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RESEARCH ARTICLE

NEW RECORDS OF WOOD ROTTING FUNGI IN PRUNED INDIAN SANDALWOOD (*SANTALUM ALBUM* LINN.) PLANTATIONS

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Abstract: This paper highlights new records of wood rotting fungi in pruned Indian sandalwood plantations.

Keywords: New records, Sandalwood, Fungi

INTRODUCTION

The Indian sandalwood tree (*Santalum album* Linn.), commonly known as the "royal tree of India", is among the most valuable and medicinally important native species found in the country. It holds a prominent place in Indian forestry, and is closely associated with Indian culture and history (Sundararaj, 2014). This tree has maintained its prestigious status as a highly regarded source of fragrance materials for over 3000 years, with a continuous history up to the present-day (Sundararaj, 2026). India has been the world's top producer and exporter of sandalwood oil since more than 5000 years ago (Hansda, 2009). But the decline in ecological integrity and ecosystem services due to rapid deforestation has led to the alarming disappearance of Indian sandalwood tree from their natural habitats, despite their remarkable adaptability to diverse ecological conditions. Since implementing the liberation policies for its cultivation, sandalwood cultivation has increased in areas outside of forests (Sundararaj et al., 2019a). Under the cultivdation of sandalwood, the trees are regularly pruned resulting in most detrimental effect on the health, growth, and productivity of sandalwood trees. The longevity of pruned plants is drastically reduced depending upon the level of pruning as they become very susceptible to decaying fungi and wood-feeding insects (Mooter and Kuhns, 1991, Mohan et al., 2022). Usually, a fungal fruiting body is an indicator of decay, but by the time fruiting bodies are produced, the fungus has already-established itself in the core and is typically revealed when the tree is broken, thrown by the wind, or felled (Kumar et al., 2022). This communication presents the first report of three wood-rotting fungal species infesting pruned sandalwood plantations, confirmed by the occurrence of characteristic fruiting bodies.

MATERIALS AND METHODS

Extensive surveys were undertaken during 2020-2025 to assess the health status of Indian sandalwood plantations covering the states of Andhra Pradesh, Karnataka, Kerala, Tamil Nadu and Telangana. The fruiting bodies of fungi observed on pruned sandalwood trees were collected, preserved and identified them with the help of fungal taxonomists.

RESULTS AND DISCUSSION

The survey revealed the infestation by several wood-rotting fungi in pruned sandalwood trees. The commonly encountered wood-rotting fungi are *Auricularia polytricha* (Mont.), *Cubamyces flavidus* (Lév.), *Daldinia concentrica* (Bolton), *Flavodon flavus* (Kl.), *Fomes* sp., *Ganoderma applanatum* (Pers.), *G. lucidum* (Lingzhi), *Hexagonia tenuis* (Fr.), *Hymenochaete* sp., *Microporus* sp., *Phellinus punctatus* (P.Karst.), *Polyporus* spp., *Porostereum* sp., *Schizophyllum commune* Fries, *Stereum gausapatum* (Fr.), *Trametes* sp. and *Trametes versicolor* (L.). Among these the infestations of the basidiomycetes *Cubamyces flavidus* and *Hexagonia tenuis*, and the agaricomycetes *Hymenochaete* sp., form the first reports on sandalwood. *C. flavidus* (Fig. 1) is one of the common wood-rotting tropical polypore in India that attacks structural and standing timbers (Banerjee ,1947), that can cause considerable damage to both sapwood and heartwood and heartwood of many tree species including Sal are ‘non-resistant’ to the attack of the fungus (Banerjee. and Samadder, 1957). *Hexagonia tenuis* (Fig. 2) causes white rot of its host (Roy and De 1979, Leite

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1994) and its infestation was found on living plant of *Nerium odorum* in Burdwan, West Bengal, and its development is not affected by diameter of the wood of host plants (De, 2018). The genus Hymenochaete (Fig. 3) encompasses more than 200 species of wood-decomposing fungi worldwide inhabiting woody hosts (Spirin et al., 2015). Hembrom, et al. (2023) recorded 134 species including species of Hymenochaete, from branches, stems or roots of trees and shrubs responsible for decaying of wood either by parasitic or saprophytic mode from Valmiki National Park, Bihar (India). Sundararaj (2026) recorded 18 and 24 species of wood-rotting fungi in standing sandalwood trees and sandalwood logs in storage, respectively. Decay by these wood-rotters result in hollowness in the wounded stem and thereby heavy loss of heartwood (Fig. 4). Pruning and other mechanical injuries significantly predispose sandalwood trees to biodeterioration by decay fungi and wood-feeders, leading to heartwood losses ranging from 22.6 to 34.5% during extraction (Sundararaj et al., 2019) and computed mean loss of 8.19% was recorded in processed wood in the Marayur sandalwood depot (Kantha Reddy et al., 2021). The science of pruning is the principle of perishing invaluable wood species (Sundararaj et al., 2022a), and it is essential to understand that mechanical damage results in permanent weakening of the tree, diminishing its value, and possible death. (Sundararaj et al., 2022b; Sundararaj & Raja Rishi, 2022).

Numerous biotic and abiotic factors contribute to the decay processes, with wounds or injuries acting as primary predisposing factors, as, regardless of the age, sandalwood virtually lacks any wound-healing mechanism, and once a wound occurs, it remains unhealed for the entire duration of the tree's life (Sundararaj, 2026). Mohanan (1994) commented that often the decay induced by wounds or injuries cannot be cured completely by any means. Heart rot resulting from decay of the central core does not immediately kill the tree; instead, affected trees continue to grow and often exhibit a healthy external appearance despite substantial destruction of standing heartwood (Mohan et al., 2022). The presence of cavities due to biodeterioration in the extracted woods plays a key role in the classification of sandalwood that is followed by the state forest departments in India, and it is the prime factor for the loss of heartwood. (Kantha Reddy et al., 2021). Hence it is almost impossible to get first class of heartwood from pruned/wounded trees. The study on the health status and heart-rot disease severity of sandalwood trees in Marayur Forest Reserves revealed that only 17% of the trees were devoid of any heart-rot symptoms (Sundararaj et al., 2022c). Contrary to pests and diseases, humans's expectations of higher wood yields through pruning were identified as the primary culprit for degrading the health, growth, productivity, and failure of sandalwood plantations. Pruning and other mechanical injuries in Indian sandalwood act as entry points for various biodeteriorating organisms, which cause loss of heartwood. Commonly used pesticides for wound dressing are largely ineffective, as many biodeteriorating agents have developed resistance. It is crucial to avoid pruning and to take all necessary precautions to prevent any other mechanical damage to live trees for preventing wood decay and loss of heartwood (Sundararaj, 2026).

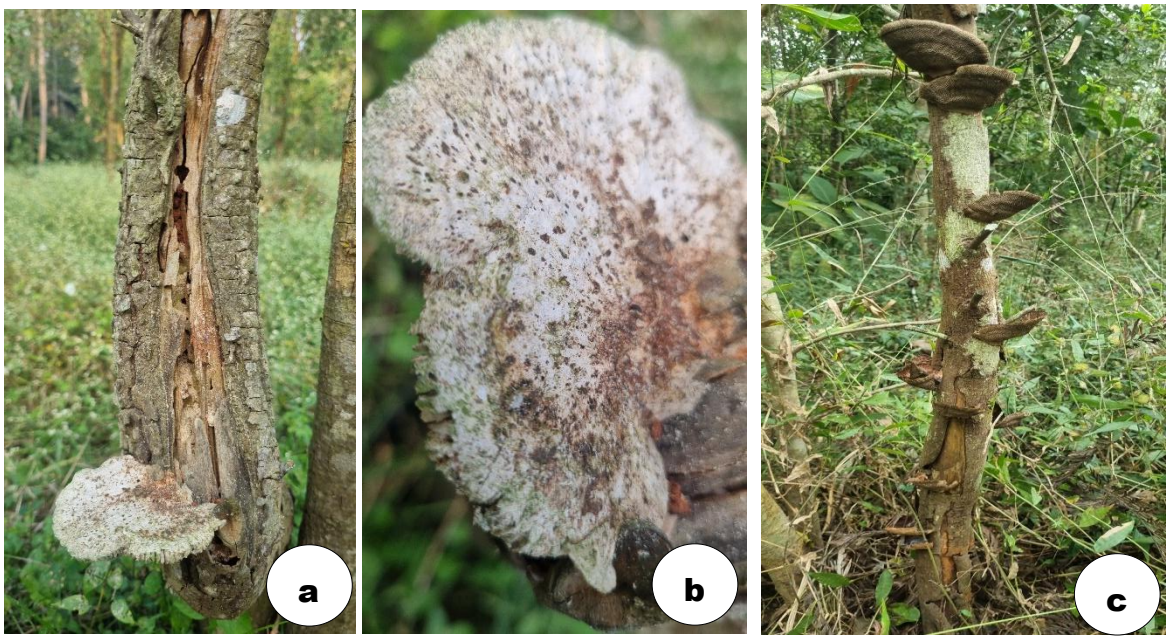

Figure 1. *Cubamyces flavidus* (a) Fruiting body on sandalwood, (b) Enlarged upper surface, (c) Enlarged under surface

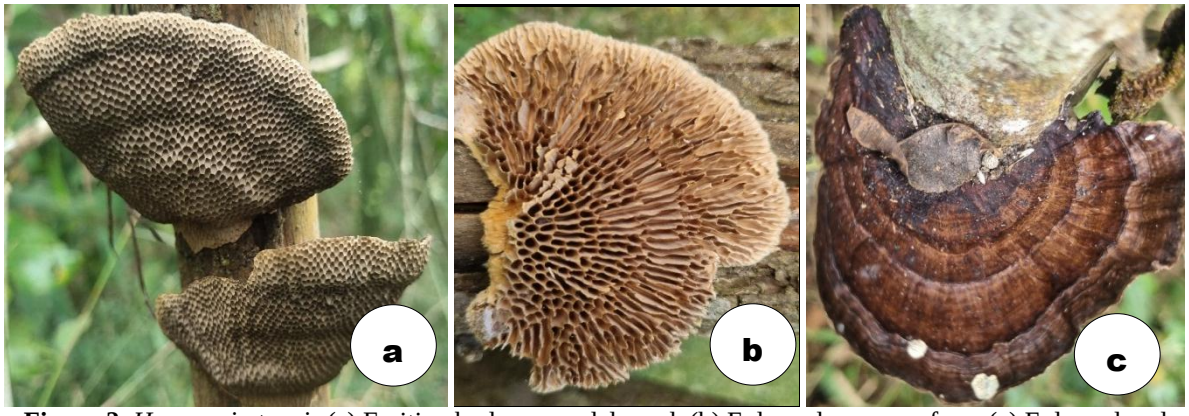

Figure 2. *Hexagonia tenuis* (a) Fruiting body on sandalwood, (b) Enlarged upper surface, (c) Enlarged under surface

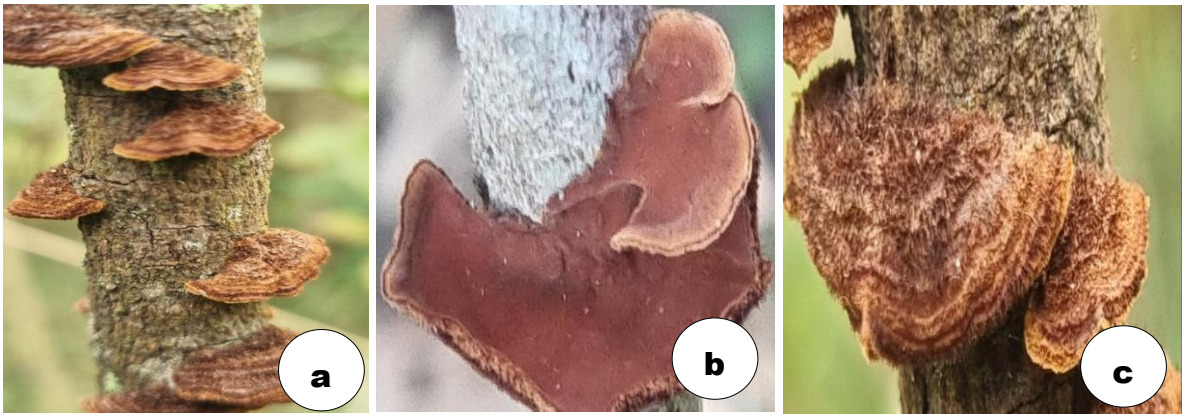

Figure 3. *Hymenochaete* sp., (a) Fruiting body on sandalwood, (b) Enlarged upper surface, (c) Enlarged under surface

