VARIABILITY IN GREATER GALANGAL (*ALPINIA GALANGA* (L.) WILD. GENOTYPES FOR YIELD AND QUALITY

By

DIVYA S.

(2020-12- 031)

THESIS

DEPARTMENT OF PLANTATION, SPICES, MEDICINAL & AROMATIC CROPS

COLLEGE OF AGRICULTURE, VELLANIKKARA,

KERALA AGRICULTURAL UNIVERSITY

THRISSUR – 680 656

KERALA, INDIA

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Submitted in partial fulfilment of the requirement for the degree of

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Faculty of Agriculture

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COLLEGE OF AGRICULTURE, VELLANIKKARA,

KERALA AGRICULTURAL UNIVERSITY

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KERALA, INDIA

2023

DECLARATION

I, **Divya S. (2020-12-031)** hereby declare that this thesis entitled **Variability in greater galangal (*Alpinia galanga* (L.) Wild. genotypes for yield and quality** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University.

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CERTIFICATE

This is to certify that this thesis entitled **Variability in greater galangal (*Alpinia galanga* (L.) Wild. genotypes for yield and quality** has been solely prepared by **Ms. Divya S. (2020-12-031)** under my guidance and supervision and that it has not been previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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LIST OF SYMBOLS

%	Per cent
/	Per
µl	Micro liter
℃	Degree Celsius
Cm	Centimeter
cm^2	Square centimeter
et al.	and others/ and co-workers
Fig.	Figure
G	Gram
Viz	Namely
L-1	Per liter
Mg	Milligram
Min	Minute
No.	Number
$Plant^{-1}$	Per plant
SE (d)	Standard error of difference
Sl. No.	Serial number
Temp.	Temperature
Wt.	Weigh
GAE	Gallic acid Equivalent

LIST OF ABBREVIATIONS

Alcl3	Aluminium chloride
ANOVA	Analysis of variance
BBCH	*Biologische Bundesanstalt, Bundessortenamt and CHemical industry*
CD	Critical difference
CRD	Completely randomized design
COA	College of Agriculture
CV	Coefficient of variation
Df	Degrees of freedom
GC-MS	Gas chromatography–mass spectrometry
H2SO4	Sulfuric acid
HCl	Hydrochloric acid
HPLC	High-performance liquid chromatography
ICAR	Indian council of agricultural research
Na2CO3	Sodium carbonate
NaNO2	Sodium nitrite
NaOH	Sodium hydroxide
SD	Standard deviation
NBPGR	National Bureau of Plant Genetic Resources
NIST MS	National Institute of Standards and Technology
RHS	Royal Horticulture Society
RPM	Revolutions Per Minute
PDA	Potato Dextrose Agar
TZC	Triphenyl tetrazolium chloride

INTRODUCTION

1. INTRODUCTION

Since ancient times, plant-based drugs have been much preferred over synthetic drugs because of their lesser side effects. About 80% of the world's population depends on the traditionalsystem of medicine, more on plant-derived drugs for primary health care, among which 30% of the medicinal plant resources are consumed by the therapeutic sector alone (Ghasi *et al*., 2000). Among the plant families, Zingiberaceae is also one of the widely used medicinal plant families consisting of a large number of medicinal plants which are well known for their usage in ethnomedicine and play a significant part in the Indian System of Medicine (Kumar *et al*., 2013).

Zingiberaceae is one of the important medicinal, ornamental, and monocotyledonous plantfamilies distributed worldwide. About 50 genera and 1300 species are concentrated in the Southand Southeast Asian region (Wu and Larson, 2000). About 22 genera and 178 species have beenidentified around India's North Eastern and peninsular regions. Northeastern parts of India alonesupport the growth of 19 genera and 88 species (Prakash and Mehrotra., 1995).

Alpinia, the largest genus in the Zingiberaceae family, was designated by renowned French botanist Charles Plumier and named after Prospero Alpino, a prominent Italian botanist of the sixteenth century. According to the Angiosperm Phylogeny Group II (APG II) system, thegenus Alpinia falls under monocotyledonous plants (Angiosperm Phylogeny Group 2003). It is amember of the order Zingiberales, subfamily Alpinioideae, and tribe Alpinieae. The genus contains 230 and 250 species spread across tropical and subtropical regions. DNA-based researchrevealed the genus to be polyphyletic, with six clades dispersed throughout the tribe Alpinieae (Kress *et al*. 2005).

Alpinia galanga (Linn.) Wild is popularly known as 'greater galangal' in English and othervernacular names for this crop are listed in Table 1.1; (Verma *et al.,* 2011).

