SINCE 2010

NAAS Rating
2012:1.3; 2013-16: 2.69
2017-2020: 3.98

IMPACT FACTOR
2019-20: 2.40; 2021:1.09

IPI Value 2.74
SJIF 6.783

Received on:
23rd August 2022
Revised on:
15th August 2022
Accepted on:
28th August 2022
Published on:
1st October 2022

Volume No.
Online & Print
152 (2022)

Page No.
01 to 04

Life Sciences Leaflets is an international open access print & e journal, peer reviewed, worldwide abstract listed, published every month with ISSN, RNI Free- membership, downloads and access.

ASSESSMENT OF FERTILITY STATUS OF SOIL OF GANDHINAGAR DISTRICT, GUJARAT, INDIA

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ABSTRACT:

Gandhinagar is the capital city of Gujarat State. The paper deals with the estimation of nutrients in soil of different talukas of Gandhinagar district. This district has four talukas with 290 villages and 10 urban habitations in the district. For the study purpose, the entire district was divided into 05 sites. One site of each taluka was selected. Total 05 sampling sites were selected to collect samples. Soil samples were collected and analysed for their physico-chemical properties like pH, EC, % Organic Carbon, Chloride (Cl), Nitrogen (N), Total Alkalinity, Calcium and Magnesium. pH showed alkaline soil. Parameters analysed in the soil of Gandhinagar district and they were recorded as per the standard, it became evident that, the soil of Gandhinagar district is fertile and suitable for the study purpose.

KEYWORDS: *Assessment, Fertility, Soil, Gandhinagart, Gujarat, India.*

INTRODUCTION:

Biologically, soil may be considered as a weathered outer crust of the earth in which remains and products of decay of living organisms are finely mingled. Ecologically, it may be defined as the part of the crust of the earth in which roots of plants are actually growing. Release of mineral elements during decomposition of litter increases fertility of soil (Charly and West, 1975). According to Marbut (1935) soil may be define

as, "the natural medium for the growth of land plants on the surface of the earth composed of organic and mineral materials." Fertile soil is the most important source for the entire living world. Apart from providing a solid substratum on which we live, the soil provides us most of our necessities through the plant and animals communities which develop on it (Asthana and Asthana, 2003). Soil testing is one of the best available tools to ascertain the physical characteristics and nutrient status of a field so as to assess the fertilizer requirement (Singh, 2007).

MATERIALS AND METHODS:

The collection was made with repeated field trips. Soil samples were collected from selected sites and analyzed for their physico-chemical parameters like pH, EC, Alkalinity, Chloride, Total Hardness, Ca, % Nitrate. The sampling was done by method of Piper (1950). Samples were analyzed as per methods suggested by Trivedy and Goel (1984).

RESULT AND DISCUSSION:

The standards by District Agriculture Plan (DAP) are given in Table – 01. The values of physico-chemical parameters analysed in present study in soil are given in Table-02. The value of pH was recorded high (8.34). pH above 7 indicates the alkaline nature of soil. EC indicates the presence of electrolytes in soil. The value of EC ranged between 0.31m.mho.cm-1 to 0.73m.mho.cm-1 in all 5 samples. Soil with EC greater than 4m.mho.cm-1 indicate the salinity in the soil (Sharma and Kaur, 1994). In present study EC ranges from 0.31m.mho.cm-1 to 0.73m.mho.cm-1 which shows that soil is not saline in nature (Karlikar, B. H. and Solanki, H. A., 2014). The value of organic carbon was highest (0.71 ppm) in S2 and lowest (0.44 ppm) in S5. Decrease in OC may be due to its high demand by living organisms (Solanki, 2001). The nitrogen value was found highest (0.052 ppm) in S2 and low (0.038 ppm) in S5. Nitrogen value was quite high (0.050 ppm) and quite low (0.40 ppm) in remain other soil samples (Table-01). The high or low value of nitrate could be correlated with soil organisms that is nitrogen fixing soil algae, as well as nitrifying and denitrifying bacteria and utilization of nitrate by plants and other living organisms including worms etc. for synthesis of amino acids (Singh, 1996 and Ahluwalia, 1999). The value of chloride was ranged between 53.7 ppm (S1) to 271.5 ppm (S3). Alkalinity in natural waters and soils is formed due to dissolution of CO2 (Trivedy and Goel, 1986). The maximum value (618.1 ppm) of alkalinity was observed in S3 and minimum value (183.3 ppm) was recorded in S4. Total hardness is mainly due to the presence of Ca and Mg hardness (Trivedi and Goel, 1986). Maximum value (251.5 ppm) of TH was obtained in S5 and minimum TH value (40.9 ppm) in S1 (Table - 01).

CONCLUSION:

The present study was carried out on 5 selected sites located at four talukas of Gandhinagar district. In present study physico-chemical characteristics of soil were estimated. Soil samples were analysed for their physico-chemical properties like pH, EC, % Organic Carbon, Chloride (Cl), Nitrogen (N), Total Alkalinity, Calcium and Magnesium. pH showed alkaline soil. Parameters analysed in the soil of Gandhinagar district and they were recorded as per the standard, it became evident that, the soil of Gandhinagar district is fertile and suitable for the study purpose.

ACKNOWLEDGEMENT:

Authors express grateful thanks to Dr. M. G. Bhatt, Principal, Government Science College, Gandhinagar and Dr. S. K. Patel, Head of Botany Department for providing all facilities and encouragement.

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Table 01: Standards (DAP Gandhinagar)

Taluka	*Parameters*	
	pH	*EC (m mho/cm)*
Gandhinagar	6.5 to 7.5	**0.25 to 0.75**
Dehgam	6.5 to 7.5	**0.25 to 0.75**
Kalol	6.5 to 7.5	**0.25 to 0.75**
Mansa	**6.5 to 7.5**	**0.25 to 0.75**

Source: Soil fertility indices (DAP) - Gandhinagar

Table 02: Physico – chemical characteristics of Soil of Gandhinagar

PARAMETERS	*Samples*				
	1	**2**	**3**	**4**	**5**
pH	8.34	8.34	8.30	8.34	8.37
EC	0.56	0.73	0.61	0.58	0.31
Organic Carbon	0.62	0.71	0.54	0.51	0.44
Nitrogen	0.05	0.50	0.40	0.50	0.038
Chloride	53.7	85.2	271.5	160.2	90.3
Alkalinity	502.6	307.0	618.1	183.3	370.5
Calcium	12.6	118.2	78.6	125.2	90.7
Magnesium	28.3	96.3	91.7	78.3	160.8
Total Hardness	40.9	214.5	170.3	203.5	251.5

Parameters in ppm, except pH, EC = m mho/cm

Behavior of ion acoustic solitons in a two-electron temperature plasma of a multi-pole line cusp plasma device (MPD)

Cite as: AIP Advances 13, 065021 (2023); doi: 10.1063/5.0141382
Submitted: 5 January 2023 • Accepted: 31 May 2023 •
Published Online: 16 June 2023

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ABSTRACT

This article presents the experimental observations and characterization of ion acoustic solitons (IASs) in a unique multi-pole line cusp plasma device (MPD), in which the magnitude of the pole-cusp magnetic field can be varied. In addition, by varying the magnitude of the pole-cusp magnetic field, the proportion of the two-electron-temperature components in the filament-produced plasmas of the MPD can be varied. The solitons are experimentally characterized by measuring their amplitude-width relation and Mach numbers. The nature of the solitons is further established by making two counter-propagating solitons interact with each other. Later, the effect of the two-temperature electron population on soliton amplitude and width is studied by varying the magnitude of the pole cusp-magnetic field. It has been observed that different proportions of two-electron-temperature significantly influence the propagation of IASs. The amplitude of the solitons has been found to be inversely proportional to the effective electron temperature (T_{eff}).

I. INTRODUCTION

Ion acoustic solitons (IASs)—the self-organized, non-linear localized structures that can propagate long distances—are widely studied in astrophysical and laboratory plasmas to understand their non-linear dynamics that describe several fundamental processes of plasma physics. A solitary wave that propagated long distances without changing its shape and speed in shallow water was first discovered in 1844 by Russels.[1] Korteweg and de Vries[2] developed the mathematical formalism of soliton dynamics, which led to significant advances in determining the properties of solitons. The soliton dynamics for plasmas were explained by Washimi and Taniuti[3] by deriving and solving the Korteweg–de Vries (KdV) equation in a plasma medium using a novel mathematical approach. Sagdeev[4] studied the arbitrary amplitude non-linear waves in plasma, highlighting many more interesting phenomena of plasma acoustic modes. Both these methods are used to analyze solitons in laboratory[5] and space plasmas.[6] The theory of solitons was further developed by Gardner,[7] obtaining an exact solution of the KdV equation by treating it as an initial value problem using the inverse scattering method. Taniuti[8] introduced the reductive perturbation principle for solving the KdV equation, which was also applicable for solving various non-linear equations apart from waves in plasma. The solution to the KdV equation for non-linear ion-acoustic waves in a plasma medium comprising negative ions had been obtained by Das and Tagare[9] and Das.[10] A comprehensive review on theoretical studies of solitons can be found in Refs. 11 and 12.

The existence of solitary waves is ubiquitous in space plasmas. Solitary waves in the magnetosphere were first observed by the S3-3 satellite[13] and subsequently confirmed and studied extensively

Characterization of Plasma Discharge in a Multi Dipole Line Cusp Magnetic Field Created by an RF Source Coupled by a Spiral Antenna

M. A. Ansari, Amit D. Patel, A. Das Alli, Prabal K. Chattopadhyay, N. Ramasubramanian, Daniel Raju, and Raj Singh

***Abstract*— A detailed characterization of RF–plasma discharge in the multi-cusp plasma device (MPD) having a multi-dipole line cusp magnetic field (MMF: B_0) has been experimentally demonstrated using the externally employed four turns high power spiral antenna. The experimentally measured input impedance (∼0.37 + j80 Ω) of the complete antenna system along with the MPD is found to be in good validation with the CST–MWS simulated result (∼0.3 + j88 Ω). The cusp magnetic field (B_0) along the MPD chamber is also validated and has a good agreement with the simulated results for the magnet current of ∼150 A. The antenna is then made to resonate at 13.56 MHz using an external L-type matching network (MN) along with an RF source. It is found that the plasma density shifts to a maximum ($n_e \sim 3.2 \times 10^{17}/m^3$) value at the argon-filled pressure of 2×10^{-3} mbar for large B_0 and RF power of 1 kW. Further, the input resistances of the antenna with a vacuum ($R_v \sim 0.27$ Ω) and plasma ($R_p \sim 0.55$ Ω) inside the MPD under the matched condition are also measured experimentally using the Rogowski coil. This concluded that the RF power coupling efficiency (η) with the Argon plasma is ∼68%. A plasma with nearly a uniform density of $\sim 2.7 \times 10^{17}/m^3$ is obtained up to a radial distance of ∼5 cm from the MPD center at the constant RF power of 800 W for the Argon-filled pressure and magnet current of 4×10^{-3} mbar and 100 A respectively.**

***Index Terms*— High power RF antenna, inductively coupled plasma (ICP), matching network (MN), plasma resistance, spiral antenna, triple Langmuir probe (TLP), vacuum shield flange (VSF).**

I. Introduction

GREAT efforts are being made toward the development of RF plasma sources for their major applications in semiconductor manufacturing industries. Other applications include plasma cleaning, ion beam etching, ion beam doping, and micro-machining. These applications require plasma sources that will operate at low pressure (1×10^{-3} to 5×10^{-2} mbar), have high plasma density (10^{17}–10^{18} m^{-3}), and have plasma homogeneity over large areas [1], [2], [3]. The low pressure is advantageous to less number of collisions in the sheath region formed around the substrate during ion etching and high-density plasma enhanced the deposition and etching efficiency. Further, controlled and confined plasma attracts the potential range of applications from industry to laboratory. MMF-confined plasma is found to be advantageous for major applications like ion sources [4], [5], [6], plasma-etching reactors [7], etc. In MMF configuration, the B_0 is approximately zero at the center of the confining region i.e., chamber while it increases radially toward the chamber surface. This type of configuration reduces the instabilities generally observed in laboratory devices, which helps to configure the high beta (β_e–the ratio of plasma pressure to magnetic pressure) plasma. It is also found that the plasma confined by MMF has a higher density as compared to the other magnetic field-confined plasma configurations [8].

RF Plasma source can be developed either by capacitively coupled RF plasma configuration (CCP) or by inductively coupled plasma (ICP) source configuration. In the case of CCP, high-density plasma can be obtained by applying high RF power to the electrodes. However, since the electrode is in contact with the plasma, this leads to higher values of the plasma potential (V_p). This will result in the bombardment of highly energetic ions on the substrate which is highly undesirable in semiconductor fabrication [9]. However, this is not the case with an ICP plasma source because there is no electrode in contact with the plasma and the propagation of the generated electrons is controlled by the changing nature of the azimuthal currents established in the plasma [10]. ICP is mainly based on the Joule heating mechanism where the electric field developed due to the RF current applied in the spiral coil accelerates the stray electrons several times due to which these electrons collide with the background particles and thereby cause power absorption.

Since the induced electric field inside the chamber turned out to be inhomogeneous, intensive studies were carried out to study the plasma parameters and electron energy distribution function (EEDF) in ICP plasma discharges [11]. Thus it is essential to study the parameters like electron density (n_e), electron temperature (T_e), and plasma floating potential

Manuscript received 20 December 2022; revised 27 January 2023; accepted 24 February 2023. The review of this article was arranged by Senior Editor F. Taccogna. *(Corresponding author: M. A. Ansari.)*

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Color versions of one or more figures in this article are available at https://doi.org/10.1109/TPS.2023.3251384.

Digital Object Identifier 10.1109/TPS.2023.3251384

Nelumbo *Vol 64 (1): (290-296) 2022*
DOI: 10.20324/nelumbo/v64/2022/169848

ISSN (Print) : 0976-5069
ISSN (Online) : 2455-376X

Eight new additions to the grass Flora of Gujarat State, Western India

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पश्चिम भारत में गुजरात राज्य के घास जातिय वनस्पति में आठ नवीन संकलन

सुजीत कुमार, आर. प्रजापति, रोहित कुमार एम. पटेल, रमेश परमर, अराधना साहु, कुमार विनोद सी. गोसावी

सारांश

गुजरात के डाहोड़ जनपद से पहली बार पोएसी के आठ जातियों- *डिमेरिया डेक्कनेंसिस* बोर., *डी. होहेनेकेरी* होस्ट. एक्स मिक., *इराग्रोस्टिस एस्पेरा* (जैक.) नीस, *ई. जेयलानिका* नीस तथा मेयेन, *इसचाइमम तुमिदम* स्टाफ एक्स बोर, *पेनिकम वालेनसी* मेज., *स्चीजाचिरियम एक्साइल* (होस्ट.) पिल्ग. एवं *ट्राइपोगोन ब्रोमोईडिज़* रोएम तथा सुल्ट. को ज्ञात कर आलेखित किया गया है।

ABSTRACT

Eight species of Poaceae viz., *Dimeria deccanensis* Bor, *D. hohenackeri* Hochst. ex Miq., *Eragrostis aspera* (Jacq.) Nees, *E. zeylanica* Nees & Meyen, *Ischaemum tumidum* Stapf ex Bor, *Panicum walense* Mez, *Schizachyrium exile* (Hochst.) Pilg. and *Tripogon bromoides* Roem. & Schult., are reported for the first time for the Gujarat state from Dahod district.

Keywords: Additions, Grassland, Dahod, Gujarat, Poaceae

INTRODUCTION

Poaceae represented by 790 genera and 12074 species (POWO, 2022) across the world. Whereas from India the Poaceae comprises 309 genera and 1760 species (Prasanna & al. 2020); Pushpa & Singh, 2020; while, Kellong & al. (2020) reported 266 genera and 1506 taxa. During the floristic exploration of Dahod district in Gujarat, authors collected interesting specimens from central Gujarat region. After critical examination of characters and review of published literature (Bor, 1960; Shah, 1978; Raghavan & al., 1981; Jani, 2014; Patel & al., 2019a; 2019b; Kellogg & al., 2020; Thoiba & Pradeep, 2021; Nagaraju & al., 2021), it was concluded that a total of eight grass species have not been reported *viz*., *Dimeria deccanensis* Bor, *D. hohenackeri* Hochst. ex Miq., *Eragrostis aspera* (Jacq.) Nees, *E. zeylanica* Nees & Meyen, *Ischaemum tumidum* Stapf ex Bor, *Panicum walense* Mez., *Schizachyrium exile* (Hochst.) Pilg. and *Tripogon bromoides* Roem. & Schult. from Gujarat. Thus, in present communication accepted name, brief description, phenology, distribution, specimen examined and photos for each species are provided for their easy identification.

TAXONOMIC TREATMENT

Dimeria deccanensis Bor., Kew Bull. 1952: 578. 1952; Bor, Grasses Burma, Ceylon, India & Pakistan 140. t. 3. f. 3.1960.

Annual. Culms 20–60 cm tall, slender, ascending, nodes hairy. Leaf sheath 5–8 cm long, terete; ligule 0.3–0.5 mm

Submitted : 01.06.2022 Accepted : 20.07.2022 Date of Publication 31.07.2022

long, membranous ciliate; leaf blade 5–10 × 0.2–0.3 cm, liner, covered with bulbous based hairs, apex acuminate. Racemes usually 2, rarely 3, straight, 5–8 cm long, rachis broader at base and narrowed at apex, flattened. Spikelets, 3–4 × 0.5–0.8 mm, narrowly oblong parallel to rachis, awned, callus glabrous. Lower glume 2.3–2.7 × 0.2–0.3 mm, sub-coriaceous, linear, puberulous, 3-nerved, apex acute to acuminate. Upper glume 3–4 × 0.4–0.7 mm, sub-coriaceous, linear-narrowly ovate, hairy, 1-nerved, 1-keeled, slightly winged at apex, acuminate. Lower lemma 2–2.5 × 0.2–0.3 mm, hyaline, narrowly elliptic, margins ciliate, 1-nerved, apex acuminate. Palea absent. Upper lemma 2.2–2.7 × 0.3–0.5 mm, membranous, linear-narrowly obovate, 1-nerved, awned from sinus; awn 10–12 mm long, geniculate, awn apex 2 toothed. Lodicules 2. Stamens 2; anther 1.3–1.5 × 0.2–0.3 mm. Pistil 1.4–1.8 mm long. Ovary 0.3 × 0.2 mm. Caryopsis 1.4–1.6 × 0.2–0.3 mm, oblong.

Flowering & *Fruiting*: October-December.

Habitat: Open grassland and rocky substratum.

Distribution: INDIA: Gujarat, Karnataka, Kerala, Maharashtra, Tamil Nadu, Endemic.

Specimen examined: Gujarat: Dahod district, Vasiya Dungari, 22°40'21.50"N, 74° 9'34.00"E, 20.11.2021, *Rohit Patel and Sujit Prajapati* GSCD0069 (Government Science College, Dhanpur).

Dimeria hohenackeri Hochst. ex Miq. in Verh. Ned. Inst. 3,4:35.1851; Hook. f. FI. Brit. Ind. 7:103. 1896; Bor, Grasses Burma, Ceylon, India & Pakistan 142. 1960.

Annual. Culms 15–60 cm tall, tufted, terete, erect or geniculate, slender, glabrous, nodes sparsely bearded. Leaf sheath 2–10 cm long, compressed, keeled, glabrous or hairy; ligule 0.3–0.5 mm long, membranous. Leaf blade 8–10.5 × 0.2–0.6 cm, liner ovate, tubercle based hairy along margins, margins ciliate, apex acute to acuminate. Racemes 3–15, 2–8 cm long, rachis filiform, trigonous, callus bearded. Pedicel 0.2–0.4 mm long. Spikelets 2.5–5 × 0.5–0.6 mm long, oblong-narrowly obovate, awned. Lower glume 2.3–4 × 0.3–0.4 mm, sub-coriaceous, narrowly elliptic, hairy, 1-nerved, 1-keeled, keels ciliate, apex acute. Upper glume 2.5–5 mm long, sub-coriaceous, linear-narrowly elliptic, 3-nerved, 1-keeled, keels ciliate, apex acuminate. Lower lemma 1.8–2.2 × 0.3–0.4 mm, hyaline, narrowly obovate, margins ciliate nerveless, apex acute. Palea absent. Upper lemma 1.8–3.2 × 0.2–0.3 mm, hyaline, linear-narrowly ovate, 1-nerved, awned; awn 9–12 mm long, apex 2-toothed. Lodicules 2. Stamens 2; anther 1.8–2 mm long. Pistil 1.3–1.5 mm long.

Flowering & *Fruiting*: October-November.

Habitat: Lateritic plateaus.

Distribution: INDIA: Gujarat, Karnataka, Kerala, Maharashtra, Endemic.

Specimen examined: Gujarat: Dahod district, Vasiya Dungari, 22°40'22.38"N, 74°9'32.97"E, 20.11.2021, *Rohit Patel and Sujit Prajapati* GSCD0074 (Government Science College, Dhanpur).

Eragrostis aspera (Jacq.) Nees, FI. Afr. Austr. 408.1841; Stapf in Hook. f. FI. Brit. Ind.7: 314.1896.

Annual. Culms 20–60 cm tall, terete, erect, simple or branched, glabrous, nodes glabrous. Leaf sheath 2–10 cm long, compressed, glabrous, bearded at mouth; ligule a fringe of long hairs; leaf blade 8–35 × 0.3–0.8 cm, liner, glabrous, apex acuminate. Panicles 15–40 cm long, lax, branches filiform, pseudo whorled, divided from near the base, bearded at base with long white hairs, scaberulous. Pedicels capillary, longer than the spikelets, scaberulous. Spikelet 2–6 × 1.6–1.8 mm, 5–14 flowered, oblong, breaking up from above downwards. Lower glume 0.6–1.4 × 0.2–0.3 mm, narrowly ovate, membranous, 1-keeled, 1-nerved, apex truncate. Upper glume 0.6–1.4 × 0.3–0.4 mm, narrowly ovate, membranous, 1-keeled, 1-nerved, apex sub-obtuse. Lemmas 1.44–1.6 × 0.5–0.6 mm, elliptic, membranous, 3-nerved, apex truncate. Palea 1.3–1.5 × 0.3–0.4 mm, hyaline, 2-keeled, keels scabrid, apex obtuse. Lodicules 2. Stamens 3; anthers 0.2–0.3 mm long. Caryopsis 0.5 × 0.4 mm, orbicular.

Flowering & *Fruiting*: September-December.

Habitat: Wasteland and open grassland.

Distribution: India: Andhra Pradesh, Gujarat, Karnataka, Madhya Pradesh, Maharashtra, Odisha, Rajasthan, Tamil Nadu; Malayasia, Sri Lanka, Africa.

Specimen examined: Gujarat: Dahod district, Rabdal , 22°50'9.81"N, 74°12'23.73"E, 11.10.2021, *Rohit Patel and Sujit Prajapati* GSCD0081 (Government Science College, Dhanpur)

Eragrostis zeylanica Nees, & Mey., Nov. Actorum Acad. Caes. Leop.-Carol. Nat. Cur. 19 (Suppl. 1): 204. 1843. *Eragrostis elongata* sensu Stapf. in Hook.f. Fl. Brit. India 7(22): 319. 1896, non Jacq. 1813.

Annuals or perennials. Culms 5–25 cm high, tufted, erect or trailing or decumbent, nodes glabrous. Leaves 2–6 × 0.1–0.2 cm, lanceolate, rounded at base; ligules fimbriate, membranous. Panicle 3–15 cm long, oblong or ovate-oblong, spreading. Spikelet 10–60-flowered, 2–3 mm wide, oblong-lanceolate, lanceolate or linear-lanceolate, sharply acute. Lower glume 1–1.5 × 0.5 mm, lanceolate, chartaceous, keeled, 1-nerved. Upper glume 1.5–2 × 0.5–2 mm, ovate-lanceolate, chartaceous, keeled,

Fig. 1: a. **Dimeria deccanensis** Bor; b. **Dimeria hohenackeri** Hochst. ex Miq.; c. **Eragrostis aspera** (Jacq.) Nees; d. **Eragrostis zeylanica** Nees & Meyen.

1-nerved. Lemma 1.5–2.5 × 1–1.5 mm, ovate-lanceolate, chartaceous, 3-nerved. Palea 1–1.5 × 0.5–1 mm, elliptic-oblong, 2-keeled, 2-nerved, ciliate along the keels. Stamens 3; anthers 0.25 mm long. Caryopsis 0.5 × 0.6 mm, ovate or orbicular.

Flowering & *Fruiting*: December-January.

Habitat: Road side and wasteland.

Distribution: India: Andaman and Nicobar Islands, Assam, Chhattisgarh, Gujarat, Kerala, Madhya Pradesh, West Bengal; Myanmar; Sri Lanka.

Specimen examined: Gujarat: Dahod district, Limkheda, 22°49'0.83"N, 73°59'35.87"E, 28.12.2021, *Rohit Patel and Sujit Prajapati* GSCD0087 (Government Science College, Dhanpur).

Ischaemum tumidum Stapf ex Bor, Kew Bull. 1951: 450. 1952; Bor, Grasses Burma, Ceylon, India & Pakistan 186. 1960.

Annual, Culms 10–30 cm tall, tufted, terete, creeping or geniculate, rooting at lower nodes, simple or sparingly branched, glabrous, nodes glabrous or villous. Leaf Sheath 2–5 cm long, compressed, glabrous; ligule 0.8–1 mm long+, membranous; leaf blade 2–10 × 0.5–1 cm, linear to narrowly elliptic-ovate, glabrous or sparsely hairy, base rounded, cordate or sagittate, narrowed into a petiole like base, apex acuminate. Racemes 2, 2–5 cm long, appressed to each other.. Joints 5–5.5 × 2–5 mm, clavate, glabrous, obovate. Spikelets 5–6 mm long, oblong-lanceolate, sessile, awned, callus glabrous or bearded. Lower glume 5–7 × 2–2.5 mm, narrowly ovate, coriaceous, glabrous or hispid with long white hairs below the middle, 2–keeled, 7–9-nearved, apex acute. Upper glume 5–7 × 1.4–1.6 mm, narrowly ovate, membranous, boat shaped, glabrous or softly hairy, 3-nerved, apex muticous. Palea 4.5–5 × 1.2–1.3 mm, oblong, hyaline, 2-nerved, apex obtuse. Upper lemma 4.5–5 × 1.3–1.5 mm, hyaline, cleft at apex into two lobes, geniculately awned from sinus; awn 15–17 mm long. Palea 4–4.5 × 1.2–1.3 mm, narrowly ovate-elliptic, hyaline, 2-keeled, 2-nerved, apex muticous. Lodicules 2. Stamens 3; anthers 3–3.2 mm long. Pedicels 08–1.2 × 1.3–1.5 mm, clavate, glabrous or ciliate along margins. Pedicelled spikelets 5–6.5 × 1.3–1.5 mm, narrowly ovate-oblong, unawned. Upper glume, lemmas and paleas similar to sessile spikelets. Upper lemma entire awn less.