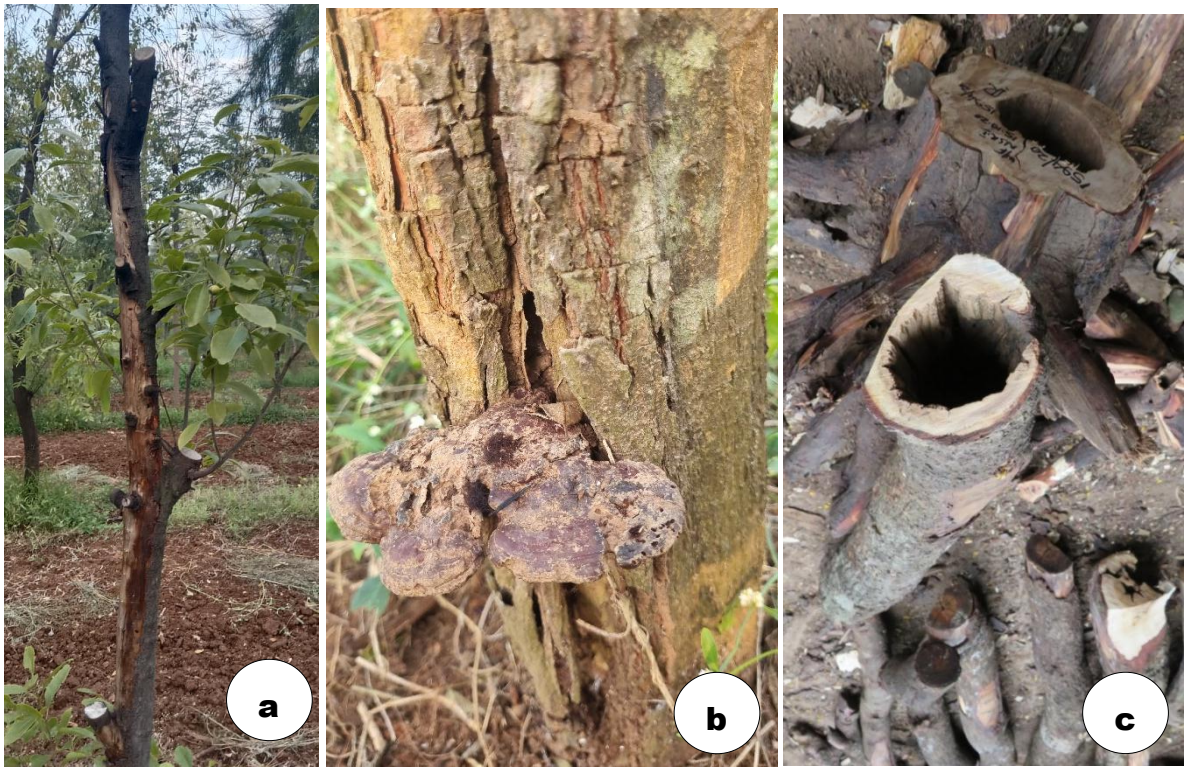

Figure 4. Impact of pruning resulting in loss of heartwood, (a) Severely pruned sandalwood, (b) Infection and establishment of wood rotting funus *Ganoderma lucidum*, (c) Visible loss of heartwood at the time of extraction

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RESEARCH ARTICLE

NEW DISTRIBUTIONAL RECORD OF PERISTYLUS CONSTRICTUS (LINDL.) LINDL. FROM TELANGANA, INDIA – WITH CONSERVATION IMPLICATIONS UNDER CAMPA

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Abstract: The genus *Peristylus* Blume (Orchidaceae) comprises about 102 species worldwide, with 36 species reported from India. During field studies of third party Evaluation of works carried out under Compensatory Afforestation Fund Management and Planning Authority (CAMPA) Scheme, conducted in August 2025, *Peristylus constrictus* (Lindl.) Lindl., a rare terrestrial orchid, was documented for the first time in Telangana from Kavadigundla village, Aswaraopet Range, Bhadradri Kothagudem District. Previously, this species was known from Northeast India, the Eastern Himalayas, Odisha, Maharashtra, and adjoining Andhra Pradesh. The discovery extends the distributional range of the species and enriches the floristic diversity profile of Telangana. The orchid was located on a moist, shaded forest floor close to an encroached habitat, highlighting its ecological vulnerability. The finding emphasizes the importance of habitat protection under CAMPA Biodiversity Conservation components and aligns with the objectives of the UN Decade on Ecosystem Restoration (2021–2030). We recommend protection of the ~200 m² orchid patch through fencing and awareness measures as a model for integrating rare species conservation (*in-situ* conservation) into landscape-level restoration programs.

Keywords: *Peristylus constrictus*, Orchidaceae, Ecosystem restoration, Biodiversity conservation

INTRODUCTION

Orchids are one of the most diverse and ecologically significant plant families, comprising over 28,000 species globally (Chase *et al.*, 2015). In India, orchids are primarily distributed in the Himalayan region, Western Ghats, Odisha, Maharashtra and Andhra Pradesh. The genus *Peristylus* Blume is widely distributed in Tropical & Subtropical Asia to Mongolia and the Pacific, includes 102 species; in India, 36 species are recorded, of which 3 species occur in Telangana (Panda *et al.*, 2025).

Peristylus constrictus (Lindl.) Lindl. is a terrestrial orchid known for its distinct inflorescence and floral morphology. The present study reports *P. constrictus* for the first time from Telangana State, thereby contributing to the floristic diversity of the region.

The discovery extends the known distribution range of the species, previously reported from Northeastern states, Eastern Himalayas, Odisha, Maharashtra, and adjoining Andhra Pradesh. Its occurrence in Aswaraopet Range highlights the ecological value of the Bhadradri Kothagudem forests and their connection to the Papikondalu Wildlife Sanctuary. However, anthropogenic pressures such as podu cultivation, habitat encroachment, and deforestation pose significant threats.

Study Area

The study area, Kavadigundla village, falls under Aswaraopet Forest Range in Palvoncha Forest Division of Bhadradri Kothagudem District, Telangana (Plate-I). The range is ecologically connected to Papikondalu Wildlife Sanctuary. Vegetation includes tropical semi-evergreen forests, tropical moist deciduous forests, and tropical dry deciduous forests (Champion & Seth, 1968).

However, the forests are severely impacted by podu-cultivation, fuel wood collection, and grazing. The *P. constrictus* population was located just 10 m from encroached forest land, indicating high vulnerability.

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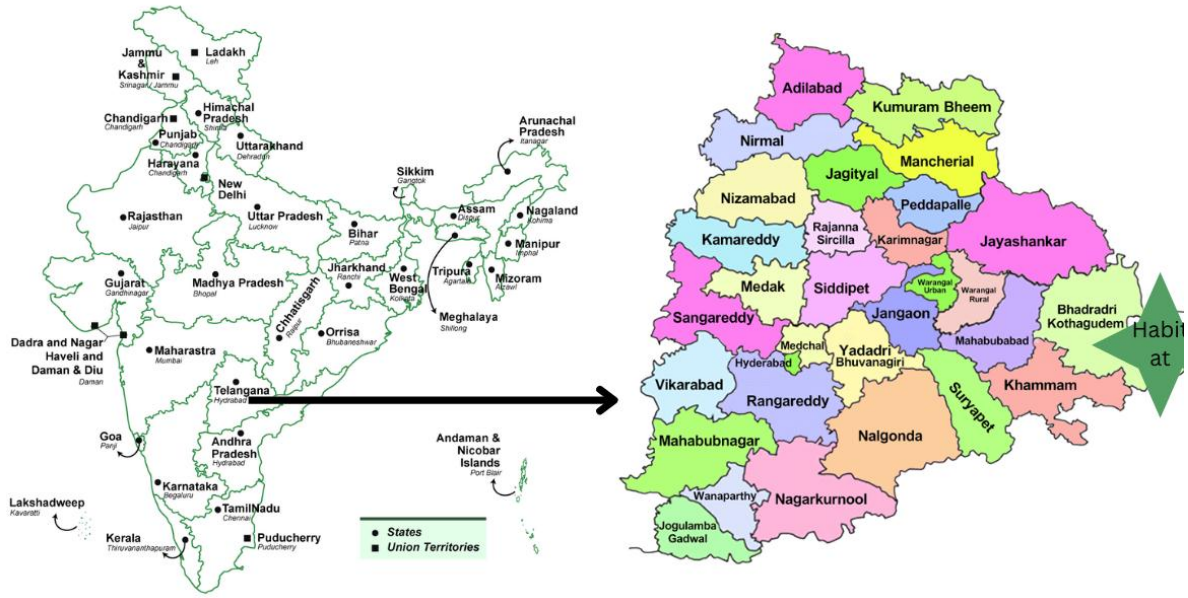
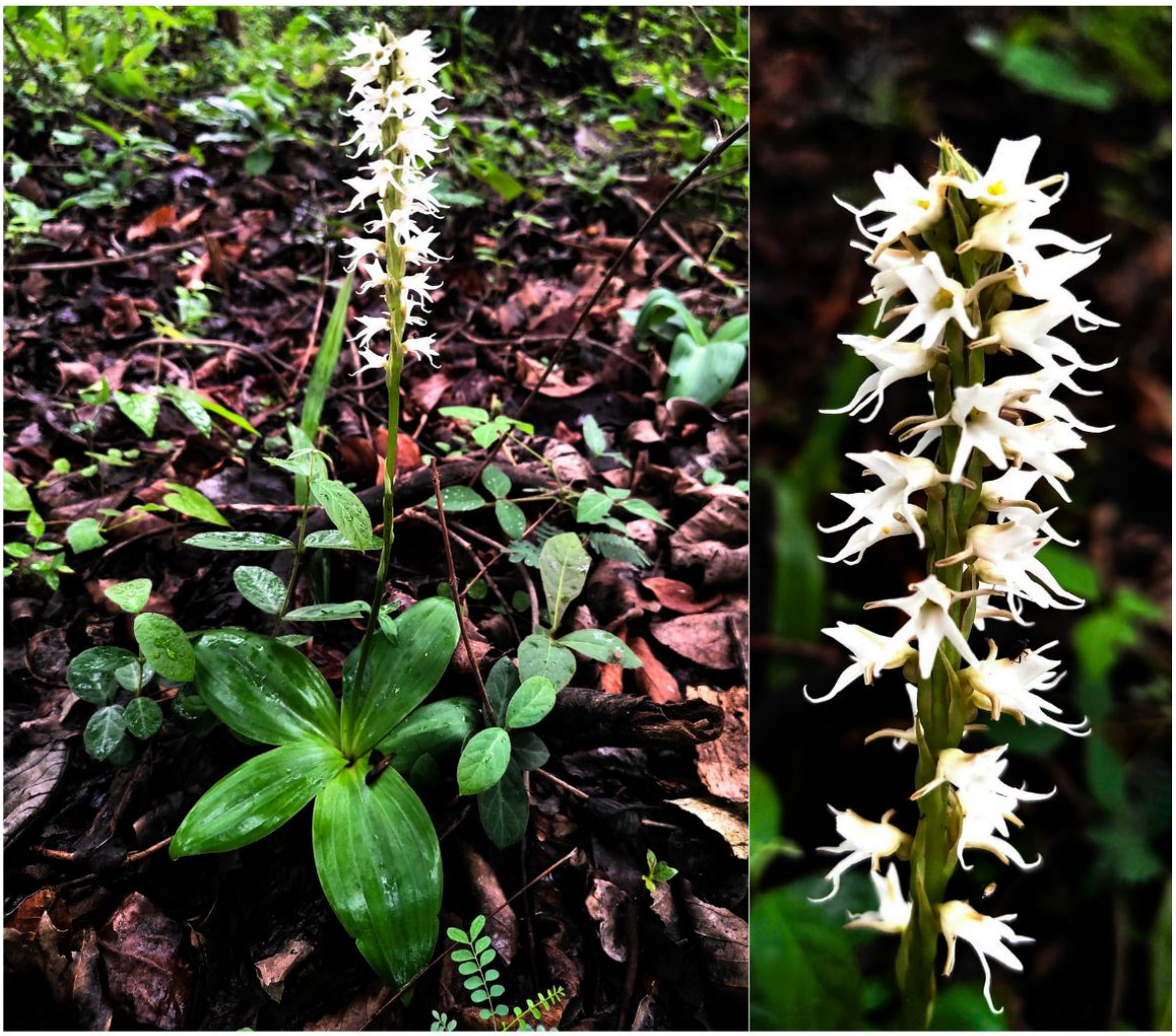
Distribution of *Peristylus constrictus*





Habitat distribution

Review and Literature

Peristylus constrictus is distributed in Bangladesh, Myanmar, Thailand, Cambodia, Vietnam, Nepal, China, and the Philippines (Flora of China, 2009). In India, it has been reported from Uttarakhand, Uttar Pradesh, Assam, Meghalaya, Nagaland, Arunachal Pradesh, and Sikkim (Misra, 2007; Jalal & Jayanthi, 2015), as well as Odisha and Maharashtra (Mudaliar, 1991; Datar & Ghate, 2018). Recent floristic surveys in Andhra Pradesh (Mahesh *et al.*, 2022) hinted at its possible occurrence due to ecological connectivity, but no previous published literature recorded it in Telangana.

