-August to mid-September, show senescence in October and dormancy till April. In the plants of lower altitudes (2300-2500 m), these events get delayed by nearly two weeks. Higher variability existing in phenological behaviour and floral traits in *A. stracheyi* seems to be the outcome of heterogeneity prevalent with regard to altitude, topography and associated climatic conditions.

Keywords: *Allium stracheyi*, Pir-Panjal, Heterogeneity, Phenological behaviour

INTRODUCTION

Allium stracheyi Baker (Amaryllidaceae), the species having distinct aroma due to its sulphur-rich compounds, is endemic to Himalaya (Dhar and Kachroo, 1983; Pandey *et al.*, 2008, 2021). Known to inhabit high altitudinal regions (2000-4000 masl) of India, Bhutan, Burma, Nepal and Pakistan (Nasir, 1975; Hooker, 1978; Shah, 2014; Tiwari *et al.*, 2014), in our country it has been reported from few pockets of Jammu and Kashmir (Gohil and Koul, 1973), West Bengal (Sharma and Aiyangar, 1961; Sen, 1974) and nearly 28 sites of Uttarakhand and Himachal Pradesh (Verma *et al.*, 2008; Pandey *et al.*, 2021; Semwal *et al.*, 2021; Kumar *et al.*, 2024).

A. stracheyi has high culinary value, with its fresh and blanched leaves along with inflorescences being used as spice (Tiwari *et al.*, 2014). These are rich in proteins, carbohydrates, phosphorous, vitamins C and E, and contain anti-inflammatory and analgesic components (Ranjan *et al.*, 2010; Maikhuri *et al.*, 2017). The indigenous people use leaf decoctions as stimulant, appetizer, carminative, expectorant and to cure digestive problems (Tiwari *et al.*, 2014; Kumar *et al.*, 2015; Agnihotri *et al.*, 2020; Chandrasekaran *et al.*, 2020). Going by these benefits, this species has high market value in India as dried *A. stracheyi* leaves from Uttarakhand are available on online platform for 245 INR/20g. This species, however, has been mentioned as vulnerable in the Red Data Book (Ved *et al.*, 2003).

While locating germplasm of this species from Pir-Panjal, we came across fifteen populations growing between 2224–3080 masl which were prolific flower producers. Literature survey (Nasir, 1975; Hooker, 1978; Pandey *et al.*, 2021) depicted this species bearing rosy-pink to pale-yellow flowers. Previously our group found such variation in flower colour in *Allium roylei* (Sharma and Gohil, 2002) as its wild plants of higher reaches (1400 masl) bear light-pink flowers and those translocated to plains (351 masl) form dirty-white flowers, with Kohli (2013) reporting inflorescences with/without bulbils. This interesting observation instigated us to study floral biology of *A. stracheyi*, detect floral variability, if present and find any association between variable forms and elevation.

*Corresponding Author

Recording timing of onset and duration of various phenological events of populations inhabiting different geographical locations is an important aspect that can aid in identifying the elevation-wise specific period during which plants produce more herbage and flowers, the products of economic use (Mir *et al.*, 2020). Therefore, studies on this aspect were also conducted.

MATERIALS AND METHODS

Distribution

The 15 populations of *A. stracheyi* located in 2021 from varying elevations (2224-3080m) of Pir-Panjal range of Jammu Himalayas belonged to Padri (3080m, 32.917°N, 75.810°E), Kondlu (2804m, 32.900°N, 75.818°E), Ganja-goth (2670m, 32.895°N, 75.810°E), Dalan (2494m, 32.932°N, 75.810°E), Khani-top (2427m, 32.938°N, 75.773°E), Thanala (2277m, 32.932°N, 75.785°E), Tiba (2781m, 33.005°N, 75.770°E), Dari (2764m, 33.006°N, 75.775°E), Dhaeri (2420m, 33.001°N, 75.776°E), Kansar (2496m, 32.965°N, 76.764°E), Sharakhi (2390m, 32.944°N, 75.788°E), Bharai (2233m, 32.997°N, 76.005°E), Ligri (2785m, 33.301°N, 76.161°E), Kedna-nallah (2396m, 33.203°N, 75.538°E) and Shiryari-top (2224m, 33.068°N, 76.009°E). For these sites, approximate area of occurrence of this species was determined alongwith number of plants growing there.

Floral biology and diversity analysis:

The plants of representative population were analysed for onset and sequence of different floral events such as sequence of emergence of floral buds, onset and duration of flower opening and fruit development and maturation followed by seed dispersal. During this analysis, tagged populations were also screened for floral variability.

Phenological studies

Various phenological events such as plant emergence, vegetative growth, flowering, fruit development and senescence have been recorded by visual inspection by frequently visiting the study sites during four seasons namely, winter (December-February), spring (March-May), summer (June-August) and autumn (September-November).

RESULTS AND DISCUSSION

Distribution detail

Presently, 15 populations from high temperate and subalpine areas of Pir-Panjal falling in Doda and Kishtwar districts were identified (Table 1). While from Doda, 13 populations were located, with six each from Padri valley and Bhaderwah forest block and one from Bhalessa. In Kishtwar district, one population each was spotted from Padder valley and Kuchal Chattroo belt. At these sites, *A. stracheyi* plants largely inhabited crevices of rocks in open meadows (Fig. 1A), sloppy grasslands (Fig. 1B) and those near streams and beneath pine trees.

Since at the studied sites Keer plants showed patchy distribution, we estimated the area within which present plants were found, though in patches in 2021. This data along with approximate number of plants seen there were counted and enlisted in Table 1.

Table 1. Habitat details and number of plants inhabiting study sites of *Allium stracheyi*

District	Locations	Population (Identity)	Alt. (masl)	Habitat	Occurrence area (km^2)	Approx. no. of plants	Inflores-cences scanned for flower colour
Doda	Padri valley and adjacent sites	Padri (Pdr)	3080	Rocky areas of meadows	1	80	35
		Kondlu (Kdl)	2804	Rocky areas of meadows	0.5	56	38
		Ganja-goth (Ggt)	2670	Undergrowth in Pine forests	0.5	15	15
		Dalan (Dln)	2494	Sloppy grasslands	0.5	5	5
		Khani-top (Ktp)	2427	Undergrowth in Pine forests	1-2	57	41
		Thanala(Thn)	2277	Open sloppy grasslands	0.5	52	34
	Villages falling in forest block	Tiba (Tib)	2781	Rockyareasof open grasslands	0.5	10	10
		Dari (Dar)	2764	Undergrowth in Pine forests	0.5	30	24
		Dhaeri (Dhr)	2420	Undergrowth in Pine forests	0.5	18	18

	of Bhaderwah	Kansar (Kns)	2496	Rocky crevices of grassland	1-2	68	37
		Sharakhi (Sha)	2390	Rocky crevices of grasslands	1-2	45	36
		Bharai (Bhr)	2233	Rocky crevices of sloppy open grasslands	0.5	69	33
	Bhalessa valley	Shiryari-top (Sht)	2224	Open sloppy grasslands	1-2	57	30
Kishtwar	Paddar Valley	Ligri (Lgr)	2785	Rocks aside stream	0.5	60	48
	Kuchal-Chatroo belt	Kedna-nallah (Kdn)	2396	Rocks in grassland near stream	0.5-1	60	49

As evident from table 1, the patches of *A. stracheyi* plants in Khani-top, Kansar, Sharakhi, Shiryari-top covered nearly 1 to 2 km^2 area whereas the remaining populations were found spread between 0.5 to 0.9 km^2. At most of these locations, nearly 45 to 80 individuals were spotted whereas at Tiba, Dari and Dhaeri, 10-18 plants and at Dalan only 5 plants were seen. It is pertinent to mention here that at Dalan and Tiba, not a single plant could be seen during subsequent visits.