Flowering & *Fruiting*: October-December.

Habitat: open grassland-rocky substratum.

Distribution: INDIA: Gujarat, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Tamil Nadu, Endemic.

Specimen examined: Gujarat: Dahod district, Rentiya, 22°54'19.47"N, 74°13'24.36"E, 27.10.2021, *Rohit Patel and Sujit Prajapati* GSCD0094 (Government Science College, Dhanpur).

Panicum walense Mez, Bot. Jahrb. Syst. 34: 146. 1904 (as 'watense'). *Panicum humile* Nees ex Steud., Syn. Pl. Glumac. 1: 84. 1854, non Thunb. ex Trin., 1826. *Panicum austroasiaticum* Ohwi, Act Phytotax. Geobot. 11: 45. 1942.

Annual. Culms 7–20 cm tall, tufted, terete, erect, slender, simple or branched, glabrous, nodes glabrous. Leaf sheath 0.5–2 cm long, sub-compressed, glabrous, margins ciliate, loose; ligule 0.5–1 mm long, narrow, fimbriate; leaf blade 1–8 × 0.2–0.4 cm, flat, linear ovate, glabrous or sparsely villous, apex acute or subacute. Panicles 1.5–10 cm long, pyramidal, lax, rachis filiform, angulate, scaberulous, branches capillary, flexuous, pedicels long, capillary, flexuous, scabrid. Spikelet 1.5–2 × 0.8–1 mm, ovate-elliptic, glabrous, acute or acuminate, tinged with purple. Lower glume ovate, 1–1.5 × 0.5–0.6 mm, membranous, glabrous, 3–5-nerved, apex acuminate. Upper glume 1.7–2 × 0.5–0.6 mm, ovate, membranous, glabrous, 3-nerved, apex obtuse. Palea 1–1.2 × 0.4–0.5 mm, elliptic, hyaline, margins inflexed, 2-keeled, apex obtuse. Upper lemma 1.2–1.5 × 0.5–0.6 mm, broadly elliptic, membranous, obscurely nerved, apex rounded. Palea 1–1.3 × 0.4–0.6 mm, elliptic-oblong, membranous, margins inflexed, glabrous, 2-keeled, apex obtuse. Lodicules 2. Stamens 3; anthers 0.6–0.8 mm long. Caryopsis 0.8–1 × 0.4–0.5 mm, elliptic oblong.

Flowering & *Fruiting*: August-November.

Habitat: Margins of ponds.

Distribution: Andaman and Nicobar Islands, Andhra Pradesh, Assam, Bihar, Goa, Gujarat, Karnataka, Kerala, Maharashtra, Orissa, Punjab, Sikkim, Uttar Pradesh, West Bengal; CHINA, MYANMAR, PAKISTAN, SRI LANKA.

Specimen examined: Gujarat: Dahod district, Rozam, 22°49'33"N, 74°10'16"E, 19.09.2021, *Rohit Patel and Sujit Prajapati* GSCD0103 (Government Science College, Dhanpur).

Schizachyrium exile (Hochst.) Stapf in Prain, Fl. Trop. Africa 9: 191. 1917. *Andropogon exilis* Hochst., Flora 27: 241. 1844; Hook. f., Fl. Brit. India 7: 166. 1896.

Annual. Culms 10–50 cm tall, terete, erect, slender, nodes glabrous. Leaf sheath 1–2.5 cm long, terete, glabrous; ligule 0.5–0.8 mm long, membranous; leaf blade 1–8 × 0.1–0.3 cm, flat, linear ovate, glabrous or covered with densely bulbous based hairs near mouth, rounded at base, apex acuminate. Racemes 2–3 cm long, solitary, very slender, concealed in narrow spathe. Joints 4–4.5 × 0.6–0.8 mm,

Fig. 2: a. **Ischaemum tumidum** Stapf ex Bor; b. **Panicum walense** Mez; c. **Schizachyrium exile** (Hochst.) Pilg.; d. **Tripogon bromoides** Roem. & Schult.

hairy. Sessile spikelet 5–6 × 0.5–1 mm, linear ovate, awned, callus shortly bearded. Lower glume 5–5.2 × 0.4–0.5 mm, coriaceous, densely villous, 2-keeled, obscurely 2-nerved, margins inflexed, apex shortly 2-toothed. Upper glume, 5–5.5 × 0.4–0.5 mm, linear ovate-sub-coriaceous, boat-shaped, 3-nerved, apex caudate acuminate. Lower lemma 1.8–2 × 0.1–0.2 mm, minute or absent, linear, hyaline, 1-nerved, acuminate. Palea absent. Upper lemma 2–2.2 × 0.3–0.5 mm, linear ovate, hyaline, cleft up to linear. Palea absent. Pedicelled spikelet 4.5–5 × 0.4–0.6 mm, represented by aristate glume.

Flowering & *Fruiting*: October-November.

Habitat: open and sloppy area with sandy substratum.

Distribution: INDIA: Andhra Pradesh, Bihar, Chhattisgarh, Gujarat, Jharkhand, Karnataka, Gujarat, Kerala, Madhya Pradesh, Maharashtra, Meghalaya, Odisha, Rajasthan, Tamil Nadu, Uttar Pradesh, West Bengal; CHINA.

Specimen examined: Gujarat: Dahod district, Kantu, 22°42'14"N, 74°07'06"E, 28.10.2021, *Rohit Patel and Sujit Prajapati* GSCD0109 (Government Science College, Dhanpur).

Tripogon bromoides Roem. & Schult., Syst. Veg., ed. 15 bis [Roemer & Schultes]. 2: 600. 1817. *Plagiolytrum calycinum* Nees, Proc. Linn. Soc. London 1: 95. 1841, nom. nud. *Triathera bromoides* Roth ex Roem. & Schult., Syst. Veg., ed. 15 bis [Roemer & Schultes]. 2: 600. 1817, nom. nud., pro syn. *Tripogon bromoides* Roem. & Schult. var. *major* Stapf ex Hook. f., Fl. Brit. India (J.D. Hooker). 7(22): 288. 1896. *Tripogon bromoides* Roem. & Schult. var. *longifolius* Hook. f., Fl. Brit. India (J.D. Hooker). 7(22): 288. 1896. *Tripogon festucoides* Jaub. & Spach, Ill. Pl. Orient. 4: 49. t. 333. 1851. *Tripogon lanatus* Hochst. ex Steud., Syn. Pl. Glumac. 1: 301. 1854. *Tripogon zeylanicus* sensu Thwaites, Enum. Pl. Zeyl. (Thwaites). 374. 1864, non Nees ex Steud., 1855. *Avena mysorensis* Spreng., Syst. Veg. (ed. 16) [Sprengel] 1: 337. 1824 ("1825"), nom. superfl. & illeg. for *Tripogon bromoides*.

Perennials. Erect, culms 30–70 cm high, nodes glabrous, slightly geniculate and 1-noded. Leaf sheaths 04–10 cm long, linear, glabrous or hairy; ligules indistinct with a tuft of 1–2 mm long hairs at apex; leaf blades 15–50 × 0.3–0.5 mm, linear-lanceolate, flat-convolute, more hairy towards collar, villous along both surfaces, apex acuminate to attenuate. Racemes 10–30 cm long, stiff, slender. Spikelets 3–7 mm long, tightly or loosely arranged in rachis; stout, glabrous or scabrid. Peduncles 10–25 cm long, glabrous. Spikelets 7–10 × 2–2.8 mm, linear, compact, dorsiventrally flattened, olive green or yellowish with purple tinge, 5–16-flowered; callus bearded, hairs 1–1.2 mm long; rachilla 1–1.2 mm long, glabrous to scabrid, almost straight, not persistent. Lower glumes 1.6–3.5 × 0.75–1.2 mm, ovate-lanceolate, notched on one side, 1-keeled, 1-nerved, keels slightly scabrid, apex acuminate. Upper glumes 2.4–5.8 × 0.5–1 mm, elliptic lanceolate, 1-keeled, 1-nerved, apex dentate, shortly aristate or awned; awns 0.3–0.8 mm long. Lemmas 2–4.4 × 1–2 mm, ovate lanceolate, 4-lobed, lateral lobes and lateral awns almost equal or sometimes reduced, 3-nerved, slightly keeled, 3-awned; awns scabrid, apex straight or geniculate, median awns 1.5–3.6 mm long, lateral awns 0.5–2.3 mm long. lateral lobes 0.3–1 mm long, margins scabrid. Paleas 2–4 × 0.5–1.2 mm, elliptic-oblanceolate, hyaline, 2-keeled, ciliate above the half, purple dotted emarginate or bi-lobed with a minute central notch at apex. Lodicules 2, 0.3–0.4 mm long, truncate. Stamens 3; anthers 1–1.2 mm long, oblong. Ovary 0.3–0.5 mm long, obovate. Caryopsis 1–1.6 × 0.3–0.4 mm, narrowly oblong-lanceolate, dark brown.

Flowering & *Fruiting*: July - December.

Habitat: Grows in grasslands, on moist slopes and in rock crevices, along forest margins and road sides at medium to high elevations.

Distribution: INDIA: Andhra Pradesh, Bihar, Gujarat, Jammu & Kashmir, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Meghalaya, Odisha, Rajasthan, Tamil Nadu, Telangana; NEPAL, SRI LANKA.

Specimen examined: Gujarat, Dahod district, Rabdal, 22°50'08"N, 74°13'00"E, 11.10.2021, *Rohit Patel and Sujit Prajapati* GSCD086 (Government Science College, Dhanpur).

ACKNOWLEDGEMENTS

Authors are also grateful to Dr. Y. S. Patel, Principal, Government Science College, Limkheda and Dr. Pankaj Solanki, Principal, Government Science College, Dhanpur, for providing facilities to undertake this work. The authors are also thankful to the staff of Baria division, Gujarat Forest Department, for their cooperation during field.

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ISSN No. 0974-035X
An indexed refereed & peer-reviewed journal of higher education
Towards Excellence
UGC-HUMAN RESOURCE DEVELOPMENT CENTRE
Gujarat University, Ahmedabad-380009, Gujarat, India

USE OF ONLINE TUTORIAL VIDEOS TO IMPROVE INFORMATION LITERACY SKILLS OF LIBRARY USERS IN LIBRARY AND INFORMATION CENTRE

Jayshree Pandya
Dr Pradipsinh Chudasma

Abstract

While learning related to entertainment such as game and social networking, users never feel that they are learning some new things, but while learning an information literacy program they become cautious. When library users are asked to express about library related services, how to search books in OPAC, how to browse online database, they try to communicate, but they may not explore very well or smoothly due to unawareness of library services, lack of information and confidence. Using online tutorial videos would be a great information to motivate the users of the library and information center to enhance or improve their information literacy skills. The main focused of the article is to spread awareness of library resources and services through online videos. The study presented and discussed in article will provide a platform to cater for library users to fulfill their requirements in present scenario.

Keywords: Information Literacy Skills; Online Tutorial Videos, Library Services, LIS, ICT

Introduction

The present study seeks to begin and continues discussions on information literacy program. In present scenario of Information and Communication Technology (ICT), every users of library must be tech-savvy and should be ready to have their knowledge about ICT and use its related things easily. therefore, the present study would be a great awareness to make library users get familiar with the library services in an easy and interesting way by using ICT. When library users are asked to express on a given library service as how to search book, periodicals in OPAC, how to search article from subscribed databases, they may not be aware from it or not show interest to speak, but library users are attracted towards online tutorial videos because of the audio-visual effect and its presentation learning process becomes more pleasurable and easier. Therefore, present study felt that online tutorial videos from library website which made in YouTube will be better to promote library services and resources and motivate the library users to learn and helping in use library resources. The present study focuses on information literacy skills of

library resources and services through using online tutorial videos from WhatsApp, YouTube and other digital as well as online modes.

Information Literacy and Literature Review

There are different types of information literacy like computer literacy, ICT literacy, media literacy, internet literacy, digital literacy, library literacy, cyber literacy etc. In the concept of library literacy program, it refers to enhance skills related to search and identified library resources and services. It provides by physically or online by ppts, online tutorial videos etc. Information literacy is not only knowledge about library resources but also how to use and understand it. According to CILIP definition of information literacy 2018, "Information literacy is the ability to think critically and make balanced judgments about any information we find and use." Information literacy related to information which are available in print, digital content, data, images and spoken words. It helps to understand legal and ethical issues related to use of information including data privacy, data protection open data and intellectual property (CILIP, 2018).

According to ANCIL (2011) "Information literacy is a continuum of skills, behaviours, approaches and values that is so deeply entwined with the uses of information as to be a fundamental element of learning, scholarship and research. It is the defining characteristic of the discerning scholar, the informed and judicious citizen, and the autonomous learner."

The term "information literacy" was first coined by Zurkowski in 1974, it's definition given by ALA (American Library Association) in 1989, the ability to determine when they need information, and know how to get information, how to evaluate and effectively use needed information (Peng, 2010). Kingori, Njiraine and Maina (2016) conducted a case study of information literacy programs in public libraries of kenya through a case study of Kenya National Library Services (KNLS), they used random sampling techniques for collecting data through face to face interviews and questionnaires. They found that major users relied on print materials while researchers used internet for research or academic work.

Chen and Lin (2011) described features of information literacy, its role in university library user education, they surveyed and reviewed publications related to information literacy, they found that well-structured information literacy program very useful for library staff, and users, librarians play important role in information literacy program with help of faculty and IT professionals. Matoush (2006) discussed and analyzed new and innovative information literacy programs at San Jose State University King Library, there are four departments in it like reference, access services, technical services and information technology. There are different types of literacy tutorials available at San Jose State University such as plagiarism tutorials, library basics, finding and searching books and articles tutorials etc. Haines and Horrocks (2006) described King's college London approach to three models of health information literacy through curriculum based, iGrid programmes and Staff information and knowledge competencies under TrainIT. They found future challenges like widening student participation initiatives, changes in

curriculum to involve more online, boosting relation between Higher education and National Health Service. Neidbala and Fogleman (2010) described library 2.0 tools for teaching information literacy to honours students. They explored features of introductory course to enhance knowledge of students about library resources, research skills and writing skills. They also described class of wiki to boom student research and academic achievement.

Bravo, Lucia and Martin (2013) conducted a survey of students, teachers and librarians, they found information literacy competences of students very useful and it applicable in their assignments, teachers and librarians need more in depth questions on citation and plagiarism. Their research has examined qualitative and quantitative study for assessing a web based library program for information literacy program. Anunobi and Ukwoma (2016) studied to determine trends, challenges and opportunities of information literacy in federal and state university libraries of Nigeria. They used survey research method for collecting data by sending questionnaire to each university through mail and telephonic interviews. They found that most of university studied yet to consolidate information literacy program of library in their universities. Baro and Zuokemefa (2011) surveyed librarians of 36 university libraries in Nigeria, they found that information literacy practices ranging from library orientations or library tour sessions for introducing searching database and information skills, how to use library resources. There are few barriers for proving information literacy program like lack interest by users, lack of facilities, low acceptance of online information literacy program etc.

Context of the Study

In the context of a poor ICT skills in library education system, very limited opportunities are being provided for information literacy. Earlier, a library user had to faced lots of hurdles to get right information or required documents when they needed. They can't use library resources because of improper orientation and information literacy program.

The library users hesitate to use online or digital library resources because of unawareness, fear, lack of ICT skills and lack of confidence, they are unable to use it. The present study serves a better way to promote and motivate the library users to use online resources, online video tutorials would great concepts and helping them to use library materials. In the world of ICT and internet, there are lots of information resources are available online. As most of the library users use Facebook, WhatsApp, cloud-based application, YouTube, sharing online videos through WhatsApp to other group. Various forwarded massages of online video tutorials in social media are effective and motivational to learn related to information literacy. In the library resource center, when the library professionals-oriented library users to use online videos, digital materials, they discussed and express core concept of searching books, periodicals and how to use online databases through remote login facility. Later, the library users can be asked to lookout online user guide with online videos and understand it. The advantage is that the library users can watch Online videos continually and use library resources when they face difficulty for any services.

Objectives of the Study

Online tutorial video of library services increases user engagement, which in turn helps to boost in searching library resources like how to reserve book online, how to check user account, how to check fine, how to use remote login service etc. If library provide reading materials related to library resources and services, they will not understand and learn properly but online video related to library services provided, they will understand it better and remember it for long time. The tutorial online videos offer users to use with their flexible time to pause/stop, start, skip or replay.

The objectives of this present study are:

1. To encourage library users to use online resources and services of library
2. To boost searching skills of library users and their confidence level, make them independent in using library resources through online videos.
3. To make aware with library services using information literacy skills and enhance their accuracy;
4. To prepare library users to DIY (Do it Yourself)

Methodology for Present Study

A group of fifty library users and a half-hour computer lab session is enough to check their information literacy skills. We have limited students; it will be suitable for the online tutorial videos. Research methodology includes the online tutorial video, tools and technique and the assessment process.

Research Design

The present study has been designed to benefit the library users to improve their accuracy in searching library resources by making them involvement in the process of information literacy program. During this present study, each group gets a different tutorial video or online video including power point presentation (PPTs), and the respective group watching videos. The library users were asking to question at the end of the session, interact with group and they express their views in terms of library online resources and services. Library users also develop their searching strategy and their confident level to search resources and also download their required materials online. In this study, the group member of library support each other if they faced any difficulties, library professional should know to teach or motivate library user to learn with information literacy program.

Procedure

The library professional distributes the library users into five groups of ten users each and allocates the different tutorial videos to each and every user. The desktop and laptop with the online tutorial video should be given to each user. Library users are asked to watch the online video and answers of the questions at the end of the session. The users of each group discuss,

express their ideas, views, and understand information literacy regarding library resources and services. Once the online videos over and users are ready, the library professional plays important role in library, they describe content of video and describe information related to online videos, so, learner solve their doubt or query related to information literacy program. Library professional appreciating and encouraging user with positive and motivate them to use library resources and services. In the end of the task, the library professional requests few libraries users to describe and express their hands on practice session and library professional ends the task with recommendations, which received from the learners. Library professional also takes feedback from the users of the library.

Time Management and Device Used

While conducting this type of hands on practice session, time is very important; otherwise, the fundamental concept of the program will be lost. First divide library users in to ten groups, sat them in to computer lab with desktop or computer and also provide information within five minutes. Only ten minutes of time given to complete watching online tutorial video of specific resources or services, time may be extended to complete the task. At the end of the session few users of each group have to describe about online videos and sharing their thought about online tutorial videos. The present study used desktop or laptop with internet connection, online tutorial video, projector, Speakers etc.

Questions and Answers Session

The question and answer session are to be organized at the end of the session related to online tutorial videos of library, the common questions are:

1. What things did you learn from online video?
2. Is this information literacy program related to online video useful or not?
3. How you rate entire task?
4. Did you find any query or doubt related to online videos which shows information regarding online resources and services?
5. What you gain from the online videos?

Online tutorial videos of how to search book, periodical, how to download article from subscribed databases of library? How to get information related fine, circulation history from their user account, how to use remote login service etc. Does user share this online video with another user?

Role of the Library and Information Professional

To meet the objectives of the present study, the library professional must be a guide, mentor and good observer. The library professional should provide computer lab with desktop or laptop device with internet connection for using online tutorial video. The library professional should give information to library users in every stage during the session. Library professional should observe users and help them for watching online videos. The library professional should

encourage the library users to complete watching video in time. Library professional should create healthy environment, so they can learn easily and interestingly. While taking feedback from the library users, the library professional must be careful not to discourage the users. The library professional has to describe the importance of information literacy program to the users of the library.

Evaluation

Evaluation is an important part of any learning program, as it opens new ideas of learning for the learners. Library users are evaluated by express their views of online videos, their interpretation and their difficulties while watching online tutorial videos of library resources and services. Library professional also observed and taking feedback for evaluation entire process.

Results

Result of present Study are as follows:

- Each library user has learned from online tutorial video related to library resources and services.
- The discussion with each other user to understand the online video from different aspects.
- The online video has generated thoughts and clear the concept to learners, now they will use library services independently.
- Their information literacy skills are developed and they confidently express and describe services of library.
- Advance search strategy and searching competence is improved gradually of library user.

Recommendations of the Study

Recommendations of the present study are as follows:

- The library professional should select attractive and easily understand online videos with different library resources and services which very useful to them in future. Before selecting each online video, library professional must check
- whether the online tutorial video achieves the objectives.
- The library professional should check and lookout the online tutorial videos well in advance and understand thoroughly. Library professional must be ready for question answer session.
- The library professional should provide healthy environment to user, also help them to clear confusion about online video.
- The library professional should provide computer lab or desktop for watching online tutorial video in library and information center.
- The library professional should guide the library user in exploring the video and motivate them in completing online tutorial video in the given time period.
- If the library users not understand properly while watching online videos, they should not be learning, but after the session, library professional should clear their doubt.

- The library professional should select the suitable time period for taking information literacy program on online video tutorial.

Conclusion

Library and information centers have some responsibilities to ensure that their library users acquire the necessary skills for independent search library resources when they need information. They have to know how to use library resources by introducing information literacy program, now a day most of the library resource centers conduct information literacy program, hence library literacy program will be very informative and attractive using different ICT based program. This study has described information literacy program among users of the library through online tutorial videos which described how to use library resources, how to check user account online, how to use remote login facility, how to search online database and print materials. As many of library community motivate for taking program that integrate information literacy across the library resources and services for users of library for easy and smooth function, increase usage of library materials and also focus for usage online databases which was subscribed by library.

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ACTA SCIENTIFIC MICROBIOLOGY (ISSN: 2581-3226)

Volume 5 Issue 7 July 2022

Research Article

Isolation of Marine Microbugs from Mangrove Ecosystem and Screening of Industrially Important Enzymes

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DOI: 10.31080/ASMI.2022.05.1094

Received: May 30, 2022

Published: June 10, 2022

Abstract

Mangrove ecosystem are known for their diverse and rich microbiota. Here human influence is limited, so novelty in bacterial diversity is more common as mangroves ecosystem comes under conservative area and human interference in restricted here. In this present study, mangrove sample like Soil, Coastal water, Roots were collected from Borivali coastal area of manori creek and Jhow Island, Maharashtra under prior permission from CCF, Mangrove Cell, Maharashtra government. Halophiles are in demand in many Biotech based company and their enzymes are equally in demand due their properties. Here in the present experiment, with the help of enriched media 30 sample were processed from which 8 sample were from Jhow Island and 22 sample from borivali coastal-line. All the samples were screened with 10% salt and 20% salt concentration (NaCl). Sample were also screened for 7pH and 9 pH growth parameters from Borivali and Jhow Island respectively. From above mention samples, total 62 Isolates were obtained, which were screened for Bio-Industrially important enzymes like Amylase, Protease and Cellulase. These enzyme have great demand when they are active in high salt concentration or alkaline pH. These enzyme may emphasize more Scientific-Entrepreneur in India.

Keywords: Mangrove Ecosystem; Halophiles; Bio-industry; Scientific-entrepreneur

Introduction

Mangrove ecosystem is known for it's diverse microbiota. The high nutritional load in mangrove sediments is due to mechanisms such as mangrove microbial activity and remineralization. This process is smoothly carried out as associated microbiota produce hydrolytic enzymes. Many researcher claim to find microorganism rich in producing crucial enzymes like Protease, Cellulase, Amylase. These enzyme play Important role in nutritional cycle of land and associated biotic forms [1]. The parameters of commercial production activities are associated to enzymatic activity in contexts with temperature, pressure, pH, and salinity changes. Industrial biotechnology is frequently used to manufacture enzymes of bacterial origin; the enzymes produced contain hydrolytic thermostable enzymes such as amylases, cellulases, proteases for the generation of bio-fuel [2]. Water covers approximately 71% of the earth's surface area, making it a magnificent blue planet. Because the salter ocean is so vast, it contains a diverse range of life forms. Halophiles are a widely distributed organism; 'Hal' means salt, and 'philos' means lovable. Their growth is affected by salt concentration. They are classified according to salt concentration and represent all three life branches, Archaea, Eukarya and Bacteria. Based on salinities, they are classified as Slight-Halophiles, Moderate-Halophiles, and Extreme-Halophiles, with sodium chloride (NaCl) concentrations of nearly 1-5 %; 5-20 %; 20-30 %, respectively. Nonhalophiles, which grow in less than 0.2M salt, and Halotolerants, that can grow

Citation: Priyanka Sawant and Jignasha Thumar. "Isolation of Marine Microbugs from Mangrove Ecosystem and Screening of Industrially Important Enzymes". *Acta Scientific Microbiology* 5.7 (2022): 35-39.

VIDYA - A JOURNAL OF GUJARAT UNIVERSITY

Peer-Reviewed, Multidisciplinary & Multilingual Journal

ISSN: 2321-1520 **E-ISSN:** 2583-3537

MORPHOLOGICAL DOMINANCE OF ISOLATES AT TWO SITES WITH LESS HUMANOID INTERFERENCE IN MAHARASHTRA, INDIA

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ABSTRACT

Products of biological origin are highly demanded in the industrial sector and because of the substantial financial gains made in the enzyme industry; more and more of these businesses are springing up. This study presents the results of processing samples collected in areas with less human interference, such as Jhow Island and the Borivali Monari Creeks. The samples were collected under the supervision of a forest officer after receiving permission from the Mangrove Cell in Maharashtra. Isolate morphology was investigated in this study. Forty-two isolates from the Borivali site shared the following characteristics: circular shape, entire margins, convex, small size colonies, smooth textures, cream pigments, opaqueness, gram positivity, and a major organism group belonging to Coccus. Twenty isolates were collected from Jhow Island, and their predominant features were as follows: circular shape, entire margins, flat elevation, punctiform colonies, smooth textures, tan pigments, opaqueness, Gram positivity, and the organism's major group belonging to Coccus. Protease, amylase, and cellulase were screened for first because of their vital role in industry. Protease producers were chosen for further testing, and using the inverted pyramid technique, the highest protease-producing isolate, Bor S17B13, was chosen for enzymatic activity. 16S rRNA sequencing was used to determine the identity of isolate Bor S17B13, and a phylogenetic tree was constructed to show that Bor S17B13 is a member of the Priestia aryabhattai strain. The gene sequence for Priestia megaterium strain B21 can be found in the National Center for Biotechnology Information database under the accession number OM743775. Protease enzymes can be used for anything from bio-industry to environmental cleanup (bio-remediation). New possibilities for scaling up enzyme production will become available as more research is done.