Thus, the present report marks the first confirmed record for Telangana Plate-I.

METHODOLOGY

The survey was undertaken as part of CAMPA Third Party Evaluation field studies in Aswaraopet Range. Specimens were collected in moist, shaded forest floor habitats. Morphological identification was carried out using orchid taxonomic keys (Misra, 2007; Jalal & Jayanthi, 2012). The specimens were photographed and cross-referenced with herbarium records.

Systematic Description

Plants robust, up to 70 cm tall. Tubers oblong, 3-4 × 0.8-1.5 cm. Stem with 4-6 tubular sheaths at base, 4-6-leaved. Leaves basal and clustered to somewhat spaced along stem, broadly elliptic, 5-13 × 3.5-6.5 cm, apex acute. Inflorescence 21-42 cm; peduncle cylindric, stout; sterile bracts several, lanceolate, to 30 mm; rachis 5-18 cm, densely many flowered; floral bracts ovate-lanceolate, 9-15 mm, exceeding ovary, apex acuminate. Flowers horizontal; sepals pale brown; petals and lip white. Dorsal sepal forming a hood, concave, 7-9 × 2-3 mm, 1-veined, apex obtuse; lateral sepals spreading, narrowly oblong-lanceolate, oblique, 7-9 × ca. 2.5 mm, 1-veined, margin incurved, apex acute to mucronate. Petals ovate-lanceolate, oblique, 9-11 × 3.5-4 mm, 3- or 4-veined, apex obtuse; lip spreading, oblong-obovate, 9-11 × 4-5 mm, ecallose, base shallowly concave, 3-lobed near middle; lateral lobes diverging at an acute angle from axis of lip, triangular to shortly oblong, slightly falcate, 2.8-5.8 mm, apex acute; mid-lobe 3-5.5 mm, slightly broader than lateral lobes, apex obtuse; spur pendulous, globose, 2-3 mm, apex rounded, neck contracted. Column ca. 1.8 mm; viscidia ovoid; rostellum deltoid, with short arms.

Phenology: June-August

Habitat: Moist, shaded forest floor at low elevation.

Success Story

Safeguarding Rare Orchids under CAMPA: First Record of *Peristylus constrictus* in Telangana

Location: Kavadigundla village, Aswaraopet Range, Bhadradri Kothagudem District, Telangana

Date: August 2025 (during CAMPA 3rd Party Evaluation field studies)

Background: Orchids are bio-indicators of healthy ecosystems. *P. constrictus*, a rare orchid, was earlier known from NE India, Odisha, Maharashtra, and Andhra Pradesh. No record existed from Telangana.

Discovery: First documented in Telangana during CAMPA studies, found just 10 m from encroached land.

Ecological Significance:

- Extends distributional range into Telangana.
- Strengthens floristic diversity profile.
- Confirms ecological connectivity with Papikondalu Sanctuary.

Challenges: Podu cultivation, fuelwood collection, grazing, and fragmentation.

Conservation Outcomes (linked to CAMPA):

- Falls under Biodiversity Conservation component.
- ~200 m² patch should be chain link fenced to prevent encroachment.
- Serves as a demonstration model of CAMPA's role in species conservation.
- Calls for habitat protection and awareness initiatives.

Link to the UN Decade on Ecosystem Restoration

The proposed fencing of the ~200 m² patch in Aswaraopet Range is not only a protective measure under CAMPA but also a step towards ecosystem restoration. By securing the orchid habitat from encroachment and grazing, this action contributes to the objectives of the UN Decade on Ecosystem Restoration (2021–2030), which emphasizes restoring degraded ecosystems for biodiversity, climate resilience, and community well-being. Protecting this micro-habitat represents a model of species-specific habitat restoration in Telangana.

Map showing the impact of Habitat Encroachment on *Peristylus constrictus* Distribution

CAMPA Components and Relevance

The discovery of *P. constrictus* is strongly aligned with CAMPA Guidelines. The table below highlights CAMPA components, activities, and their relevance.

Component	Activities	Relevance to Orchid Site
Artificial Regeneration (AR)	Raising plantations	Improves vegetation cover in Aswaraopet and Papikondalu landscape
Assisted Natural Regeneration (ANR)	Protecting natural patches	Supports natural orchid regeneration
Soil & Moisture Conservation	Check dams, percolation tanks	Maintains moisture vital for orchids
Protection Measures	Fire control, fencing, watchtowers	Fencing ~200 m² orchid site near encroachment
Wildlife & Biodiversity Conservation	Species plans, awareness	Core component; ensures rare orchid survival
Catchment Area Treatment	Watershed management	Prevents degradation of moist habitats
Miscellaneous Activities	Awareness, research, training	Community awareness and documentation

ACKNOWLEDGMENT

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RESEARCH ARTICLE

GROUNDWATER QUALITY ASSESSMENT, CHARACTERIZATION AND MAPPING FOR DADRI-I BLOCK OF CHARKHI DADRI DISTRICT FOR IRRIGATION PURPOSE

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Abstract: Survey, characterization, and classification of groundwater quality across Dadri-I block of Dadri districts of Haryana was conducted, involving the collection and analysis of 20 groundwater samples The samples were tested in the laboratory for electrical conductivity (EC), pH, and concentrations of major cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) and anions (CO_3^{2-}, HCO_3^-, Cl^-, SO_4^{2-}). Based on EC, Sodium Adsorption Ratio (SAR), and Residual Sodium Carbonate (RSC), the samples were classified into irrigation water quality classes according to the criteria of the Central Soil Salinity Research Institute (CSSRI), Karnal, which include good quality water, three saline subclasses, and three alkali subclasses. Most samples in the good water class showed a dominance of Na^+ followed by Ca^{2+} and Mg^{2+}, while Cl^- was the dominant anion, followed by HCO^- and CO^{2-}. Spatial distribution maps for EC, pH, SAR, and RSC were developed using ArcMap GIS (version 9.3.1), and their intersection helped to generate a groundwater quality map for irrigation purposes, allowing calculation of area under each water quality class. The results showed that 45% of the samples belonged to the good quality class, followed by 35% in marginally saline (B1),15% in high SAR saline and 5% in marginally alkali (C1) categories.

Keywords: Groundwater, SAR, RSC, Cations, Anions, Spatial variability

INTRODUCTION

In India, the scarceness of water is burning issue which is going to escalate because of climate change. This is unquestionable particularly in arid and semi-arid areas of the country owing to the vagaries of monsoon and paucity of surface water. During the past few decades, the competition for economic development linked with the population boom and urbanization has led to the substantial changes in land use thereby ensue more demand of water for agriculture, household and industrial activities (Nag and Das, 2014). Besides air, water is the inexpensive raw material available in the world. The exceptional physical and chemical characteristics determine the use of the water. The utilizable water supply is not adequate to irrigate the arable area. Therefore, efforts are required to amplify the chances of water for irrigation in agriculture (Sharma, 2005; Ahamed *et al*., 2013). The exploitation and contamination with numerous chemical and biological sources has lead to retreating of worldwide surface water sources thereby increasing tremendous pressure on groundwater resources (Singh *et al*., 2006; Bhat *et al*., 2016). The quality of groundwater encompasses the physical, chemical and biological features of groundwater. The suitability of groundwater for different uses mainly reckons on its quality, therefore, evaluation of groundwater is a major concern (Packialakshmi *et al*., 2011). Since, groundwater is the main source of irrigation in arid and semi-arid regions of the world, thus, farming is restricted due to dearth of suitable irrigation water. The quality of irrigation water profoundly impacts crop production and has strong bearing on physical and chemical properties (Jalali, 2010).

On account of being devoted to agricultural usage, the groundwater quality must be appraised to protect public health and environment. Accordingly, comprehensive groundwater quality supervision is an effective tool not only to assess the suitability of groundwater for irrigation but also to assure a competent management of water resources. It is imperative that the natural resources should be used judiciously not only for the welfare of current population but also to satisfy the needs and ambition of future generations for overall sustainable development of the society. Ground water is one of the valuable resource for which a planned approach is needed (Jain *et al*., 2012). The chemical make-up of groundwater determines its suitability for different uses which require various standards. The quality of groundwater depends upon distinct natural (precipitation, rock-water interaction, geology, geomorphology etc) and anthropogenic (agriculture, industry, domestic, land use etc.) activities that eventually make the groundwater vulnerable.

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Vulnerability is the characteristic of the aquifer to receive and carry contaminant from anthropogenic sources (Vrba and Zoporozec, 1994; Adhikary *et al*., 2014). The quality of water for agricultural purposes is ascertained by examining the effect of water on superiority and yield of the crops in addition to distinctive changes in the soil (FAO, 1985; Zinabu *et al.*, 2010). The problems confronted in soil which are used as a base for evaluation of water quality are those associated to salinity, water infiltration rate, toxicity and set of other heterogenous problems (Richardson, 1954; Zinabu *et al.*, 2010). Therefore, the quality of groundwater monitoring is obligation because we need good quality water for irrigation purposes in order to prevent the secondary salinization. Keeping in view the aforementioned facts, the present study was undertaken to evaluate the quality of groundwater for irrigation purposes in Dadri-1 block of district Charkhi dadri district of Haryana.

MATERIALS AND METHODS

The study area of block Dadri -I falls in the district Charkhi Dadri, Haryana and is surrounded by district Bhiwani, Mhandergarh,Jhajjar, Rohtak and Rewari. The climate of study are can be classified as tropical steppe, semiarid and hot (above 40^{o}C in May and June) which is mainly dry with very hot summer and cold winter (near about 7^{o}C in January) except during monsoon season when moist air of oceanic origin penetrates into the district. The soils of the block are sandy to sandy loam in texture. The dominating cropping system in this region cotton-wheat and pearlmillet -mustard under sprinkle irrigation the other main crops grown in the area are jowar, bajra, culsterbean and gram.

In order to assess water quality of the study area, 20 groundwater samples were collected to cover the entire study area and locations were recorded using hand held GPS. The location map of the sampling point is presented in Fig 1.

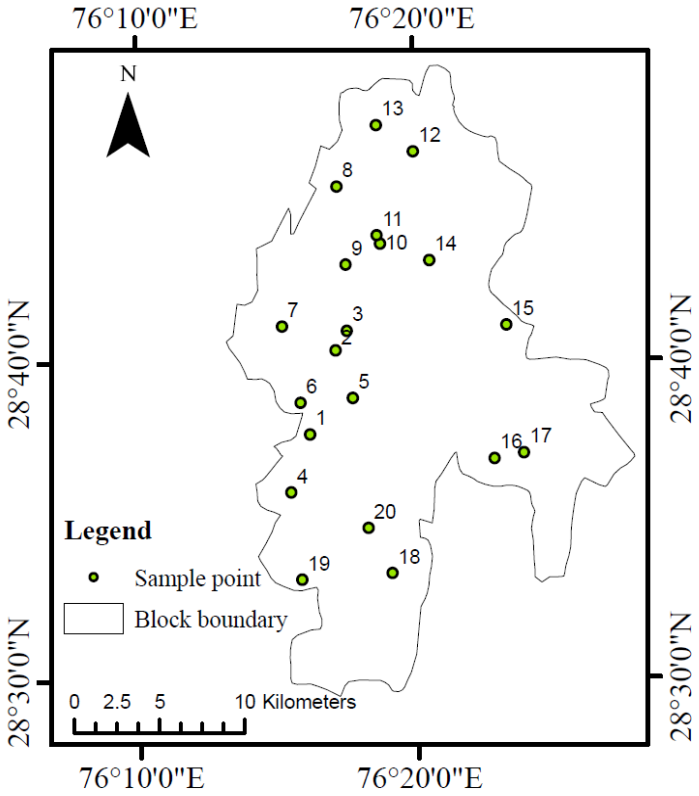

Fig.1: Location map of sampling points in Dadri 1block

Sampling was carried out using pre-cleaned plastic bottles, which were rinsed thrice with sample water prior to sample collection. Before analysis of groundwater, the instruments were calibrated in accordance with the manufacturer's recommendations. The chemical analysis was accomplished as per the standard methods relevant to the analysis of groundwater (Table1). Electrical Conductivity (EC) was measured by conductivity meter and pH by pH meter. Sodium (Na^{+}) and potassium (K^{+}) were measured by flame photometer. Calcium and magnesium were determined with standard EDTA solution titrimetrically. Carbonate and bicarbonate were estimated by titration with H_2SO_4, Chloride by titrating against standard silver nitrate ($AgNO_3$) solution. The colorimetric analysis of sulphate and nitrate was done by spectrophometer.