Floral biology

The studied perennial plants of *A. stracheyi* above ground bear flat glaborous leaves (Fig. 1C) and underground differentiate tunicated bulbs having a distinct rhizome (Fig. 1D). With the onset of flowering season, a single long scape emerges which supports young inflorescences. After some time, spathe of young inflorescence ruptures followed by exposure of floral buds. Then the flowers start opening sequentially in morning and evening hours. Expansion of flower is followed by unfolding of one tepal and marginal opening of bud. Nearly after 2 hours, one stamen emanates (Fig 1E) followed by other two (Fig. 1F). After some time, floral bud is fully expanded and all the six stamens are visible. Subsequentially, filaments start elongating, first three in a sequence in a short period of time. In just open flowers, anthers appear at different levels with respect to stigma (Table 2). In a sample of 20 flowers, those with 3 long+3 short filaments (Fig. 1G) were preponderant followed by the flowers having 4 long+2 short (Fig. 1H), 2 long+4 short (Fig. 1I) and 1 long+3 medium+2 short filaments (Fig. 1J).

Table 2. Different patterns of appearance of anthers with respect to stigma in *A. stracheyi*

Stage	Appearance of anthers with respect to stigma			
Long+Short filaments	3Long+3Short	4Long+2Short	2Long+4Short	1Long+3Medium+2Short
Percentage of flowers	70	15	10	5
Flower no./ Total	14/20	3/20	2/20	1/20
Fig.	1G	1H	1I	1J

At these stages, style is short nearly at the level of anthers of short filaments. As soon as, filaments attain maximum length, three were seen dehisced and three undehisced (Fig. 1K), followed by dehiscence of the remaining three. The flowers having six long stamens showed distylous condition (Figs. 1L, M). That is flowers having short (4mm) and long styles (8mm) were found at the same stage of development.

Allium roylei is another species whose trimerous flowers bear three long and three short stamens (Kohli, 2013) though it depicts tristylous condition. The complete opening of flower takes about 20-24 hrs and its longevity remains for 4-5 days. Nearly after 7 days, tepals wither and ovaries start swelling. Within next 10 days, fruit get matured followed by seed dispersal.

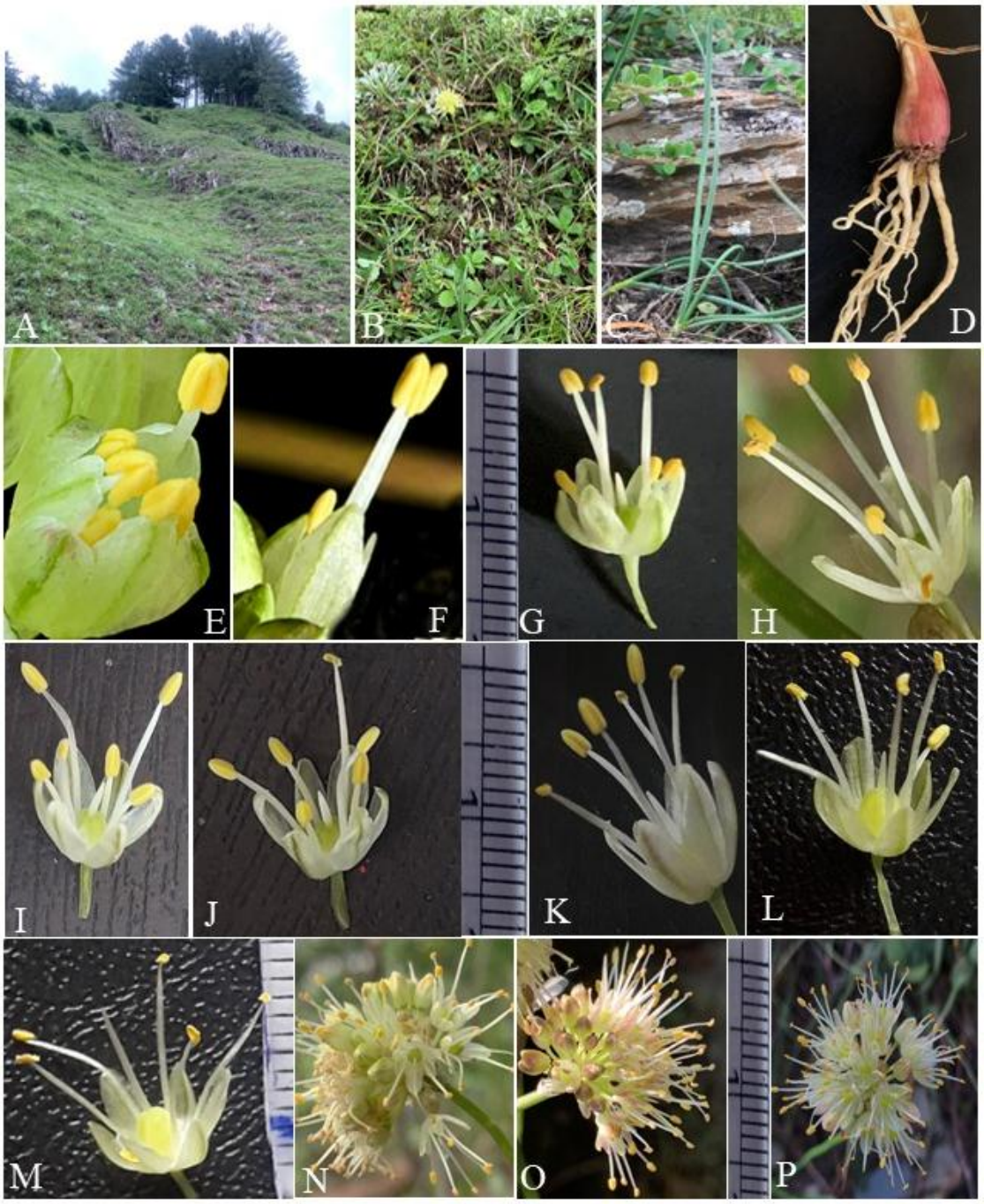

Figure 1. Plants of *A. stracheyi* growing in (A) open meadows; (B) sloppy grasslands; (C) leaves; (D) bulb; (E-F) buds showing flower opening sequence; Flowers with variable stamens length (G) 3 long plus 3 short; (H) 4 long and 2 small; (I) 2 long and 4 small and (J) 1long plus 3 intermediate and 2 short; (L) 6 long filaments bearing 3 dehisced and 3 undehisced anthers; (L) flowers with long and (M) short style. Inflorescences bearing (N) pale-yellow; (O) light pink and (P) pale-yellow plus light pink flowers

Floral variability

Current studies revealed that the flower colour of *A. stracheyi* plants slightly vary at different elevations (Fig. 2). While flowers of 5 populations (Dha, Dln, Kns, Thn and Bhr) of lower elevations (2200-2400 m) are pale-yellow barring Shiryar-top having more pink flowers, those of remaining populations growing at (Pdr, Kdl, Lgr, Sha, Tib, Kdn, Ggt, Dar, Ktp) higher altitudes (2400-3000 m) contain more plants with light pink-flowers except Ganja-goth (Figs. 1N-P).