Key words: Marine, Mangroves, Protease, Halophile, Extremophile

INTRODUCTION

Extremophile microorganisms that have adaptation to a diverse range of conditions in the natural world are subjected to debates in scientific community. Scientists are interested in the bioactive constituents produced by organisms that have adapted to survive under extreme conditions for probable utility in the fields of biofuels, many areas in medicine and agriculture(Amoozegar et al., 2003), (J. Thumar et al., 2010).

Halotolerant organisms are those that can endure high salt concentrations while also surviving in low to zero salt concentrations and producing metabolites (J. T. Thumar & Singh, 2007), (Vijay et al., 2018)(Oliveira de Veras et al., 2018). Both sea sands and green algae have yielded a wide range of halophilic microorganisms. In this way, there are numerous bacteria that are either mildly halophilic or exceptionally halotolerant in the ocean. The predominant varieties of colonies that evolved on agar plates were allotted by numbering taxonomy to the belongs to the genus Salinivibrio , Pseudomonas spp., Alcaligenes group, Acinetobacter, and Flavobacterium in a study of Spanish intermediate salt concentration (between 15% and 30% salts) ponds . They flourished in a salty environment of 10% but were discovered at salt levels of up to 25%. Below 15% salt, Salinivibrio species predominated, but beyond 15% salt, bacteria belonging to the Pseudomonas, Alcaligenes and Alteromonas groups were overwhelmingly prevalent. Below 30% salt, Gram-positive cocci were predominantly observed, whereas Flavobacterium and Acinetobacter were uniformly distributed in lower numbers (Arahal et al., 2002). These organisms are divided into three groups based on the salt concentration required for optimal growth: extreme halophiles, moderate halophiles, and slight halophiles. Various sulfur-oxidizing, sulfate-reducing, homo-acetogenic, methanogenic, hetero-trophic bacteria and archaea, including aerobic representatives of Archaea associated with the genera Halo-bacterium, Natrono-bacterium, Haloferax, and Haloarcula, as well as several species belonging to the Bacteria and Eukarya, are found in the oxygen - deficient areas in the sediment below.

Acta Scientific Pharmaceutical Sciences (ISSN: 2581-5423)

Volume 7 Issue 3 March 2023

Research Article

Marine-derived Producer Bor S17B13 and its Response to Variations in Salt (NaCl) Concentration and pH in the Growth Medium

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Received: January 17, 2023

Published: February 14, 2023

Abstract

In the current investigation, our primary focus is on the synthesis of extracellular protease, and we aim to identify the factors that produce the most favorable results. Bor S17B13 is a halo-tolerant, gram +ve, bacillus sp. isolated from soil associated mangrove vegetation in the Indian state of Maharashtra. Based on the sequencing of the 16s rRNA gene, it was discovered that strain Bor S17B13 had a connection to the Priestia aryabhattai strain. It is interesting to note that Bor S17B13 reveals a biphasic growth pattern despite having two different sources of nitrogen and just one source of carbon. During the second log phase, which was also the time in which lavish growth was noted and the maximum level of protease production was recorded at the same time, The growth rate achieved with an inoculum concentration of 9% v/v was optimal. Optimization for the isolate revealed that it can grow on 0-20% NaCl concentrations and even produce the protease enzyme, confirming the Bor S17B13 isolate's halotolerant nature. Maximum production of protease was at 0% NaCl (w/v) (171 U/ml), and optimum growth was also seen at the same concentration. Growth and protease activity were greatest at pH 7 (172 U/ml), but they can grow in the pH range of 7 to 9. When up-scaling a product to an industrial level, the use of a specialised medium for a possible isolate plays a very important and critical function. This helps to avoid economic losses and significantly lowers the manufacturing costs of the enzyme product.

Keywords: Mangrove Ecosystem; Halotolerant; Protease; NaCl; Growth pH; Optimization

Introduction

Microorganisms produce enzymes such as protease, cellulase, and amylase. Proteases from them have wide applications in the agricultural, medicine, chemical processing, cleansing, and paper-pulp industries. The global market for enzymes is expected to rise by $9 billion by 2027, according to GVR (Grand View Research report ID: 9781680388442). Microbial enzymes' importance in safe, eco-friendly, cost-effective biotechnological processes are fueling demand. Bio-fuel, cleaning supplies, livestock feed, and foodservice will drive industry growth over the course of the period.

Proteolytic proteases produced by bacteria appear to be the most well investigated enzyme since enzymology was first developed. These enzymes have attracted attention not only for the crucial roles they play in metabolic processes, but also for the extensive ways in which they are put to use in numerous commercial ventures [1]. Due to the fact that these microbial proteases possess all of the desirable features for industrial applications, they are favored over proteases derived from plants and animals [2]. Proteases obtained from these microorganisms are widely diluted for use in a variety of commercial settings and are majorly extra cellular

ACTA SCIENTIFIC MICROBIOLOGY (ISSN: 2581-3226)

Volume 6 Issue 3 February 2023

Research Article

Impact of Substrate pH and Enzyme-Substrate Incubation Time on Protease from a New Halo-tolerant Bor S17B13 Found in the Mangroves of Western India

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Received: January 17, 2023

Published: February 02, 2023

Abstract

Since enzymes are bioactive chemicals, they are of interest to scientists all over the world. Demand for microbial enzymes is rising rapidly across a wide range of sectors as their importance in ensuring the security, sustainability, and efficiency of biotech processes becomes more widely recognised. Media optimization is a powerful tool to enhance production capacity from biological sources (microorganisms). In this present study, samples were collected from mangrove-rich sources such as Borivali Monari Creek and Jhow Island, Maharashtra, western India. Samples collected were mangrove-associated soil, mangrove root, and sea water. The type of sampling was random. Samples were collected with prior permission from the mangrove cell maharashtra government. A total of 30 samples were processed, from which 62 isolates were obtained. These isolates were all screened for industrially important enzymes (protease, amylase, and cellulase), and the best protease producer was selected for further studies. The method of optimization was "ONE VARIABLE AT A TIME OVAT" and method is a well-known optimization technique. Optimization was carried out for parameters like inoculum size, pH of the growth medium, various carbon sources, varied nitrogen sources, and metal ions and cations. Casein was used as substrate, and casein was prepared in various pH buffers for the enzyme assay. At 5 minutes after incubation of enzyme substrate mixture, enzyme activity was at its peak, and substrate degradation in the medium was minimal as compare 65 minutes set. Enzyme activity decreased dramatically as substrate pH increased (between 11 and 12 pH). This optimization supports maximizing the yield of protease production and is of great benefit to the biotech industry. The present study shows that with time stability of enzyme reduces and residues of degraded substrate increases within the reaction mixture. Here in our experiment variation in substrate pH were studied, which showed that protease from Bor S17B13 isolate has optimum enzyme activity at substrate pH 10 at highly alkaline pH then 10 pH, activity of enzyme was reduced. A phenomenon called dissociation of substrate (casein) was seen at casein solution 13pH.

Keywords: Mangrove Ecosystem; Halotolerant; Protease; Bor S17B13; Substrate pH; Enzyme Substrate; ES

Introduction

Researchers from all over the globe are interested in enzymes because of their role as bioactive molecules. The role that microbial enzymes play in safe, eco-friendly, and cost-effective biotech processes is driving an ever-increasing and ever-expanding demand for them in a variety of industries and other uses. From 2020 to 2027, the worldwide market for industrial enzymes is projected to expand from its 2019 valuation of USD 5.6 billion at a CAGR (compound annual growth rate) of 6.4% according to GVR (Grand View Research, report ID: 9781680388442). The industry is expected to grow throughout the forecast period as a result of growing product demand from and use in sectors such as biofuel,

Citation: Priyanka Sawant and Jignasha Thumar. "Impact of Substrate pH and Enzyme-Substrate Incubation Time on Protease from a New Halo-tolerant Bor S17B13 Found in the Mangroves of Western India". *Acta Scientific Microbiology* 6.3 (2023): 03-09.

Ann. For. Res. 65(1): 10908-10937, 2022
ISSN: 18448135, 20652445

ANNALS OF FOREST RESEARCH
https://www.e-afr.org/

MANGROVE ENDOPHYTIC FUNGI: A TREASURE OF BIOACTIVE COMPOUNDS AGAINST INFECTIOUS DISEASE

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Abstract
Mangrove harbours a large number of unique fungal communities known as manglicolous fungi. Fungal endophytes are attracting much attention and increasingly renowned, as their host plants are traditionally used for the medicinal purposes. Endophytic fungi reside within the plant tissues and maintain a strong symbiotic relationship. The relationship between endophytes and their host plants has fascinated many among especially for their ability to produce chemically diverse bioactive natural products. Additionally, they have great values for research and broad prospects for the development of biopharmaceutical and biomedical agents. A mission for discovering novel chemical compounds of pharmaceutical importance has drawn attention towards the mangrove ecosystem, which offers unique biodiversity. The current study attempts to provide a comprehensive account on the mangrove fungal endophytic community residing in a unique habitat and being such a rich source of novel bioactive compounds.
Key Words: Mangrove Ecosystem, Marine Fungi, Secondary metabolites

1 Introduction

A mangrove thrives in beachfront territories, estuaries of sub-tropical and tropical atmospheres and for the most part, contains woody plants. Among the marine biological systems, mangroves possess second as far as environment multifaceted nature, which incorporates bushes, plants, palms, trees, etc., that are all around familiar with endurance capacity in new just as the salt condition. They produce brace uncovers that rise of the mud, which help these plants to secure oxygen and water required for endurance. Mangroves are essentially tropical vegetation and are one of the most beneficial environments and represent 9 orders, 20 families, 27 genera and about 70 species [1]. Mangrove vegetation covers 18 million hectors of coastal areas worldwide. It is considered as the most productive ecosystem [2]. Amongst which total mangrove cover in India is estimated to about 2.7% compared to global mangrove cover[3]. Sundarban possessing the highest mangrove cover in India, while Gujarat, with a coastline of about 1650km (which harbours 1140 Sq.km of mangroves), supports the second largest tidal forest of India [4]. Mangroves are even considered as the hub of extremophiles, many studies have been reported on extremophilic diversity of Kutch and their contribution towards the synthesis of natural metabolites like enzymes

or secondary metabolites[5]–[9]. Additionally, mangroves are even reported to possess various medicinal properties[10].

1.1 Pharmaceutical potential of Mangrove endophytes

Mangrove forests are located in a unique and extreme environment which is generally an interface between land and sea [11].The soil of the mangrove ecosystem has such unique characteristics that it favors the growth of microorganisms[11]. A special term, 'endophyte' is used when we refer to microorganisms residing in plants. They could be considered as bacteria or fungi or actinomycetes, which spend their whole or part of their life cycle in the inter or intracellular tissues of different plant parts (stems, petioles, roots, leaves) without causing any apparent diseases to their host plants[12]. Plants harbor a diverse array of endophytes. The extensive communication between endophytes and their host plants is however hidden and their roles towards those plants are fascinating. For example, the endophytic community not only benefits the plants but also our environment. For instance, endophytes help their plants to grow healthy by producing growth hormones, take part in phytoremediation, biodegradation and nutrient cycling which subsequently reduce debris load in our environment[13].

Mangrove endophytic fungi represent the second largest ecological group of the marine- derived fungi[14]. Marine fungi have salt-tolerance capacity and are able to degrade organic matters[15]. Some endophytes are even capable to change the type of interaction along with their host throughout their lifecycle[16]. Mangrove derived fungi are found to be a rich source of unique and novel secondary metabolites[17]. Especially Mangrove endophytic fungi are considered to play a major role in the synthesis of novel bioactive compounds[18]. Thus, they are an interesting source of novel principal structures for medical applications.

This review describes endophytes from mangroves and their diverse compounds with bioactivities reported in the past decade. It also highlights the traditional medicinal uses and recent investigations on bioactivities of common mangroves, in the hope that this would assist in narrowing down the most suitable source material for isolation of endophytes.

2 Bioactive compounds from mangroves

The common chemical constituents present in the mangroves are aliphatic alcohols and acids, amino acids, alkaloids, carbohydrates, carotenoids, hydrocarbons, free fatty acids including polyunsaturated fatty acids, lipids, pheromones, phorbol esters, phenolics and related compounds, steroids, triterpenes and their glycosides, tannins and other terpenes [19]. Several common mangroves of tropical and subtropical regions, their traditional uses and bioactivity are given in Table1.1. The percentage distribution of bioactive metabolites reported from mangrove endophytic fungi for various activities[20] are mentioned in Fig.1.1. The total number of compounds and their bioactivity reported in this review are mentioned in Table 1.2. The detailed overview of compounds with their potential of antimicrobial, Anti-Diabetic, Anti-Oxidant, Anti-inflammatory and cytotoxicity are mentioned in Table 1.3 and 1.4

Figure 1.1: Percentage distribution of bioactive metabolites reported during the period from 2013 to 2019 (Up to June) from mangrove endophytic fungi for various activities
[20]

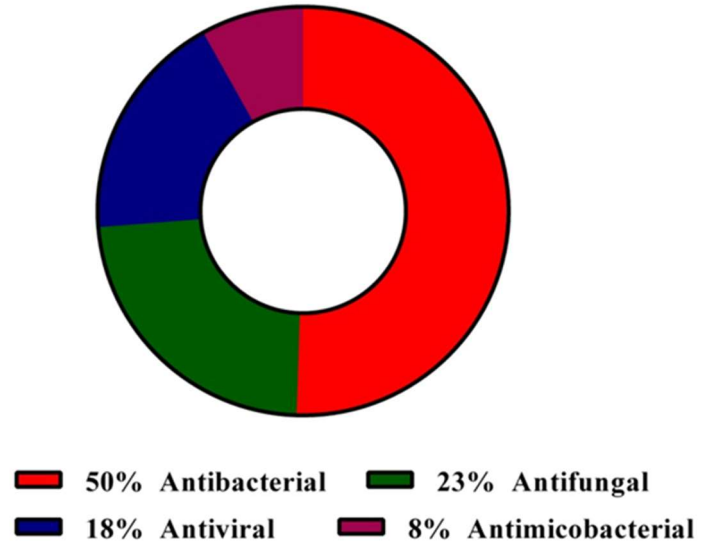

Table: 1.1: Traditional use and in vitro bioactivity of Mangroves

Species of Mangrove	Traditional uses	Bioactivity
Avicennia officinalis	diuretic,Aaphrodisiac, cure for hepatitis,leprosy [10]	antibacterial [21]
Avicennia marina	Cure for skin diseases [22]	antifungal [24] cytotoxic[23] antibacterial [23]
Aegiceras corniculatum	Cure for diabetes, asthma, rheumatism, fish poison [10]	antifungal,piscicidal [10] antioxidant [25] anti-inflammatory[25] hepatoprotective [25] antinociceptive [26] antidiabetic [27]
Acanthus ilicifolius	Treatment for asthma, hepatitis, paralysis, diuretic, dyspepsia, leprosy,	anti-viral [10] muscle

	anti-inflammatory, rheumatic pains,analgesic, leishmanicidal [10],[28]	relaxant, , central nervous system depressant, antipyretic, hypnotic, anti fungal [29] anticancer [30] antioxidant [31][32] anti-inflammatory[32] antinociceptive [33] anti ulcer [34]
Ceriops decandra	anti hemorrhage, Aastringent, to treat ulcers, hepatitis, pain [28]	antioxidant [31] anti fungal [29] anti nociceptive [35] anti bacterial [36] anti inflammatory [37] anti diabetic [38]
Excoecaria agallocha	Uterotonic,fish poison, conjunctivitis, dart poison, treatment of epilepsy, toothache, dermatitis, leprosy, hematuria [10][28]	anti bacterial [21] anti microbial , neuropharmacological effect and cytotoxic [39][40] antioxidant [41] anti allergic [41] anti fungal [29] anti-HIV[42] biocidal effects on marine organisms and phytoplankton, piscicidal [43]
Heritiera littoralis	Mosquito control, fish toxicant, cure for diarrhea, [10]	antimycobacterial, Insecticidal, antioxidant, antifungal [44]
Rhizophora mucronata	Treatment of ulcers, elephantiasis, hepatitis, haematoma, as febrifuge [10]	anti HIV [42] antibacterial [45]
Rhizophora apiculata	Aastringent, nausea, and vomiting for diarrhoea,,antiseptic, cure for typhoid antihaemorragic, [10]	antibacterial [21] anti HIV[42]
Rhizophora mangle	Treatment of diabetes, bruises, dysentery, elephantiasis, angina, boils, fungal infections, diarrhoea, malarial fever, leprosy,	insecticidal [46] anti diabetic [47] anti ulcer [48] antioxidant [49]

	plaster for fractured bones, tuberculosis, antiseptic [10]	
Sarcolobus globosus	relief for rheumatism, dengue fever[50]	thrombolytic [51] cytotoxic [51]
Sonneratia caseolaris	to treat piles,hemorrhages, sprain poultices [28][10]	anti fungal [29] antioxidant [52] anti diabetic[53] bactericidal [54] anti nociceptive[55] anti allergic [56]
Sonneratia alba	sprain and swelling [10]	anti bacterial [45]
Thespesia populnea	Treatment of malaria [10]	anti bacterial, cytotoxic, anti steroidogenic[57][58]
Xylocarpus granatum	relief from dysentery, ,cholera, malaria, diarrhea, inflammation and other abdominal problems [28]	anti microbial [36] antioxidant [59] anti diarrheal [60]

The role of marine derived microorganisms mostly for the production of the secondary metabolite is insignificant[61]. Due to increasing drug resistance in the pathogenic microorganism and with the increasing incidents of infectious diseases, a need for the search of novel antimicrobial compounds has increased many folds. Fungi is major decomposer and distributed in various habitats like animals, soil, deep sea, plants, polar regions as well as deep sea sediments.

Despite of playing a critical role in biogeochemical cycles, it can even produce a wide variety of therapeutic compounds. Since past few years, plant endophytic fungi have proved to be a most promising source of bioactive compounds and many unusual compounds, with antimicrobial potential, have been reported[62].Screening of endophytic fungal metabolites led to the discovery of novel as well as the rediscovery of previously known metabolites. For this reason, interest in bioprospecting of antimicrobial compound production from fungi, belonging to less or unexplored regions like mangroves, has been increased.

The bioactive compounds produced by the endophytic fungi of mangroves were reviewed by few authors [63][64]. Amongst the marine fungi, mangrove fungi are able to produce bioactive compounds of various chemical classes such as terpenes, chromones, coumarins, polyketides, alkaloids and peptides,etc.[65].

High-performance, efficient, pharmacological testing increases the prospect of identifying novel microbial therapeutic agents. Also, it is the most promising source of unusual antimicrobial, anticancer, anti-inflammatory, antioxidant compounds and anti-diabetic compounds[66].

2.1 Antibacterial Compounds

A study on mangrove endophytic fungus Stemphylium sp. 33231, led to the discovery of two novel stemphol sulfates, stemphol A (1) and stemphol B (2). These compounds were capable to prevent the growth of a variety of gram-positive and gram- negative pathogenic bacteria with MIC values ranging from 0.6–10 μg/ml. [67]. Similarly, two novel chlorinated preussomerins, chloro-preussomerins A and B (3 - 4) isolated from leaf endophytic fungus showed effective antibacterial activity against S. aureus[68]. A novel biphenyl derivative, 5,5-dimethoxybiphenyl-2,2-diol (5) along with a known compound, altersolanol B (6) were characterized from Phomopsis longicolla HL-2232 The compounds showed potent antibacterial activity against V. parahaemolyticus and V. Anguillarum[69].

An endophytic fungi Talaromyces amestolkiae YX1 were isolated from Kandelia obovata possessed two new benzofurans, (7-8) which were moderately able to inhibit the growth of S.aureus, S. epidermidis, E. coli, and B. subtilis [70]. A chlorinated bioactive compound, 20-acetoxy-7- chlorocitreorosein (9) were obtained from Penicillium citrinum HL-5126 which showed substantial antibacterial activity against V. parahaemolyticus and S.aureus [71]. Similarly, Penibenzophenone A (10), a novel bioactive metabolite was extracted from Penicillium citrinum HL-5126. It exhibited moderate activity against S. aureus [72].

A mangrove endophytic fungus Penicillium aculeatum was found to produce chromone derivative, (2′S*)-2-(2′-hydroxypropyl)-5-methyl-7, 8-dihydroxy-chromone (11), along with bacillisporin A (12), bacillisporin B (13). Compounds were effective to inhibit the growth of Salmonella sp. and B.subtilis[73]. Acropyrone (14) displayed antibacterial activity against P. aeruginosa and B. subtilis with MIC values of 25 and 50 μM, respectively and ampelanol (15) inhibited the growth of B. subtilis and S. aureus with MIC of 25 and 50 μM. The compounds were extracted from Phomopsis sp. HNY29-2B [74]. Leptospyranonaphthazarin A (16) was extracted from Leptosphaerulina sp. SKS032. The compound showed potent antibacterial activity against Staphylococcus aureus and K. Peneumoniae[75].

Another mangrove endophytic fungus Pestalotia sp. led to the extraction of xylitol (17) oxysporone (18). These compounds were able to overcome arbekacin resistance in a variety of strains of MRSA[76].2-(hydroxymethyl)-3-propylphenol (19) and (−)-brassicadiol (20), obtained from Aspergillus sp. ZJ-68 were effective to inhibit the proliferation of S. aureus, E. coli, and B. subtilis with a MIC value of 4.15 - 12.5 μg/ml[77].

Three novel metabolites dichlorodiaportintone (21), desmethyldichlorodiaportin (22) and dichlorodiaportin (23), obtained from Ascomycota sp. CYSK-4, displayed moderate antibacterial activity against a range of pathogenic bacteria [78]. Colletotric A-B (24-25) were retrieved from Phoma sp. SYSU-SK-7 exhibited strong antimicrobial activity against pathogenic organisms like P. aeruginosa, MRSA and B. subtilis[79]. Fusolanone B (26) showed promising activity with MIC value 6.25 μg/ml against V. parahaemolyticus which was obtained from Fusarium solani associated with the root of Rhizophora apiculata Blume [80].

A potent mangrove endophytic fungus Pseudopestalotiopsis theae was found to be the source of cytosporins V-W (27-28). The compounds showed moderate antibacterial activity against drug-resistant A. Baumannii[81]. Another potent mangrove endophytic fungus Epicoccum nigrum SCNU-F0002 led to the extraction of radicinol derivative and 1-(4-hydroxy-2-methoxybenzofuran-5-yl) butan-1-one (29-30). These compounds displayed significant inhibitory activity against a range of pathogenic bacteria [82].
A plant pathogenic fungus, Colletotrichum gloeosporioide was found to possess novel compounds, (2S)-2,3-dihydro-5,6-dihydroxy-2-methyl-4H-1-benzopyran-4-one (31), and 4-ethyl-3-hydroxy-6-propenyl-2H-pyran-2-one (32).The compounds were potent enough to inhibit the growth of a range of pathogenic bacteria[83]. One of the potent compound, Cowabenzophenone A (33) was extracted from Aspergillus terreus, which showed potent antibacterial activity against a range of gram-positive and gram-negative pathogenic bacteria. [66].

Three novel compounds, dichlorodiaportin (34) were obtained from Aspergillus sp. HN15-5D isolated from leaves of Acanthus ilicifolius located at Hainan Island, China. The compound exhibited moderate inhibitory activity against S.aureus and B.subtilis[84]. Pestalotiopisorin B (35), a novel isocoumarin derivative were obtained from Pestalotiopsis sp. isolated from Rhizophora stylosa. The compound showed moderate antibacterial activity against E. coli and P. aeruginosa[85].

2.2 Anti-fungal Compounds

Botryospyrones A-C (36-38), novel isocoumarin derivatives and a new tryptamine, (3aS, 8aS)-1-acetyl-1, 2, 3, 3a, 8, 8a-hexa- hydropyrrolo [2,3b] indol-3a-ol (39) was extracted from Botryosphaeria ramosa L29. Where, natural tryptamine (39) was found to be more effective against F. oxysporum, P. italicum, and F. graminearum.[86]. The fungus Aspergillus terreus isolated from Bruguiera gymnorrhyza produced a potent bioactive compound Cowabenzophenone A (33). The compound showed showed potent antifungal activity against C.albicans, C.gloeosporioides and A. niger[66].
Penicoffeazine A (40) elucidated as unusual δ-lactone from Penicillium coffeae MA-314 which displayed antifungal activity against Fusarium oxysporum f. sp. momordicae nov. f. and Colletotrichum gloeosporioides [87]. Fusarihexin A-B (41-42) along with cyclo (L- Leu-L-Leu-D-Leu-L-Leu-L-Val) (43) significantly inhibited three plant pathogens, Colletotrichum

gloeosporioides, C. musaeand F. oxysporum. The compoundswere obtained from Fusarium sp. R5 associated with M. bontioides [88].Reinvestigation of secondary metabolites from Penicillium chrysogenum V11 led to the isolation of novel chaetoglobosin, penochalasin K(44). Which was also able to significantly inhibit the growth of R. solani and C. gloeosporioides and MIC of 6.26 and 3.13 μM[89].

2.3 Cytotoxic Compounds

One of the potent bioactive compound, Cowabenzophenone A (33) was extracted from Aspergillus terreus. The compound was explored for cytotoxicity and were found to be potential enough against a HCT 116 colon cancer cell line with an IC50 value of 10.1 μM[66]. A new polyketide derivative, cytosporins W (28) was obtained from Pseudopestalotiopsis theae associated with Rhizophora racemosa.The compound displayed significant cytotoxicity against L5178Y[81]. Phomopsol A (45) and a known polyketide (46) was extracted from Phomopsis sp. xy21 Which were positively screened for neuroprotective effect against corticosterone induced injury [90].