Table 1. Methods used for estimation of different hydrochemical parameters of groundwater in the study area

Parameters	**Method used**
pH	Glass electrode (Richards, 1954)
EC (Electrical Conductivity)	Conductivity Bridge method (Richards, 1954)
Na^+ (Sodium)	Flame Photometric method (Osborn and Johns, 1951)
K^+ (Potassium)	Flame Photometric method (Osborn and Johns, 1951)
Ca^{2+} (Calcium)	EDTA titration method (Richards, 1954)
Mg^{2+} (Magnesium)	EDTA titration method (Richards, 1954)
CO_3^{2-} (Carbonate)	Acid titration method (Richards, 1954)
HCO_3^-(Bicarbonate)	Acid titration method (Richards, 1954)
Cl^- (Chloride)	Mohr's titration method (Richards, 1954)
NO_3^- (Nitrate)	Spectrophotometric method (Richards, 1954)
SO_4^{2-} (Sulphate)	Turbidity method using $CaCl_2$(Chesnin and Yien, 1950)

The water quality indices viz., SAR (Richards, 1954) and RSC (Eaton, 1950) are calculated as:

a) $$SAR = \frac{Na^+}{\sqrt{\frac{Ca^{2+} + Mg^{2+}}{2}}}$$

b) $$RSC\left(meq\ l^{-1}\right) = \left(HCO_3^- + CO_3^{2-}\right) - \left(Ca^{2+} + Mg^{2+}\right)$$

Based on EC, SAR and RSC, water samples were classified into different categories as per the classification of All India Coordinated Research Project (AICRP) on management of salt affected soils and use of saline water in agriculture (Gupta *et al*., 1994).

RESULTS AND DISCUSSION

In the Dadri I block, the electrical conductivity (EC) of the water samples ranged from 0.41 to 12.60 dSm^{-1} with a mean of 2.85 dSm^{-1} (Table 2).

Table 2. Range and mean of different water quality parameters in Dadri 1 block

Sr. No.	**Quality Parameter**	**Range**	**Mean**
1	pH	7.20-8.23	7.88
2	EC (dSm^{-1})	0.41-12.60	2.85
3	RSC ($me\ l^{-1}$)	0.00-2.60	0.46
4	SAR ($mmol\ l^{-1})^{1/2}$	4.67-12.61	10.29
5	Ca^{2+} ($me\ l^{-1}$)	0.20-14.22	1.97
6	Mg^{2+} ($me\ l^{-1}$)	0.60-28.40	4.80
7	Na^+ ($me\ l^{-1}$)	3.30-77.20	20.49
8	K^+ ($me\ l^{-1}$)	0.10-3.20	0.72
9	CO_3^{2-} ($me\ l^{-1}$)	0.00-1.40	0.54
10	HCO3− (me l-1)	0.40-7.90	2.87
11	Cl− (me l-1)	3.90-111.30	23.71
12	SO42− (me l-1)	0.60-1.90	0.89

To study the spatial distribution of EC in the whole block, a spatial variability map was prepared by using Arc GIS through the interpolation of the available data at 20 sampling point fig 2.

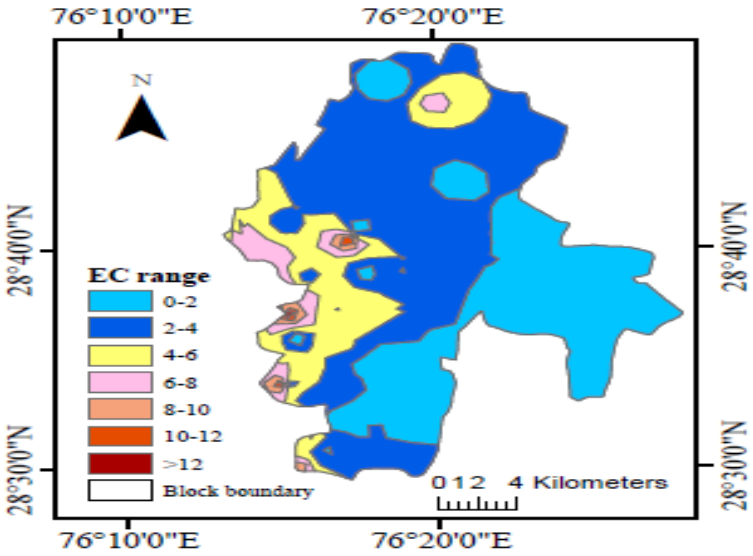

Fig.2: Spatial variable map for EC of groundwater in Dadri 1block

The pH of the water samples ranged from 7.20 to 8.23 with a mean of 7.88. Bhat *et al*. (2016) reported pH in range of 7.19 - 9.72 in Gohana block of Sonipat district. Kumar *et al*. (2013) reported that EC varied from 0.79-9.38 dSm^{-1} in Lakhan Majra Block of Rohtak district. Gagandeep *et al*. (2017) reported that the mean chemical composition and related quality parameters in different EC classes of block Palwal and percent distribution of sample in different EC classes. The sodium adsorption ratio (SAR) of ground water samples ranged from 4.67 12.61 (mmol $l^{-1})^{1/2}$ with a mean value 10.29 (mmol $l^{-1})^{1/2}$. Spatial variable map for SAR of groundwater in Dadri I block presented in Fig.3 Bhat *et al*. (2016) reported that SAR varied from 4.03-24.16 (mmol $l^{-1})^{1/2}$ in groundwater of Gohana block, Haryana. Isaac *et al*. (2009) ascertained that the SAR of soil solution is increased with the increase in SAR of irrigation water which eventually increases the exchangeable sodium of the soil.

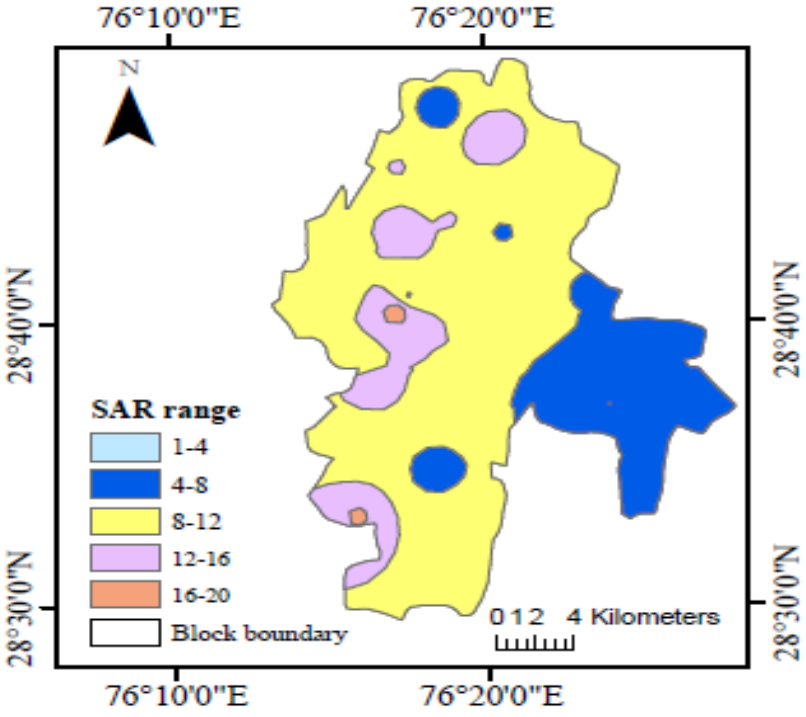

Fig.3: Spatial variable map of SAR of groundwater in Dadri 1

The RSC ranged from 0.00 to 2.60 mel^{-1} with a mean value of 0.46 mel^{-1}. Spatial variable map for RSC of groundwater in Dadri I block presented in Fig.4 Naseem *et al*. (2010) reported that pH, EC and SAR of the irrigation water are significantly influenced by RSC.

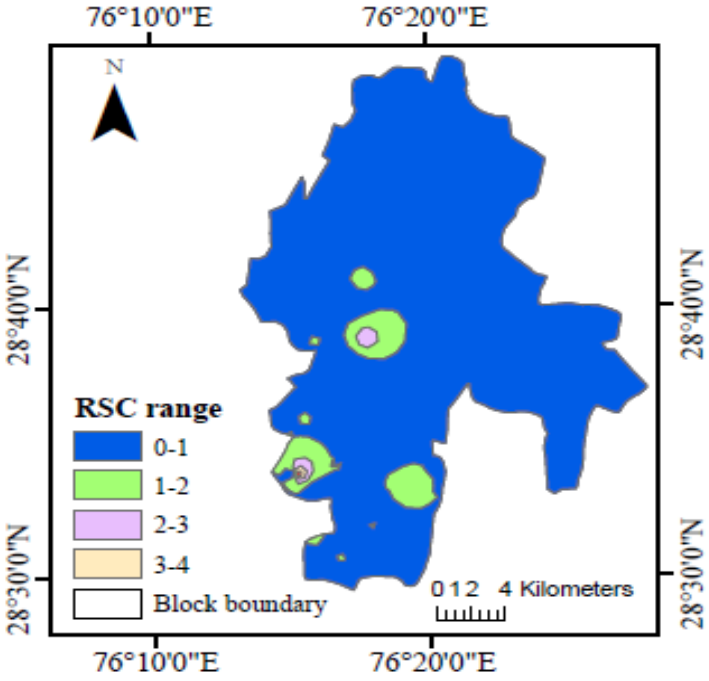

Fig.4: Spatial variable map of RSC of groundwater in Dadri 1

In case of anions, chloride was the dominant anion with the maximum value of 111.30me/l and the minimum value of 3.90 me/l was recorded. Bicarbonate ranged from 0.40 to 7.90 mel^{-1} with a mean value 2.87 me l^{-1}. The mean values for CO_3^{2-}, HCO_3^-, Cl^- and SO_4^{2-} were found to be 0.54, 2.87, 23.714 and 0.89 me/l, respectively (Table 2). Among cations, Na^+ was highest and also varied widely from 3.30 to 77.20 me/l (Table 2), followed by magnesium (0.60–28.40 me/l) and calcium (0.20-14.22 me/l). Average values for Na^+, Mg^{2+}, Ca^{2+} and K^+ were 20.49, 4.80, 1.97 and 0.72 me/l, respectively (Table 2). The mean cationic composition was observed in order of $Na^+ > Mg^{2+} > Ca^{2+} > K^+$ likewise the anionic composition was observed in order of $Cl^- > HCO_3^- > SO_4^{2-} > CO_3^{2-}$. The reasons for carbonate (CO_3^{2-}) and bicarbonate (HCO_3^-) concentrations in groundwater can be ascribed to carbonate weathering as well as from the dissolution of carbonic acid in the aquifers.

Kumar *et al*. (2013) analyzed groundwater quality of Lakhan Majra Block of Rohtak district and reported that the order of abundance of cations was $Na^+ > Mg^+ > Ca^+ > K^+$ and those of the anions were $Cl^- > HCO_3^- > SO_4^{2-} > CO_3^{2-}$. The presence of sodium in groundwater primarily results from the chemical decomposition of feldspars, feldspathoid and some iron, magnesium minerals. The amount of Na^+ ions in the water predicts the sodicity danger of the water (Singh, 2000).

According to All India Coordinated Research Project (AICRP) on management of salt affected soils and use of saline water in agriculture classification, out of 20 water In Dadri I block of Charkhi Dadri district 45.00, 35.00, 15.00 and 5.00 per cent samples were found in good, marginally saline, High SAR saline and marginally alkali categories (Table3), respectively.

Table 3. Ground water quality classification of Dadri-1 Block

Water quality	Class	Percentage
Good	A	45.00
Marginally saline	B1	35.00
Saline	B_2	0.00
High SAR saline	B_3	15.00
Marginally alkali	C1	5.00
Alkali	C2	0.00
Highly alkali	C3	0.00
Total	20	

The spatial distribution map using GIS techniques of Dadri 1 block is presented in fig 5.