Table 1.1: Vernacular names of *Alpinia galanga*

Sl.no	**Language**	**Names**
1	*Malayalam*	*Arattha, Kolinji,*
2	*Kannada*	*Dhumarasmi*
3	*Tamil*	*Pera-rattai*
4	*Sanskrit*	*Mahabaracach, Sugandha Vacha, Rasna*
5	*Telugu*	*Pedda-dhumpa*
6	*Hindi*	*Kulanjan*

This crop has become naturalized in several parts of South East Asia (Ravindran *et al*., 2012). The plant is commonly found in Egypt, Malaysia, India, Sri Lanka, Indonesia, China, andthe Gulf (Jatoi *et al*., 2006). It is one of the endemic species in the Western Ghats that grows widely in tropical and subtropical regions (Pooja *et al*., 2020), and frequently cultivated in Konkan and North Kanara regions (Chouni and Paul, 2018; Shetty and Monisha, 2015).

Alpinia is a rhizomatous, perennial herb that reaches a height of about 2.5 to 3 m. The rhizome is very prominent and aromatic. Externally, it is reddish brown and internally reddish- white. The pseudo stem is erect, green, and formed by the rolling of leaves; the leaf sheath is pubescent with a robust tillering habit. It will reach a maximum height of 129.4 cm with more than 48 tillers per clump and 13 leaves per tiller (Trimanto *et al*., 2021; Verma *et al*., 2011). Leavesare oblong, alternate, shiny, leathery dark green color on the dorsal surface and light green coloron the ventral side with short petiole around 1cm.

Flowers are greenish white born on the terminal compound panicle with a pleasant smell3-4cm long; sepals three in number, light green color; labellum is showy white with pink color markings; calyx tubular; corolla has distinctly clawed lips. stamens are white /slightly yellowishin color; stigma is white. flowering is seen from May to July. Fruit takes 5 to 6 months for maturation, fruit type is a capsule, and the seeds are small dark brown to black color; covered bywhite aril; seeds are embedded in 3 locules, but one locule will not contain any seeds. Each locule has two seeds, a total of four seeds in a capsule. The active growth cycle of the plant ends in December later months it dries off and regenerates after receiving monsoon rain.

The plant is successfully grown in sandy loam soils and humid tropical climates. It can also be grown in open areas with sufficient rainfall. No named variety has been released so far. Rhizome splits are frequently used for propagation. Rhizomes are planted in the southern part ofIndia in April and May, but in the hilly northeast, the best season is from mid-February to mid- April. The number of rhizome slices needed for propagation depends on the distance between crops and the time of harvest (Tonwitowat, 2008). For a 1-year crop, 5.5 tonnes per hectare of rhizome sets are required at 30×30cm spacing, whereas for a two-year crop, 3.5 tonnes of sets require at the spacing of 45×30 cm (Pooja *et al*., 2020).

Intermittent watering is given during the growth phase to keep the soil from being too dry. One-year crop and two-year crop yields of fresh rhizomes were reported to be 23.93 and 82.91 tonnes per hectare, respectively. But drying lowers the yield to 5.65 and 22.65 tonnes of dry rhizome per hectare (Kumar, 2019). When the crop turns 12 months old plant startsdrying. It is the correct stage for harvesting rhizomes. The harvested rhizomes have an oil contentof between 0.32 per cent and 0.4 per cent on a dry-weight basis. Usually, it takes one or two years for the cropto be ready for harvest. But it can be maintained as a perennial to preserve the genetic material. Harvesting the rhizomes at 42 months after planting gives high rhizome yield (45.4 t/ha) and oil (127.4 liters/ha) yields, and for obtaining oil of good quality (27.1% cineole [eucalyptol]). (Joy *et al*., 2001).

Generally, the rhizomes are used as a spice, a source of essential oil, and seasoning fish in pickling. In Tamilnadu, dried rhizomes are used as food. Flowers and young shoots are used inthe form of vegetables or as a spice. Seed is used in emaciation and mouth cleaning and it stimulates the digestive power and appetite. It is also used as a purgative Kumar (2019). It can beadded to alcoholic and alcoholic-free beverages as an alcohol booster or alcohol replacer (Gupta.,2010).

Alpinia rhizomes are mainly used in dietary intake and the traditional medicine system, *viz.* Ayurveda, Unani, Chinese, and Thai folk medicine. It has a pungent, fiery, and spicy taste and a ginger-like fragrance. It has been discovered to have a variety of medicinal properties, including immune-stimulant, anti-fungal, antioxidant, anti-amoebic, anti-diabetic, anti-inflammatory, anti-cancer, analgesic, anti-allergic, anti-

ulcer, anti-dermatophytic, anti- bacterial, and many more. Pharmacological studies discovered the presence of several flavonoids, terpenes, and tannins, in rhizome extracts, which are responsible for medicinal activity (Ghosh and Rangan, 2013). The pungency in galangal extract is mostly due to the presence of 1'S-1'- acetoxychavicol acetate, which also influences the biological activities (Chudiwal *et al*., 2010).