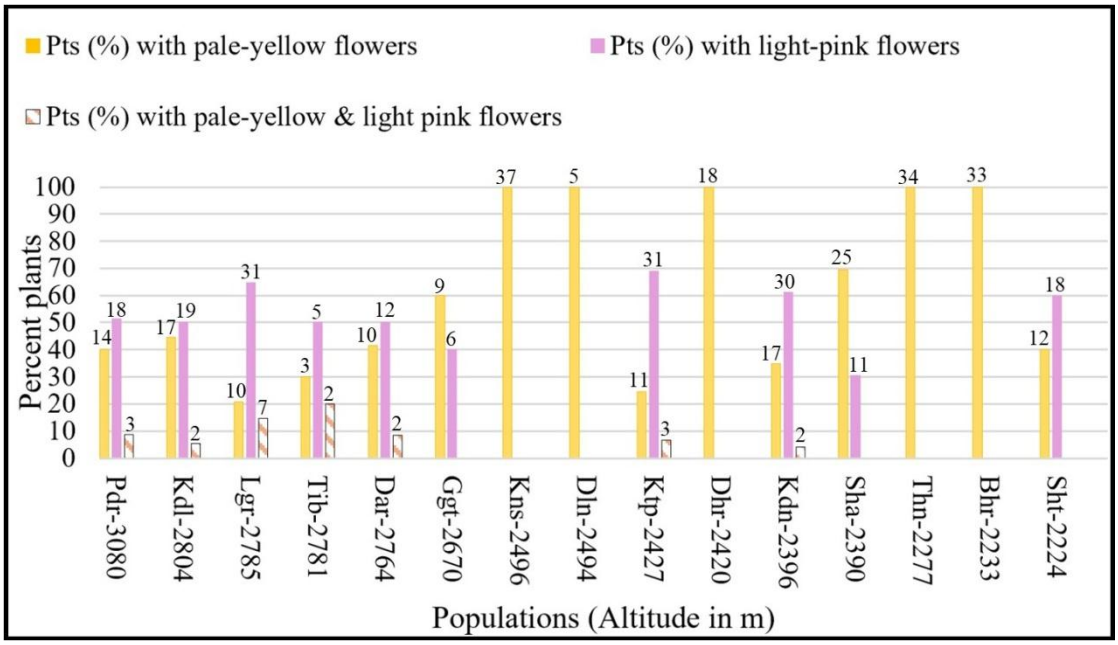

Figure 2. Graph showing plants (%) with pale-yellow, light pink and both pale-yellow and light-pink flowers

Earlier, Nasir (1975) and Pandey *et al.,* (2021) documented pink to pale-yellow flowers of *A. stracheyi*, but without mentioning occurrence of specific flower type at distinct elevation range. We (Sharma and Gohil, 2002) also found in *Allium roylei*, plants bearing light-pink flowers at higher elevations (1800m) and dirty-white in plains (351m). Higher variability witnessed in floral traits in *A. stracheyi* seems to be the outcome of heterogeneity with regards to altitude, topography, temperature, relative humidity, ecology and soil conditions.

Phenological characteristics

Recording of phenological events such as initiation and duration of flowering, fruiting and senescence in medicinal herbs which are valued primarily for foliage, provides an idea about suitability of specific season for its harvesting (Mir *et al.,* 2020, Lalmuanpuii *et al.,* 2020). Duration of different biological events and timing of onset varied in plants of high temperate and sub-alpine zones (Fig. 3). The perennial plants of higher reaches (2600-3100 m) show vegetative growth from April ending to July. They flower from 1st to 3rd week of August, develop fruits from mid-August to mid-September. show senescence in October and dormancy till April, followed by emergence of juvenile plants in April. In the plants of lower altitudes (2300-2500 m), these events get delayed by nearly two weeks. Occurrence of *Allium* plants at different habitats of varying elevations with some differences in the duration of phenological events is indicative of their high adaptability to varying climatic conditions (Duchoslav, 2009).

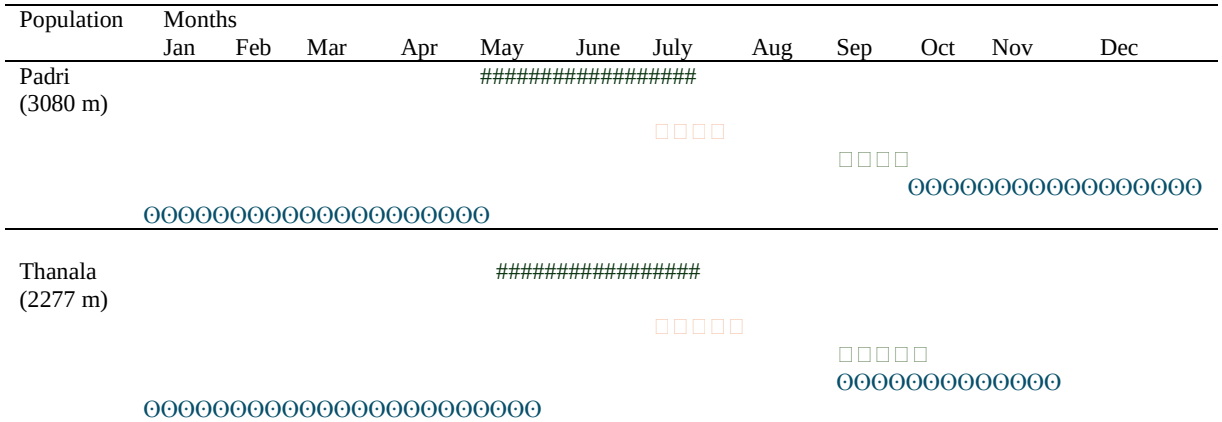

Figure 3. Phenological events of emergence and vegetative growth (###), flowering (□□□□), fruiting (□□□□) and senescence (ΘΘΘ) in *A. stracheyi* plants found at 2277 and 3080 m

As such, current studies are indicative of significant effect of altitude and associated climatic conditions on floral traits and phenological behaviour of *A. stracheyi*.

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RESEARCH ARTICLE

CURVULARIA LEAF SPOT - AN IMPORTANT DISEASE OF COTTON CAUSED BY *CURVULARIA LUNATA* (WAKKER) BOEDIJN

Payal Kodavala[1]*, Prashant B. Sandipan[2], R.K. Patel, Satish Kumar Sain, Kishor Sharma, Navya Sharma and Vedansh Sharma

[1]Department of Plant Pathology, N. M. College of Agriculture, Navsari Agricultural University, (NAU), Navsari, Gujarat, India.
[2]Main Cotton Research Station (MCRS), Navsari Agricultural University (NAU), Surat 395 007 (Gujarat), India
Email: payalkodavla1309@gmail.com

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Abstract: Cotton (*Gossypium hirsutum* L.) is one of the most important fiber crop playing a key role in the economic and social scenario of the globe. It is also known as "The white gold" or "The king of fibers". It is a premier cash crop of our country and belongs to the family *malvaceae*. Curvularia leaf spot appear initially as small circular brown to brownish black spot surrounding with yellow, later it become dark yellow to brown hallow surrounding to brownish black spots and in severe cases, the leaves turned yellow colour and detached easily from the branch resulting in the defoliation. The survey was conducted in the cotton growing regions of Surat, Bharuch and Narmada districts of South Gujarat in the year 2024-25 to examine the presence of *Curvularia lunata* (Wakker) Boedijn pathogen on cotton plants and to record the observation on per cent disease intensity and per cent disease incidence The maximum mean per cent disease intensity was found in the Bharuch district with 32.64 per cent, while the lowest disease intensity of *Curvularia lunata* was found in Surat district with 17.45 per cent and the maximum per cent disease incidence was found in Bharuch district with 12.33 per cent, while the lowest per cent disease incidence was found in Surat district with 7.22 per cent.