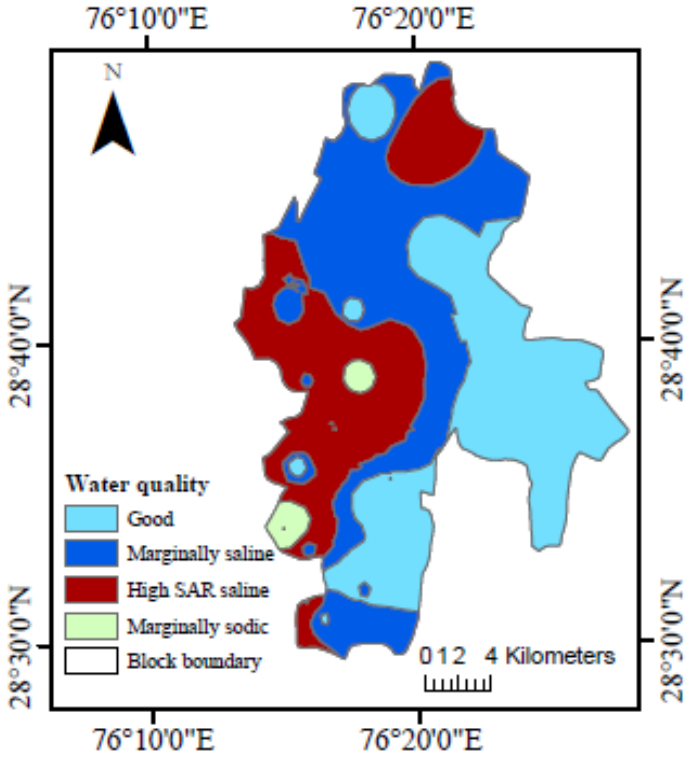

Fig.5: Spatial variable map of groundwater quality in Dadri I of Charkhi Dadri district

CONCLUSION

The groundwater analysis showed that various constituents are in permissible limits, therefore, the groundwater in Gulha block can be used for irrigation purpose without any harm. In Guhla block, anions were found in order of HCO_3^- > Cl^-> SO_4^{2-}> CO_3^{2-} > NO_3^- and cations followed the order Na^+ > Mg^{2+}> Ca^{2+}> K^+. However, at some places where the water is of doubtful category, care is to be taken to use the water for irrigation. The spatial distribution maps generated for various physico-chemical parameters using GIS techniques could be valuable for policy makers for initiating groundwater quality monitoring in the area.

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RESEARCH ARTICLE

BOTANICAL AND ORGANIC APPROACHES FOR IMPROVING SEED QUALITY OF MUNGBEAN UNDER YELLOW MOSAIC DISEASE PRESSURE

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Abstract: Mungbean (*Vigna radiata* (L.) Wilczek) is a nutritionally important pulse crop, but its productivity is severely constrained by Yellow Mosaic Disease (YMD), a whitefly-transmitted viral disease-causing substantial yield and seed quality losses. The present study evaluated eco-friendly management options for YMD through field experiments conducted at Kanpur, during crop seasons (summer and Kharif) for two consecutive years (2024-2025). Fifteen treatments comprising botanical extracts, organic formulations, micronutrients, a chemical insecticide, and an untreated control were tested on two cultivars namely DGGV2 and Soorya, using a randomized block design. Botanical extracts (5% v/v) were applied as uniform foliar sprays. In the cultivar DGGV2, severe YMD incidence resulted in uniform infection across treatments, precluding treatment-wise differentiation. In Soorya, numerical reductions in disease severity and unhealthy seed parameters were observed with *Calotropis procera* leaf and flower extracts; however, these differences were statistically non-significant in summer but were found significant in the Kharif season. Notably, seed weight was significantly influenced by treatments in both seasons and years. Application of *C. procera* leaf extract consistently recorded the highest average seed weight per plant weight (3.51-3.57 g in summer and 3.54-3.56 g in Kharif) indicating improved seed filling and grain development. Overall, the study demonstrates that while *C. procera* extracts may not substantially reduce visible YMD severity under moderate disease pressure, they can significantly enhance seed weight and quality. These findings highlight the potential of Calotropis-based botanicals as components of integrated, environmentally sustainable YMD management strategies in mungbean.

Keywords: YMD, Mungbean, Botanicals, Management, Seed weight

INTRODUCTION

Mungbean (*Vigna radiata* (L.) Wilczek) is a short-duration grain legume with a typical crop cycle of 60–70 days and a strong capacity to perform across a wide spectrum of agro-climatic environments. These attributes have contributed to a steady expansion of its cultivation, particularly throughout South and Southeast Asia (Huang *et al.*, 2024). Global production of mungbean exceeded 6.5 million tonnes in 2022, with India, Myanmar, and China together accounting for more than 70% of total output (Dikr, 2023). During the last decade, the cultivated area under mungbean in Southeast Asia increased by nearly 23%, a trend largely driven by its low input requirements and suitability for resource-efficient farming systems (Sehrawat *et al.*, 2024). As a result, mungbean has become a key component of food and nutritional security strategies in developing regions of Asia, Africa, and Latin America (Dai *et al.*, 2024). The crop is believed to have originated in India, and its genome size, estimated to range between 494 and 579 megabases (Mb), indicates substantial genetic diversity that offers significant scope for crop improvement and breeding programs (Yin *et al.*, 2024). Mungbean establishes an efficient symbiotic association with Rhizobium species, enabling biological nitrogen fixation and reducing reliance on synthetic nitrogen fertilizers (Huppertz *et al.*, 2023). From a nutritional perspective, mungbean seeds exhibit high digestibility (approximately 85-90%) and are rich in essential nutrients, including B-complex vitamins such as folate and thiamine, high-quality protein (about 20-24%), and antioxidant flavonoids such as vitexin and isovitexin (Yin *et al.*, 2024). Compared with several other widely consumed legumes, including soybean and lentil, mungbean contains relatively lower levels of anti-nutritional factors such as phytic acid and lectins, thereby enhancing its nutritional value and consumer acceptability (Chen *et al.*, 2024).

In recent years, the frequency and intensity of plant disease outbreaks have increased markedly under shifting climatic conditions, posing a serious

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challenge to global food security. In mungbean, several viral diseases including Yellow Mosaic Disease (YMD) recognized as the most devastating across South Asia (Singh *et al.*, 2018). The occurrence of YMD on mungbean was first documented at the Indian Agricultural Research Institute (IARI), New Delhi(Nariani, 1960). YMD in pulse crops is associated with four principal begomoviruses, namely mungbean yellow mosaic virus (MYMV), dolichos yellow mosaic virus (DoYMV), mungbean yellow mosaic India virus (MYMIV), and horsegram yellow mosaic virus (HgYMV), which are collectively referred to as legumoviruses (LYMVs) (Qazi *et al.*, 2007; Naimuddin *et al.*, 2016). Transmission of these viruses occurs exclusively through the whitefly *Bemisia tabaci* in a circulative, non-propagative mode, with no evidence of spread via seed, soil, or mechanical means. Under severe epidemic conditions, YMD can result in yield losses of up to 85% in mungbean, and its incidence continues to extend into previously unaffected geographic regions (Karthikeyan *et al.*, 2014; Deepa *et al.*, 2019).

Infection by YMD initially manifests as small chlorotic specks on newly emerged leaves. These lesions gradually enlarge and merge, giving rise to the characteristic yellow mosaic pattern with irregular green and yellow patches, often accompanied by leaf deformation (Nene, 1973; Dhingra and Chenulu, 1985; Deepa *et al.*, 2019). With disease progression, leaves may become completely yellow, followed by drying, wilting, and premature leaf drop under severe infection. Affected plants show a pronounced decline in flowering and pod set, and the pods that develop frequently contain fewer seeds that are poorly filled, shrivelled, or malformed(Nene, 1973; Dhingra and Chenulu, 1985). Collectively, these symptoms result in substantial reductions in both seed quality and overall yield.

Effective management of YMD relies on an integrated disease management strategy that combines suppression of the whitefly vector through chemical and biological interventions with the use of host plant resistance. Recent studies have drawn attention to plant-derived products, particularly neem (*Azadirachta indica*), which possess bioactive constituents such as azadirachtin with insecticidal activity and nimbin with reported antiviral properties, thereby offering environmentally sustainable options for YMD control. Azadirachtin disrupts insect growth, feeding, and reproduction, making neem highly effective against whiteflies and other YMD vectors. Neem oil and seed kernel extracts at concentrations of 3–10% significantly reduce vector populations and YMD incidence, with efficacy comparable to some synthetic insecticides. Neem extracts, including nimbin, exhibit broad-spectrum antimicrobial activity, including antiviral effects, which may contribute to direct suppression of plant viruses. Field studies show that foliar application of neem oil or seed kernel extract can lower YMD incidence by up to 40% and improve yield and seed quality in crops like mungbean, urdbean, and cucurbits(Sethuraman *et al.*, 2001; Saravanan, 2006; Kumar *et al.*, 2021; Hashmi *et al.*, 2024). In view of the considerable economic importance of YMD and the severe losses in mungbean associated with its outbreaks, the present study was conducted to assess the field performance of selected commercial botanicals, organic formulations, and conventional chemical insecticides in managing YMD in mungbean.

MATERIALS AND METHODS

Experimental Site and Design

Field experiments were conducted for two consecutive years 2024-2025 during the summer and Kharif seasons to evaluate the efficacy of plant extracts, organic products, micronutrients, and a chemical insecticide against yellow mosaic disease (YMD) at the experimental fields of ICAR-Indian Institute of Pulses Research (ICAR-IIPR), Kanpur, India. After standard land preparation, micro-plots of 3×2 m^2 were laid out, each consisting of eight rows with inter-row spacing of 30 cm and intra-row spacing of 10 cm. Two mungbean varieties, DGGV2 (susceptible to YMD) and Soorya (also known as IPM 512-1; resistant to YMD), were sown together following a randomized block design (RBD) with three replications (Figure 1a-b). The treatments (n=15) designated as T1 to T15, comprised foliar applications of seven botanical extracts, four organic products, two micronutrients, one insecticide, and an untreated control (Table 1).

Preparation of Botanical Materials

Fresh plant materials used in the study included leaves of Cannabis (*Cannabis sativa*), Calotropis (*Calotropis procera*), Kadamb (*Neolamarckia cadamba*), Marigold (*Tagetes erecta*), Lantana (*Lantana camara*), Castor (*Ricinus communis*), and flowers of *Calotropis procera*. Approximately 500 g of each plant material was collected, washed three times with distilled water, and shade-dried until completely moisture-free. The dried materials were ground into a fine powder using a mixer blender and stored in airtight containers for subsequent use.

Extraction of Botanical Crude Extracts

Ten grams of air-dried plant powder were mixed with 100 mL of 80% methanol in clean glass bottles and incubated on a rotary shaker at 190–220 rpm for 24 h at room temperature. After incubation, the extracts were filtered and the filtrates were collected, which was further concentrated by evaporating three-fourth of its original volume. The concentrated extracts were stored at 4 °C in airtight bottles as stock solutions and subsequently diluted to 5% (v/v) with distilled water for use in field applications.

Treatment application

The seeds of both cultivars (DGGV2 and Soorya) were treated with Rhizobium culture {@ 0.2 % (v/w)} and Trichoderma (@10 g/Kg) by mixing uniformly, prior to sowing. Further, treatments (T1-T15) were applied as foliar sprays at 20, 35, and 50 days after sowing (DAS) using a hand-operated sprayer. Care was taken to ensure uniform coverage of foliage in all plots during each spray schedule.

Disease severity and seed quality assessment

Disease severity was assessed as described in a previous study (Yadav *et al.*, 2021), on five tagged plants per plot, randomly selected at uniform spatial intervals following foliar application of treatments to ensure representative sampling. At physiological maturity, the tagged plants were harvested individually and threshed separately. Pods were collected, and seeds obtained from these plants were pooled and weighed to estimate total seed weight, and the average seed weight per plant was derived by dividing the pooled weight by five. From the pooled harvest per treatment, random samples of 100 seeds were drawn across three replications to determine hundred seed weight. These samples were further examined for seed quality assessment. Unhealthy seeds were identified based on virus-associated morphological and physical abnormalities characteristic of yellow mosaic disease (YMD), including shrivelling, poor or incomplete seed filling, reduced size, surface wrinkling, discoloration (pale yellow to brown), deformation, and loss of seed lustre as described in previous studies (Nene, 1973; Dhingra and Chenulu, 1985). Seeds exhibiting one or more of these symptoms were classified as unhealthy, separated from healthy seeds, and quantified. Both the number and cumulative weight of unhealthy seeds were recorded to assess the impact of YMD on seed quality.

Statistics

Replicated data recorded for disease severity, seed count and seed weight were subjected to statistical analyses using R program (R Core Team, 2024).

Table 1. Detail of treatments.

S.no	Treatments	Treatment Detail	Plant tissue	Doses
1	T1	Foliar spray of Cannabis leaf extract	Leaves	5% (v/v)
2	T2	Foliar spray of Calotropis leaf extract	Leaves	5% (v/v)
3	T3	Foliar spray of Calotropis flower extract	Flowers	5% (v/v)
4	T4	Foliar spray of Kadamb leaf extract	Leaves	5% (v/v)
5	T5	Foliar spray of Marigold leaf extract	Leaves	5% (v/v)
6	T6	Foliar spray of Castor leaf extract	Leaves	5% (v/v)
7	T7	Foliar spray of Lantana camara leaf extract	Leaves	5% (v/v)
8	T8	Foliar spray of Panchgavya		3% (v/v)
9	T9	Foliar spray of Jeevamurat		500ml/ha
10	T10	Foliar spray of Beejamurat		200ml/kg
11	T11	Foliar spray of Nimbicidine LC50		5ml/lit
12	T12	Foliar spray of Zinc oxide		75 ppm
13	T13	Foliar spray of Ferric oxide		75 ppm
14	T14	Foliar spray of Imidacloprid 17.8%		0.5ml/litre
15	T15	Control (Water)		-

RESULTS

Disease severity under different treatments

In the highly susceptible mungbean genotype DGGV2, YMD severity reached 100% across all treatments during both seasons (summer and Kharif) due to a severe disease outbreak. The intense infection adversely affected pod formation and seed development, resulting in uniformly unhealthy seeds across treatments. Consequently, treatment-wise effects on seed quality parameters could not be distinguished in DGGV2, and further seed analysis for this genotype was excluded from the study.