An isocoumarin derivative, aspergisocoumrins A and B (47-48) were obtained from Aspergillus sp. HN15-5D which showed effective inhibitory activity against human cancer cell lines[84]. Flavoglaucin(49), a potent bioactive compound isolated from Aspergillus sp. AV-2 showed effective toxicity towards Caco-2 cells with IC50 value of 2.87 μM [91]. Phomapyrrolidone A and Phomapyrrolidone C (50-51) were isolated from Didymella sp. CYSK-4 which exhibited moderate cytotoxicity against wide range of human cancer cell lines with IC50 value of 4.2-7.8 μM [92]. epi-isochromophilone II (52), a known azaphilone derivatives along with isochromophilones D(53) displayed inhibitory activities against several renal carcinoma cell lines. While, isochromophilones D(53) exhibited cytotoxicity against 786-O cells [93].

Botryorhodine H (54) was extracted from the mangrove endophytic fungus Trichoderma sp. 307 by co-culturing with Acinetobacter johnsonii B2. The compound displayed significant cytotoxicity against rat prolactinoma MMQ and rat pituitary adenoma GH3 cell lines [94]. Penicisulfuranols A−C (55-57), epipolythiodioxopiperazine (ETP) alkaloids were obtained from Penicillium janthinellum HDN13-309. Where, the compounds showed significant cytotoxicity against HeLa and HL-60 cell lines[95].5-deoxybostrycoidin (58), and fusaristatin A (59) was obtained from Diaporthe phaseolorum SKS019. The compounds exhibited inhibitory activity against NCI-H460 and MDA-MB-435 and HCT11cell lines [96].

2,4-Dihydroxy-6- nonylbenzoate(60) was extracted from Lasiodiplodia sp. 318# isolated from E. agallocha. The compound showed potent cytotoxicity against MMQ and GH3 with IC50 values of 5.29 and 13.05 μM respectively[97]. Penochalasin K (44) also exhibited cytotoxicity against MDA-MB-435, SGC-7901 and A549 cells with IC50 values of 4.65 ,5.32 and 8.73 μM respectively [89]. Demethylincisterol A3 (61) were obtained from Pestalotiopsis sp. showed significant cytotoxicity[98]. One of the potent mangrove endophytic fungi, Annulohypoxylon sp.

obtained from Rhizophora racemosa was explored to produce daldinone I (62) which significantly inhibited the growth of Jurkat J16 and Ramos, a human leukemia and lymphoma cell lines[99].

Ethyl-2,4-dihydroxy-6-(80-hydroxynonyl)-benzoate, a lasiodiplodin(63) was isolated from Lasiodiplodia sp. 318# displayed inhibitory activity against human leukaemia cell lines likeTHP1, A549, HepG2, HCT-116, and MDA-MB-435[100]. Also, two novel chlorinated preussomerins, chloro- preussomerins A and B (3 and 4) exhibited potent cytotoxicity against A549 and MCF-7 human cancer cell lines [68]. Phomopsichalasin G (64), a new cytochalasin was obtained from Phomopsis spp. xy21 and xy22. Phomopsichalasin G (64) displayed significant cytotoxicity against a range of human cancer cell lines [101]. Penochalasin I (65) and Penochalasin J (66) new chaetoglobosins were obtained from Penicillium chrysogenum V11 associated with Myoporum bontioides. The compounds displayed potent cytotoxicity against MDA-MB-435, SGC-7901 and A549 cells[102].

Rhizovarins A, B and E (67-69), three new indole-deterpeneswere obtained from Mucor irregularis isolated from Rhizophora stylosa. The compounds inhibited the growth of human cancer cell lines like A-549 and HL-60. [103]. Penibenzophenone B (70) was extracted from Penicillium citrinum HL-5126 isolated from Bruguiera sexangula var. rhynchopetala from South China Sea. The compound showed moderate cytotoxicity against human A549 cell lines [72]. Campyridones D (71) and ilicicolin H (72) were extracted from Campylocarpon sp. HDN13-307 from the root of Sonneratia caseolaris. Both of these compounds showed significant cytotoxicity against Hela cell lines with IC50 value of 8.8 μM and 4.7 μM [104].

Table: 1.2 Bioactivity of Various Compounds

Sr. No.	Name of the Compound	Bioactivity	Reference
1	Stemphol A (**1**) and Stemphol B (**2**)	Antibacterial Activity	[67]
2	Chloro- preussomerins A and B (**3 - 4**)	Antibacterial Activity, Cytotoxicity	[68]
3	5,5′-dimethoxybiphenyl-2,2′-diol (**5**), altersolanol B(**6**)	Antibacterial Activity	[69]
4	Benzofurans (**7-8**)	Antibacterial Activity	[70]
5	20-acetoxy-7- chlorocitreorosein (**9**)	Antibacterial Activity	[71]
6	Penibenzophenone A (**10**)	Antibacterial Activity	[72]

7	(2′S*)-2-(2′-hydroxypropyl)-5-methyl-7, 8-dihydroxy-chromone (**11**), bacillisporin A(**12**), bacillisporin B(**13**)	Antibacterial Activity	[73]
8	Acropyrone (**14**), Ampelanol (**15**)	Antibacterial Activity	[74]
9	leptospyranonaphthazarin A (**16**)	Antibacterial Activity	[75]
10	xylitol (**17**), oxysporone (**18**)	Antibacterial Activity	[76]
11	2-(hydroxymethyl)-3-propylphenol (**19**) and (−)-brassicadiol(**20**)	Antibacterial Activity	[77]
12	dichlorodiaportintone (**21**),desmethyldichlorodiaportin (**22**) and dichlorodiaportin (**23**)	Antibacterial Activity,Anti-inflammatory	[78]
13	colletotric A (**24**), colletotric A(**25**)	Antibacterial Activity	[79]
14	Fusolanone B (**26**)	Antibacterial Activity	[80]
15	Cytosporins V-W (**27-28**)	Antibacterial Activity, Cytotoxicity	[81]
16	Radicinol derivative and 1-(4-hydroxy-2-methoxybenzofuran-5-yl) butan-1-one(**29-30**)	Antibacterial Activity	[82]
17	(2S)-2,3-dihydro-5,6-dihydroxy-2-methyl-4H-1-benzopyran-4-one (**31**), 4-ethyl-3-hydroxy-6-propenyl-2H-pyran-2-one (**32**)	Antibacterial Activity	[83]
18	Cowabenzophenone A (**33**)	Antibacterial, Antifungal Activity,Cytotoxicity,Antidiabetic Activity, Anti-inflammatory	[66]
19	Dichlorodiaportin (**34**)	Antibacterial Activity	[84]
20	Pestalotiopisorin B (**35**)	Antibacterial Activity	[85]
21	Botryospyrones A-C (**36-38**), (3aS, 8aS)-1-acetyl-1, 2, 3, 3a, 8, 8a-hexa- hydropyrrolo [2,3b] indol-3a-ol(**39**)	Antifungal Activity	[86]
22	Penicoffeazine A(**40**)	Antifungal Activity	[87]

23	Fusarihexin A (**41**), Fusarihexin B (**42**), cyclo(L- Leu-L-Leu-D-Leu-L-Leu-L-Val) (**43**)	Antifungal Activity	[88]
24	Penochalasin K(**44)**	Antifungal Activity,Cytotoxicity	[89]
25	Phomopsol A (**45**) , 3-(2,6-dihydroxyphenyl)-4-hydroxy-6-meth- yl-isobenzofuran-1(3H)-one(**46)**	Cytotoxicity	[90]
26	Aspergisocoumrins A and B (**47-48**)	Cytotoxicity	[84]
27	Flavoglaucin(**49)**	Cytotoxicity	[91]
28	Phomapyrrolidone A and Phomapyrrolidone C (**50-51**)	Cytotoxicity	[92]
29	*epi*-isochromophilone II (**52**) isochromophilones D (**53**)	Cytotoxicity	[93]
30	Botryorhodine H (**54**)	Cytotoxicity, Anti-Diabetic Activity	[94]
31	Penicisulfuranols A−C (**55-57)**	Cytotoxicity	[95]
32	5-deoxybostrycoidin (**58**), and fusaristatin A (**59**)	Cytotoxicity	[96]
33	2,4-Dihydroxy-6- nonylbenzoate(**60)**	Cytotoxicity	[97]
34	Demethylincisterol A_3 (**61**)	Cytotoxicity	[98]
35	daldinone I (**62**)	Cytotoxicity	[99]
36	lasiodiplodin(**63)**	Cytotoxicity	[100]
37	Phomopsichalasin G (**64)**	Cytotoxicity	[101]
38	Penochalasin I (**65**) and Penochalasin J (**66**)	Cytotoxicity	[102]
39	Rhizovarins A,B and E (**67-69)**	Cytotoxicity	[103]
40	Penibenzophenone B(**70)**	Cytotoxicity	[72]

41	Campyridones D (**71**) and ilicicolin H (**72**)	Cytotoxicity	[104]
42	Asperchalasine I (**73**)	Anti-Oxidant Activity,Antidiabetic Activity	[105]
43	(+)-epicoccone C ((+)-**74**), (-)-epicoccone C ((-)-**74**), epicoccone D (**75**), epicoccone E (**76**), epicolactone A (**77**)	Anti-Oxidant Activity, Anti-diabetic Activity	[106]
44	(−)-(7S)-10-hy- droxysydonic acid(**78**)	Anti-Oxidant Activity	[107]
45	Asperisocoumarins A and C (**79-80**)	Anti-Oxidant Activity	[108]
46	(±)-(4R*,5S*,6S*)-3-amino-4,5,6-trihydroxy-2-methoxy-5-methyl-2-cyclohexen-1-one (**81**), (±)-(4S*,5S*)-2,4,5-trihydroxy-3-methoxy-4-methoxycarbonyl-5-methyl-2-cyclopenten- 1-one (**82**)	Anti-Oxidant Activity	[109]
47	Asperpanoid A (**83**)	Antidiabetic Activity	[77]
48	Penicieudesmol F(**84**)	Antidiabetic Activity	[110]
49	Colletotric B (**85**), 3-hydroxy-5-methoxy-2,4,6-trimethylbenzoic acid (**86**), colletotric C (**87**), chaetochromone D (**88**) and 8-hydroxy-pregaliellalactone B (**89**)	Antidiabetic Activity	[79]
50	Botryorhodine C,D,G (**90-92**)	Antidiabetic Activity	[94]
51	Lasiodiplactone A (**93**)	Antidiabetic Activity, Anti-inflammatory	[111]
52	(+)-asperglactam A (**94**), (−)-asperglactam A (**95**) and 7-deoxy- 7,14-didehydrosydonol (**96**)	Antidiabetic Activity, Anti-inflammatory	[112]
53	Peniisocoumarins C,G,I and J (**97-100**)	Antidiabetic Activity, Anti-*Mycobacterial*	[113]

54	(R)-2-chloro-3-(8-hydroxy- 6-methoxy-1-oxo-1H-isochromen-3-yl)propyl acetate (**101**), along with dothiorelone C (**102**) and cytosporone A (**103**)	Antidiabetic Activity	[114]
55	Asperisocoumarins C, E and F(**104-106**)	Antidiabetic Activity	[108]
56	botryorhodines E–G (**107-109**)	Antidiabetic Activity	[115]
57	aspergifuranone (**110**)	Antidiabetic Activity	[116].
58	Isoaustinol (**111**), dehydroaustin (**112**) and dehydroaustinol (**113**)	AchE inhibitors	[117]
59	Arisugacin B (**114)**, territrem C (**115**) and terreulactone C (**116)**	AchE inhibitors	[118]
60	asperophiobolins H (**117)**	Anti-*Mycobacterial, Anti-inflammatory*	[119]
61	diaporisoindole A (**118**) tenellone C (**119**)	Anti-*Mycobacterial*	[120]
62	Talaramide A(**120)**	Anti-*Mycobacterial*	[121]
63	(±)-asperlone A (**121**), (±)-asperlone B (**122**) and (−)-mitorubrin (**123**)	Anti-*Mycobacterial*	[122]
64	Talanaphthoquinone A(**124)** and 6-[1-(acetyloxy)ethyl]-5-hydroxy-2,7- dimethoxy-1,4-naphthalenedione (**125**)	Anti-inflammatory	[123]
65	cis-(3R,4S)-3,4-Dihydro-3,4,8 trihydroxynaphthalen-1(2H)-one (**126)**	Anti-inflammatory	[124]
66	Asperophiobolin D, I, J (**127-129),** 21-deoxo-21- hydroxy-6-epi-ophiobolin G (**130**), ophiobolin Q (**131**) and ophiobolin U (**132)**	Anti-inflammatory	[119]
67	1,2-dehydro-terredehydroaustin (**133**)	Anti-inflammatory	[125]
68	Amestolkolides A and B (**134-135)**	Anti-inflammatory	[126]

69	botryosphaerin-B (**136**)	Anti-inflammatory	[127]
70	Diaporindenes A−D (**137-140)**	Anti-inflammatory	[128]

2.4 Antioxidant Compounds

Chemical analysis of mangrove derived fungi Mycosphaerella sp. SYSU-DZG01, which was isolated from Bruguiera located at Hainan, China, resulted in isolation of novel bioactive metabolites like, asperchalasine I (73). The compounds showed positive antioxidant activity against DPPH, showing EC50 value range 16.3 to 85.8 μM.[105]. Similarly, (+)-epicoccone C ((+)-74), (-)-epicoccone C ((-)-74), epicoccone D (75), epicoccone E (76), epicolactone A (77) displayed higher antioxidant activity than the positive controls with an IC50 values 10.2 to 15.3 reported by[106].Sesquiterpenoids, (−)-(7S)-10-hy- droxysydonic acid(78) showed weak antioxidative activity against DPPH radical with an IC50 value of 72.1 μM [107].

A new metabolites, asperisocoumarins A and C (79-80) displayed weak DPPH radical scavenging activity with EC50 values of 125 and 138 μM.[108].With the further investigation of Myoporum bontioides associated root endophytic Alternariasp. R6 led to isolation of (±)-(4R*,5S*,6S*)-3-amino-4,5,6-trihydroxy-2-methoxy-5-methyl-2-cyclohexen-1-one (81) and (±)-(4S*,5S*)-2,4,5-trihydroxy-3-methoxy-4-methoxycarbonyl-5-methyl-2-cyclopenten- 1-one (82) which showed potent ABTS scavenging activities [109].

Table: 1.3 Antimicrobial and Cytotoxicity of compounds

No.	Source Organism	Plant Source	Target	MIC/ IC_{50} value	References
(1, 2)	*Stemphylium sp.* 33231	*Brguiera sexangula var. rhynchopetala* (South China Sea)(healthy leaf)	*E.coli, B.cereus, B. subtilis, M. tetragenus*, *K. rhizophila* and *S. aureus*	0.6–10 μg/ml	[67]
(3,4)	*Lasiodiplodia theobromae* ZJ-HQ1	*Acanthus ilicifolius* (South China Sea) (healthy leaf)	*S. aureus* A549 and MCF-7	6.2 and 3.2 μg/ml 5.9-8.9 μM	[68]
(5,6)	*Phomopsis longicolla* HL-2232	*Brguiera sexangula var. rhynchopetala* (South China Sea) (healthy leaf)	*Vibrio parahaemolyticus* ,*Vibrio anguillarum*	2.5 to 40 μg/ml.	[69].
(7-8)	*Talaromyces amestolkiae* YX1	*Kandelia obovata* (Fresh Tissue) (South China Sea)	*S.aureus, S. epidermidis, E. coli,* and *B. subtilis*	25–50 μg/ml	[70]

(**9**)	*Penicillium citrinum* HL-5126	*Bruguiera sexangula var. rhynchopetala* (South China Sea)	*V. parahaemolyticus,S. aureus*	10 µM and 22.8 µM	[71]
(**10**)	*Penicillium citrinum HL-5126*	*Bruguiera sexangula var. rhynchopetala* (South China Sea)	*S. aureus*	20 µg/ml	[72]
(11-13)	*Penicillium aculeatum*	*K. candel* (Leaves) (Guangdong province, China)	*Bacillus subtilis, Salmonell*	0.13 ± 0.02 µM, 2.00 ± 0.02 µM	[73]
(14,15)	*Phomopsis sp.* HNY29-2B	*A. ilicifolius* (South China Sea) (Branch)	*P. aeruginosa ,B. subtilis, S. aureus*	25 and 50 µM	[74]
(16)	*Leptosphaerulina sp.* SKS032	*Acanthus ilicifolius (South China Sea) (Branch)*	*Staphylococcus aureus, K. peneumoniae*	25 and 50 µg/ml	[75]
(17,18)	*Pestalotia sp.*	*Heritiera fomes* Buch –Ham(Sundarbans, Bangladesh)	*MRSA strains: XU212,ATCC25923, SA1199B,EMRSA-15,MRSA340702*	128 - 128,128-64,64-32,64-32,128-64 µg/ml	[76]
(19,20)	*Aspergillus sp.* ZJ-68	*Kandelia candel* (South China Sea) (Healthy Leaves)	*S. aureus, E. coli,* and *B. subtilis*	4.15, 8.3 and 12.5 µg/ml	[77]
(21-23)	*Ascomycota sp.* CYSK-4	*Pluchea indica* (Branch)(Guangxi Province, China)	*S. aureus, B. subtilis, E. coli, K. pneumoniae and A. calcoaceticus*	25–50 µg/ml, 15.8 µM	[78]
(24-25)	*Phoma sp. SYSU-SK-7*	*Kandelia candel* (South China Sea) (Branch)	*B. subtilis, P. aeruginosa, MRSA*	1.67- 6 µg/ml	[79]
(26)	*Fusarium solani*	*Rhizophora apiculata Blume* (Root) (Hainan Province, China)	*V. parahaemolyticus*	6.25 µg/ml	[80]
(27,28)	*Pseudopestalotiopsis theae*	*Rhizophora racemosa* (Root) (Lagos, Nigeria)	*A.baumannii,* mouse lymphoma cell line L5178Y	50 and 100 µM 3.0 µM	[81]
(29,30)	*Epicoccum nigrum* SCNU-F0002	*Acanthus ilicifolius* L. (Fruit) (Guangdong province, China)	*S. aureus, B. subtilis, E. coli, P. aeruginosa, and S. enteritidis*	25 - 50 µg/ml	[82]
(31,32)	*Colletotrichum gloeosporioide*	*Ceriops tagal* (Hainan island, China)	*M. tetragenus, S. aureus, S. albus, B. cereus,* and *B. subtilis*	12.5-25 µg/ml	[83]
(33)	*Aspergillus terreus*	*Bruguiera gymnorrhyza*(SriLanka)(leaf, stem, flower, bark and fruit)	*B. subtilits*, *S. aureus* and MRSA *P. aeruginosa*, *E. coli* , *C. albicans, C. gloeosporioides and A. niger* HCT 116, α-glucosidase, THP-1	1- 4 µg/ml 2-4 µg/ml 10.1 µM, 7.8 µM, 12.1 µg/mL	[66]

(34)	*Aspergillus sp*. HN15-5D	*Acanthus ilicifolius* (Leaf) (Hainan Island, China)	*S.aureus* and *B.subtilis*	25 µg/ml	[84]
(35)	*Pestalotiopsis sp.*	*Rhizophora stylosa* (Twigs)(Hainan Island, China)	*E. coli* and *P. aeruginosa*	12.5µg/ml and 50µg/ml	[85]
(36-39)	*Botryosphaeria ramosa* L29	*Myoporum bontioides* (Leaves)(Leizhou Peninsula, China)	*F. oxysporum, P. italicum,* and *F. graminearum, F. graminearum*	28.6-900.0 µg/ml	[86]
(40)	*Penicillium coffeae* MA-314	*Laguncularia racemosa* (Leaves)(Hainan Island, China)	*Fusarium oxysporum f. sp. momordicae nov. f.* and *Colletotrichum gloeosporioides*	5 µM	[87]
(41-43)	*Fusarium sp. R5*	*M. bontioides* (South China) (leaves, leaf veins, stems, barks, and roots)	*C. gloeosporioides, C. Musae,and F. oxysporum*	14-91 µM	[88]
(44)	*Penicillium chrysogenum* V11	*Myoporum bontioides* (Vein) (Leizhou Peninsula, China)	*R. solani, C. gloeosporioides,C. musae and P. italic* MDA-MB-435, SGC-7901 and A549	6.13-392.23 µM 4.65 ,5.32 and 8.73 µM	[89]
(45-46)	*Phomopsis sp.* xy21	*Xylocarpus granatum* (Leaves) (Thai)	PC12 cells	5.0−40.0 µM	[90]
(47-48)	*Aspergillus sp.* HN15-5D	*Acanthus ilicifolius* (Hainan Island, China) (Leaves)	MDA-MB-435, HepG2, H460, MCF10A	4.98-43.70 µM	[84]
(49)	*Aspergillus sp.* AV-2	*Avicennia marina* (Leaves) (Hurghada, Egypt)	Caco-2 cells	2.87 µM	[91]
(50-51)	*Didymella sp.* CYSK-4	*Pluchea indica* (Branch) (South China sea)	MDA-MB-435, MDA-MB-231, SNB19, HCT116, NCI-H460, and PC-3	4.2-7.8 µM	[92]
(52-53)	*Diaporthe sp.* SCSIO 41011	*Rhizophora stylosa* (Hainan Province, China)	ACHN, OS-RC-2, and 786-O	3.0 to 4.4 µM 8.9-14 µM	[93]
(54)	*Trichoderma sp.* 307	*Clerodendrum inerme* (stem bark) (Guangdong Province, China)	MMQ and GH3	3.09 and 3.64 µM.	[94]
(55-57)	*Penicillium janthinellum* HDN13-309	*Sonneratia caseolaris* (Hainan Province, China) (Roots)	HeLa and HL-60 0.5 and 0.1 to 3.9 µM	0.1 to 3.9 µM	[95]
(58,59)	*Diaporthe phaseolorum* SKS019	*Acanthus ilicifolius*	NCI-H460 and MDA-MB-435 and HCT116	5.32 to 34.8 µM	[96]
(60)	*Lasiodiplodia sp.* 318[#]	*E. agallocha(Guangdong Province, China)*	MMQ and GH3	5.29 and 13.05 µM	[97]
(61)	*Pestalotiopsis sp*.	*Rhizophora mucronata*	Hela, A549 and HepG	0.17 to 14.16 nM	[98]
(62)	*Annulohypoxylon sp*.	*Rhizophora racemosa* (Cameroon) (Fresh Fruits)	Jurkat J16 and Ramos	6.6 and 14.1 µM	[99]

(63)	*Lasiodiplodia sp.* 318[#]	*Excoecaria agallocha* (Guangdong Province, China)	THP1, A549, HepG2, HCT-116, and MDA-MB-435	10.13-39.74 μM	[100]
(64)	*Phomopsis spp*. xy21 and xy22	*Xylocarpus granatum* (Leaves)(Trang Province, Thailand)	MDA-MB-231, A549, A2780, HCT-8 and HCT- 8/T	3.4-8.6 μM	[101]
(65-66)	*Penicillium chrysogenum* V11	*Myoporum bontioides* (Vein) (South China Sea)	MDA-MB-435, SGC-7901 and A549 cells	7.32-37.70 μM	[102]
(67-69)	*Mucor irregularis QEN-189*	*Rhizophora stylosa* (Hainan Island, China) (Stems)	A-549 and HL-60.	5.0-11.5 μM	[103]
(70)	*Penicillium citrinum* HL-5126	*Bruguiera sexangula var. rhynchopetala*(South China Sea)	A549	15.7 μg/mL	[72]
(71-72)	*Campylocarpon sp.* HDN13-307	*Sonneratia caseolaris* (Roots)	Hela cell lines	8.8 μM and 4.7 μM	[104]

2.5 Anti-diabetic Compounds

An endophytic fungus Epicoccum nigrum SCNU-F0002 was explored to produce the potent compounds like four novel bioactive compounds including (+)-epicoccone C ((+)-74), (-)-epicoccone C ((-)-74), epicoccone D (75), epicoccone E (76), epicolactone A (77) which showed an excellent α-glucosidase inhibitory effect than acarbose[106]. Asperchalasine I (73) were extracted from Mycosphaerella sp. SYSU-DZG01 associated with fruit of Bruguiera located at Hainan, China. The compound displayed good α-glucosidase inhibitory activity with IC50 values of 17.1 μM.[105]. Cowabenzophenone A (33) even exhibited an α-glucosidase inhibitory activity with IC50 value of 7.8 μM[66].

A novel metabolite Asperpanoid A (83) was extracted from Aspergillus sp. ZJ-68. The compound showed effective α-glucosidase activity through a standard in vitro assay with an IC50 value 12.4 μM [77]. Penicieudesmol F (84) were extracted from Penicillium sp. J-54 showed α-glucosidase with IC50 value of 2.27 mM [110]. Colletotric B (85), 3-hydroxy-5-methoxy-2,4,6-trimethylbenzoic acid (86), colletotric C (87), chaetochromone D (88) and 8-hydroxy-pregaliellalactone B (89) were extracted from Phoma sp. SYSU-SK-7 displayed α-glucosidase inhibitory activity with the IC50 values of 36.2-90.6 μM[79].

A novel depsidones, botryorhodine H (54), C, D, G (90-92) were obtained by co-cultivating Trichoderma sp. 307 with Acinetobacter johnsonii B2. The compounds displayed significant α-glucosidase inhibitory activity[94]. Lasiodiplactone A (93), obtained from Lasiodiplodia theobromae ZJ-HQ1.showed a potent α-glucosidase inhibitory activity with IC50 value of 29.4 μM [111]. A pair of 3-arylisoindolinone enantiomers, (+)-asperglactam A (94), (−)-asperglactam A (95) and 7-deoxy- 7,14-didehydrosydonol (96) displayed moderate α- glucosidase inhibitory activity with IC50 values of 7.5-60.1 μM [112].