Presently this crop is gaining importance in the pharmacological field and is to be used asa future potent drug for many diseases. Because of growing demand, the plant is facing greater threat to its survival in its natural habitat, and the population of this plant is also decreased exponentially in the last few years in its natural habitat due to improper harvesting and poor plantmultiplication. Hence, proper conservation of this species in a systematic way for furtherutilization is essential before it is lost forever (Parida *et al*., 2011). *Alpinia galanga* is found in the wild/semi-domestic state of Kerala and is reported to be exported from the hilly tracts of Southern Kerala. Despite its versatile uses as a spice as well as medicine, substantial research hasnot been done on the species. Kerala Agricultural University has taken up geotagging of this species recently. Keeping all the above aspects in view, the present investigation entitled "Variability in greater galangal (*Alpinia galanga* (L.) Wild. genotypes for yield and quality" has taken up with the following objectives:

- Evaluation of genotypes of greater galangal for yield
- Evaluation of genotypes of greater galangal for quality
- Evaluation of genotypes of greater galangal for anti-microbial properties.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Alpinia galanga is a less explored medicinal plant used in dietary intake and the traditionalmedicine system. It is also widely used in the industrial sector since it contains various bioactivecomponents. However, this plant has entered the endangered medicinal plant list because of improper harvesting methods, deforestation, and the industry's high dependency on natural resources. In India, commercial cultivation of this crop is mainly confined to the south Indian states like Kerala, Karnataka, and Tamil Nadu. Since there is no large-scale cultivation in Kerala,it is essential to investigate and evaluate accessions that would result in selecting suitable accessions for commercial cultivation. The present study mainly concentrates on the morphological characterization of different accessions for yield, quality, and antimicrobialproperties of different genotypes. Some of the literature specific to *Alpinia galanga* are collected and reviewed in this chapter under these headlines.

2.1 Taxonomy, distribution, and morphology

2.2 Phytoconstituents and uses of *Alpinia galanga*

2.3 Antimicrobial activity

2.1 TAXONOMY, DISTRIBUTION, AND MORPHOLOGY

2.1.1. Taxonomic classification of *Alpinia galanga*

Botanical information on *Alpinia galanga* (Ramanunny *et al*.,2022)

Kingdom : Plantae
Phylum : Magnoliophyta
Class : Liliopsida
Order : Zingeriberales
Family : Zingiberaceae
Subfamily : Alpinioideae
Tribe : Alpiniaiea
Genus : Alpinia
Species : galanga

Alpinia was first categorized by Plumier, but it was given its name in honor of the Italianbotanist Prospero Alpino, who lived in the 16th century. This genus is a member of the order Zingiberales, the subclass Zingiberidae, and the division Magnoliophyta (Angiospermae) (Kress *et al*., 2005).

Six tribes and four subfamilies had been identified in a new taxonomy based on phylogeny: the Siphonochiloideae (Siphonochileae), Tamijioideae (Tamijieae), Alpinioideae (Alpinieae, Riedelieae), and Zingiberoideae (Zingibereae, Globbeae) (Kress *et al*., 2002).

The Zingiberaceae family is classified into four tribes based on morphology: Alpinieae, Hedychieae, Zingibereae, and Globbeae (Larsen *et al*., 1998).

According to Smith's (1990) classification, *A. galanga* and *A. conchigera* are grouped together in the same subgenus Alpinia, even though they are in distinct sections.

2.1.2 Current status of the crop

According to prior studies, India was major exporter of galangal, alongside nations like Thailand and Indonesia (Rao *et al*., 2011.) Over 100 tonnes of fresh rhizomes and roughly 30 tonnes of dried rhizomes were imported into the Netherlands each year (Scheffer and Jansen, 1999).

In the U.S., galangal root oil and root oleoresin have the regulatory status of generally recognized as safe (GRAS) by Parid *et al*. (2011).

The vast medical benefits, widespread applications, overuse, and high demand of *A. galanga* are the causes that put its survival in its native habitat in danger. Due to scarcity of sufficient, high-quality plant materials that are disease-free, germplasm conservation and storageare challenging aspects. Another significant issue for the preservation of germplasm is the high susceptibility of this plant to several diseases like bacterial wilt and rhizome rot (Nayak, 2002; Singh *et al*., 2011).