In the resistant variety Soorya, YMD severity in the untreated control was low during the summer season (6.67% in 2024 and 6.93% in 2025) but increased during the Kharif season (11.85% in both years). Across seasons, *Calotropis procera* was the most

effective treatment, with 5% leaf extract applied as foliar spray in summer reducing disease severity to 4.67% (2024) and 5.33% (2025), and foliar spray in Kharif further lowering severity to 5.18% (2024) and 2.96% (2025), followed by Calotropis flower extract (**Tables 2 and 3**). All other botanical, organic, micronutrient, and chemical treatments resulted in comparatively higher YMD severity, while the insecticide Imidacloprid performed poorly relative to botanical treatments. However, treatment differences were statistically non-significant in both years and seasons, indicating that these reductions represent numerical trends rather than statistically distinct treatment effects.

Effect on number of unhealthy seed count and seed weight per 100 seed

In Soorya, the number and weight of unhealthy seeds per 100 seeds showed clear numerical variation among treatments in both summer and Kharif seasons across 2024 and 2025. In summer, plots treated with 5% *Calotropis procera* leaf extract recorded the lowest unhealthy seed counts (13.67 and 16.00) and lowest unhealthy seed weight (0.56 g in both years), followed by Calotropis flower extract, whereas the untreated control had the highest unhealthy seed counts (21.67 and 24.33) and highest unhealthy seed weight (0.91 g and 0.76 g), indicating greater proportions of shriveled and poorly filled seeds under unmanaged YMD conditions. A similar pattern was observed in Kharif, where Calotropis leaf extract again produced the lowest unhealthy seed counts (14.33 and 15.33) and lowest unhealthy seed weight (0.58 g and 0.59 g), while the untreated control recorded the highest values (23.33 and 22.00 unhealthy seeds; 0.72 g and 0.67 g unhealthy seed weight). Across both seasons, Imidacloprid-treated plots consistently showed relatively higher unhealthy seed counts and weights than botanical treatments (Tables 2 and 3). However, for both years differences among treatments were statistically non-significant in summer, whereas it was significant in the Kharif season.

Effect of treatments on seed weight per plant

Average seed weight per plant was significantly influenced by treatments in both summer and Kharif seasons during 2024 and 2025. Across seasons, seed treatment followed by foliar application of 5% *Calotropis procera* leaf extract consistently produced the highest seed weight (3.51–3.57 g in summer and 3.54–3.56 g in Kharif), remaining statistically superior to most other treatments (Tables 2 and 3). Calotropis flower extract also resulted in higher seed weights and was statistically comparable to the best treatment group. In contrast, the untreated control recorded the lowest seed weights (3.33–3.35 g in both seasons and years). The significant improvement in seed weight under Calotropis-based treatments reflects improved seed filling and a reduced negative impact of YMD on grain development.

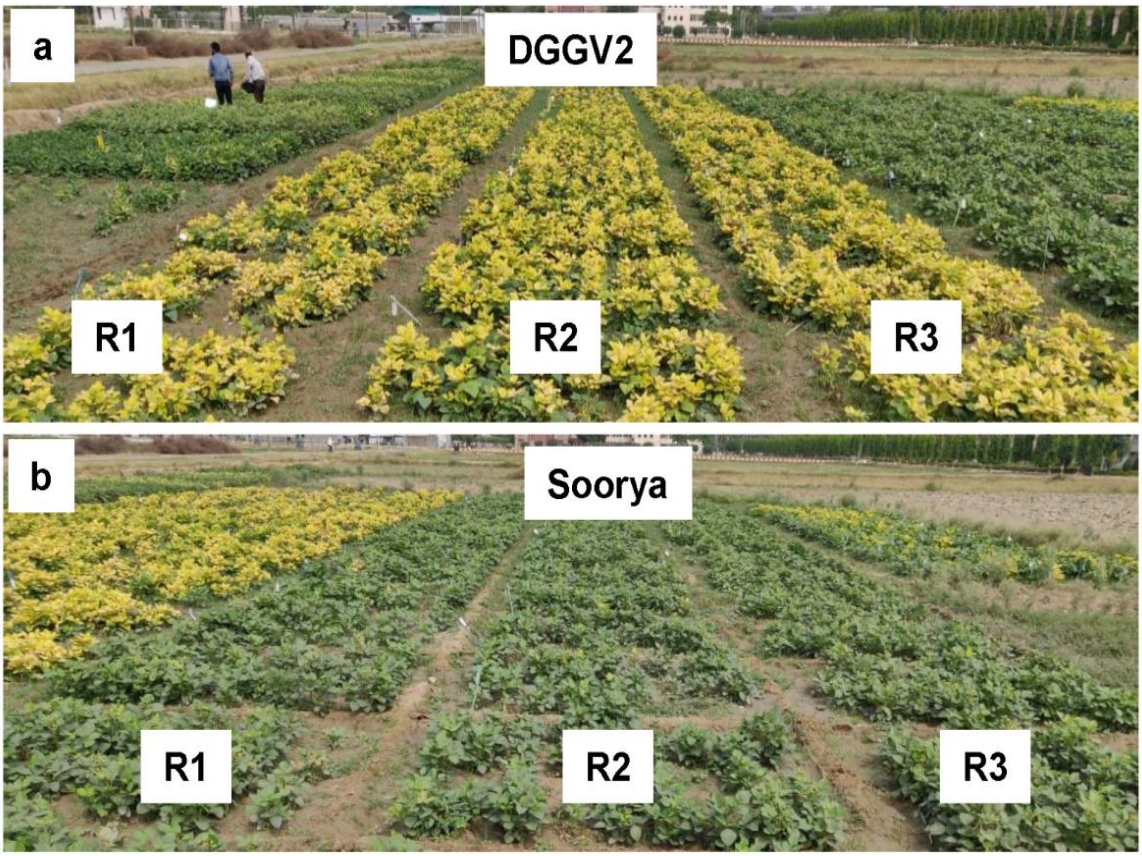

Figure 1 (a) Field layout with three replications (R1-R3) of 15 treatments considered in this study on DGGV2, and (**b)** Soorya. (**c**) Unhealthy and **(b)** healthy seeds of Soorya (IPM512-1) Mungbean studied in this study.

DISCUSSION

Yellow Mosaic Disease (YMD) remains one of the most destructive viral diseases of mungbean, particularly under agro-climatic conditions that favor whitefly proliferation. The present study assessed the field performance of selected botanicals, organic formulations, micronutrients, and a chemical insecticide for YMD management, with emphasis on disease severity and seed quality attributes.

The uniformly high disease severity observed in the susceptible genotype DGGV-2 across all treatments underscores the vulnerability of highly susceptible cultivars under severe epidemic pressure. The inability of any treatment to mitigate YMD effects in this genotype suggests that once systemic infection is established, external interventions exert limited influence on disease expression and seed health. This reinforces the central role of host resistance as the cornerstone of YMD management, as consistently reported in earlier studies (Nariani, 1960; Karthikeyan *et al.*, 2014; Naimuddin *et al.*, 2016; Mishra *et al.*, 2020). This response is further explained by the experimental conditions, as the ICAR-IIPR, Kanpur fields are a known hotspot for mungbean yellow mosaic India virus (MYMIV), the principal causal agent of YMD, resulting in intense and uniform disease pressure (Akram *et al.*, 2024).

In the resistant variety Soorya, application of *Calotropis procera* extracts resulted in numerical reductions in disease severity; however, these differences were not statistically significant, indicating limited observable effects under moderate disease pressure. Similar patterns have been documented in other crops, where *C. procera* extracts showed strong disease-suppressive activity under controlled conditions. For instance, aqueous and methanolic extracts significantly reduced Fusarium wilt severity in tomato and chickpea, with higher concentrations producing stronger effects, including up to 83.6% reduction in tomato under greenhouse conditions (Abo-Elyousr *et al.*, 2022; Zubairi *et al.*, 2025). Field studies in potato and wheat have also reported reductions in disease severity and yield improvement following *C. procera* treatments, although treatment effects were sometimes non-significant in resistant genotypes or under moderate disease intensity (Naz *et al.*, 2018; Abdul-Karim and Hussein, 2024; Hussain *et al.*, 2024).

The absence of statistically significant differences in disease severity among treatments in Soorya suggests that host resistance restricted symptom development, resulting in comparable disease pressure across treatments. Unhealthy seed count and unhealthy seed weight followed trends similar to disease severity, with Calotropis leaf extract treatments recording lower numerical values and untreated controls showing the highest levels. However, these differences were also statistically non-significant, indicating modest treatment effects on seed health under prevailing field conditions. This close association between disease severity and unhealthy seed formation aligns with earlier reports that YMD impairs seed quality primarily through disruption of photosynthesis and assimilate translocation during pod filling (Nene, 1973; Dhingra and Chenulu, 1985).

In contrast, average seed weight per plant was significantly influenced by treatments in both years, highlighting seed weight as a more sensitive indicator of treatment response under YMD stress. Although *C. procera* leaf extract treatments did not consistently produce significant reductions in visible disease severity, they were associated with improved seed filling and grain development. Similar physiological benefits have been reported in wheat, where *C. procera* leaf extracts increased grain number per spike, 100-grain weight, and overall yield, accompanied by enhanced photosynthetic pigments, protein content, phenolics, and defense-related enzyme activity (Naz *et al.*, 2018). In addition, optimal concentrations of *C. procera* extracts have been shown to improve seed

germination and seedling vigor, likely due to bioactive compounds such as phenolics and flavonoids, although excessive concentrations may exert inhibitory effects (Al-Zahrani and Al-Robai, 2007; Yau *et al.*, 2022). Induction of plant defense responses, including antioxidant enzymes and pathogenesis-related proteins, may further reduce stress intensity and indirectly support improved seed development even when disease suppression is limited (Naz *et al.*, 2018).

The relatively poor performance of imidacloprid in reducing disease severity and improving seed quality parameters may reflect increasing resistance in *Bemisia tabaci*, limited residual activity, or insufficient suppression of viruliferous adults prior to virus transmission. Similar declines in neonicotinoid efficacy have been reported in pulse-growing regions with prolonged insecticide use (Karthikeyan *et al.*, 2014; Naimuddin *et al.*, 2016), emphasizing the limitations of sole reliance on chemical control.

Overall, the consistent numerical superiority and statistically significant improvement in seed weight observed with *Calotropis procera* leaf extract indicate its potential as a botanical component in integrated YMD management strategies. While reductions in disease severity and unhealthy seed parameters were not statistically significant, the positive influence on seed development suggests meaningful agronomic benefits. Integration of botanical treatments with host resistance may enhance crop performance while reducing dependence on synthetic insecticides. Further studies on active phytochemicals, their modes of action, and multi-location validation would strengthen the case for Calotropis-based formulations in sustainable mungbean production systems.

AUTHOR'S CONTRIBUTION

AR and DK wrote the original manuscript. MA conceived the idea and supervised the study. AR performed the analysis and DK prepared the illustrations. MA edited and reviewed the manuscript. All authors read and approved the final version of the manuscript.

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Table 2. Effect of different treatments on yellow mosaic disease severity and seed health of mungbean (Soorya) in the summer season.