Keywords: Cotton, Survey, *Curvularia*, Intensity, Incidence

INTRODUCTION

Cotton is the world's most widely grown fibre crop, which belongs to the genus *Gossypium* in the family *Malvaceae* (Anonymous, 2017). It is oldest among the commercial crops of the world and also known as “THE KING OF FIBERS” and “WHITE GOLD”. It has delicate, white, soft and fluffy fibre that is made of about 87 to 90 per cent of cellulose. Cotton has been cultivated as over thousands of years for both the food and fibre. It is versatile crop, its fibre is used as raw material in textile, pulp and paper industries and oil extracted from the cotton seed is used in food, cosmetics, chemicals and pharmaceuticals (Proto *et al*., 2000).

The symptoms of Curvularia leaf spot appear initially as small circular brown to brownish black spot surrounding with yellow hallow, later it become dark yellow to brown hallow surrounding to brownish black spots (Joshi *et al*., 2023).

Curvularia is a wide spread air borne facultative weak pathogen, which mostly survives as a saprophyte in tropical and sub-tropical areas. It is a dematiaceous, filamentous fungus. *Curvularia* spp. are darkly pigmented fungi with spores (conidia) efficiently adapted for most aerial dissemination. Some species have caused devastating disease epidemics of important food crops such as rice, wheat and maize (Sivanesan, 1987). The Curvularia leaf spot injures or kills the leaf tissues and thereby reduces the area of chlorophyll which involved in photosynthesis. If considerable leaf area is killed, then vigour and yields are reduced drastically.

Unfortunately, in our efforts for maximization of crop production by evolving various operations in crop production system very often tends to increase the potential disease hazards of some diseases which are either new or of major importance to the crop, Curvularia leaf spot disease is one example of a minor disease gaining such importance. Anamorph of *Curvularia lunata* is *Cochliobolus lunatus* (Nelson and Haasis, 1964) has been known to cause a leaf spot of cotton in India (Sharma and Chauhan, 1985). This fungus is found throughout the tropics and has a wide host range in tropical countries of South East Asia such as Thailand, Cambodia, Vietnam, Indonesia and Nepal. Looking into the occurrence of the Curvularia leaf spot disease in cotton crop, it has the potential to spread drastically over a large area.

Curvularia leaf spot is becoming an important disease in the cotton growing areas of Gujarat. So, a field survey was conducted to collect the information

*Corresponding Author

on the severity of Curvularia leaf spot disease of cotton in the selected districts of South Gujarat.

MATERIALS AND METHODS

Survey of Curvularia Leaf Spot Disease

A roving survey of intensity and incidence of Curvularia leaf spot disease of cotton was conducted during *kharif*, 2024 in August to December.

The multistage random sampling survey was carried out in Surat, Bharuch and Narmada districts of Gujarat. For this purpose, three taluka in each district was taken and in each taluka three villages was taken for the disease assessment.

Calculation of Per cent Disease Intensity (PDI)

In each field, five spots selected randomly and total of 25 plants were assessed 4 bottoms, 4 middle and 2 top leaves in term of 0-5 scale as given by (Sheoraj, 1989).The PDI was calculated by using following formula given by McKinney (1923)**.**

$$\text{PDI} = \frac{\text{Sum of all the diseases rating}}{\text{Total no. of leaves observed} \times \text{Maximum grade}} \times 100$$

Calculation of Per cent Disease Incidence (DI)

Intensity of Curvularia leaf spot disease was examined on the basis of percentage of severity of disease. Similarly, the per cent disease incidence of Curvularia leaf spot was calculated by using the following formula given by Wheeler, 1969.

$$\text{PDI} = \frac{\text{No. of Plants infected}}{\text{Total no. of plants examined}} \times 100$$

The disease reaction was recorded as per the grading system as following:

To record the development of the disease, a scale was used in which the plants were classified into the following (0-5) classes.

Table 1. The disease reaction grade for Curvularia leaf spot disease

Disease rating	Description
0	Plant free from Curvularia leaf spot
1	Infection in traces on lower leaves, covering up to 10 per cent leaf area
2	Slight infection, some spot on leaves 11-25 per cent leaf area, stem showing slight infection, bolls free
3	Moderate infection, covering 26-50 per cent leaf area, middle leaves showing severe infection, bolls usually free
4	Heavy infection, 51-70 per cent leaf area, bolls slightly infected
5	Severe infection, 71-100 per cent leaf area, stems and bolls severely infected defoliation common

RESULT AND DISCUSSION

A roving survey was carried out in the year 2024-2025 to know the presence of Curvularia leaf spot pathogen on the cotton plants and recorded the observations on per cent disease intensity (PDI) and disease incidence (DI) in the cotton growing regions of South Gujarat. In South Gujarat region, three districts were selected *viz*., Surat, Bharuch and Narmada. In each district, three taluka were taken and in each taluka three villages were taken for the disease assessment. Five spots/places in each field were chosen to determine the severity of the disease.

A total of 25 plants were evaluated from each field to calculate the per cent disease intensity and 100 plants were examined to determine the per cent disease incidence. The severity of the condition was determined by using a 0-5 point disease assessment scale. The survey was conducted for the severity, incidence, distribution and spread of the disease as the results are provided in the chapter.

The data collected from the roving survey in randomly chosen fields across various villages of South Gujarat are presented in Table: 2, Table: 3 and Photo: 4.1.

Chokhvada,Umarpada, Surat

Bhilvada, Mangrol, Surat

Chopadvav, Sagbara, Narmada

Chitrol, Nandod, Narmada

Kathodara, Bharuch, Bharuch

Moriyana ,Valiya,Bharuch

Photo 4.1. Survey of Curvularia leaf spot disease in South Gujarat during the year 2024-2025

Per cent Disease Intensity of Curvularia Leaf Spot Disease of Cotton

The survey was carried out at various locations for the intensity of disease, where the cotton crop was at different growth stages during the field visit. The data of Curvularia leaf spot disease for *kharif*, 2024 was described in Table: 2. It was revealed from the table that the disease intensity ranged from 9.50 to 35.10 per cent during the year 2024. The highest mean disease intensity 33.43 per cent was recorded at Bharuch taluka of Bharuch district and the minimum mean disease intensity 14.66 per cent was recorded at Mangrol taluka of Surat district.