Pooja *et al*. (2020) reported that seed propagation is not a commercial technique of propagation for reproducing the species in the wild, hence this species has been

declared under the red list of medical plant species reported by FRLHT (Foundation for Revitalisation of Local Health Tradition- 1997).

2.1.3 Geographical distribution

Alpinia galanga is frequently found in places like Malaysia, Egypt, Sri Lanka, Indonesia,India, and the Arab gulf. It grows in open, sunny areas, forest areas, and scrub. It is frequently grown in Sri Lanka's low and midlands (Arambewela and Wijesinghe, 2006).

The plant thrives well in Gujarat, Goa, Western Ghats, Mysore, and some other SoutheastAsian countries (Malik *et al*., 2016).

The Alpinia plant was described by Kumar and Maya (2021) as a rhizomatous herb that grows in dry deciduous forests. It is grown in the southern Indian states of Kerala, Karnataka, and parts of Tamil Nadu.

According to Hanh *et al.* (2014) most of the Alpinia spp. prefers to grow in wet and shady places like under the forest canopy, along grassy slopes, or roadsides but *Alpinia oblongifolia* (*A. Chinensis*) and *Alpinia hainanensis* prefer 30 to 1500 m altitude.

Alpinia genus can be found in many tropical and subtropical areas, including the AndamanIslands, Australia, Burma, Caroline Islands, China, Fiji, India, Indochina, Indonesia, Japan, New Guinea, New Caledonia, Malaysia, Philippines, Sri Lanka, Solomon Islands, Samoa, and Thailand (Smith, 1990: Ma *et al.,* 2017).

2.1.4 Morphological characters

In the field of plant taxonomy, morphological features can be employed to aid in speciesidentification (Windarsih *et al*., 2021). According to Susetyarini *et al*. (2020), even among members of the same species, morphological characteristics might vary in form and structure.

The morphological characteristics of plants can be employed as diagnostic or key factors when describing, classifying, and resolving taxonomic issues.

Environmental,positional, and juvenile factors are responsible for variations in the morphological characters of zingibers (Iroka *et al*., 2015).

Ponmozhi and Kalaiselvi (2011) evaluated three varieties of galangal for their morphological, anatomical, and biochemical characteristics. The varieties exhibited significant degree of variation for the morphological traits observed.

Tonwitowat *et al.* (2008) evaluated the agronomic traits in red, yellow, and white galangal cultivars that were collected from various parts of Thailand. The findings revealed that, practicallyall of red galangal agronomic traits were higher at harvest (eight months after planting) includingplant height 149.5 cm, LAI 8.2, and total fresh weight of plant and rhizome, 2.9 and 1.8 kg per plant.

Mathew *et al*. (2014) compared the morphological and anatomical features of *A. galanga*and *A. calcarata*. They observed that the rhizomes of *A. galanga* were profusely branched and yellow in color as against the creamy yellow colored and less branched rhizomes of *A. calcarata*.

Pooja *et al*. (2020) tested the genotypic variability, heritability, and genetic progress for yield and yield-contributing traits in fourteen *Alpinia galanga* (L.) Wild. accessions at the ICAR-IIHR in Bengaluru. They found that the genotypic coefficient of variation was less than the phenotypic coefficient of variation in all the characters studied. The PCV and GCV were higher for the characters like petiole length, inflorescence length, and leaf area. As per broad sense heritability values, traits including petiole length (83.35%), plant height (74.47%), leaf length (68.5%; equal to leaf area), inflorescence length (68.03%), and pedicel length (64.87%) had strong heritabilities. Petiole length (52.72%), inflorescence length (31.26%), leaf area (29.38%),and pedicel length (23.47%) were the features with the highest estimations of GAM.

Parida *et al*. (2011) carried out experiment on *Alpinia galanga* plantlets that were grown in tissue culture and transplanted into the field, and assessed for phenotypic traits. The experimentrecorded plant height (73 ± 2.54 cm), number of tillers (15.6 ± 1.18), leaf length (34.6 ± 2.03cm), leafwidth (4.8 ± 0.6cm) and rhizome yield as (84.2 ± 1.9gm) for conventionally propagated plants, while it was (66 ± 1.2cm), (21.0 ± 0.9),

(36.2 ± 0.8cm), (5.2 ± 0.6cm), (98.3 ± 1.1gm) respectively for the same characters in micro propagated plants.