A novel isocoumarins, peniisocoumarins C,G,I and J (97-100) showed effective α-glucosidase inhibitory activity with IC50 value of 38.1 to 78.1 μM [113]. A novel isocoumarin, (R)-2-chloro-3-(8-hydroxy- 6-methoxy-1-oxo-1H-isochromen-3-yl)propyl acetate (101), along with dothiorelone C (102) and cytosporone A (103) were were extracted from Penicillium sp. YYSJ-3 isolated from Heritiera littoralis.The compounds exhibited better α-glucosidase inhibitory activity than a positive control 1-deoxynojirimycin. [114]. Asperisocoumarins B(104),asperisocoumarins E and F(105-106) were obtained from Aspergillus sp. 085242 .The compounds showed moderate activity against α-glucosidase with IC50 values ranging from 52.3 to 95.6 μM [108].New depsidones, botryorhodines E–G (107-109) were extracted from Meyerozyma guilliermondii. The compounds displayed potent α-glucosidase inhibitory activity Where, (108) was found to be noncompetitive inhibitors of α-glucosidase based on kinetic studies [115]. Similarly,aspergifuranone (110) was also found to be noncompetitive inhibitors of α-glucosidase [116].

Table: 1.4 Bioactivity of Anti-Diabetic, Anti-Oxidant, Anti-inflammatory Compounds

Compound No.	Source Organism	Plant Source	EC_{50} / IC_{50} value	References
(73)	*Mycosphaerella sp.* SYSU-DZG01	*Bruguiera (South China Sea)* (Fruit)	16.3 to 85.8 μM, 17.1 μM	[105]
(74-77)	*Epicoccum nigrum SCNU-F0002*	*Acanthus ilicifolius L.*(Fruit) (Guangdong province, China)	10.2 to 15.3 μM, 32.3 to 63.3 μM	[106]
(78)	*Aspergillus sp. xy02*	*Xylocarpus moluccensis* (Trang Province, Thailand) (Leaves)	72.1 μM	[107]
(79-80)	*Aspergillus sp. 085242*	*Acanthus ilicifolius* (Guangxi Province, China)(Roots)	125 and 138 μM	[108]
(81-82)	*Alternariasp.* R6	*Myoporum bontioides* (Roots) (Guangdong Province, China)	8.19 and 16.09 μM	[109]
(83)	*Aspergillus sp. ZJ-68*	*Kandelia candel* (Leaves) (Guangdong Province, China)	12.4 μM	[77]
(84)	*Penicillium sp.* J-54	*Ceriops tagal* (Hainan province,China) (Leaves)	2.27 mM	[110]
(85-89)	*Phoma sp.* SYSU-SK-7	*Kandelia candel* (Guangxi Province, China) (Branch)	36.2-90.6 μM	[79]
(90-92)	*Trichoderma sp.* 307	*Clerodendrum inerme* (Guangdong Province, China) (stem bark)	8.1 to 54.1 μM	[94]
(93)	*Lasiodiplodia theobromae ZJ-HQ1*	*Acanthus ilicifolius* (Leaves) (South China Sea)	29.4 μM 23.5 μM	[111]
(94-96)	*Aspergillus versicolor SYSU-SKS025*	*Excoecaria agallocha* (Guangxi province, China) (Branch)	7.5-60.1 μM. 12.5 μM	[112]

(97-100)	*Penicillium commune QQF-3*	*Kandelia candel* (Fruit) (Guangdong Province, China)	38.1 to 78.1 µM 20.7 µM	[113]
(101-103)	*Penicillium sp.*YYSJ-3	*Heritiera littoralis* (Guangdong Province, China) (Stem)	100.6-309.6 µM/L.	[114]
(104-106)	*Aspergillus sp.* 085242	*Acanthus ilicifolius* (South China Sea) (Root)	52.3 to 95.6 µM	[108]
(107-109)	*Meyerozyma guilliermondii*	*Kandelia obovata* (Roots) (Guangdong Province, China)	9.8-15.4 µM	[115]
(110)	*Aspergillus sp. 16-5B*	*Sonneratia apetala* (Leaves) (Hainan Island, China)	9.05 µM.	[116]
(111-113)	*Aspergillus sp.* 16-5c	*S. apetala* (Hainan Island, China) (Leaves)	0.40-3.00 µM	[117]
(114-116)	*Penicillium sp.* SK5GW1L	*Kandelia candel* (Guangxi province, China) (Leaves)	0.028-0.03 µM	[118]
(117)	*Aspergillus sp.*	*Kandelia candel (*Guangdong Province, China) (Leaves)	19 µM, 21 µM	[119]
(118-119)	*Diaporthe sp*. SYSU- HQ3	*Excoecaria agallocha* (Branch) (Guangdong Province, China)	4.3 and 5.2 µM	[120]
(120)	*Talaromyces sp.* (HZ-YX1)	*Kandelia obovata* (South China Sea) (Leaves)	55 µM	[121]
(121-123)	*Aspergillus sp.* 16-5C	*S. apetala (*Hainan Island, China) (Leaves)	3.99-4.32 µM	[122]
(124-125)	*Talaromyces amestolkiae*	*Kandelia obovata* (Fruit) (South China Sea)	1.7-3.9 µM	[123]
(126)	*Penicillium citrinum* QJF	*Kandelia candel* (Twig)(Hainan, China)	44.7 µM	[124]
(127-132)	*Aspergillus sp.*	*Kandelia candel* (Guangdong Province, China) (Leaves)	14-42 µM	[119]
(133)	*Aspergillus terreus*	*Kandelia obovata*	42.3 µM.	[125]
(134-135)	*Talaromyces amestolkiae* YX1	*Kandelia obovata* (Leaves) (Guangdong Province, China)	30 and 1.6 µM	[126]
(136)	*Botryosphaeria sp.*	*Kandelia candel* (South China Sea) (Fruit)	1.12 µM	[127]
(137-140)	*Diaporthe sp.* SYSU-HQ3	*Excoecaria agallocha* (Branches) (Guangdong Province, China)	4.2 to 9.0 µM	[128]

2.6 Acetylcholinesterase (AchE) inhibitors

Isoaustinol (111), dehydroaustin (112) and dehydroaustinol (113) were obtained from Aspergillus sp. 16-5c. The compounds exhibited significant acetylcholinesterase (AchE) inhibitory activity with IC50 value of 2.50, 0.40 and 3.00µM,[117]. Arisugacin B (114), territrem C (115) and terreulactone C (116) were extracted from Penicillium sp.SK5GW1L showed strong inhibitory activity against acetylcholinesterase (AchE)[118].

2.7 Anti-Mycobacterial Compounds

One of the newophiobolin-type sesterterpenoid, asperophiobolins H (117) displayed inhibitory action against Mtb protein tyrosine phosphatase B (MptpB).The compound was obtained from Aspergillus sp. isolated from the fresh leaves of Kandelia candel[119]. Similarly, new isoprenylisoindole alkaloid, diaporisoindole A (118) along with precursor, tenellone C (119) showed activity against MptpB with IC50 values of 4.3 and 5.2 μM. [120]. Talaramide A (120), new alkaloid was extracted from Talaromyces sp. (HZ-YX1) isolated from Kandelia obovata. The compound showed significant inhibitory activity against Mycobacterial PknG with an IC50 value of 55 μM[121].

Peniisocoumarins G (98), novel isocoumarin showed potent MptpB inhibitory activity with an IC50 value of 20.7 μM was reported by [113]. (±)-asperlone A (121) and (±)-asperlone B (122), racemic dinaphthalenone derivatives along with (−)-mitorubrin (123) were obtained from Aspergillus sp. 16-5C showed potent inhibitory activity against Mycobacterium tuberculosis protein tyrosine phosphatase B (MptpB)[122]

2.8 Anti-inflammatory Compounds

Talanaphthoquinone A(124) and 6-[1-(acetyloxy)ethyl]-5-hydroxy-2,7- dimethoxy-1,4-naphthalenedione (125), two of the potent bioactive compounds were derived from Talaromyces amestolkiae. The compounds strongly inhibited LPS-induced inflammatioion [123].A potent bioactive compound, Cowabenzophenone A(33) even showed an effective anti-inflammatory activity against a human monocytic cell line THP-1 [66]. cis-(3R,4S)-3,4-Dihydro-3,4,8 trihydroxynaphthalen-1(2H)-one (126) wasobtained from Penicillium citrinum QJF exhibited moderate anti-inflammatory activity by reducing the release of NO in LPS-induced RAW264.7 cells without causing cytotoxicity to the cells[124]. Asperophiobolin H(117), Asperophiobolin D,I,J(127-129),21-deoxo-21- hydroxy-6-epi-ophiobolin G (130), ophiobolin Q (131) and ophiobolin U (132) were extracted from Aspergillus sp. Showed effectiveinhibition against LPS induced NO production in RAW 264.7 marcrophage cells [119].

Desmethyldichlorodiaportintone (22) obtained from Ascomycota sp. CYSK-4 showed a good anti-inflammatory activity against NO production in LPS induced RAW 264.7 cells[78]. Also, one new bioactive compound, 1,2-dehydro-terredehydroaustin (133) obtained from Aspergillus terreus exhibited weak activity against NO production in LPS induced RAW 246.7 mouse macrophages[125]. Amestolkolides A and B (134-135) was extracted from Talaromyces amestolkiae YX1 was found to inhibit NO production in LPS activated in RAW264.7 cells [126]. Lasiodiplactone A (93) was obtained from Lasiodiplodia theobromae ZJ-HQ1 showed potent anti-inflammatory activity against NO production [111]. 7-deoxy- 7,14-didehydrosydonol (96) also displayed inhibitory potential against NO production in RAW 264.7 macrophages with IC50 value of 12.5 μM [112].

Anti-inflammatory (136) was obtained from Botryosphaeria sp. exhibited moderate anti-inflammatory effect against COX-2 with the IC50 value of 1.12 mM [127]. Diaporindenes A−D

(137-140) were obtained from Diaporthe sp. SYSU-HQ3 displayed significant activity against NO production with IC50 value of 4.2 to 8.5 μM [128].

3 Conclusion

Mangrove endophytic fungi are of significant interest because of their unusual potential of synthesizing diverse secondary metabolites. In the present review, we have described 140 bioactive metabolites, isolated from mangrove endophytic fungi, out of which compound 33 displayed antibacterial, anti-inflammatory, cytotoxicity, anti-diabetic, antifungal activities. Also, the compounds, 86-92 showed antidiabetic Activity and anti-inflammatory Activity. All these compounds exhibit a distinct chemical structure. The literature surveys have shown that majority of the researchers focus on novel chemical compound production without aiming towards their bioactive potential, so, if it would be looked forward then it may help the process of drug discovery. In vivo studies must be encouraged rather than in vitro studies. As compared to the bioactive compounds obtained from plants, microbial compounds allow easy compound recovery and also favor manipulation for pharmaceutical production. Till now only a 1/4th portion of novel compounds and bioactivity potential have been explored. Indeed, mangrove endophytic fungi can emerge as a source of the compounds with novel applications.

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ISSN 2515-8260 Volume 09, Issue 07, 2022

Isolation and Identification of an endophytic fungi associated with mangroves of Kutch, Gujarat, India

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Abstract: Mangroves is one of the chief ecosystem which thrives in tropical and subtropical intertidal zones. It plays an important role in safeguarding beaches and rivers from prefunding sea waves, air pollutants and storms. Since these plants can withstand high biotic and abiotic stress, they can be a source of novel biotechnologically useful products. Mangroves are considered as the most productive ecosystem harboring a variety of flora and fauna. It is a home to a huge number of unique fungal communities known as manglicolous fungi. In the current study mangroves of unexplored location of Kutch, Gujarat, India was investigated for the presence of endophytic fungi. Total 13 different fungi were isolated from various root samples. The fungi were identified based on morphological characteristics. Where, *Penicillium sp, Aspergillus sp. and Alternaria sp.* was the most commonly found genera.

Keywords: *Avicennia marina*, Endophytic fungi, Mangroves

1. Introduction: Mangroves are also known as halophytes, found in estuarine habitat, protecting shorelines and streams from harsh waves and storms. Mangrove vegetation covers 18 million hectors of coastline globally. It is said to be the most productive ecosystem [1]. In comparison to worldwide mangrove cover, overall mangrove cover in India is estimated to be at 2.7% [2]. Sundarbans has the greatest mangrove cover in India, whereas Gujarat, with a coastline of around 1650 km (and 1140 Sq.km of mangroves), has India's second biggest tidal forest [3]. The soil of the mangrove ecosystem has such unique characteristics that it favors the growth of microorganisms [4]. An endophytic microorganisms are the one which reside asymptomatically in the plant tissue without causing any harm to the host [5]. The extensive communication between endophytes and their host plants is however hidden and their roles towards those plants are fascinating. They assist their plants to grow healthy by creating growth hormones. They also participate in phytoremediation, biodegradation, and nutrient cycling, which reduces debris load in our environment [6]. Most of the mangrove fungi are marine and some of these are terrestrial in nature. Generally, mangrove roots, twigs or stems when submerged in water often houses marine fungus into it. While the terrestrial fungi are often found in the branches and upper section of the roots [7]. Mangroves have natural ability to withstand difficult environmental conditions like high salt concentration, anaerobic habitat, high temperature and strong wind velocity. Mangrove forests create a significant quantity of detritus in the form of leaf litter, twigs, bark, timber, inflorescence, and other

International Journal of Biological Macromolecules 238 (2023) 124051

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Review

Solvent tolerant enzymes in extremophiles: Adaptations and applications

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ARTICLE INFO

Keywords:
Extremophiles
Non aqueous enzymes
Solvent stability
Immobilization
Adaptations

ABSTRACT

Non-aqueous enzymology has always drawn attention due to the wide range of unique possibilities in biocatalysis. In general, the enzymes do not or insignificantly catalyze substrate in the presence of solvents. This is due to the interfering interactions of the solvents between enzyme and water molecules at the interface. Therefore, information about solvent-stable enzymes is scarce. Yet, solvent-stable enzymes prove quite valuable in the present day biotechnology. The enzymatic hydrolysis of the substrates in solvents synthesizes commercially valuable products, such as peptides, esters, and other transesterification products. Extremophiles, the most valuable yet not extensively explored candidates, can be an excellent source to investigate this avenue. Due to inherent structural attributes, many extremozymes can catalyze and maintain stability in organic solvents. In the present review, we aim to consolidate information about the solvent-stable enzymes from various extremophilic microorganisms. Further, it would be interesting to learn about the mechanism adapted by these microorganisms to sustain solvent stress. Various approaches to protein engineering are used to enhance catalytic flexibility and stability and broaden biocatalysis's prospects under non-aqueous conditions. It also describes strategies to achieve optimal immobilization with minimum inhibition of the catalysis. The proposed review would significantly aid our understanding of non-aqueous enzymology.

1. Introduction

Biocatalysts are widely used for a range of applications in many biotechnological industries. In general, enzymes are a good choice in industrial bioprocesses due to their biodegradability, cost-effectiveness, and generation of non-toxic by-products. The enzymes must have high catalysis and stability across various unfavorable conditions, including extremes of temperatures, pH, surfactants, inhibitors, chelators, and solvents. Non-aqueous enzymology deals with solvent-stable enzymes. The advantages of non-aqueous biocatalysis have propelled the search for solvent-tolerant organisms and enzymes. Non-aqueous reactions enhance the solubility of the hydrophobic substrates, shift the reaction equilibrium from hydrolysis to synthesis, and reduce side reactions, such as hydrolysis, polymerization, and racemization, besides reducing the risk of microbial contamination [1]. The biotransformation reactions in organic solvents must be productive, highly selective, and require only a few steps to synthesize the desired product [2,3]. However, the most significant problem of biocatalysis in organic solvents is the non-native nature of organic solvents for enzymatic functions [4,5]. Enzymes are folded according to their function in the aqueous solutions, while their surrounding residues commonly interact with water molecules. Thus, non-aqueous biocatalysis encounters several significant challenges for the large-scale applications, such as high cost of the enzyme, protein fragility, and exceedingly low enzyme activity [6]. The effects of organic solvents on enzymes are mainly manifested in five ways; conformational changes within enzymes, loss of enzyme-bound water essential for activity, competitive inhibition by organic solvent molecules, changes in substrate solubility, and stabilization of charge transition states. Compared to non-polar solvents, polar organic solvents penetrate enzymes more profoundly and can disrupt secondary and tertiary structures [7]. Besides, polar solvents can easily strip off essential water molecules, adversely affecting the structure and function of the protein.

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https://doi.org/10.1016/j.ijbiomac.2023.124051
Received 21 December 2022; Received in revised form 5 March 2023; Accepted 12 March 2023
Available online 16 March 2023

INDIAN JOURNAL OF SCIENCE AND TECHNOLOGY

RESEARCH ARTICLE

Statistical Optimization for the Production of Antimicrobial Compounds Produced by Mangrove Endophytic *Aspergillus sp.*

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OPEN ACCESS

Received: 02-05-2023
Accepted: 19-05-2023
Published: 03-06-2023

Citation: Trivedi NS, Thumar J (2023) Statistical Optimization for the Production of Antimicrobial Compounds Produced by Mangrove Endophytic *Aspergillus sp.*. Indian Journal of Science and Technology 16(22): 1614-1623. https://doi.org/10.17485/IJST/v16i22.1027

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Funding: None

Competing Interests: None

Published By Indian Society for Education and Environment (iSee)

ISSN
Print: 0974-6846
Electronic: 0974-5645

Abstract

Objectives: The purpose of this study was to investigate the antibacterial properties of an endophytic fungus, *Aspergillus sp.*, isolated from the roots of the *Avicennia marina* belonging to an unexplored location in Kutch, Gujarat, India. The study aimed to optimize various factors to enhance the production of antimicrobial compounds. **Methodology:** Antimicrobial activity was performed using the agar-well diffusion method, and the results were compared with the standard antibiotic streptomycin as a positive control and DMSO as a negative control. The classical one-factor-at-a-time (OFT) approach was used to optimize various factors such as temperature, pH, culture media, carbon and nitrogen sources, and solvents to enhance antimicrobial production. Response Surface Methodology (RSM) was used to statistically optimize the production of antimicrobial compounds. **Findings:** The investigation revealed that the endophyte exhibited remarkable inhibitory action against sensitive strains of *Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Bacillus cereus, and Staphylococcus aureus*. The most suitable conditions for the production of antimicrobials were; an inoculum size of $1*10^5$ in potato dextrose medium at a temperature of 25°C and pH 6, with an incubation period of 12 days. Starch and ammonium chloride acted as the best carbon and nitrogen sources, respectively. The use of RSM resulted in a 1.46-fold increase in the antimicrobial activity against *Pseudomonas sp.*, with an increase in the zone of inhibition from 18.36mm to 26.8mm. **Novelty:** The study highlights the unique antimicrobial potential of an unexplored endophyte derived from *Avicennia marina* roots in Kutch, Gujarat, India. RSM played a crucial role in detecting optimum conditions for the production of antimicrobial compounds, resulting in a significant increase in activity. Its application emphasizes the importance of advanced statistical techniques in improving our understanding of complex biological systems.

Vegetos (2023) 36:163–172
https://doi.org/10.1007/s42535-023-00582-9

RESEARCH ARTICLES

Characterization and antibacterial activity of endophytic fungi isolated from *Avicennia marina*

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Received: 14 April 2022 / Revised: 2 January 2023 / Accepted: 18 January 2023 / Published online: 27 January 2023

Abstract

In the present study, we investigated the antibacterial activity of potent endophytic fungi isolated from the roots of *Avicennia marina*, a mangrove species collected from the Gulf of Kutch, Gujarat, India (23°01′58.6"N 70°09′27.3"E). About thirteen fungal endophytes were isolated from the roots of *A. marina* utilizing Czapek dox agar medium (10% w/v, NaCl and pH, 7.2). Antibacterial activity against selected pathogenic bacterial test cultures was checked by agar well diffusion method using ethyl acetate extracts of 3 weeks old fungal cultures. Very strong antibacterial activity was recorded against a diverse range of bacterial pathogens, including *Shigella flexneri, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus cereus and Klebsiella pneumoniae*. Six fungal isolates, which displayed the antibacterial activities, were subjected to the molecular identification. The rRNA internal transcribed spacer (ITS) sequences with high similarity indexes were obtained from GenBank. The phylogenetic analysis of these fungal isolates was performed using the maximum likelihood method, which showed that the selected six fungal isolates belonged to *Alternaria, Aspergillus* and *Penicillium*. Three fungal extracts of isolate TR-1 10(7), KR-1 10(14) and TR-2 10(32) displayed broad-spectrum antibacterial activity. Among the three isolates, the crude extract of *Penicillium sp.* TR-1 10(7) showed broad-spectrum activity against all the test cultures used in the study. The findings suggest that the isolated endophytic fungi from *A. marina* could be a reservoir of unusual bioactive metabolites. This is the very first report on the antibacterial potential of endophytic fungi, derived from *Avicennia marina*- belonging to the Gulf of Kutch (Western India) to the best of our knowledge.

Keywords Antibacterial · *Avicennia marina* · Bioprospecting · Endophytic fungi · UPGMA

Abbreviations

ITS	Internal transcribed spacer
UPGMA	Unweighted pair group method with arithmetic mean
PCR	Polymerase chain reaction
ML	Maximum likelihood method
MIC	Minimum inhibitory concentration

Introduction

Mangrove vegetation covers 18 million hectare of coastline around the world. It is one of the most productive ecosystems found between tropical and subtropical areas of land and the coastal sea regions (Spalding 1997). Compared to global mangrove cover, the total mangrove cover in India is estimated to be around 2.7% (Giri et al. 2011). These forests are uniquely adapted to harsh tides, severe airstreams, high temperatures, oxygen deficiency, muddy soil, and high saline conditions (Kathiresan 2010). Surrounded by the Arabian Sea, Gujarat is located on India's west coast with the second largest tidal forest in India, following Sunderbans (Bhatt et al. 2009). It has the most extended coastline in India, with approximately 1650 km, supporting a diverse range of marine vegetation and wildlife.

Mangroves are known to possess various medicinal properties (Bandaranayake 2002). Earlier studies showed that all parts of mangroves, including the bark, stems, leaves etc., can be used for different therapeutic purposes such as antibacterial,

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Received: 27 July 2022 | Revised: 30 August 2022 | Accepted: 5 September 2022

DOI: 10.1002/ces2.10163

International Journal of Ceramic Engineering & Science

RESEARCH ARTICLE

Enhancement of bioactivity of collagen–hydroxyapatite nanocomposite on He^+ ion implantation

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Abstract

This study focuses on the effect of He^+ ion implantation on the structural, microstructural, and bioactivity of the collagen–hydroxyapatite (Cg/HAP) nanocomposite for orthopedics as well as vascular stent applications. The material is successfully synthesized by a surfactant-mediated approach and has been investigated using X-ray diffraction (XRD), energy-dispersive spectroscopy, scanning electron microscope, and bioactivity analysis techniques. The presence of the characteristic peaks of collagen in the Fourier transform infrared spectrum confirms the successful synthesis of Cg/HAP nanocomposite. Observation of a blueshift reduction in the intensity and broadening of the peaks corresponding to the asymmetric stretching mode of PO_4^{-3} (1034–1083 cm^{-1}) on the HAP addition indicates a strong interaction between the HAP nanoparticles and collagen. Energy-dispersive spectroscopy analysis confirmed the phase of protein content within the sample. On ion implantation, a reduction in the crystalline size is observed from the XRD analysis. The enhancement in the bioactivity of implanted Cg/HAP composite is based on electronic loss. The electronic interaction through various processes makes the surface of the composites charged leading to more surface deposition activity from simulated body fluid studies, which is further confirmed by the higher surface roughness of the implanted samples.

KEYWORDS
bioactivity, composites, hydroxyapatite, nanomaterials

1 | INTRODUCTION

Hydroxyapatite (HAP) and collagen are major components of bone, that is, 70%–80% HAP and 20%–30% collagen.[1] The majority of multicellular animals are having collagen (a family of fibrous proteins). In mammals, collagen constitutes almost 25% of the total protein mass. The HAP is nontoxic, osteoconductive, having anti-inflammatory and lower thrombogenesis behavior.[2,3] Therefore, it is highly preferred as a grafting or implanting material in orthopedic surgeries or as a coating material for drug delivery on cardiovascular stents.[4,5] The stent coating should not only remain undamaged at the time of deformations taking place during stent crimping and implantation but also

should remain biologically inert after implanting it into the arteries.[3] This is very difficult to manage with HAP due to its poor tensile strength and brittleness. However, some literature reviews suggest that the presence of collagen with the HAP in a coating or grafting material not only improves the healing time of a bone but also reduces the brittleness of HAP due to its high degree of elasticity.[6,7] Therefore, the collagen–HAP composite may find applications in orthopedic as well as vascular stent materials. Moreover, it has been observed that the amorphous HAP materials are more degradable and adjustable with the host than the crystalline ones when selected as grafting or filling material in orthopedics and dentistry.[8,9]

Surface characteristics are more easily influenced by ion beam irradiation. This technique has improved the adhesion and densification of HAP thin films,[10] the osteointegration property of HAP,[11] and the bioactivity of the HAP.[12] Moreover, the effect of swift heavy ions irradiation on fluoroapatite pallets has been studied to explore its suitability for nuclear waste treatment.[13] This is the surface modification technique in which the bulk property remains unaffected.

Suzuki et al.[14] reported the irradiation of collagen-coated polystyrene and polytetrafluoroethylene tubes with lower energy He^+, Ne^+, and Kr^+ ions. They carried out in vitro endothelial cell attachment and in vivo graft patency test on Mongrel dogs. Here the He^+ ion–irradiated samples are proved to be having more patency due to lesser platelet adhesion and better cell attachment.[14] This kind of property leads to antithrombogenicity, which may be due to the destruction of specific ligands of collagen or reduction in plasma protein adsorption at the particular fluence of He^+ ion.[15] The details are discussed well in the literature.[16]

Recently, Joshy et al.[17] studied the effect of swift heavy silicon ion irradiation on HAP. TiO_2-nanoparticle-reinforced dense polycrystalline bovine HAP bioceramic for dental implants is also reported recently.[18] Enhancement of HAP dissolution through structure modification by Krypton ion irradiation is observed by Zhu et al.[19]

2 | EXPERIMENTAL PROCEDURES

The HAP nanoparticles were synthesized by the surfactant-mediated approach as discussed by Jogiya et al.[20]

For the synthesis of Cg/HAP nanocomposites, 1.91-g (20 wt% of precursors used for HAP) collagen (A-grade from SRL) was added to the solution of calcium nitrate hexahydrate ($Ca(NO_3)_26H_2O$) before the addition of potassium dihydrogen phosphate (KH_2PO_4) and stirred for 3 h at the temperature of 60°C, thereafter the procedure mentioned in literature was carried out.