Among three districts, the highest mean PDI 32.64 per cent was recorded in Bharuch district. The maximum PDI ranged from 00.00 to 35.10 per cent

was recorded in the Chiklota village of Valia taluka of Bharuch district and Kambodiya village of Netrang taluka with 0.00 to 26.30 per cent was in the tune. The lowest mean PDI 17.45 per cent was in Surat district. In Surat district, the highest PDI ranged from 00.00 to 33.10 per cent was found in Chokhvada village of Umarpada taluka and lowest PDI was observed in Hathoda village of Mangrol taluka with 0.00 to 9.50 per cent of Curvularia leaf spot disease.

In Narmada district, mean PDI was 18.86 per cent was recorded whereas, Kundiamba village of Dediyapada taluka of Narmada district, the highest PDI ranged from 00.00 to 31.30 per cent was recorded and the minimum PDI was observed in Chopadvav village of Sagbara taluka in the tune of 0.00 to 10.60 per cent. Overall, Curvularia leaf spot disease was observed during middle and the late stages of crop growth on the leaves of cotton plant (Table: 3, Fig. 1, Fig. 2).

Table 2. Survey of Curvularia leaf spot disease intensity in different villages under South Gujarat during the year 2024-25

Sr. No.	District	Taluka	Village	Variety/ Hybrids	GPS Location	PDI range (%)	Mean disease intensity (PDI) of Taluka
1.	**Surat**	Mangrol	Bhilvada	BG II	21.48'21"°N 73.21'02"°E	00.00-19.30	14.66
			Amkhuta	BG II	21.41'44"°N 73.21'91"°E	00.00-15.20	
			Hathoda	BG II	21.42'53"°N 72.99'09"°E	00.00-9.50	
		Olpad	Saroli	BG II	21.11'41" °N 72.53'41" °E	00.00-11.20	15.30
			Masma	BG II	21.17'46" °N 72.45'42"°E	00.00-16.50	
			Asnabad	BG II	21.19'35" °N 72.45'13"°E	00.00-18.20	
		Umarpada	Pinpur	BG II	21.44'54" °N 73.48'35"°E	00.00-22.60	22.40
			Umargot	BG II	21.43'73" °N 73.52'29"°E	00.00-11.50	
			Chokhvada	BG II	21.43'33" °N 73.54'79" °E	00.00-33.10	
						District mean	**17.45**
2.	**Bharuch**	Valia	Chiklota	BG II	21.63'38" °N 73.31'46" °E	00.00-35.10	32.60
			Moriyana	BG II	21.64'89" °N 73.33'95" °E	00.00-30.50	
			Hirapor	BG II	21.35'22" °N 73.08'39"°E	00.00-32.20	
		Netrang	Netrang	BG II	21.38'24" °N 73.21'37" °E	00.00-33.10	31.90
			Kelvikuva	BG II	21.37'15" °N 73.21'23"°E	00.00-31.30	
			Kambodiya	BG II	21.36'12" °N 73.21'40"°E	00.00-26.30	
		Bharuch	Derol	BG II	21.46'06" °N 72.55'57" °E	00.00-32.80	33.43
			Kathodara	BG II	21.31'56" °N 72.53'45" °E	00.00-34.50	
			Nabipur	BG II	21.48'34" °N 73.01'50"°E	00.00-32.00	
						District mean	**32.64**
3.	**Narmada**	Nandod	Chitrol	BG II	21.49'11" °N 73.26'30"°E	00.00-16.50	18.36
			Virsingpura	BG II	21.53'32" °N 73.39'07"°E	00.00-23.90	
			Akuwada	BG II	21.40'19" °N 73.08'09"°E	00.00-14.70	
		Dediyapad	Jargam	BG II	21.55'23" °N	00.00-12.50	20.93

		a			73.61’88”°E		
			Kundiamba	BG II	21.52’36” °N 73.63’09”°E	00.00-31.30	
			Ghankhetar	BG II	21.60’24” °N 73.59’77”°E	00.00-19.00	
		Sagbara	Chopadvav	BG II	21.33’62” °N 73.44’60”°E	00.00-10.60	17.30
			Dudhaliver	BG II	21.33’13” °N 73.43’53”°E	00.00-19.50	
			Amiyar	BG II	21.33’08” °N 73.45’12”°E	00.00-21.80	
						District mean	**18.86**

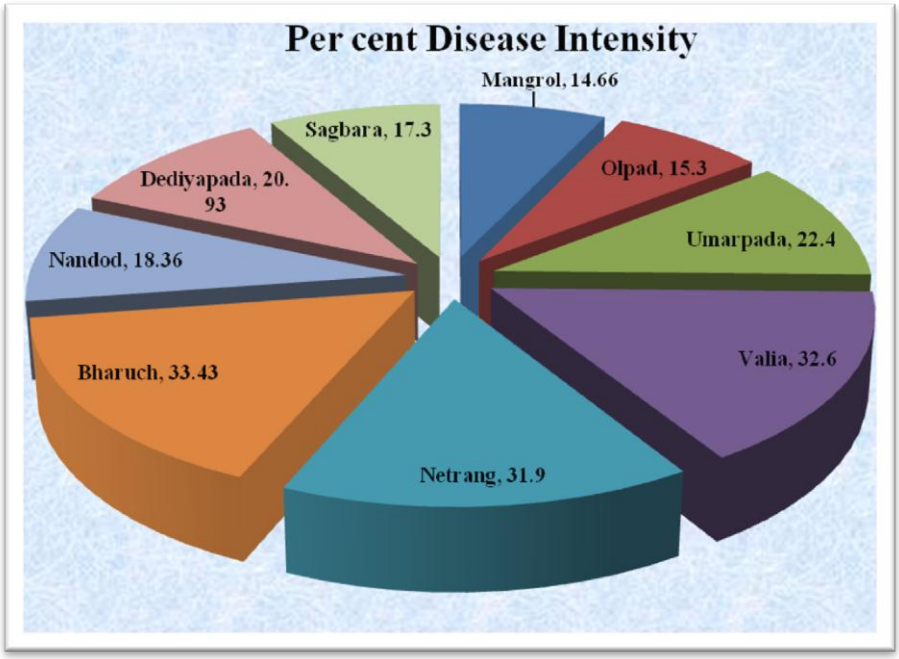

Fig. 1. Mean per cent disease intensity of Curvularia leaf spot of cotton in different taluka of South Gujarat during the year 2024-25

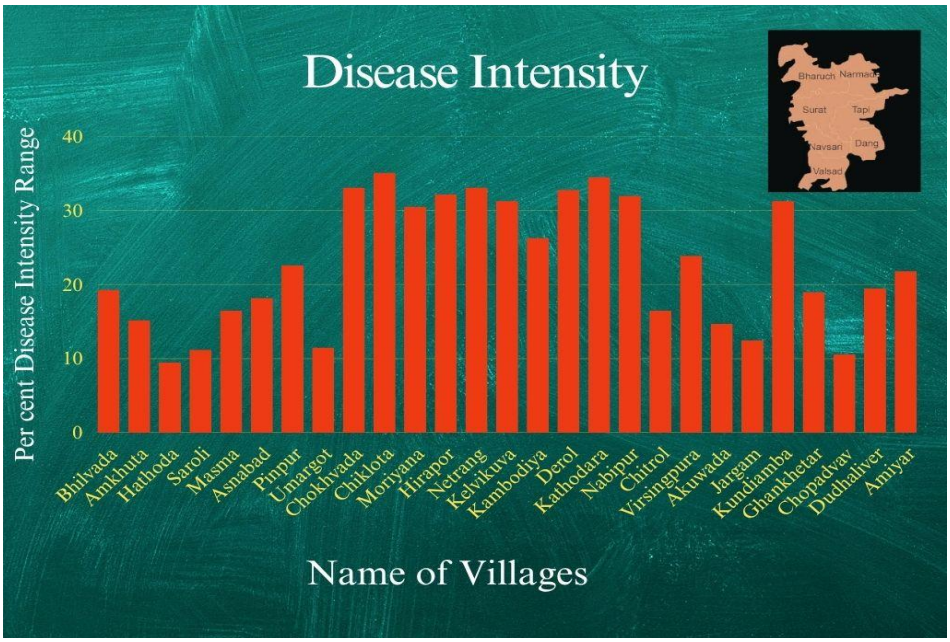

Fig. 2. Range of per cent disease intensity of Curvularia leaf spot of cotton in different villages of South Gujarat during the year 2024-25