Treatment	Disease Severity (%)		Number of unhealthy seed /100 seed harvested		Seed weight (g) of unhealthy seeds		Total average seed weight/ plant (g)	
	2024	2025	2024	2025	2024	2025	2024	2025
T1	5.93±1.28	5.93±1.28	16.67±1.53	16.67±6.81	0.70±0.06	0.60±0.19	$3.45±0.04^{bcd}$	$3.45±0.02^{g}$
T2	4.67±0.00	5.33±1.28	13.67±2.52	16.00±2.65	0.56±0.11	0.56±0.05	$3.51±0.02^{a}$	$3.57±0.01^{a}$
T3	5.67±2.22	5.63±1.28	15.00±1.00	17.67±1.53	0.60±0.07	0.59±0.09	$3.51±0.01^{a}$	$3.56±0.01^{ab}$
T4	6.67±0.00	6.67±2.22	16.00±1.00	20.67±4.04	0.66±0.06	0.64±0.05	$3.39±0.04^{ef}$	$3.49±0.04^{def}$
T5	5.93±1.28	5.93±1.28	15.33±6.66	20.67±5.69	0.64±0.28	0.67±0.23	$3.43±0.04^{cde}$	$3.50±0.03^{cde}$
T6	6.67±2.22	6.67±2.22	18.67±2.89	19.00±2.65	0.73±0.03	0.66±0.10	$3.42±0.04^{de}$	$3.48±0.02^{efg}$
T7	6.67±2.22	6.67±2.22	18.33±4.04	20.00±2.65	0.77±0.17	0.67±0.04	$3.43±0.04^{cde}$	$3.50±0.03^{de}$
T8	6.67±0.00	5.93±1.28	14.67±3.79	20.67±2.52	0.62±0.16	0.67±0.04	$3.46±0.01^{abcd}$	$3.45±0.02^{fg}$
T9	5.93±1.28	5.93±1.28	15.67±2.31	21.67±1.15	0.63±0.15	0.64±0.02	$3.43±0.07^{cde}$	$3.51±0.04^{cde}$
T10	6.67±0.00	6.67±0.00	15.33±1.53	21.00±1.00	0.64±0.06	0.66±0.01	$3.47±0.02^{abcd}$	$3.48±0.06^{efg}$
T11	6.67±2.22	6.67±2.22	15.67±1.53	21.67±1.53	0.62±0.14	0.66±0.02	$3.44±0.02^{cde}$	$3.47±0.04^{efg}$
T12	5.67±0.00	5.23±1.28	15.00±2.65	18.67±3.51	0.59±0.05	0.63±0.14	$3.48±0.02^{abc}$	$3.54±0.03^{abc}$
T13	5.23±1.28	5.73±1.28	15.12±4.36	18.60±2.00	0.60±0.18	0.64±0.03	$3.51±0.02^{ab}$	$3.53±0.02^{bcd}$
T14	5.89±1.28	6.49±1.28	18.67±7.51	23.33±1.53	0.78±0.32	0.62±0.14	$3.46±0.04^{abcd}$	$3.47±0.01^{efg}$
T15	6.67±0.00	6.93±1.28	21.67±7.37	24.33±0.58	0.91±0.31	0.76±0.23	$3.35±0.02^{f}$	$3.33±0.02^{h}$
F stat	0.48^{NS}	0.43^{NS}	0.85^{NS}	1.80^{NS}	0.85^{NS}	0.42^{NS}	6.08**	23.30**
SE(m)	0.67	0.67	2.24	1.70	0.10	0.07	0.02	0.01
SE(d)	0.95	0.95	3.17	2.41	0.14	0.1	0.03	0.02
CV(%)	18.33	19.02	23.26	14.44	24.53	18.57	0.90	0.59

NS= Non-significant, **= $p<0.01$

Table 3. Effect of different treatments on yellow mosaic disease severity and seed health of mungbean (Soorya) in the Kharif season.

Treatment	Disease Severity (%)		Number of unhealthy seed /100 seed harvested		Seed weight (g) of unhealthy seeds		Total average seed weight/ plant (g)	
	2024	2025	2024	2025	2024	2025	2024	2025
T1	7.40±0.73^{cd}	7.41±0.72^{b}	16.66±0.88^{c}	18.00±0.57^{bc}	0.65±0.00^{bc}	0.64±0.01^{ab}	3.42±0.01^{cd}	3.45±0.02^{bcd}
T2	5.18±0.74^{ef}	2.96±0.74^{d}	14.33±0.33^{d}	15.33±0.66^{cd}	0.58±0.00^{f}	0.58±0.00^{e}	3.56±0.00^{a}	3.54±0.01^{a}
T3	6.66±0.00^{de}	5.18±0.74^{c}	15.33±0.88^{cd}	16.00±1.00^{cd}	0.59±0.03^{ef}	0.59±0.00^{de}	3.53±0.00^{ab}	3.50±0.00^{ab}
T4	8.88±0.00^{b}	7.41±0.75^{b}	18.66±1.66^{b}	16.66±1.20^{cd}	0.62±0.00^{de}	0.64±0.00^{ab}	3.48±0.01^{abc}	3.47±0.01^{abc}
T5	6.66±0.00^{de}	8.15±0.74^{b}	18.33±2.33^{b}	17.66±0.66^{bc}	0.64±0.00^{cd}	0.64±0.00^{ab}	3.47±0.01^{bcd}	3.47±0.01^{abc}
T6	8.88±0.00^{b}	7.41±0.73^{b}	17.66±0.88^{bc}	16.00±0.57^{cd}	0.65±0.00^{bc}	0.63±0.01^{bc}	3.48±0.00^{abc}	3.46±0.01^{bcd}
T7	6.66±0.00^{de}	8.15±0.74^{b}	16.66±0.33^{c}	18.00±0.57^{bc}	0.64±0.00^{cd}	0.61±0.00^{cd}	3.45±0.01^{cd}	3.44±0.01^{cd}
T8	8.14±0.72^{bc}	8.15±0.73^{b}	17.00±1.00^{c}	16.66±0.66^{cd}	0.64±0.00^{cd}	0.62±0.00^{cd}	3.43±0.00^{cd}	3.47±0.00^{abc}
T9	7.40±0.74^{cd}	8.00±0.68^{b}	18.00±1.55^{bc}	18.00±0.57^{bc}	0.65±0.00^{bc}	0.64±0.00^{ab}	3.46±0.02^{bcd}	3.49±0.00^{abc}
T10	8.14±0.74^{bc}	7.41±0.74^{b}	18.00±1.52^{bc}	18.00±0.57^{bc}	0.66±0.00^{b}	0.62±0.00^{cd}	3.48±0.00^{abc}	3.47±0.00^{abc}
T11	8.88±0.00^{b}	7.95±0.66^{b}	19.33±0.88^{b}	19.66±0.82^{b}	0.65±0.00^{bc}	0.63±0.00^{bc}	3.48±0.03^{abc}	3.45±0.02^{bcd}
T12	4.44±0.00^{f}	4.98±0.54^{c}	16.00±0.57^{cd}	15.33±0.33^{cd}	0.61±0.00^{de}	0.58±0.00^{e}	3.50±0.00^{abc}	3.46±0.01^{bcd}
T13	6.66±0.00^{be}	7.41±0.74^{b}	16.66±0.88^{c}	16.00±0.57^{cd}	0.61±0.00^{de}	0.60±0.00^{de}	3.50±0.00^{abc}	3.42±0.01^{cd}
T14	8.14±0.74^{bc}	8.11±0.72^{b}	20.66±0.88^{a}	21.33±0.88^{a}	0.69±0.00^{a}	0.66±0.00^{a}	3.46±0.01^{bcd}	3.43±0.00^{cd}
T15	11.85±0.74^{a}	11.85±0.74^{a}	23.33±1.20^{a}	22.00±0.57^{a}	0.72±0.00^{a}	0.67±0.00^{a}	3.35±0.01^{d}	3.34±0.00^{d}
F stat	13.76**	7.02**	3.98**	7.57**	16.62**	14.45**	10.90**	9.63**
SE(m)	0.47	0.73	1.10	0.73	0.00	0.00	0.01	0.01
SE(d)	0.67	1.04	1.56	1.04	0.01	0.01	0.02	0.01
CV(%)	10.79	17.27	10.76	7.25	2.50	1.87	0.75	0.66

**= $p<0.01$

Table 4. Pooled observations of effect of different treatments on yellow mosaic disease severity and seed health of mungbean **(**Soorya**)** in the summer season

Treatment	Average Disease Severity (%)	Percent decrease over control	Average Number of unhealthy seed /100 seed harvested	Percent decrease over control	Average Seed weight (g) of unhealthy seeds	Percent decrease over control	Average (Summer) seed weight/ plant (g)	Percent decrease over control
T1	3.58±0.82	19.55	16.67±1.80	6.33	0.65±0.05	0.18	3.44±0.00	0.11
T2	3.21±0.98	27.87	14.83±1.07	8.17	0.55±0.03	0.28	3.55±0.01	0.22
T3	3.95±0.78	11.24	16.33±0.76	6.67	0.59±0.02	0.24	3.53±0.00	0.20
T4	4.07±0.95	8.54	18.33±1.43	4.67	0.65±0.02	0.18	3.43±0.02	0.10
T5	3.45±0.82	22.47	18.00±2.51	5.00	0.65±0.09	0.18	3.46±0.01	0.13
T6	4.19±0.82	5.84	18.83±1.01	5.17	0.69±0.03	0.14	3.45±0.01	0.10
T7	3.70±0.97	16.85	19.17±1.30	3.83	0.72±0.05	0.11	3.46±0.00	0.13
T8	3.70±0.89	16.85	17.67±1.78	5.33	0.64±0.04	0.19	3.46±0.02	0.13
T9	3.45±0.82	22.47	18.67±1.49	4.33	0.63±0.03	0.20	3.47±0.03	0.14
T10	3.95±0.87	11.24	18.17±1.35	4.83	0.65±0.01	0.18	3.46±0.00	0.13
T11	3.70±0.97	16.85	18.67±1.45	4.33	0.63±0.03	0.20	3.45±0.01	0.12
T12	3.82±0.90	14.16	19.83±1.40	3.17	0.61±0.04	0.24	3.51±0.00	0.18
T13	3.95±0.70	11.24	19.00±1.52	4.00	0.62±0.05	0.21	3.51±0.01	0.18
T14	3.45±0.56	22.47	21.00±2.23	2.00	0.70±0.09	0.13	3.46±0.00	0.13
T15	4.45±0.69	0.00	23.00±2.00	0.00	0.83±0.10	0.00	3.33±0.01	0.00
F stat	1.76**		1.70**		1.04**		14.08**	
C.D	1.33		4.15		0.16		0.03	

SE(m)	0.33		1.47		0.05		0.01	
SE(d)	0.66		2.07		0.08		0,01	
CV(%)	21.68		18.54		22.03		0.94	

**= $p<0.01$

Table 5. Pooled observation of effect of different treatments on yellow mosaic disease severity and seed health of mungbean (Soorya) in the Kharif season

Treatment	Average Disease Severity (%)	Percent decrease over control	Average Number of unhealthy seed /100 seed harvested	Percent decrease over control	Average Seed weight (g) of unhealthy seeds	Percent decrease over control	Average (Summer) seed weight/ plant (g)	Percent decrease over control
T1	7.40±0.00	37.55	17.33±1.02	5.33	0.64±0.02	0.06	3.44±0.01	0.11
T2	4.07±1.12	65.65	14.83±0.85	7.84	0.58±0.04	0.12	3.55±0.00	0.22
T3	5.92±0.74	50.04	15.67±2.21	7.00	0.59±0.02	0.11	3.52±0.01	0.19
T4	8.14±0.74	31.31	17.67±1.23	5.00	0.63±0.05	0.07	3.47±0.03	0.14
T5	7.40±0.74	37.55	18.90±1.64	3.77	0.64±0.05	0.06	3.47±0.02	0.14
T6	8.14±0.74	31.31	16.83±1.45	5.84	0.64±0.03	0.06	3.47±0.00	0.14
T7	7.40±0.74	37.55	17.33±1.56	5.34	0.62±0.06	0.08	3.44±0.01	0.11
T8	8.14±0.00	31.31	16.83±1.49	5.84	0.63±0.04	0.07	3.45±0.00	0.12
T9	7.70±0.30	35.02	18.00±1.53	4.67	0.65±0.05	0.05	3.47±0.02	0.14
T10	7.77±0.37	34.43	18.00±1.82	4.67	0.64±0.04	0.06	3.47±0.03	0.14
T11	8.40±0.47	29.11	19.50±1.91	3.17	0.64±0.06	0.06	3.46±0.01	0.13
T12	4.74±0.28	60.00	15.67±1.29	7.00	0.60±0.07	0.10	3.48±0.01	0.15
T13	7.03±0.37	40.68	16.33±2.11	6.34	0.60±0.03	0.10	3.47±0.02	0.15
T14	8.12±0.02	31.48	21.00±1.85	1.67	0.67±0.06	0.03	3.45±0.01	0.12
T15	11.85±0.00	0.00	22.67±1.78	0.00	0.70±0.09	0.00	3.33±0.03	0.00
F stat	0.94**		9.28**		29.26**		18.18**	
C.D	1.24		1.91		0.01		0.02	
SE(m)	0.43		0.67		0.00		0.01	
SE(d)	0.62		0.95		0.00		0.01	
CV(%)	14.15		9.57		2.20		0.72	

**= $p<0.01$

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SHORT COMMUNICATION

EFFECT OF GENERATIONS OF CULTURE ON YIELD AND BIOLOGICAL EFFICIENCY OF *VOLVARIELLA VOLVACEA*

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Abstract: Successive sub-culturing of mushroom strains may lead to physiological degeneration and yield instability. The present investigation evaluated the effect of successive culture generations on spawn performance, yield attributes, and biological efficiency of *Volvariella volvacea* (paddy straw mushroom). Five culture generations (G0–G4), derived from a single high-performing isolate, were assessed under controlled cultivation conditions. Parameters studied included mycelial growth rate on PDA, spawn colonization time, primordia initiation, yield components, biological efficiency, and contamination incidence. Early generations (G0–G1) showed faster colonization, lower contamination, and significantly higher yield and biological efficiency. Later generations (G3–G4) exhibited delayed spawn run, increased variability in fruiting behavior, and symptoms of physiological degeneration. The results demonstrate progressive loss of culture vigor with repeated sub-culturing and highlight the importance of limiting serial transfers. Adoption of systematic strain preservation and rejuvenation practices is recommended to maintain consistent productivity in commercial cultivation of *V. volvacea*.