The powdered sample of Cg/HAP was pressed into round pellets (10 mm × 1 mm) by applying 2-t pressure. The pellets were implanted with 250-keV He^+ ions using the facility available at the Inter University Accelerator Centre, New Delhi, India. At the 0.5-μA current, these samples were implanted with various ion fluences (10^{12}, 10^{13}, and 10^{14} ions/cm^2) and labeled as 12Cg/HAP, 13Cg/HAP, 14Cg/HAP, respectively, whereas the pristine sample was labeled as Cg/HAP. The uniformity of irradiation is maintained by scanning the magnetic scanner on the samples. The range of He^+ ions in HAP was calculated using the SRIM 2016 program and found to be 1.5 μm. The nuclear and electronic energy losses were 8.0×10^{-3} and 1.3 keV/nm, respectively.

The samples were characterized by CHN, FT-IR, X-ray diffraction (XRD), scanning electron microscope (SEM), AFM, and bioactivity study.

2.1 | CHN analysis

The elemental (CHN) analysis was performed to investigate the presence of collagen in the samples. The results indicated 6.25% protein content within the samples, which is extremely lower than the desired ratio. The observation of the lower ratio could be due to the difference in solubility of precursors used to synthesize HAP and the collagen itself or may be due to the least attachment of collagen itself occurring at the surface of HAP.

2.2 | FT-IR spectroscopic study

The representative FT-IR absorption spectra of HAP and Cg/HAP composite are shown in Figure 1. It can be observed from the FT-IR spectrum of HAP nanoparticles that the absorption peak at 500–650 cm^{-1} indicates the bending mode of PO_4^{-3}. In the FT-IR spectrum of Cg/HAP nanocomposite, the peaks indicating the presence of amide bands (due to collagen) are found at 3274 and 2918 cm^{-1},[21] which are getting overlapped with the characteristic peak of O–H of the HAP and resulted in a broadened band near 3400 cm^{-1}. The absorption corresponding to amide I (1639 cm^{-1}) is getting merged with the vibration of H_2O at a wave number of 1630 cm^{-1},[22] whereas the symmetric stretching of the NH_3 group is observed at 1484 cm^{-1}. The band corresponds to symmetrical stretching of CO_2 is also observed at 1460 cm^{-1}. Comparing the spectrum of Cg/HAP composite with that of the pure HAP, it is seen that the addition of collagen to the HAP results in blueshift and reduces the intensity and broadening of the peaks corresponding to the PO_4^{-3} asymmetric stretching mode (1034–1083 cm^{-1}), which is probably indicating a certain

TABLE 1 Powder-X-ray diffraction (XRD) analysis table

Sample	Unit–cell parameter ($\alpha = \beta = 90, \gamma = 120°$) $a \pm 0.05$ Å	$b \pm 0.05$ Å	$c \pm 0.05$ Å	Average crystallite size (nm)	Crystallinity (%)	Cell volume (Å^3) ±0.15
Cg/HAP	9.28	18.83	6.88	33 ± 2.2	50.6 ± 3.4	1202.71
12Cg/HAP	9.30	18.88	6.88	13 ± 1.8	32.7 ± 2.5	1080.74
13Cg/HAP	9.30	18.88	6.99	13 ± 2.3	19.1 ± 3.1	1098.29
14Cg/HAP	9.30	18.83	6.88	14 ± 1.5	23.2 ± 3.5	1077.87

Note: Where, α, β, and γ are lattice angles and a, b, c are lattice parameters.
Abbreviation: HAP, hydroxyapatite.

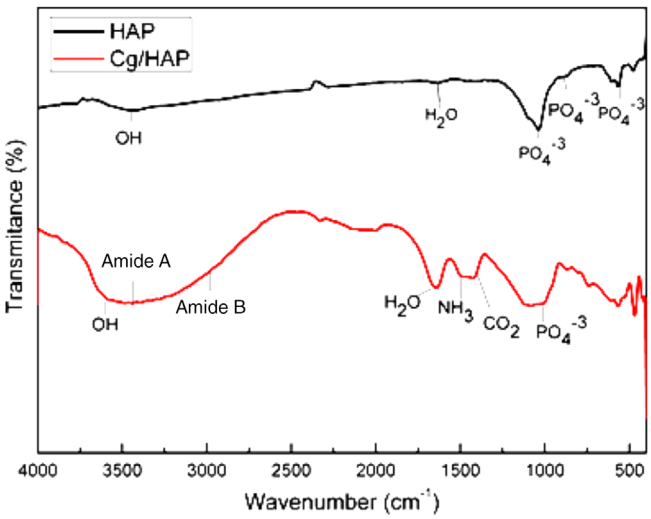
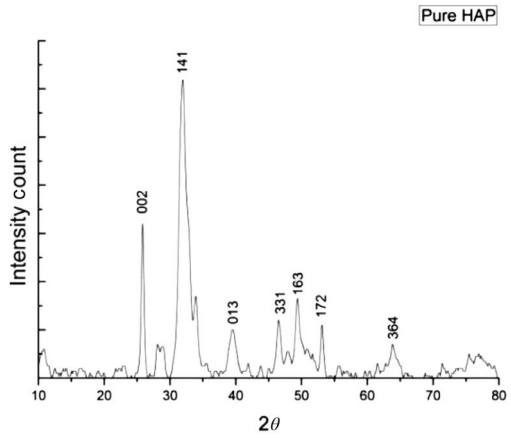


FIGURE 1 The FT-IR spectra of pure hydroxyapatite (HAP) and Cg/HAP composites

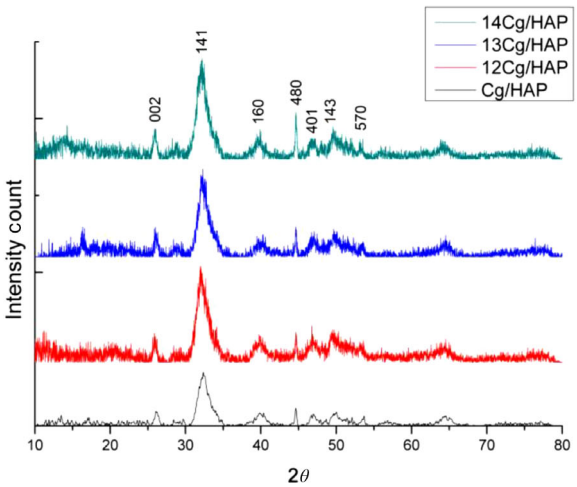
degree of interaction between the HAP nanoparticles and collagen. It is also hypothesized that the broadening of the said peak might be caused by the merging of amide III (1250 cm^{-1}) and PO_4^{3-} asymmetric stretching (1084–1034 cm^{-1}). The symmetric stretching mode of PO_4^{-3} can be observed as a tiny peak near about 960–970 cm^{-1} in both spectra.[23] Thus, the presence of the characteristic peaks of collagen in the FT-IR spectrum confirms that the Cg/HAP nanocomposite has been successfully synthesized.

2.3 | Structural analysis

The powder XRD patterns of the pure HAP and He^+-implanted Cg/HAP nanocomposite and the XRD pattern of the pristine sample correspond to the pattern reported by Zhou et al.,[21] as shown in Figure 2. The separate phase of collagen is not observed in it. Even after implantation, no extra peak suggesting any kind of phase reversal is observed in any of the samples. The average crystallite size is estimated using Scherrer's formula, and the remaining data are fitted using powder-X software, which is shown in Table 1. As seen in the table, there is a noticeable change in



(A) **XRD pattern of pure HAP.**



(B) **XRD patterns of Cg/HAP and He^+ implanted Cg/HAP samples.**

FIGURE 2 (A) X-ray diffraction (XRD) pattern of pure hydroxyapatite (HAP); (B) XRD patterns of Cg/HAP and He^+-implanted Cg/HAP samples

cell parameters and in, hence, cell volume too. All the samples indicated a monoclinic structure. The crystallinity was calculated using a standard formulation given by

$$\text{Crystallinity} = 100 \times C/(A + C) \quad (1)$$

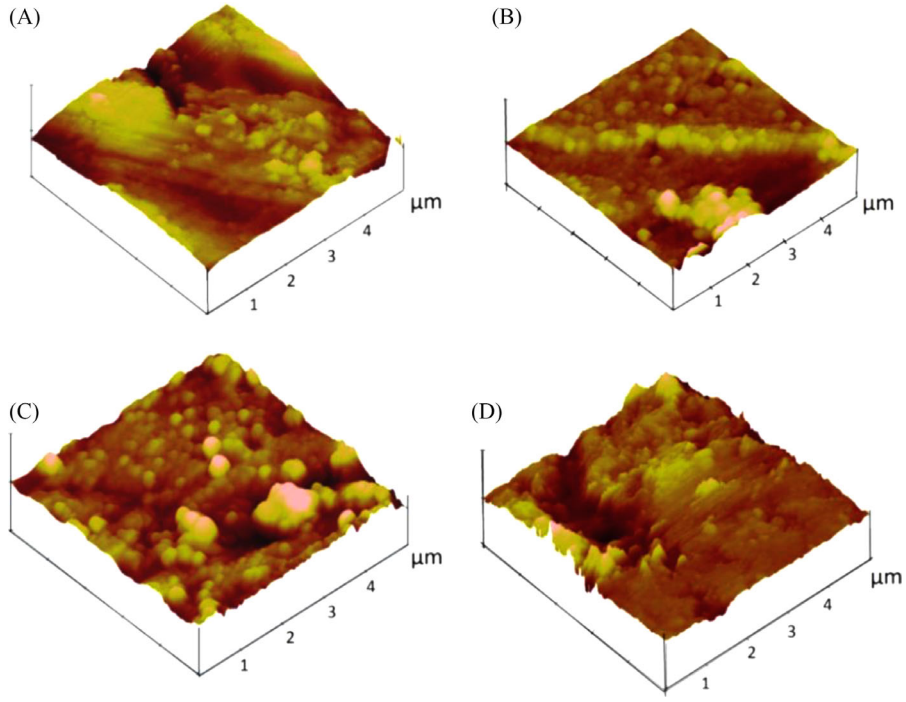


FIGURE 3 AFM images of (A) Cg/hydroxyapatite (HAP), (B) 12Cg/HAP, (C) 13Cg/HAP, and (D) 14Cg/HAP samples, respectively

where *C* is the crystalline area, and *A* is the background, that is, the amorphous area. The values of *C* and *A* were calculated by using the software Powder-X. The crystallinity of the samples tapers off with the increasing fluence rate. The energetic He^+ might be distorting the regular periodic arrangement of atoms within the material, which may be the reason for the reduction in crystallinity.

2.4 | AFM analysis

Figure 3 shows the AFM images of the samples. As shown in Table 2, the particle size and roughness are found to be reduced for the samples 12Cg/HAP and 13Cg/HAP, whereas for the 14Cg/HAP, the roughness, as well as particle size, is found to be increased, which may be due to the local heating caused by the implanted ions above the melting point of the material.[24] Here, it is important to note that the HAP has high thermal stability. Therefore, it is assumed that the melting of collagen might occur at the higher fluence followed by the rapid quenching, and hence, resulting in the larger particle size and roughness of the sample. The hillock kinds of structures can be seen in images (B) and (C) of Figure 3, whereas a large crater is observed in the 14Cg/HAP sample.

TABLE 2 Roughness and size estimation from AFM

Fluence	Roughness (nm) ±1%	Size (nm) ±1%
Cg/HAP	48.81	37.45
12Cg/HAP	32.29	23.30
13Cg/HAP	27.49	19.52
14Cg/HAP	86.46	58.86

Abbreviation: HAP, hydroxyapatite.

2.5 | SEM analysis

Figure 4 shows the SEM images of the pristine and implanted samples. In the pristine sample of Figure 4A, the bi-morphologies, namely, the needle and spherical types, are observed within the nanoscale. According to Wulff's[25] theorem, the process of recrystallization occurs in such a way that the total energy of the system should remain minimum. In the present experiment, the energy transfer from the implanted He^+ to the target sample causes the enhancement in the surface energy. Hence, to balance that additional energy and gain a stable morphology, particle agglomeration occurs, which resulted in the spherical morphology. This kind of shape evolution during the implantation takes place due to the recrystallization phenomenon.[26] Baldwin et al.[27] reported the action of the He plasma inducing a drastic change in the surface morphology. Ghicov et al.[28] found that the ion implantation at the rate 1×10^{16} ions/cm^2 destroyed the morphology of the self-organized TiO_2 nanotube layers. The modification in the morphology of nanoparticles is also observed due to change in the growth rate in a particular direction.[29,30] It is

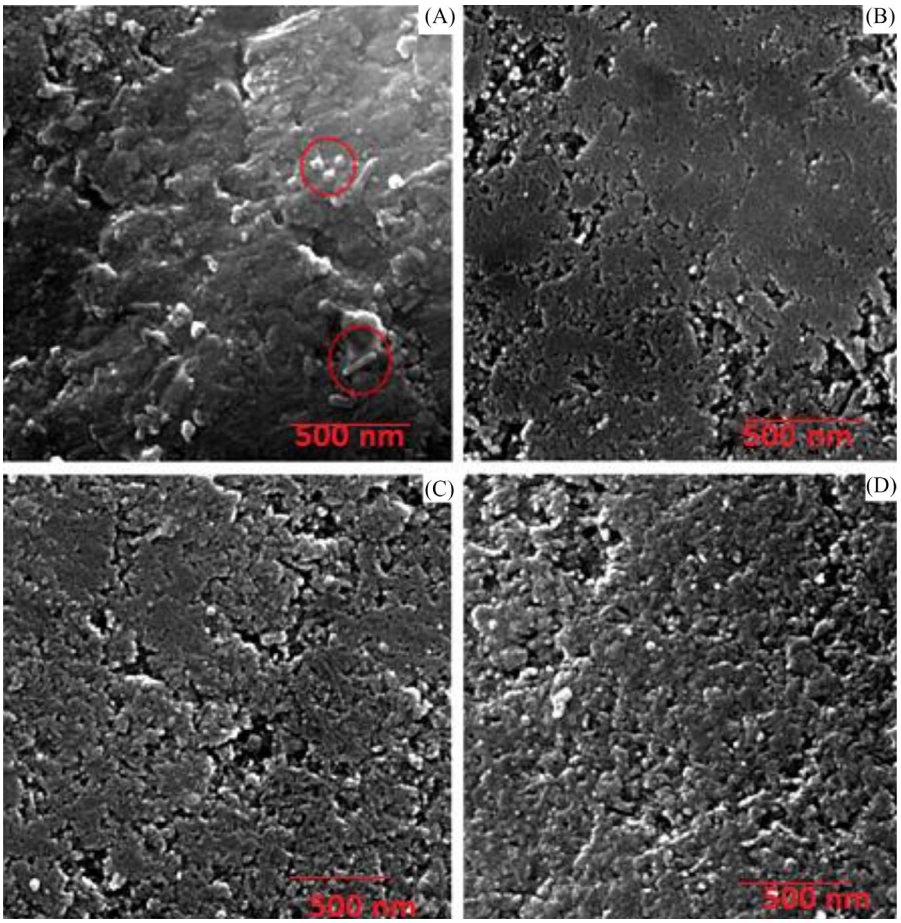


FIGURE 4 Scanning electron microscope (SEM) images of (A) Cg/hydroxyapatite (HAP), (B) 12Cg/HAP, (C) 13Cg/HAP, and (D) 14Cg/HAP samples, respectively

hypothesized that the implanted He^+ ion delivered energy to the sample, which causes the rearrangement of atoms within the sample.

It is possible that during this rearrangement, the maximum growth occurs in the {10} facet instead of {11}. This phenomenon may be responsible for morphological changes.

2.6 | SBF studies

Cziko et al.[31] reported in vitro bioactivity comparison of some HAP-based materials by using simulated body fluid (SBF). Nouri et al.[32] synthesized calcium phosphate nanoparticles using an SBF solution and observed that the samples were found more suitable for gene delivery than that of the water-based one. Li et al.[11] reported the crystallization of ACP (amorphous calcium phosphate) on the surface of SBF. Tank et al.[4] reported the enhanced bioactivity of metal ion–doped HAP nanoparticles. It is also observed that the morphology of the surface-formed particles depends on factors like pH, temperature, and ion substitution at the C site of the HAP indicating OH^- position. However, Kim et al.[33] stated that the process is highly dependent on parameters, such as surface area, the structure of the surface, and material density. Liang et al.[34] observed a higher bioactivity of Mg ion–implanted zirconia and titanium in SBF. They found that the surface roughness contributes to higher bioactivity because the higher values of roughness and porosity provided more favorable microenvironments and three-dimensional sites for nucleation. Thus, bioactivity is a surface-sensitive analysis, and implantation by an energetic ion modifies the surface in terms of increased roughness or structural distortion within a few μm depths of the target sample. Therefore, it is essential to carry out the bioactivity of the pristine and implanted samples.

Figure 5 shows the microscopic images of the pure and implanted Cg/HAP pellets before and after soaking in SBF for the duration of 7 and 14 days. From Figure 5 it is clear that the particles are not observed on the first day. However, the clear growth of particles can be seen on day 7 and up to the 14th day with the layer of particles turned into a fine bunch after agglomeration. It is also marked that the

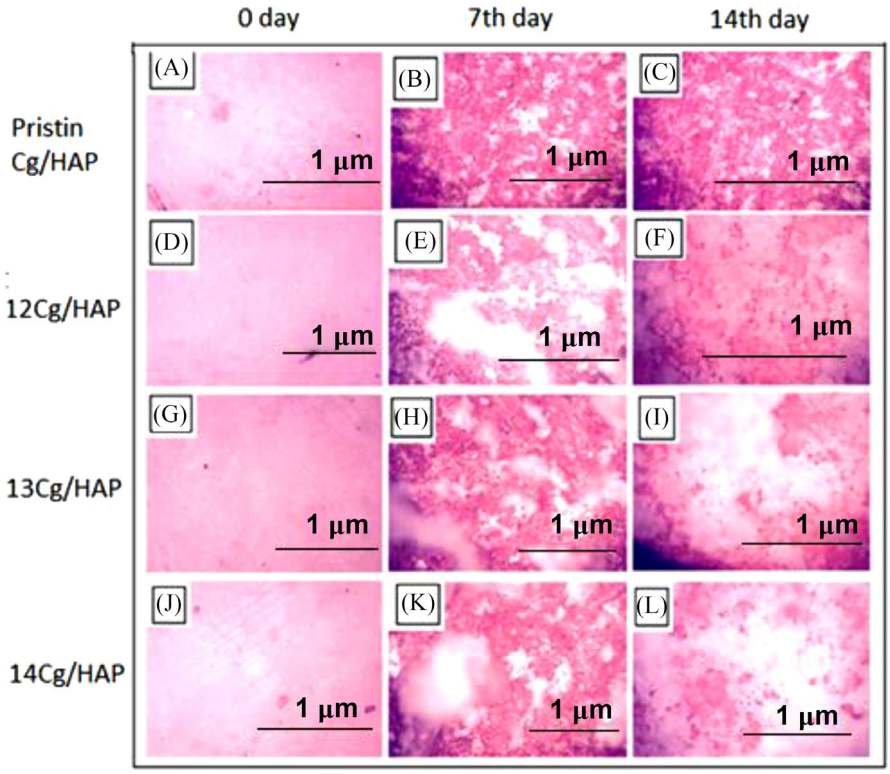


FIGURE 5 Microscopic images of the pellets before (A, D, G, J) and after soaking in various simulated body fluids for 7 days (B, E, H, K) and 14 days (C, F, I, L) respectively. (White particles represent the apatite phase.)

implanted samples exhibit a better bioactivity by having a thick bunch of particles on their surfaces compared to the pristine. The phenomenon of apatite formation is highly based on surface negativity.[35] By comparing the data of implanted samples with the pristine, one can notice that the implanted samples are exhibiting better bioactivity in terms of having a thick bunch of particles on the surface. The reason behind this phenomenon can be the increase in roughness of the samples by the energetic He^+ ion. Further, on the ion implantation, the NH_3 ion converts into the NH_2 ion.[13] Thus, the number of negative ions increases on the surface leading to an increase in surface negativity; this can be one of the reasons for the increase in bioactivity.[36] The bioactivity of the pristine and implanted samples is compared. One can notice that the pristine samples show less bioactivity, whereas it is quite high and found to be increasing with time in the case of implanted samples.

Bioactivity is nothing but the ability of a material to form a bond with the tissues in the living body without causing toxic effects. Here, the in vitro bioactivity study has been carried out using simulated body fluid (SBF). The samples were immersed in SBF for 15 days, and the new layer of apatite was forming more rapidly and growing to the extent with an increasing fluence rate was noticed. Thus, one can say that the increase in bioactivity of the implanted samples compared to the pristine is observed. This can be explained by more roughness after implantation resulting in a more energetic surface or the implanted ions He^+ may obtain electrons from proline and glycine molecules of collagen and get neutralized. In this process, the proline and glycine are expected to become positively charged and attract PO_4^{3-} and OH^- ions from the SBF along with Ca^{2+} enhancing the formation of apatite. These several processes related to electronic loss or electronic interaction lead to an enhanced activity of HAP formation. This complex interaction may be responsible for the enhanced bioactivity in the SBF solution of the ion beam implanted samples.

3 | CONCLUSION

The Cg/HAP nanocomposites were synthesized by a surfactant-mediated approach. The elemental analysis confirmed the presence of protein content within the sample. The FT-IR studies confirmed the presence of functional groups related to the collagen and HAP phases. On He^+ ion implantation, the structural distortion took place, and as a result, a decrease in the crystallite size and crystallinity of the Cg/HAP nanocomposite was observed. Initially, for the lower ion fluence of He^+ ions, the roughness and particle size were found to be decreased. But, for

the highest fluence, the roughness and particle size were found to be increased. The morphological changes were caused by the rearrangement of particles in such a way that the growth rate was more in {10} facet instead of {11} due to the implantation of energetic He^+ ions and recrystallization occurring as its outcome. The SBF studies showed improvement in the bioactivity of the implanted samples compared to the pristine ones. The enhancement in the bioactivity of implanted Cg/HAP composite is explained based on electronic loss and electronic interaction through various processes making the surface of the composites charged leading to more surface deposition activity from SBF compared to the pristine one. Moreover, the surface roughness of the implanted samples enhances this.

ACKNOWLEDGMENTS

The authors are thankful to the Head, Department of Physics, for his support, the Director, IUAC, New Delhi, for the use of ion implantation, AFM and SEM facilities, Professor Pawan Kumar Kulriya from the School of Physical Sciences, Jawaharlal Nehru University, Delhi for the technical discussion, UGC, New Delhi, for the financial assistance under DRS-SAP and DST for the FIST, and the author (BVJ) for the UGC-SRF under the Meritorious Student's Scheme.

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How to cite this article: Jogiya BV, Tank KP, Joshi MJ. Enhancement of bioactivity of collagen–hydroxyapatite nanocomposite on He^+ ion implantation. Int J Ceramic Eng Sci. 2022;4:408–415. https://doi.org/10.1002/ces2.10163

Nelumbo *Vol 64 (1): (314-315) 2022*
DOI: 10.20324/nelumbo/v64/2022/168539

ISSN (Print) : 0976-5069
ISSN (Online) : 2455-376X

Microchloa R. Br. (Poaceae): A new generic record to the Flora of Gujarat, India

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माइक्रोक्लोआ आर.ब्र. (पोएसी): गुजरात के वनस्पति संपदा में एक नवीन वंशपरक अभिलेख

रोहित कुमार एम. पटेल, सुजीत कुमार आर. प्रजापति और कुमार विनोद सी गोसावी

सारांश

वंश माइक्रोक्लोआ आर.ब्र. का जाति एम. इंडिका (एल. एफ.) पी.ब्यूब. को साथ गुजरात से पहली बार संग्रह किया गया है जो इस राज्य के पादप संपदा के लिए एक नवीन वंशपरक अभिलेख है। इस शोधपत्र में *माइक्रोक्लोआ* इंडिका का आसानी से पहचान करने के लिए छायाचित्रों सहित विवरण प्रस्तुत किया गया है साथ ही इसके क्षेत्रात्मक वितरण का भी उल्लेख किया गया है।

ABSTRACT

The genus *Microchloa* R. Br. with a species *M. indica* (L.f.) P. Beauv. has been collected for the first time from the Gujarat state, which forms a new generic record to the flora of the state. The present paper provides a description, and photographs of *Microchloa indica* for easy identification and its distribution note in India.

Keywords: Genus, Gramineae, Gujarat, *Microchloa*, new record

INTRODUCTION

Genus *Microchloa* (tribe Chlorideae and subfamily Pooideae) comprises six species globally (Clayton & al., 2022). In India, the genus is represented by two species, namely *M. indica* (L.f.) P. Beauv. and *M. kunthii* Desv. (Bor, 1960; Kellogg & al., 2020; Prasanna & al., 2020; Nagaraju & al., 2021). Both species can be easily identified by annual and perennial habits (Potdar & al., 2012; Chorge & Prasanna, 2021).

During the floristic exploration of the Dahod district in Gujarat, authors came across an interesting grass species, which was collected for further identification. After a critical examination of characters, consultation of literature its identity was confirmed as *Microchloa indica* (L.f.) P. Beauv. The species was previously reported from Andhra Pradesh, Bihar, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Odisha, Tamil Nadu and West Bengal, however the genus has not so far been reported from Gujarat state (Shah, 1978; Raghavan & al., 1981; Jani, 2014), making this the first record for the state. Thus, in the present communication detailed description, photos and distribution of *Microchloa indica* (L.f.) P. Beauv. is given below.