Per cent Disease Incidence of Curvularia leaf spot Disease of Cotton

Data pertaining to the percentage of disease incidence of Curvularia leaf spot of cotton in three districts of South Gujarat are presented in Table: 3. It is evident from the data that the disease incidence prevails in all the cotton growing areas of South Gujarat with the incidence varying from 3.00 to 19.00 per cent at various locations surveyed. The maximum disease incidence 19.00 per cent was recorded at Hirapor village of Valia taluka of Bharuch district followed by 16.00 per cent at Derol village of Bharuch taluka and 14.00 per cent at Kelvikuva village of Netrang taluka of Bharuch district (Table: 3, Fig. 3, Fig. 4).

In Surat district, the maximum per cent disease incidence was recorded in Hathoda village with 12.00 per cent followed by Amkhuta village with 10.00 per cent of Mangrol taluka and Pinpur village with 10.00 per cent of Umarpada taluka of Surat district, respectively.

In Narmada district, the highest 15.00 per cent disease incidence was observed in Chitrol village of Nandod taluka followed by Chopadvav village of Sagbara taluka with 13.00 per cent and 11.00 per cent in Kundiamba village of Dediyapada taluka of Narmada district (Table: 3, Fig. 3, Fig. 4).

Among all the three districts surveyed, the highest Curvularia leaf spot mean disease incidence 12.33 per cent was recorded in Bharuch district followed by Narmada district with 8.00 per cent. In Surat district, the lowest mean incidence 7.22 per cent disease was recorded (Table: 3).

The present work was more or less corroborate with the research findings reported by Singh (2006) carried out a roving survey on the severity of Curvularia leaf spot disease of cotton at Oilseed Research Farm, Kalyanpur, Kanpur of C.S. Azad University of Agriculture and Technology, Kanpur and farmers' fields. He reported that the disease severity ranged from 1.0 to 36.0 per cent. Shirsath *et al.* (2018) also reported the incidence of leaf spot disease on cotton caused by *Curvularia verruculosa* Ellis. The disease incidence and disease severity index (DSI) were 36.00 and 49.38 per cent, respectively. Jatwa *et al*. (2021) conducted an extensive and regular survey in the month of September for the two crops to record Curvularia leaf spot incidence. Distribution and prevalence of Curvularia leaf spot disease of maize was observed in the five districts *viz*., Udaipur, Chittorgarh, Bhilwara, Banswara and Dungarpur. In *kharif* 2017 and 2018, disease prevalence was ranged between 91.66 to 34.28 per cent in all the surveyed areas and the severity scale was in the tune of 1.50 to 8.00 which, indicates that the disease was present in traces to severe form in most of the surveyed areas. During the survey, leaf spot disease was found prevalent in the three major districts of Gujarat as Panchmahal, Dahod and Mahisagar. They revealed that Curvularia leaf spot (CLS) disease severity varied from 13.12 to 55.12 per cent.

Table 3. Survey of Curvularia leaf spot disease incidence in different villages of cotton under South Gujarat during the year 2024-25

Sr. No	District	Taluka	Village	Variety/ Hybrids	GPS Location	Total no of plant examined	Total no of infected plant	Per cent Disease Incidence (PDI) (%)	Mean disease Incidence of Taluka (%)
1.	**Surat**	Mangrol	Bhilvada	BG II	21.48'21"°N 73.21'02"°E	100	8	8.00	10.00
			Amkhuta	BG II	21.41'44"°N 73.21'91"°E	100	10	10.00	
			Hathoda	BG II	21.42'53"°N 72.99'09" °E	100	12	12.00	
		Olpad	Saroli	BG II	21.11'41" °N 72.53'41" °E	100	5	5.00	5.00
			Masma	BG II	21.17'46" °N 72.45'42"°E	100	3	3.00	
			Asnabad	BG II	21.19'35" °N 72.45'13"°E	100	7	7.00	
		Umarpada	Pinpur	BG II	21.44'54" °N 73.48'35" °E	100	10	10.00	6.66
			Umargot	BG II	21.43'73" °N 73.52'29"°E	100	6	6.00	
			Chokhvada	BG II	21.43'33" °N 73.54'79" °E	100	4	4.00	
								District mean	**7.22**
2.		Valia	Chiklota	BG II	21.63'38" °N 73.31'46" °E	100	11	11.00	14.00

	Bharuch		Moriyana	BG II	21.64’89” °N 73.33’95” °E	100	12	12.00	
			Hirapor	BG II	21.35’22” °N 73.08’39”°E	100	19	19.00	
		Netrang	Netrang	BG II	21.38’24” °N 73.21’37” °E	100	12	12.00	11.33
			Kelvikuva	BG II	21.37’15” °N 73.21’23”°E	100	14	14.00	
			Kambodiya	BG II	21.36’12” °N 73.21’40” °E	100	8	8.00	
		Bharuch	Derol	BG II	21.46’06” °N 72.55’57” °E	100	16	16.00	11.66
			Kathodara	BG II	21.31’56” °N 72.53’45” °E	100	11	11.00	
			Nabipur	BG II	21.48’34” °N 73.01’50”°E	100	8	8.00	
								District mean	**12.33**
3.	**Narmada**	Nandod	Chitrol	BG II	21.49’11” °N 73.26’30”°E	100	15	15.00	8.00
			Virsingpura	BG II	21.53’32” °N 73.39’07”°E	100	4	4.00	
			Akuwada	BG II	21.40’19” °N 73.08’09”°E	100	5	5.00	
		Dediyapada	Jargam	BG II	21.55’23” °N 73.61’88”°E	100	9	9.00	9.00
			Kundiamba	BG II	21.52’36” °N 73.63’09”°E	100	11	11.00	
			Ghankhetar	BG II	21.60’24” °N 73.59’77”°E	100	7	7.00	
		Sagbara	Chopadvav	BG II	21.33’62” °N 73.44’60”°E	100	13	13.00	7.00
			Dudhaliver	BG II	21.33’13” °N 73.43’53”°E	100	3	3.00	
			Amiyar	BG II	21.33’08” °N 73.45’12”°E	100	6	6.00	
								District mean	**8.00**