Keywords: *Volvariella volvacea*, Paddy straw mushroom, Culture generation, Spawn quality

INTRODUCTION

Volvariella volvacea, commonly known as the paddy straw mushroom, is an important tropical edible fungus widely cultivated in South and Southeast Asia. Productivity and economic viability of cultivation depend strongly on spawn quality. Repeated sub-culturing (serial transfers) of fungal strains can lead to genetic drift, physiological degeneration, or accumulation of contaminants, affecting yield and cultural characteristics. While degeneration and culture aging are well-documented in several cultivated mushrooms (e.g., *Pleurotus spp.*, *Agaricus bisporus*), systematic information for *V. volvacea* under standard spawn production practices is limited.

This study aims to quantify the influence of successive culture generations on spawn performance and crop yield and to provide practical recommendations for spawn handling in small-to-medium scale farms and institutional labs.

Objectives

1. To evaluate mycelial growth, spawn run and fruiting performance of *V. volvacea* across five successive generations (G0–G4) of culture.
2. To quantify changes in yield parameters (biological efficiency, average fruit weight, number of fruit bodies) and contamination rate across generations.
3. To make practical recommendations for spawn production and strain maintenance for growers.

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MATERIALS AND METHODS

Fungal material and isolation

A high-performing field isolate of *Volvariella volvacea* (labelled Vv-01) was obtained from a commercial grower near Bilaspur and purified by single-hyphal-tip transfer on potato dextrose agar (PDA). The founding culture (G0) served as the progenitor for generation series.

Generation scheme

Successive generations were produced by the following scheme:

- **G0** — founding, freshly purified single-hyphal-tip culture (master culture).
- **G1** — single transfer of G0 mycelium to fresh PDA (7–10 days incubation) then to grain spawns.
- **G2** — single transfer from G1 fruitbody tissue or from G1 PDA to fresh PDA, then to spawn substrate.
- **G3** — similarly transferred from G2.
- **G4** — transfered from G3.

Each generation used aseptic technique. All transfers followed identical incubation conditions (28±1°C; 70–80% RH) and were completed within 4 weeks per generation to standardize environmental effects.

Spawn production

Grain spawn used: sorghum grain soaked 12 h, boiled until al dente, drained, mixed with 2%

calcium carbonate, dispensed into 500 mL polypropylene bottles (200 g grain per bottle). Bottles were autoclaved at 121°C for 20 min and cooled. Each generation's inoculum consisted of 5 bottles of well-colonized PDA (or agar plugs) per 10 spawn bottles. Spawn was incubated at 28°C until full colonization.

Parameters recorded during spawn production:

- Time to 50% and 100% colonization (days)
- Contamination rate (%) per generation (number of bottles contaminated/total)

Substrate preparation and cropping

Substrate: chopped paddy straw (local variety) pasteurized by wet-heating in hot water at 60–65°C for 6 hours, drained to ~65% moisture, and filled into perforated polythene bags (5 kg wet straw per bag). Spawn rate: 2% (w/w) fresh straw basis. For each generation, 20 replicate bags were inoculated.

Cultivation conditions: incubation at 30±2°C in dark until spawn run (white mycelial cover), then low-light conditions (12 h photoperiod 500–1000 lux), humidity 85–90%, and temperature 30–33°C for fruiting (typical for tropical cultivation of *V. volvacea*). Pinning was induced by cold shock when applicable (brief exposure to 18–20°C) or by maintaining high humidity depending on local practice.

Data collection

For each replicate bag across generations, the following were recorded:

- Spawn run time (days to full colonization)
- Days to primordia initiation (from inoculation)
- Number of fruiting flushes observed within 30 days after primordia
- Total fresh weight of mushrooms per bag (g)
- Number of fruit bodies per bag
- Average fruit body weight (g) = total fresh weight / number of fruit bodies
- Biological efficiency (BE) = (total fresh weight of mushrooms / dry weight of substrate) × 100
- Incidence of abnormal fruiting or degeneration symptoms (thin stipes, small cap, delayed maturation)

Statistical analysis

Data were analyzed using one-way ANOVA with generation as the main factor. Post-hoc comparisons used Tukey's HSD. Contamination rates were analyzed using chi-square tests. Significance threshold set at $p < 0.05$. Where required, data were log-transformed to meet ANOVA assumptions.

RESULTS AND DISCUSSION

Spawn colonization and contamination

Culture generation significantly influenced spawn colonization rate and contamination incidence (Table 1). Early generations (G0–G1) colonized grain rapidly with minimal contamination, whereas later generations showed progressively slower colonization and higher contamination rates (χ^2, $p < 0.05$). Reduced competitive ability of physiologically aged mycelium may explain the increased contamination in later generations.

Fruiting behavior and degeneration

Later generations (G3–G4) exhibited delayed primordia initiation, uneven flushes, and increased frequency of malformed or aborted fruit bodies. Days to primordia initiation increased from 9.5 ± 1.2 days in G0 to 13.2 ± 2.1 days in G4, indicating progressive loss of physiological vigor.

Yield and biological efficiency

Yield and biological efficiency declined significantly with advancing culture generations (Table 2). G0 and G1 recorded the highest yields and BE, while G3 showed the lowest performance. Although G4 exhibited relatively higher mean BE, this was associated with high variability among replicates, indicating unstable performance rather than true recovery. Similar degeneration-related yield declines have been reported in other edible fungi.

Table 1. Effect of culture generation on spawn colonization and contamination rate in *Volvariella volvacea*

Culture generation	Time to 100% colonization (days)	Contamination (%)
G0	7.0 ± 0.7	2
G1	7.5 ± 0.8	3
G2	8.2 ± 0.9	5
G3	9.0 ± 1.1	8
G4	9.8 ± 1.3	12

Values are mean ± SD.

Table 2. Effect of culture generation on yield and biological efficiency of *Volvariellavolvacea*

Culture generation	Yield (g bag^{-1})	Fruit bodies (no. bag^{-1})	Avg. fruit wt (g)	BE (%)
G0	950 ± 85	42 ± 5	22.6	18.0 ± 3.4
G1	920 ± 95	40 ± 6	23.0	16.8 ± 4.1
G2	840 ± 110	36 ± 7	23.3	13.6 ± 4.8
G3	760 ± 140	33 ± 10	23.0	10.4 ± 6.0
G4	680 ± 160	29 ± 12	23.4	17.2 ± 7.2

Values are mean ± SD; differences significant at $p < 0.05$.

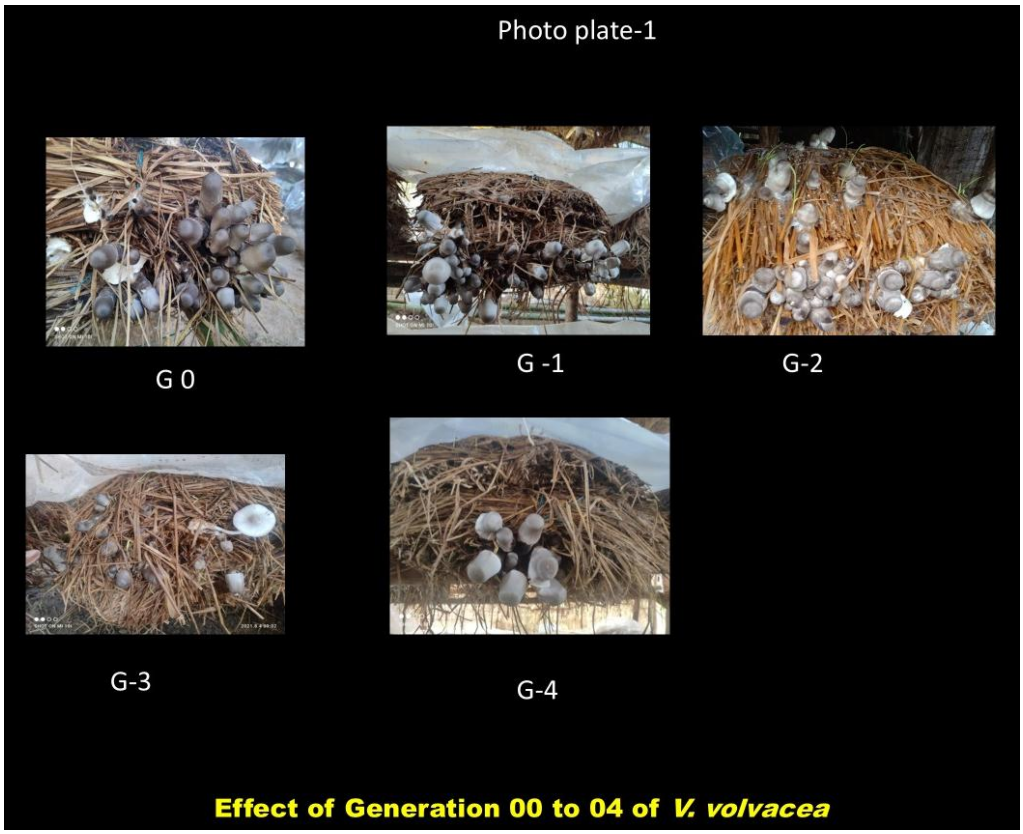

Fig.1. Effect of generation on yield in *Volvariella volvacea*

Repeated sub-culturing of fungal mycelia on artificial media is widely recognized as a major cause of strain degeneration in cultivated mushrooms. Degeneration manifests as reduced mycelialvigor, altered colony morphology, delayed primordia initiation, abnormal fruit bodies, and overall decline in yield potential (Chang and Buswell, 1996; Royse *et al.,* 2017). At the cellular level, degeneration has been linked to chromosomal instability, mitochondrial DNA rearrangements, epigenetic modifications, and reduced expression of lignocellulolytic enzymes essential for substrate colonization. In *Volvariella volvacea*, degeneration is often observed during routine spawn multiplication, particularly when cultures are repeatedly transferred without proper rejuvenation. Chang (1978) and Quimio (1982) emphasized that over-aged or repeatedly sub-cultured *V. volvacea* strains show erratic fruiting behavior, reduced fruit body size, and poor cropping uniformity. Oei (2005) further noted that spawn derived from aged cultures often exhibits delayed spawn run and increased contamination susceptibility. Evidence from other cultivated mushrooms supports the negative impact of serial sub-culturing. In *Pleurotus ostreatus*, repeated transfers resulted in slower substrate colonization, reduced enzyme activity, and lower biological efficiency (Sharma *et al.,* 2013). Similarly, degeneration in *Lentinula edodes* has been associated with delayed pinning, malformed fruit bodies, and reduced cropping cycles. In *Agaricus bisporus*, strain senescence has been linked to mitochondrial genome instability and reduced competitive ability during compost colonization. To mitigate degeneration, several strain preservation and rejuvenation techniques have been recommended, including periodic re-isolation from young and healthy fruit bodies, storage on agar slants under refrigeration, mineral oil preservation, sterile distilled water storage, and cryopreservation in liquid nitrogen. Stamets (2000) and Royse *et al.* (2017) emphasized that maintaining a master culture system and limiting the number of serial transfers are essential practices for sustaining long-term productivity in commercial mushroom cultivation. Despite these recommendations, quantitative studies evaluating the effect of defined successive culture generations on yield and biological efficiency of *V. volvacea* under standardized cultivation conditions are scarce. The present investigation addresses this gap by systematically assessing physiological performance, yield parameters, and degeneration symptoms across five successive generations (G0–G4) of pure culture.

CONCLUSION

Successive culture generations (G2–G4) of *Volvariella volvacea* produced through serial sub-

culturing showed measurable declines in spawn vigor, delayed fruiting, increased contamination, and reduced yield and biological efficiency compared to the founding culture (G0). Occasional higher values in later generations were inconsistent and associated with high variability. To sustain productivity, serial transfers should be minimized, master cultures maintained and periodic rejuvenation through fruit body re-isolation adopted.

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