TAXONOMIC TREATMENT

Microchloa indica (L.f.) P. Beauv., Ess. Agrost. (1812); Bor, Grass. Burma, Ceylon, India, Pakistan 473.1960; Bhattacharya (Sunanda Moulik), Grasses Bamboos India 2: 569. 1997; Prasanna & al., Poaceae in Mao & Dash (eds.) Fl. Plants of India- an annotated checklist – Monocotyledons 395. 2020. *Nardus indica* L.f., Suppl. Pl. 105. 1782. *Microchloa setacea* R. Br., Prodr. Fl. Nov. Holland 208. 1810. (Fig. 1)

Annual. Culms terete, 5–15 cm high, erect; nodes glabrous. Leaf sheaths glabrous or hairy, 1 –1.5 cm long; ligule ciliate, membranous; leaf-blades linear, 1–3 cm long, glabrous, acute at apex, margins serrulate. Inflorescence linear, slender, curved secund spike, 3–6 cm long; peduncle filiform, 2–5 cm long. Spikelets sessile,

Submitted : 19.02.2022 Accepted : 25.06.2022 Date of Publication 31.07.2022

Fig. 1: Habit of *Microchloa indica* (L.f.) P. Beauv.

oblong-lanceolate, 2–2.3 × 0.4–0.5 mm; callus bearded. Lower glume oblong-lanceolate, 2–2.2 × 0.4–0.5 mm, acute at apex, chartaceous, 1-nerved, margins infolded. Upper glume narrowly ovate-elliptic, 2–2.2 × 0.4–0.5 mm, membranous, glabrous, margin inflexed, 1-nerved, apex acute. Lemma elliptic-oblong, 1.5–1.8 × 0.4–0.5 mm, chartaceous, 3-nerved, densely hairy on dorsal side, margins incurved, acuminate at apex. Palea narrowly elliptic-oblong, 1.3–1.5 × 0.2–0.3 mm, apex 2-toothed, hyaline, 2-nerved, 2-keeled; keels ciliate. Lodicules 2, membranous. Stamens 3. Caryopsis 0.8–1 × 0.3–0.4 mm, elliptic-oblong.

Flowering & *Fruiting*: August-October.

Habitat: Open arid barren uplands and rocky gravelly soil.

Specimen examined: INDIA: Gujarat, Dahod district, Zabu, 22° 40' 17" N, 74° 09' 25" E, *Rohit Patel* and *Sujit Prajapati* GSCD006526/11/2021 (Government Science College, Dhanpur)

Distribution: INDIA: Andhra Pradesh, Bihar, Gujarat (present study), Karnataka, Kerala, Madhya Pradesh, Maharashtra, Odisha, Tamil Nadu, West Bengal; SOUTH-EAST ASIA, EAST AND WEST AFRICA, AUSTRALIA and introduced in SOUTH AMERICA.

ACKNOWLEDGEMENTS

The authors are thankful to Baria division, Gujarat Forest Department, for permission to undertake the study. Authors are also grateful to Dr. Y. S. Patel, Principal, Government Science College, Limkheda, and Dr. Pankaj Solanki, Principal, Government Science College, Dhanpur, for providing facilities to undertake this work.

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Open Access
Volume 5 Issue 1
Research Article

Journal of Soil Science and Plant Physiology
ISSN: 2694-6157

PUBTEXTO

Changes in Phytohormones Levels in Normal and *Ochrobactrum anthropi* Infected Almond (*Prunus dulcius*) Seeds: A Novel Mechanism of Plant Microbe Interactions

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Article Info

Article History:
Received: 09 January, 2023
Accepted: 14 January, 2023
Published: 19 January, 2023

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DOI: https://doi.org/10.36266/JSSPP/158

Abstract

In this experiment, normal and infected almond seeds were tested for germination and the estimation of endogenous hormonal changes *in vivo* and the obtained results are confirmed with *in vitro* data. The almond seeds were separated into 7-9 groups according to their size. All selected categories of seeds were studied for changes in their endogenous hormones (IAA, PAA, GA, ABA) level using antibodies raised against each. It was observed that IAA and PAA levels remained relatively higher in infected seeds compared to normal seeds. Plant pathogens reduced crop yield significantly by damaging cell metabolism and functions using various mechanisms in the host plants. From the infected seeds, the bacterium was isolated and identified by 16S rDNA technique as *Ochrobactrum anthropi*. In infected seeds, GA and ABA levels dropped down and in some groups it was undetectable. *In vitro* assay was performed using almond seed and various concentrations of *O. anthropi* to confirm the relationship between degree of seed infection and changes in the endogenous hormonal levels. The results suggest that hormone conjugates hydrolytic properties of the pathogen regulates the concentrations of the endogenous growth hormones in infected almond seeds.

Keywords: Almond, Homones; IAA; *Ochrobactrum anthropi*; PAA

Abbreviations

IAA – Indole acetic acid, PAA – Phenyl acetic acid, GA – Gibbrellic acid, ABA –Abscisic acid, DMF –Dimethyl formamide, DEAE–Diethyl aminoethyl cellulose.

Introduction

Almond *Prunus dulcis* L. belongs to the family Rosaceae, is an economically important plant for its soft embryo. It is one of the oldest nut crops of the world with many pharmaceutical and commercial values [1]. Phytohormones play a key regulatory role in the seed growth and development [2, 3]. Phytohormones are responsible for development of sink size in *Cajanus cajan*, soybean and rice seeds [4,5]. In these studies, it is observed that auxins participate in cell number and cell size regulations while ratio of GA and ABA is important for net accumulation of dry matter in the seed. However, no such reports are available for almond seed.

Further, substantial decrease in yield of almond seeds is reported due to pathogens which ultimately lead to economic loss. *Botryosphaeria dothidea* is causing band canker in almond fruit and *Alternaria* species is causing leaf spot of almond [6,7]. The presence of bacterial pathogen in almond seeds is rather scanty, we have observed it in contaminated almond embryo. To understand the mechanism of pathogenecity of the organism is the important target for the researcher to reach the solution. Bacterial pathogens affect crop yield about 15% using various mechanisms. A set of genes expressed by pathogens, synthesized array of proteins; and are categorize in to type I - IX secretion systems [8]. Among these the type II secretion system (T2SS) in gram-negative bacteria function for secretion of diverse toxins, surface associated virulence factors, wide range of hydrolytic enzymes that hydrolyze macromolecules of the host cells and helped pathogen in infection. Many workers has reported proteins that hydrolyze polysaccharides [9-12]; however, the reports on hormones hydrolyses are rather scanty. In this study, we report a novel mechanism that hydrolyze plant hormone conjugates and released free form of the hormones.

Planting of infected seed may result in a widespread distribution of the disease within the crop and an increased number of initial infection sites from which the disease can spread. We have isolated and identified bacterium from infected seed material. In many cases the infected seeds are not able to germinate due to metabolic disturbance. We have checked fluctuation in endogenous hormonal level in vivo due to infection and compared it with the normal seeds. For estimation of endogenous hormones, antibody against each hormone is developed previously is used for the immunoassay. To confirm the obtained results we have done in *vitro* analysis. Presence of hydrolytic enzymes in pathogen is known [13], we first time report here for hormone conjugates hydrolysis which changes hormonal levels in the almond seeds.

Materials and Methods

Collection of Almond Seeds

The almond seeds were purchased from the local market are washed thoroughly under running tap water and blotted dry. The embryo was dissected from the seeds and tough seed coat removed. Amongst these seeds, about 10% were infected. Each individual embryo was weighed and on the basis of size and weight variations, the seeds were classified into seven or nine groups (Table 1). Each categories of groups, contained minimum 10 seeds.

Table 1: *Variation in size of normal and, infected almond seeds.*

Almond seed size group	Normal seed weight (gm.)	Infected seed weight (gm.)
G1	0.5-0.7	0.7-0.8
G2	0.7-0.8	0.9-1.0
G3	0.8-0.9	1.0-1.1
G4	0.9-1.0	1.1-1.2
G5	1.0-1.1	1.2-1.3
G6	1.1-1.2	1.3-1.4
G7	>1.2	1.4-1.5
G8	-	1.5-1.6
G9	-	>1.6

Raising of Antibodies against PGRs

The specific antibodies against each hormone were raised previously are used in this study [4]. Fresh PGR-casein conjugate for each hormone was prepared according to Monpara et al. and used in the assay [5].

In vivo Extraction of Hormones from the Almond Seeds

All categories of the seeds with different weight groups were separately crushed with liquid nitrogen. From the frozen samples 500mg powder was mixed with 5 ml of 80% methanol containing 100 mg ascorbic acid as an antioxidant. The mixture was incubated for 48h in dark. The mixture was centrifuged at 10,000 g for 10 min and supernatant was collected. Pellets were washed twice with 80% methanol and pooled supernatant was collected and kept for evaporation in dark. Final volume of the samples (10 ml) was prepared with phosphate buffer saline (pH 7.2) and directly used for the estimation of IAA, PAA, GA KiN and ABA.

Estimation of Endogenous Hormones from Normal and Infected Almond Seeds

Endogenous level of hormones viz. IAA, PAA, KiN, GA and ABA were estimated by a comparatively more sensitive and specific technique i.e. indirect ELISA. PGR-casein conjugate (300 µl) was coated on ELISA plate and incubated overnight at 4ºC, followed by washing with PBS-T. The next step involved was blocking of free protein binding sites of well with egg albumin and incubated for 1h at 37ºC. Antibodies against each PGRs mixed with samples were coated and incubated for 3h at 37ºC. Finally, the plate was coated with anti-Rabbit IgG, tagged with peroxidase and the color was developed using O- phenylene diamine as a substrate. The reaction was terminated by addition of 1M sulfuric acid (50 µl). After each coating, the ELISA plate was washed thoroughly with PBS containing 0.05 % tween-20.

In vitro Estimation of Endogenous Hormones

For the extraction of hormones, five hundred mg of dry seed powder was taken and mixed with five different concentrations of activated bacteria (1.0 to 5.0 ml broth culture OD 0.6, at 660nm) in a final volume 5ml. The mixture was incubated at room temperature for 48 hr. in dark. Equal volume of 80% methanol was added to this mixture and samples were centrifuged at 10,000g for 10 min for extraction of hormones. Supernatant was collected and kept in dark for methanol evaporation. From the evaporated samples final volume (5 ml) was prepared with phosphate buffer saline, pH 7.2. Estimation of the released hormones was performed by indirect ELISA as mentioned above. Endogenous level of hormones estimated from the seed powder was expressed, as µg/gm of dry seed powder. Control was prepared without inoculation with bacteria. Calibration curve of IAA, PAA, GA, KiN and ABA was plotted with standard in a range of 50-500 ng/well. In addition, the samples with internal standard (approx.50ng) used for sensitivity test and assay corrections. All assays are performed in triplicates and mean values are calculated.

Identification of Bacteria from Infected Seeds

The bacteria were initially identified on the basis of biochemical tests and sugar fermentation behavior as described in Bergy's Manual of Determinative Bacteriology and 16s rDNA sequencing was perform for molecular identification.

DNA Extraction from the Bacteria

Bacteria were isolated from the infected almond seeds and identified by 16S rDNA sequencing. Bacteria were inoculated with Nutrient broth and grown overnight on shaker. 1.5 ml culture was taken in 2.0 ml tube and centrifuged at 12,000 g for 10 min. Bacterial cells were re-suspended in 500 µl of 10mM Tris- EDTA buffer, and treated with 30µl SDS (10% W/V) and 2µl Proteinase K (10 mg/ml). It was mixed well and incubated for 1 h at 37ºC. Then 20µl of Cetyltrimethyl ammonium bromide (10%, W/V) and 100µl of NaCl (5M) were added, and incubated for 10 min at 65ºC. DNA was purified by two 1:1 extractions in which Chloroform: Isoamyl alcohol (24:1) and Phenol: Chloroform: Isoamyl alcohol (25:24:1) were used. The mixture was then precipitated with isopropanol, washed with ethanol (70%), and dissolved in Tris-EDTA buffer. The quality and concentration of the DNA was confirmed by measuring optical density 260/280 (nm) ratio.

16S rDNA Gene Amplification

The 16S rDNA gene was amplified using universal primer pair 8F (5'-AGAGTTTGATCCTGGCTCAG- 3') and 1525R (5'-ACGGCTACCTTGTTACGAC-TT-3'. PCR reaction: A master

mix solution consisting of 2.5µl 10X buffer (10mM Tris-HCl pH 9.0, 50 mM KCl, 0.1% Triton X-100), 1.5 mM MgCl2, 200 µM each deoxynucleoside triphosphate, 10µM primer and 1U of Taq DNA polymerase, 200ng bacterial DNA, in total volume of 25 µl was prepared. Profile of PCR was: initial denaturation 95ºC - 5 min, followed by 35 cycles: denaturation 95ºC - 30 s, annealing 52ºC - 45 s, extension 72ºC - 2 min and final extension 72ºC - 12 min. Amplified DNA fragments were separated by electrophoresis through 1.5% low melting agarose gel. DNA fragments were eluted from low-melting temperature agarose gels. The band of interest was excised with a sterile blade, placed in a microcentrifuge tube, frozen at -20ºC, and then melted. TE-saturated phenol was added to the melted gel slice and the mixture was again frozen and then thawed. After this second thawing, the tube was centrifuged and the aqueous layer removed to a new tube. The DNA was concentrated by ethanol precipitation.

Sequencing of 16s rDNA Gene

The eluted PCR products were sequenced using a Big Dye Terminator V3.1 Cycle Sequencing Kit using ABI 3130 genetic analyzer. The sequencing reaction required 1µl of Premix, 10 pmol of sequencing primer and 200ng of the PCR product template in a total volume of 10 µl. 16S rDNA partial sequence was determined using 8F and 1525R sequencing primers. All sequencing reactions were performed using the VeritiTM Thermal Cycler with 45 cycles of denaturation (95ºC - 30 s), annealing (52ºC - 20 s) and extension (60ºC - 4 min).

Nucleotide Sequence Accession Number

The nucleotide sequences determined in this study have been submitted to the NCBI Gen Bank database (JF816283).

Results and Discussion

In the present study, infected almond seeds failed to germinate, unlike normal (Figure 1).

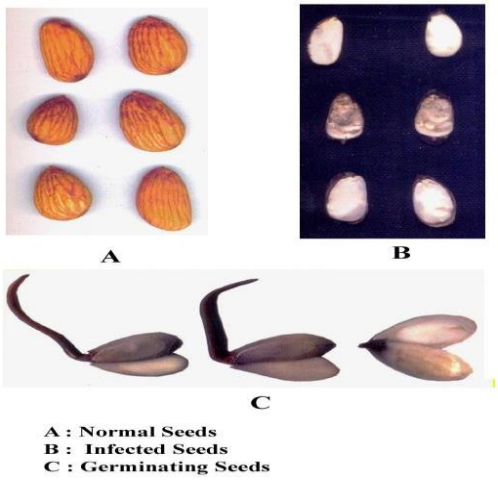

Figure 1: *Variation in almond seeds.*

Seed germination and dormancy are complex physiological processes that are controlled by a range of developmental and external cues [14,15]. The plant growth regulators i.e. auxins, gibberellins (GAs), and abscisic acid (ABA) play an important role in the germination processthat leads to mature fruit and viable mature seeds production [2,3,16]. Changes in endogenous levels of hormones showed differences in normal, and infected seeds. IAA, PAA, GA and ABA were detected in all groups of normal seeds (Figure 2a, 2b, 2c and 2d).

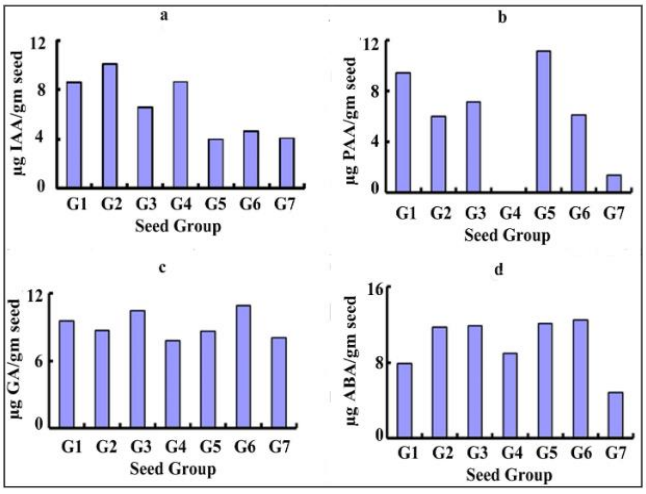

Figure 2*: Changes in IAA (a), PAA (b), GA (c) and ABA (d) in normal almond seeds.*

Notably, in infected seeds both the forms of auxin; IAA and PAA were five to ten times higher as compared to normal seeds (Figure 3a, 3b); Whereas changes in GA and ABA levels were not observed in all groups of infected seeds (Figure 3c, 3d).

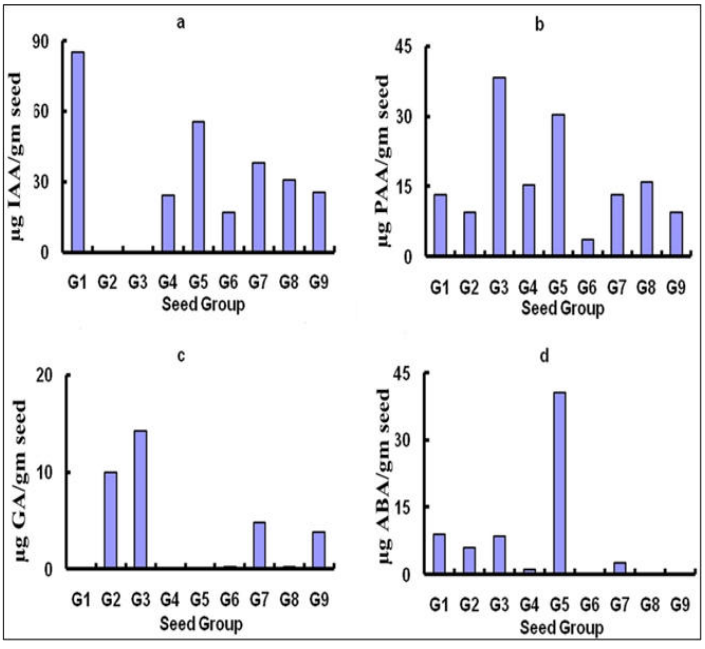

Figure 3: *Changes in IAA (a), PAA (b), GA (c) and ABA (d) in infected almond seeds.*

Extracellular pathogens and proteobacteria harbor a group of proteins such as phospholipases, cellulases, pectinases, proteases, lipases, and toxins are included in type II secretion systems which

cause cell damage and disease of the plant. The infected material damaged gradually followed by controlled secretion of virulence factors occurs with increased bacterial growth at appropriate site of the host [17]. Hormone conjugation with sugars, amino acids, peptides and proteins, and food reserveis documented [18-20]. It was interesting to notice that IAA and PAA levels remained excessively higher in infected seeds compared to normal seeds. In infected seeds there might be conjugated forms of auxins which are released due to infection. In plants, endogenous hormones are present in three forms; free, conjugated andoxidized. Conjugate, the addition of low molecular weight compounds, represents a mechanism to regulate the cellular level of 'active' hormones by generating products with low biological activity [21]. Seeds contain phytohormones in conjugated form [22] which are released during germination [23].

It was observed that during seed germination, due to infection, the pathogenic bacteria are changing the pattern of nutrient translocation within the seed, and causing net influx of nutrients, to satisfy their demands [24]. Thus, infection site becomes a strong metabolic sink. Plant hormones can be considered to be important signals in establishing relationships between plant and microbes [25]. Hydrolases are the enzymes which release free hormones from the conjugated form and thus are likely to play an important role in regulating free hormonal levels. These hydrolases have been detected inbacteria and in variety of plants [26,27]. Several reports stated that other phytohormones (IAA, GA and zeatin) are produced by various bacteria living in association with plants [28]. To address this, the organisms were isolated from the infected seeds and identified. From the 16S rDNA techniques it was found that the infection in the almond seed was due to *Ochrobactrum anthropi*. *O. anthropi* is recognized as an emerging opportunistic pathogen, although relatively little is known about its pathogenesis and factors contributing to its virulence [29,30]. Ubiquitous gram-negative *Ochrobactrum* strains are widely distributed in soils and aqueous environments, where they biodegrade aromatic compounds [31], organophosphorus pesticides [32], and other hydrocarbons [33] and remove heavy metal ions such as chromium and cadmium [34].

The present study showed the release of conjugated form of hormones by *O. anthropi* might have disturbed the hormonal status in the almond seeds and thus failed to germinate. The presence of iodole- amide conjugate hydrolyzing enzyme from the *Enteobacter agglomerans is* reported [26,35]. This indole-3-acetyl-L-aspartic acid hydolase hydrolysed IAA-L-Asp conjugate only and had very high substrate specificity. Similarly, if the pathogen is known it may help to understand the mechanism of the pathogenecity in almond host. To confirm the obtained results, *in vitro* experiment was performed to analyze changes in endogenous hormones by *O. anthropi*. *In vitro* experiments showed that almond seed powder treated with *O. anthropi* showed larger amounts of IAA, PAA, GA and ABA compared to control (Figure 4).

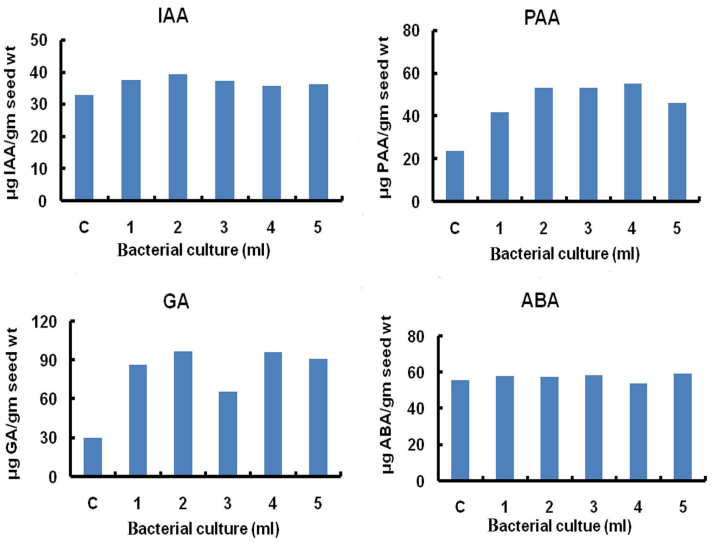

Figure 4: *Conjugated IAA, PAA, GA and ABA released by Ochrobactrum anthropi C - control sample indicates free form of IAA, PAA, GA and ABA, 1 - 5 indicates conjugated form of IAA, PAA, GA and ABA.*

This result showed that bacterial cells have ability to release conjugated form of IAA, PAA, GA and ABA. Control sample showed only free form endogenous hormones while test samples showed amount of conjugated seed hormones released by the bacteria also (Figure 4). The difference between control and test sample remained higher in GA and PAA. These results showed that *O. anthropi* possessed hydrolytic enzyme(s) that might release conjugated hormones from the seed powder during the treatment. From the results we conclude infection by *O. anthropi* in almond seed may inhibit seed germination by mechanism ofchanging the hormonal status in the plant and the organism has capacity to hydrolyze the conjugated formof hormones in the seed powder.

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Journal of Applied Sciences

ISSN 1812-5654

science alert

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OPEN ACCESS Journal of Applied Sciences

ISSN 1812-5654
DOI: 10.3923/jas.2023.87.94

Research Article

Functional Characterization and Shelf-Life of Freeze-Dried Cells of *Lactobacillus rhamnosus* Fb in Carrier Media and Chocolate Formulations

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Abstract

Background and Objective: Probiotics are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host". The present study focused on the characterization of diverse formulations prepared with freeze-dried *Lactobacillus rhamnosus* Fb and evaluation of their shelf life along with their functionality and viability. **Materials and Methods:** Survival of *L. rhamnosus* Fb cells freeze-dried with or without carrier medium in simulated gastrointestinal conditions and during storage at 4°C was evaluated. Moreover, freeze-dried cells were added to the chocolate mixture. Formulated chocolates were evaluated for antigenotoxic activity against 4-NQO and MNNG using SOS-Chromotest. Statistical differences among the mean viability values were analysed by One-Way Analysis of Variance (ANOVA). **Results:** Experimental evidence implicated that the carrier medium during the freeze-drying process did not exert any significant influence on the viability of the cells, but during storage, help to preserve the viability and probiotic activity. The combination of skim milk with sucrose or lactose as a carrier medium helped in the retention of $\geq$80% viability during 4 months of storage. Freeze-dried cells (7.13 log CFU g^{-1}) mixed in chocolate, remained viable in simulated gastrointestinal conditions. New insight of the chocolate formulation containing *L. rhamnosus* Fb is the reduction of genotoxicity of food-borne mutagens (directing-acting carcinogens/mutagens) 4-nitroquinoline-1-oxide (81%) and N-methyl-N'-nitro-N-nitrosoguanidine (68%), evaluated using SOS-Chromotest. **Conclusion:** Hence, freeze-dried cells retained viability and functional activities, therefore, promoting the use of viable freeze-dried cells in the formulation of health-promoting indigenous functional food products.

Key words: Antigenotoxic activity, chocolate, freeze-drying, *Lactobacillus rhamnosus*, MNNG, 4-nitroquinoline-1-oxide, probiotics

Citation: Pithva, S., P. Ambalam, J. Dave and B.R.M. Vyas, 2023. Functional characterization and shelf-life of freeze-dried cells of *Lactobacillus rhamnosus* fb in carrier media and chocolate formulations. J. Appl. Sci., 23: 87-94.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 25, No. (1) : 2023 : 124-129

ISSN-0972-3005

DOI No.: http://doi.org/10.53550/AJMBES.2023.v25i01.019

PRODUCTION OF BIOACTIVE COMPOUNDS BY *GEOTRICHUM CANDIDUM*

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(Received 12 August, 2022; Accepted 9 October, 2022)

Key words: *G. candidum, GABA, Prebiotics, Cholesterol, Enzymes, Bioactive compounds*

Abstract–The focus of the present study was to evaluate the production of bioactive compounds from *G. candidum*. *G. candidum* is used as a starter culture in dairy industry for cheese ripening. *G. candidum* produces various beneficial enzymes like amylase, lipase, gelatinase, glutaminase, L-asparaginase and glutamate decarboxylase. Furthermore, strain showed prebiotic utilization, GABA and cholesterol lowering activity. *G. candidum* can be used to prepare cheese that will confer additional benefit to the consumers.

INTRODUCTION

Bioactive compounds are produced by a wide range of microorganisms (Shukla, 2015), but currently, just a few of these possibilities are known. *Galactomyces geotrichum*, often known as *G. geotrichum*, is a little-known mold that is employed as a starter or nonstarter culture in the manufacturing of numerous cheeses around the world (Chaves-López *et al.,* 2017). According to the literature, *G. geotrichum* can produce peptides that inhibit the angiotensin I converting enzyme (Grygier *et al.,* 2017).