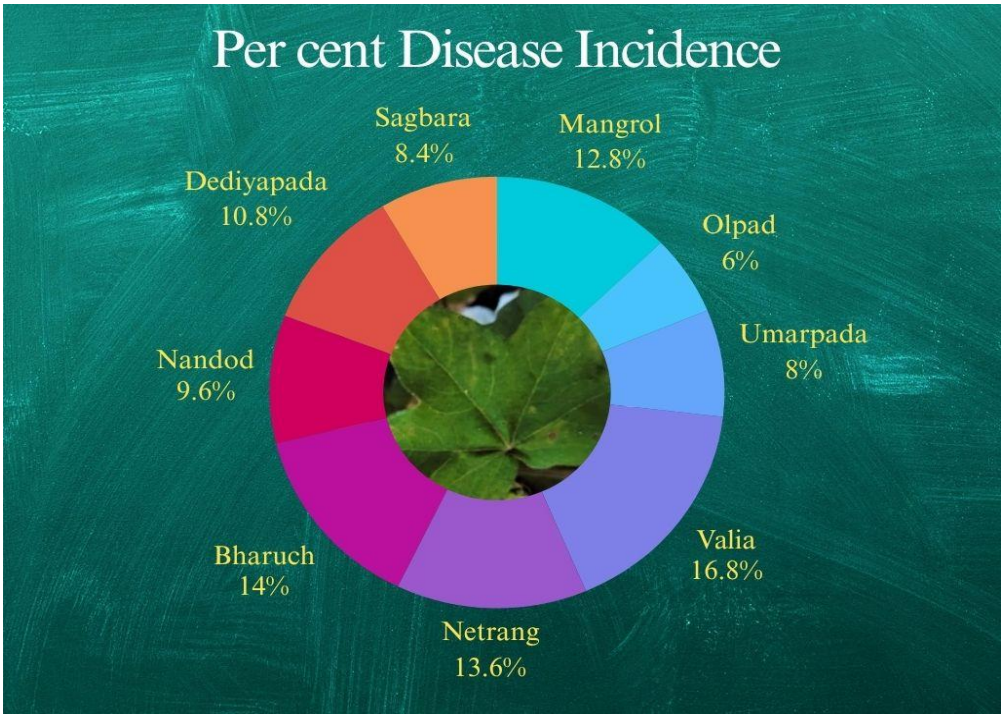

Fig. 3. Disease Incidence of Curvularia leaf spot of cotton in different talukas of South Gujarat during the year 2024-25

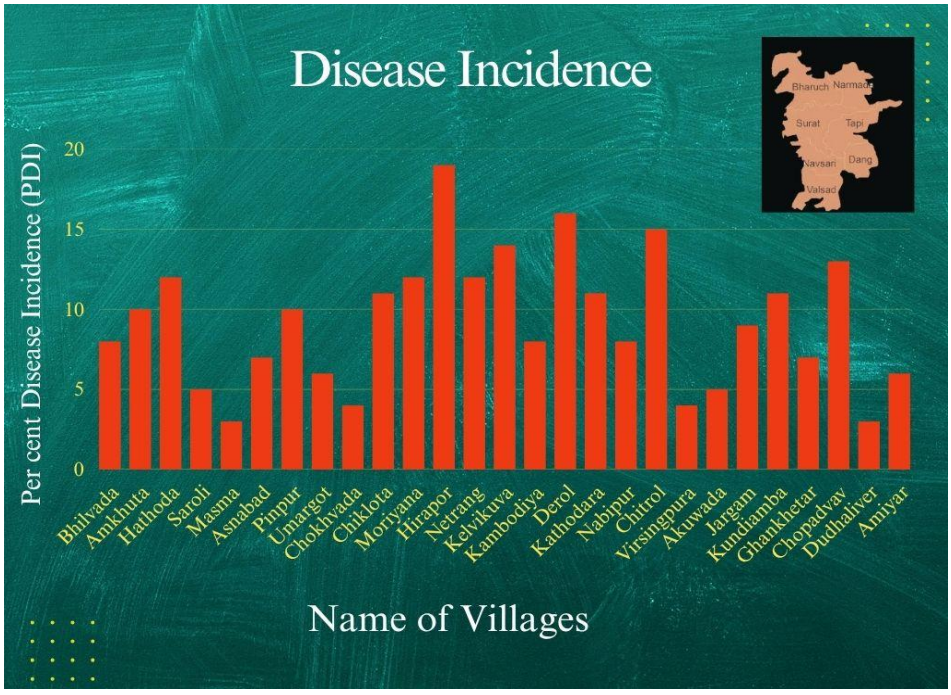

Fig. 4. Disease Incidence of Curvularia leaf spot of cotton in different talukas of South Gujarat during the year 2024-25

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RESEARCH ARTICLE

VALIDATION OF AN ICP-OES METHOD FOR DETERMINATION OF HEAVY METALS IN LONG PEPPER (*PIPER LONGUM* L.) AND ASSESSMENT OF MARKET SAMPLES

C. Beena*

AICRP on MAP & B, College of Agriculture, Vellanikkara, Kerala state, Pin-680656
Email: beenac2@gmail.com

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Abstract: The long pepper (*Piper longum* L.) is an essential medicinal spice commonly used in traditional healthcare. However, due to the growing threat of environmental pollution, the concentration of heavy metals in such medicinal plant materials poses a significant hazard. Therefore, an analytical technique based on ICP-OES which was developed for the quantitative determination of Cd, Cr, Pb, and Ni concentrations was validated in the long pepper samples. The method was tested for several validation parameters, which included linearity, LOD (limit of detection), LOQ (limit of quantification), accuracy, precision, and repeatability. Calibration curves obtained in the study showed excellent linearity with R^2 values ranging from 0.9988 to 0.9994. LOD and LOQ values determined in the analysis were found to fall within the ranges 0.05–0.20 µg/L and 0.15–0.60 µg/L, respectively. Results obtained in recovery studies ranged between 97.8% and 99.2%, and RSD was not greater than 3%. Determination of cadmium, lead, chromium and nickel in both farm and market samples showed that Cromium and Nickel were within permissible limits, whereas Cadmium and lead could not be detected at all.

Keywords: *Piper longum*, ICP-OES, Heavy metals, Method validation, Medicinal plants

INTRODUCTION

Medicinal plants continue to play a significant role in traditional and modern healthcare systems. Among them, long pepper (*Piper longum* L.), a member of the family Piperaceae, is widely valued for its therapeutic properties, including digestive enhancement, respiratory support, and anti-inflammatory activity. Its fruits are extensively used in Ayurvedic formulations as well as in spice mixtures (Suresh *et al*., 2011).

Despite medicinal importance, the safety of plant-derived materials is increasingly challenged by environmental contamination. Heavy metals such as cadmium (Cd), chromium (Cr), lead (Pb), and nickel (Ni) can enter plant systems through contaminated soils, irrigation water, agrochemicals, and industrial emissions. These elements tend to accumulate in plant tissues and may pose serious health risks upon consumption. (Sulaiman *et al*., 2024).

In this context, reliable analytical methods are essential for monitoring heavy metal contamination in medicinal plants. Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) is widely recognised for its capability in multi-element analysis due to its sensitivity, precision, and rapid detection (Senila, 2024).

However, the reliability of analytical data depends on proper method validation. Parameters such as linearity, detection limits, accuracy, precision, and reproducibility must be systematically evaluated to ensure the suitability of the method (Ilieva *et al*., 2018).

The present study was therefore undertaken to validate an ICP-OES-based method for the determination of Cd, Cr, Pb, and Ni in Long pepper and to assess their levels in selected farm and market samples of *Piper longum.*

Materials and methods

Instrumentation

Heavy metal analysis was carried out using an ICP-OES system (Optima 8000, Perkin Elmer). The instrument was operated under optimised conditions to ensure accurate measurements following the procedure in the operation manual of the instrument.

Operating conditions:

- RF power: 1500 W
- Plasma gas flow: 12 L/min
- Nebulizer flow: 0.7 L/min
- Auxiliary gas flow: 0.2 L/min
- Sample flow rate: 1 mL/min

Analytical wavelengths:

- Cd: 228.802 nm
- Cr: 267.716 nm
- Ni: 231.604 nm
- Pb: 220.353 nm

Sample preparation

Long pepper samples (dried fruits) were collected from local herbal markets of Kerala and one sample collected from the experimental farm of Kerala Agricultural University, Vellanikkara. Samples were

*Corresponding Author

oven- dried at 50°C and ground into a fine powder using a stainless steel grinder. The powdered material was sieved through a 0.5 mm mesh to ensure uniformity of powdered sample prior to analysis.

Acid digestion

All glassware and plastic ware were thoroughly cleaned by soaking in 10% potassium permanganate followed by 10% nitric acid for 24 hours, and rinsed with ultrapure water to minimise contamination.

Each plant sample (0.5 g) was subjected to wet digestion using a mixture of nitric acid (HNO_3) and perchloric acid in a 9:4 ratio. The digestion process was continued until a clear solution was obtained, filtered, then diluted to 50 mL with ultrapure deionised water.

Standard stock solutions (100 mg/L) of heavy metals Cadmium, Chromium, Lead and Nickel were diluted appropriately to prepare calibration standards. Blanks and reference standards and plant samples were analysed by feeding to the ICP OES instrument alongside the samples following the instrument manual guidance.

Prepared calibration curves using standard solutions. Known concentrations of Cd, Cr, Pb, and Ni added to long pepper samples to prepare spiked samples. Recovery % and % RSD calculated. One sample from KAU farm and 3 market samples were analysed for heavy metals using above procedure.

RESULTS

Method validation

Table 1. Linearity (R^2)

Element	Concentration range (ug/L)	R^2
Cadmium	0.5-100	0.9994
Chromium	1-100	0.9988
Lead	1-100	0.9992
Nickel	1-100	0.9989

Table 2. LOD and LOQ

Element	LOD (µg/L)	LOQ (µg/L)
Cadmium	0.05	0.15
Chromium	0.10	0.30
Lead	0.20	0.60
Nickel	0.11	0.33

Table 3. Accuracy and Precision

Element	Spike level (µg/L)	Recovery %	RSD %
Cadmium	10	98.5	2.5
Chromium	50	97.8	2.3
Lead	50	99.2	2.7
Nickel	50	98.7	2.6

Table 4. Repeatability and Reproducibility

Element	Mean concentration (µg/L)	RSD%(intra- day)	RDS% (inter-day)
Cadmium	9.85	2.4	3.1
Chromium	48.9	2.2	3.0
Lead	49.6	2.5	3.2
Nickel	49.4	2.3	3.1

Table 5. Real sample analysis:

Heavy metal content in Long pepper samples analysed

Parameter	FSSAI limit MRL (mg/kg)	Market Sample-1 (mg/kg)	Market Sample.2 (mg/kg)	Market Sample.3 (mg/kg_)	Experimental Farm Sample.4 (mg/L)
Cadmium	1.5	BDL	BDL	BDL	BDL
Chromium	1.0	0.89	1.00	0.90	0.45
Nickel	1.5	0.90	1.50	0.80	0.55
Lead	2.0	BDL	BDL	BDL	BDL

BDL-Below detectable level, MRL-Maximum residue limit

Linearity

Calibration curves were prepared over appropriate concentration ranges for each element. A strong linear relationship was observed, with correlation coefficients (R^2) exceeding 0.998 for all elements, confirming excellent linearity (Table 1.)

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The method exhibited low detection and quantification limits, indicating high sensitivity suitable for trace-level analysis of heavy metals in plant samples (Table 2).

Accuracy and precision

Accuracy was assessed through spike recovery experiments. Recovery values ranged between 97.8% and 99.2%, demonstrating that the method provides reliable quantification .Precision, expressed as relative standard deviation (RSD), remained below 3%, indicating good repeatability of measurements (Table 3).

Repeatability and reproducibility

Both intra-day and inter-day variations were minimal, with RSD values below 5%. This confirms that the method is consistent and reproducible under routine laboratory conditions (Table.4).

Analysis of real samples

Four long pepper samples, including one farm sample and three market samples, were analysed. Cadmium and lead were found to be below detectable levels in all samples analysed. Chromium and nickel were detected in measurable quantities; however, their concentrations were generally within acceptable safety limits as per regulatory standards of FSSAI. (FSSAI,2011).

The results (Table.5) suggested that the analysed samples are largely safe with respect to heavy metal contamination, although continuous monitoring and more sample analysis are remains necessary.

DISCUSSION

The present study demonstrated that the ICP-OES method developed for the determination of Cd, Cr, Pb, and Ni is both reliable and analytically robust in Long pepper analysis. The high linearity (R^2 > 0.998), low detection limits, and satisfactory recovery values obtained in this study are in close agreement with earlier reports on ICP-OES-based heavy metal analysis in various plant matrices. (Fahad *et al.,* 2025).

For example, one study looking at plant products with ICP-OES also found similar straight-line results and could easily spot tiny amounts of metals after breaking down the samples with acid. This shows how good this method is for complicated plant materials. Likewise, when people analyzed many elements in medicinal plants, ICP-OES consistently performed well for a bunch of them, including Cd, Cr, Ni, and Pb (Castro *et al*., 2009).

Our recovery data (97.8–99.2%) were also in line with earlier tests, where adding known amounts of metals back into plant samples and then analysis, confirmed that the method is accurate and reliable.

Other similar studies have also pointed out that ICP-OES effectively detected very small amounts and is very sensitive when checking environmental and plant samples, making it a good choice for keeping an eye on trace metals (Parvathy *et al*.,2020).

In some studies toxic metals were found being too low to even detect. This could mean there's not much pollution around, or that farming practices are really good. In this study, we couldn't find any cadmium or lead in samples tested, which is a good sign with regard to safety .But then, some other studies on medicinal plants showed that how much heavy metal builds up can change a lot. It depends on where the plant grew, what kind of soil it was in, and what people were doing in that area.

In the tested samples chromium and nickel were present but their levels were within safe limits. There was a bit more of them in the market samples compared to the one from the farm. This difference might be because of how the plants were handled after harvest, how much they were exposed to the environment, or if they came from different growing areas. Even with this variation, all the levels we found were within safe limits, meaning the samples are safe to consume when it comes to heavy metal contamination.

CONCLUSION

The ICP-OES method validated in this study worked well for finding heavy metals in long pepper samples. It proved to have straight lines on graphs, could find very tiny amounts, recovered a lot of what was there, and was precise. Absence of cadmium or lead in the samples, is really encouraging for food safety. While chromium and nickel were present, their amounts stayed within safe limits.

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