GABA is a nonprotein amino acid that acts as a major inhibitory neurotransmitter in the mammalian central nervous system (Schousboe & Waagepetersen, 2007). Furthermore, GABA has hypotensive, calming, and diuretic properties and can help prevent diabetes (Li and Cao, 2010). L-asparaginase is a clinically acceptable anti-cancer agent that has been used in combination with other agents in the treatment of acute lymphoblastic leukaemia (mainly in children), reticle sarcoma, Hodgkin disease, acute myelocytic leukaemia, acute myelomonocytic leukaemia, chronic lymphocytic leukemia, lymphosarcoma and melanosarcoma chemotherapy (Abbas *et al.,* 2010). Lipases are used in a variety of industries, including food, degreasing formulation, dairy, medicine, detergents, and fine chemical synthesis (Gupta *et al.,* 2004). *G. candidum* lipase research is limited; however, it is of particular importance due to its vast industrial demand and use in the food sector (Kocabiyik and Ozel, 2007).

The focus of the present study was to evaluate the production of bioactive compounds from *G. candidum*. Various enzymes like amylase, lipase, gelatinase, glutaminase, asparaginase and glutamate decarboxylase, furthermore prebiotic utilization, antimicrobial activity and antibiotic susceptibility. The potential of *G. candidum* to biosynthesize GABA and cholesterol lowering activity was also studied.

MATERIALS AND METHODS

Yeast strain

G. candidum, proprietary strain Gc2 of Enformtech, New Zealand, was grown on glucose, peptone, and yeast extract (GPY) agar plates and incubated at 30°C for 24 h. Activated culture was inoculated in 100 mL GPY medium, incubated at 30°C for 24 h and used for biomass production.

Cholesterol removal assay

The method of Gilliland *et al.* (1985) was used to estimate cholesterol removal by *G. candidum.*

Indian Forester, **148**(9) : 924-930, 2022
DOI: 10.36808/if/2022/v148i9/166036

ISSN: 0019-4816
eISSN: 2321-094X

Status and Grass Species Diversity of Bandheli

Reserve Grassland of Panchmahal District in Gujarat State, India

In Gujarat, a total of 8,490 km^2. area under grassland falls in the eight districts. However, a systematic inventory of grassland is not yet done in the state. The present study deals with the systematic inventory of grass species diversity and characterization of Bandheli Reserve Grassland. As a result of the present investigation, a total of 60 grass species have been reported. Among the recorded 60 grass species, 18 were highly palatable, 22 are moderately palatable, nine are least palatable, and 11 are unpalatable. This entire grassland is divided into major types based on the dominant grass and its composition. This zonation will be used in its management and improvement. This grassland facing threats of converting into woodland by tree growth, i.e., out of 754.04 ha, 304 ha area covered by trees and around 100 ha is degraded. The zonation map will be helpful in the management and improvement of this grassland.

Key words: Bandheli, Grass species, Species composition, Threats

Bandheli Reserve Grassland is spread over a 754.04 ha area and supports 60 grass species with high productivity. However, it faces the threat of converting grassland into woodland.

Introduction

The natural and semi-natural pastureland, rangeland, scrubland, and steppe configuration are dominated by grasses, and grass-like plants called the Grassland ecosystem (Blair *et al*., 2014). Such an ecosystem covered approximately 3.5 billion ha in 2000, representing 26% of the world's land area and 20% of the world's soil carbon stocks (FAOSTAT, 2009; Ramankutty *et al*., 2008). This ecosystem has closely co-evolved with grazing ungulates and plays a significant role in farming history (Stebbins, 1981). The grassland ecosystem provided various fundamental goods and services, including food and forage, climate regulation, biogeochemical cycles, erosion prevention, carbon storage, and cultural services. It is the backbone for the pastoral communities (Ramankutty *et al.*, 2008; White *et al.*, 2000 and Bengtsson *et al.,* 2019). The grassland-based income generation activities like milk production and beef contribute 27% and 23%, respectively, indicating its significant role in the economy by providing livelihood to about 1 billion people (FAO, 2006). Various cereals such as wheat, rye, maize, rice, millets, sorghum, *etc.,* are crucial to human beings' survival from wild grassland components. Besides, it forms an essential habitat for breeding, 0igrating, and wintering birds, ideal conditions for many soil fauna, and rangelands for wild herbivores (Verma and Prakash, 2007).

In the context of India, Grassland constitutes one of the major biomes. India's grassland ecosystem is categorized into five major types (Dabadghao and Sankaranarayan, 1973), in which *Sehima-Dichanthium* and *Dichanthium-Cenchrus-Lasiurus* type are found in Gujarat state. In India, approximately 30 to 43 million pastoralists are wholly dependent on livestock and grasslands for their livelihood (Rahmani, 1992). In Gujarat, the grassland area extended to 8,490 km^2 (4.33%) of the total geographical location. This area falls under the eight districts with a majority (41.23%) spread in Kachchh (SAC, 2002). Despite the enormous ecological and economic significance, there has not been any systematic

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Received August, 2021
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inventory or characterization of a grassland ecosystem in the country or the state of Gujarat (Dixit *et al*., 2001).

Wildlife Institute India, ENVIS center on wildlife and protected areas published a bulletin on the grassland habitats of India (Rawat and Adhikari, 2015), the factors affecting ecology and management was discussed, especially on the disappearance of native and palatable grass species by exotic and non-palatable species (Vijay *et al.,* 2015). It is observed that habitat alteration by the change in species composition is a prominent phenomenon in the deterioration of the grassland ecosystem. Hence documentation of species diversity of grassland has become most crucial for planning and management perspective. Therefore, here is an attempt to document the grass species diversity, demarcation of the degraded area, species composition-based zonation, and suggest the management and improvement strategies for Bandheli reserve grassland.

Material and Methods

Study area

The Bandheli Grassland falls under the Godhra forest division of Gujarat state spread area an area of 754.04 ha, surrounded by Segwa, Vansia, Bakhkhar, Dumelav, Kevadia, Dholi, and Kanajia villages.

Bandheli grassland is one of the potential reserve grasslands of the state, having an average of 11-12 lakh kg per annum, and last year in 2019-20, it was 14.10 lakh kg productions. The climate of this district is characterized hot in summer and not much severe cold in winter. The number of rainy days and rainfall is very irregular and erratic. The temperature ranges from 10-45^{0}C while last year, Godhra received an 897 mm rainfall, which was reasonable compared to its average, which varies from 400 mm to 800 mm.

The general vegetation composition and type are *Cymbopogon-Themeda-Heteropogon* dominated by *Cymbopogon martini, Themeda triandra, T. quadrivalvis* and *Heteropogon contortus*. In individual pockets, highly palatable grasses are like *Dichanthium annulatum, D. coricosum*, and *D. foveolatum* also found. It is crucial to note that the composition found is to be in a transitional stage of succession due to unavoidable natural and human-induced factors.

Methods

The detailed methodology used for the present study is as follow:

Collection and identification of grass species: Hence, the present study focuses only on the grasses of Bandheli Grassland; we collected maximum grass specimens during their active growth period, *i.e.*, September to November 2020, by making several trips. We collected all the required specimen for further analysis in polythene bags and marked them with field numbers. We also took enough photographs of the same. Collected grass specimens were appropriately dried and processed with blotting papers and newspapers for future analysis. Herabrium of the all the collected specimen were prapered and deposited at Herbarium of Government Science College, Dhanpur. The collected grass specimens were critically diagnosed for features, described, and photographed. The crucial part of grass to establish their identity, spikelets were dissected carefully and identified with available literature. The important floras used for identification were "The Bombay Grasses" (Blatt. and McCann., 1935), "Grasses of Burma, Ceylon, India, and Pakistan" (Bor, 1960), "The Flora of the Presidency of Bombay, Vol. III" (Cooke, 1901-1908), "Flora of Gujarat state" (Shah, 1978).

Mapping of grass species for Zonation: The entire grassland area dominated by the four grass species *viz*. *Cymbpogon martini*, *Themeda triandra*, *T. quadrivalvis* and *Heteropogon contortus.* Hence it is planned to divide into the various zones based on the above four dominant species. With the Range forest officer and other frontline staff, the entire area was explored to mark the zone based on the dominant species. We used the circular route and other *Kachchh* roads passing through it to prepare for the monitor, management, and protection in this zonation activity. The large-sized image was captured from online open-source, Google Earth, and prints on A2 size paper. The printed image was kept with us while exploring the entire area and mark at different levels. A total of around 100 points were checked for species composition. Later finalized the map of different zone based on the dominant species. The Range Forest Officer also provided his technical inputs in preparing the map of zonation.

Further, macro-level land use cover with the help of the satellite imageries of 4th Oct. 2020 was also classified under four major land use cover categories *viz.* grass cover, tree cover, water, and open area.

Results and Discussion

Grass species diversity in the grassland ecosystem is the key components to measure the its health. This ecosystem provides various good and services to maintain the natural balance as well as well being of humankind, which includes food and forage, climate regulation, biogeochemical cycles, erosion prevention, carbon storage, and cultural services (Ramankutty *et al.*, 2008; White *et al.*, 2000 and Bengtsson *et al.,* 2019). Focusing on the significance of balancing nature, it is crucial to maintain its ecological integrity for sustainable development. Results of the present investigation discussed as follow:

Diversity of Grass species in Bandheli Grassland

Based on the floristic enumeration, it was found that the Bandheli Grassland is rich in grass species diversity. The first known systematic enumeration was done during the preparation of working plan-2008 by S. N.

Tyagi, and later Gandhi studies the grasses for its morpho-anatomical characterization in 2018. The present study is also documented the grass species diversity. In working plan-2008, 35 grass species were reported, while Gandhi (2018) said 48 grass species from Bandheli. During the present investigation, 60 grass species have been reported (Table 1 and Annexure 1).

Table 1: No. of Grass species recorded by different workers

S.No.	Name of Author	Year	No. of grass species recorded
1	S.N. Tyagi	2008	35
2	Dhara Gandhi	2018	48
3	Patel and Meena	2021	60 (Present study)

A cumulative account of species diversity based on the above three workers records, a total of 82 grass species have been reported from 2008 to 2020. Out of the total reported 82 species, Nine (9) species were mentioned in the Working plan, which was not reported later by two researchers. Gandhi (2018) and by the present study, a total of 10 and 12 new grass species have been added to grass diversity of the study area. It shows the grass species richness of Bandheli, which is very high concerning the area of extend (*i.e.,* 754.04 ha) compared to other state and nation grasslands.

Among the recorded 60 grass species during the present study, 18 species are highly palatable, 22 are moderately palatable, and nine are least palatable. A

Annexure 1: List of grass species reported and its palatability of Bandheli grassland

S.No.	Species Name	Working Plan (2008)	Dhara (2018)	Present study (2020)	Palatability
1	*Acrachne racemosa* (B.Heyne ex Roth) Ohwi		•	•	Moderate
2	*Alloteropsis cimicina* (L.) Stapf		•	•	Moderate
3	*Apluda mutica* L.	•	•	•	Low
4	*Aristida adscensionis* L.	•	•	•	Un-palatable
5	*Aristida funiculata* Trin. & Rupr.	•	•	•	Un-palatable
6.	*Aristida redacta* Stapf			•	Un-palatable
7	*Arthraxon lanceolatus* (Roxb.) Hochst.	•	•	•	Moderate
8	*Bothriochloa pertusa* (L.) A.Camus	•	•	•	High
9	*Brachiaria eruciformis* (Sm.) Griseb.		•		High
10	*Capillipedium huegelii* (Hack.) A.Camus		•		Moderate
11	*Cenchrus biflorus* Roxb.			•	High
12	*Cenchrus ciliaris* L.	•	•	•	High
13	*Cenchrus setigerus* Vahl.	•			High
14	*Chloris barbata* Sw.	•	•		Low
15	*Chloris virgata* Sw.		•	•	Moderate
16	*Chrysopogon fulvus* (Spreng.) Chiov.	•		•	High
17	*Coix lachryma-jobi* L.	•			High
18	*Cymbopogon martini* (Roxb.) W.Watson	•	•	•	Low
19	*Cynodon dactylon* (L.) Pers.	•	•	•	High
20	*Dactyloctenium aegyptium* (L.) Willd.	•	•	•	High
21	*Dactyloctenium scindicum* Boiss.			•	Moderate
22	*Desmostachya bipinnata* (L.) Stapf		•	•	Un-palatable
23	*Dichanthium annulatum* (Forssk.) Stapf	•	•	•	High
24	*Dichanthium caricosum* (L.) A.Camus	•		•	High
25	*Dichanthium foveolatum* (Delile) Roberty		•	•	High
26	*Digitaria abludens* (Roem. & Schult.) Veldkamp		•	•	Moderate
27	*Digitaria ciliaris* (Retz.) Koeler	•	•	•	Moderate
28	*Digitaria longiflora* (Retz.) Pers.			•	Moderate
29	*Dinebra retroflexa* (Vahl) Panz.	•			Un-palatable
30	*Echinochloa colona* (L.) Link	•	•	•	Moderate
31	*Echinochloa crusgalli* (L.) P.Beauv.		•		Moderate
32	*Eleusine indica* (L.) Gaertn.		•	•	High
33	*Eragrostiella bachyphylla* (Stapf) Bor		•		Moderate
34	*Eragrostis cilianensis* (All.) Janch.			•	Moderate
35	*Eragrostis ciliaris* (L.) R. Br.			•	Moderate
36	*Eragrostis pilosa* (L.) P.Beauv.		•		Moderate
37	*Eragrostis tenella* (L.) P.Beauv. ex Roem. & Schult.	•	•	•	Moderate
38	*Eragrostis tremula* Hochst. ex Steud.			•	Moderate
39	*Eragrostis viscosa* (Retz.) Trin.		•	•	Moderate
40	*Eriochloa procera* (Retz.) C.E.Hubb.		•	•	Un-palatable

S.No.	Species Name	Working Plan (2008)	Dhara (2018)	Present study (2020)	Palatability
41	*Heteropogon contortus* var. *contortus* sub var. *genuinus* Domin	•	•	•	Moderate
42	*Heteropogon contortus* var. *contortus* sub var. *typicus* Domin	•	•	•	Moderate
43	*Heteropogon ritchiei* (Hook.f.) Blatt. & McCann		•	•	Moderate
44	*Imperata cylindrica* (L.) Raeusch.		•		Un-palatable
45	*Ischaemum molle* Hook. f.	•			High
46	*Ischaemum pilosum* (Willd.) Wight	•			High
47	*Iseilema laxum* Hack.	•		•	High
48	*Melanocenchris jacquemontii* Jaub. & Spach	•	•	•	Low
49	*Mnesithea granularis* (L.) de Koning & Sosef	•	•	•	High
50	*Ophiuros exaltatus* (L.) Kuntze	•			Un-palatable
51	*Oplismenus burmannii* (Retz.) P.Beauv.			•	Low
52	*Oropetium thomaeum* (L.f.) Trin.			•	Un-palatable
53	*Oropetium villosulum* Stapf ex Bor		•	•	Un-palatable
54	*Panicum trypheron* Schult.		•	•	Moderate
55	*Panicum maximum* Jacq.		•		Low
56	*Panicum miliaceum* L.		•		Low
57	*Pennisetum pedicellatum* Trin.			•	High
58	*Perotis indica* (L.) Kuntze			•	Moderate
59	*Phragmites karka* (Retz.) Trin. ex Steud.			•	Un-palatable
60	*Pseudoraphis spinescens* (R.Br.) Vickery			•	Un-palatable
61	*Saccharum spontaneum* L.			•	Un-palatable
62	*Sachoenefeldia gracilis* Kunth.		•	•	Low
63	*Sehima ischaemoides* Forssk.	•			High
64	*Sehima nervosum* (Rottler) Stapf	•		•	High
65	*Sehima sulcatum* (Hack.) A.Camus	•			High
66	*Setaria pumila* (Poir.) Roem. & Schult.	•		•	Moderate
67	*Sorghum halepense* (L.) Pers.	•	•		Low
68	*Sporobolus coromandelianus* (Retz.) Kunth		•		Low
69	*Sporobolus diander* (Retz.) Beauv.		•	•	Low
70	*Sporobolus indicus* var. major (Buse) Baaijens			•	Low
71	*Thelepogon elegans* Roth	•			Moderate
72	*Themeda cymbaria* Hack.		•		Moderate
73	*Themeda laxa* (Andersson) A.Camus	•		•	High
74	*Themeda quadrivalvis* (L.) Kuntze		•	•	High
75	*Themeda tremula* (Nees ex Steud.) Hack.			•	Moderate
76	*Themeda triandra* Forssk.	•	•		High
77	*Tragus mongolorum* Ohwi		•	•	Low
78	*Tripogon jacquemontii* Stapf			•	Low
79	*Urochloa distachya* (L.) T.Q.Nguyen			•	High
80	*Urochloa panicoides* P.Beauv.			•	High
81	*Urochloa reptans* (L.) Stapf		•	•	Moderate
82	*Vetiveria zizanioides* (L.) Nash		•	•	Un-palatable

total of 11 grass species are unpalatable out of the recorded 60 species.

Dominant Grass species and Zonation: It is observed that *Cymbopogon martini*, *Themeda triandra* and *T. quadrivalvis* and *Heteropogon contortus* are the most dominant grass species having an average height >100 cm. However there are all the major dominant grasses species occurs in pure patch having less than 30% presence of other grass species especially dense patches of *Cymbopogon martini; Themeda triandra* and *T. quadrivalvis* while *Heteropogon contortus* var. *contortus* sub var. *genuinus and H. contortus* var. *contortus* sub var. typicus having around 50% other co-species of grasses. To delineate the patches of dominant grass species as discussed in methodology, authors have marked them on Google earth image and calculated the approximate area of it's extend. For the Zonation, four major categories have been identified *viz Cymbopogon martini* (Rosha) dominant, *Themeda triandra* and *T. quadrivalvis* (Bhothi) dominant, *Heteropogon contortus* var. *contortus* sub var. *genuinus* (Nani Sukli) and *H. contortus* var. *contortus* sub var. typicus (moti sukli) dominant, and degraded area. Based on the information collected during the field by

measuring the area of extend for the above-mentioned grass species and consultation with officials and frontline staff, the following image has been created on Google Earth to measure the area of extend. The area of different patches will be useful in drawing grassland improvement prescriptions (Fig. 1).

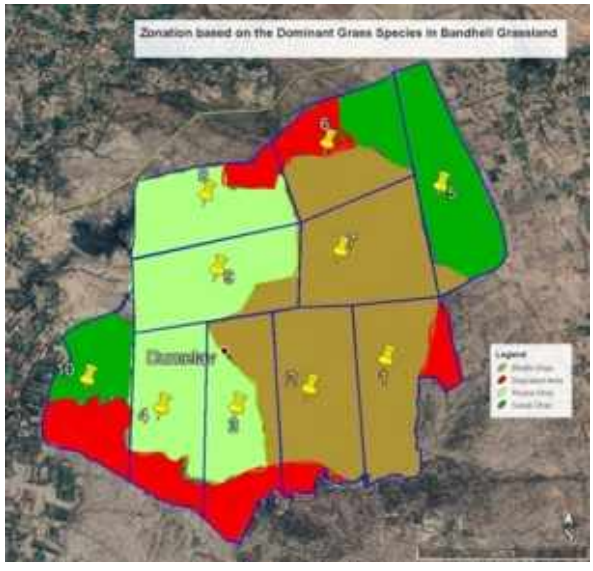

Fig. 1: Zonationi based on the dominant grass species in Bandheli grassland

The area under each zone is mentioned below the table (Table 2) and found around 300 ha area, *i.e*., nearly 40% area is under *Themeda triandra* and *T. quadrivalvis*. (Bhothi) cover followed by the area under *Cymbopogon martini* (Rosha), which is around 200 ha. The area under *Heteropogon contortus* (Sukli) distribution is about 125 ha, and approximately 130 ha area is degraded. A good number of water sources are also present in it, *viz*. check dams, streams/rivulets, and natural ponds.

Land use land cover: In the last two working plans prepared by the forest department, tree growth issues in this grassland have been raised. The major concern to raise this issue is that the grass production area is gradually getting down, and this grassland is meant for grass production. Hence an attempt was made to know the land use land cover of this grassland using the latest satellite imagery. The imagery of 4th Oct. 2020 has been used to estimate land use and land cover of Bandheli grassland. The processed image categorized four major land-use types which area Grass dominated area, Tree cover, Open area, and water. The NDVI has been calculated for the different land-use types, which shows the area under grass cover is 320 ha, area under tree cover is 304 ha, open area is 104 ha, and area under water is 24 ha. Here it is important to note that a significant area is under water which may lead to a higher water table (CGWB, 2014). This may lead to growing taller grass species as well as an increase in tree cover of Bandheli.

Threats

Grassland ecosystem is one of the most threatened natural ecosystems of the world as it received immense natural and anthropogenic pressure. Such pressure leads to degradation and a reduction in its productivity. The pressure faced by Bandheli grassland area categorized as natural as well as human-induced as follow:

Anthropogenic pressure: Grassland ecosystem is the backbone for the pastoral community; however, in the vicinity of Bandheli grassland, locals do animal husbandry as agriculture-dependent. Hence, they also depend on open and free grazing during the dry period. Seven villages fall in the close vicinity of Bandheli, having livestock rearing as a vital occupation for their livelihood and holding cumulatively around 20,000 buffalo and cow (Census, 2019). Hence it is very much clear that this grassland will have immense pressure on grazing. To minimize the grazing pressure, the forest department imposed various protection measures to protect grass from grazing during its growth period. This free grazing animal serves as vector to introduce non-palatable grass, herbs and other woody species in it by their dung and sticking on their body. Certain non-desirable species have vigorous growth patterns which hinder the growth of important species. Sometimes heavy open grazing also leads to deterioration of topsoil and its porosity.

Natural threats: Among the natural threats, the invasion of exotic or alien species is the most significant threat to the various natural ecosystems. Sometimes habitat conditions favor the growth of non-alien species, which may negatively impact the natural balance or

Table 2: Area of extend by various dominant grass species in Bandheli grassland

S.No.	Legend color	Dominant grass species	Distributed in coupe No.	Area (Approx.) in Ha
1	Brown	*Themeda triandra* and *T. quadrivalvis*	1, 2, 3, 6, 7 and 9	280
2	Light Green	*Cymbopogon martini*	3, 4, 8 and 9	200
3	Dark Green	*Heteropogon contortus* var. *contortus* sub var. *genuinus and H. contortus* var. *contortus* sub var. typicus	5, 6 and 10	125
4	Red	Degraded area	1, 3, 4, 6, 8 & 10	130
5	water	-	-	19
	Total area			**754**

productivity of a specific ecosystem. In Bandheli, a total of five species have been reported having a negative impact on the grassland ecosystem, which are as follow:

1. ***Neuracanthus sphaerostachyus*** (Ganthera) - Non- alien and native to West and South India.
2. ***Hyptis suaveolens*** (Gandhelu) - Alien and native to Tropical America.
3. ***Holarrhena pubescens*** (Kado) - Non-Alien and wide distribution.
4. ***Senna tora*** (Kuvandio) - Non-Alien and native to Asia.
5. ***Senna uniflora -*** Alien and native to Tropical South America.

As mentioned, invasive species are gradually occupying grasses' areas by vigorous growth, which adversely affects grass productivity. This is one of the immediate attention requiring threats to grassland.

Abundant tree growth is also a crucial threat to the grassland as it occupies an area and inviting other non-desired species as it serves as a host to many bird species. *Terminalia crenulata* and *T. elliptica (*Sadad) and *Soymida febrifuga* (Rohan) is the tree species that favor grass growth under it, while the negative impact of *Acacia catechu* (Kher) and *A. leucophloea* (Rhinjdo) has been observed. Dense tree cover maintains soil moisture level and groundwater table, leading to growing tall and unpalatable grass species.

Conclusion

As results of Present investigation a total 60 grass species have been reported from the Bandheli Reserve Grassland of Panchmahal-Gujarat. Among the recorded 60 grass species 18 species are highly palatable, 22 are moderately palatable, 09 are least palatable and 11 are unpalatable. Based on the zonation, around 280 ha area under *Themeda triandra* and *T. quadrivalvis* followed by area under *Cymbopogon martini.* The area under *Heteropogon contortus* var. *contortus* sub var. *genuinus* and *H. contortus* var. *contortus* sub var. typicus is around 125 ha and the grasses growing in this zone is more palatable compare to others. Approximately 130 ha area reported as degraded and can be improve to enhance its productivity. Based on the Landuse land cover map it was found that around 304 ha areas is under tree growth and around 24 Ha area is under water (seasonal and perennial sources). This Zonation and land use land cover based information will be used in better management and improvement of this crucial grassland.

गुजरात राज्य के पंचमहल जिले में स्थित बांधाली आरक्षित घासीया भूमि कि स्थिति एवं घास प्रवासी विविधता

आर.एम. पटेल एवं एम.एल. मीणा

सारांश

गुजरात के कुल आठ जिलों में 8490 वर्ग किमी घासीया भूमि फैली हुआ है। बांधाली की इस घासीया भूमि की जाति–विविधता को व्यवस्थित सूची अब तक नहीं बनाई गई है। वर्तमान अध्ययन घास प्रजातियों की विविधता की व्यवस्थित सूची और बंधेली रिजर्व घास के मैदान के लक्षण वर्णन से संबंधित है। वर्तमान जांच के परिणामस्वरूप, कुल 60 घास प्रजातियों को सूचना मिली है। दर्ज की गई 60 घास प्रजातियों में से 18 अत्यधिक खाद्य थी। 22 मध्यम रूप से खाद्य है। 9 (नौ) सबसे कम खाद्य है और 11 अनुपयुक्त है। इस पूरे घास के मैदान को प्रभावी घास की प्रजातियों के आधार पर बांटा गया है। इस बटवारे का उपयोग इसके प्रबंधन और सुधार में किया जायेगा। वृक्षों की वृद्धि से वनभूमि में परिवर्तित होने का समाना कर रहा है, यानी 754.04 हेक्टेयर में से 304 हेक्टेयर क्षेत्र पेड़ों से आच्छादित है और लगभग 100 हेक्टेयर खराब हो गया है।

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