Variations in the tillers per plant in *Alpinia galanga* genotypes were reported by Tonwitowat (2008); it ranged from 22 to 45 per plant. Joy *et al*. (2006) found that applying FYMat the rate of 15t/ha resulted in 3.5 suckers per plant for *kaempferia rotunda.*

Kasarkar and Kulkarni (2011) studied the phenology of two Alpinia species and three Zingiber species. They recorded the leaf length and breadth, ranging from 21.2cm to 6.46cm respectively.

Kadam *et al*. (2012) screened galangal roots for physicochemical, microscopic, macroscopic, and phytochemical parameters to ensure the purity of the drug. As per the macromorphological perspective, the *Alpinia galanga* root has numerous branches, a bitter taste,and an aromatic odor; 5–10 cm long and 3-5 mm in diameter. Additional characteristics revealedthat the unpeeled roots had a rough fibrous surface and a shape that was roughly cylindrical witha slight taper at one end.

Trimanto *et al*. (2021) reported the key taxonomic morphological features of *Alpinia galanga* and also described the results of qualitative characteristics like flower color, leaf shape,and seed color in their studies.

Divya (2008) and also by Devi and Raj (2019) reported the qualitative and quantitative morphological characters in *Kaempferia galanga,* one of the medicinal species of Zingiberaceae family.

Windarsih *et al*. (2021) studied and documented the all qualitative and quantitative morphological features of 13 Zingiberaceae species gathered from Serang District, Indonesia.

Sabu (2006) documented all of the morphological aspects of plants in the Zingiberaceae family, including both qualitative and quantitative morphological characteristics of flowers, fruits, and rhizomes.

Kasarkar and Kulkarni (2011) described the phenological aspects of the three Zingiber species and two Alpinia species. They observed the changes in their life cycles, including rhizome sprouting to maturity, flower bud opening to anthesis, fruit development to dehiscence, and bud opening to flower anthesis. Results showed that flowering was observed in the month of June to August, and June to November was best for seed production. Flower opening occurred in the earlymorning, and the crop was vegetatively active up to November. Later it dried off.

According to Kumar (2019), *Alpinia galanga* flowering takes place between May and June, while fruiting happens between August and September. According to Sabu (2006), *Alpinia galanga* shows flowering and fruiting from April to December.

Zhang *et al*. (2003) investigated the floral biology and breeding system of *Alpinia blepharocalyx* identified in Yunnan Province, South West China. Furthermore, the floral morphs and stigmatic moments of the flower before and after pollination were also reported in the study.

Mangily and Sabu, (1992) described the detailed key taxonomic morphological features of *Alpinia Roxb*. (Zingiberaceae) with respect to floral biology and rhizome characters. Floweringphenological stages, floral biology, and pollination behavior, and pollinator visit patterns of *Alpinia mutica Roxb.*, were reported by Aswani and Sabu (2015).

There is only limited information available on the seed dormancy of members of the Zingiberales (Baradwaj *et al*., 2016**)**. When seeds were subjected to 20/10°C day and night temperatures; 86.7% germination was obtained and seeds began germinating after six weeks. After dry storage for four months, seeds were incubated at (30/20°C); they began to sprout in the sixth week and 93.3 per cent of the seeds germinated after 18 weeks under this temperature.When the seeds were kept dry for four months, and later treated with 50 mg/l gibberellic acid (GA3) germination started after two weeks at 30/20°C.

Wu *et al*. (2014) conducted studies to identify the seeds of seven Zingiberaceae species. Their fruits and seeds were classified based on microscopic characteristics.

Based on the results,it was possible to identify seven species based on the anatomical features of the seed coat seen intransverse sections.

Alpinia conchigera (subgenus Dieramalpinia) and *Alpinia galanga* (subgenus Alpinia) both had traits in common, including the shape of the leaf margin, the lack of hypodermis, and the presence of hypodermal fibers in the midrib and petiole. Studies on the anatomy of fruits andseeds had revealed that *A. galanga* is related to species in the subgenus (Dieramalpinia) and is close to *A. conchigera* (Liao and Wu, 1996).

2.2 PHYTOCONSTITUENTS AND USES OF *Alpinia galanga*

Alpinia galanga was reported to contain, essential oils, glycosides, tannins, phenol, carbohydrates, and monoterpenes. Gallic acid, galangoisoflavonoid, zerumbone, glycoside, β- sitosterol, alpinin, galangin, and kampferide had been identified from various portions of *A. galanga* in recent years (Kaushik *et al*., 2011).

2.2.1 Bioactive components and phytochemistry of *Alpinia galanga*

From the rhizome of *Alpinia galanga*, Matsuda *et al*. (2003) isolated nine knownphenylpropanoids and hydroxy benzaldehyde (1’S-1’-acetoxy chavicol acetate and 1'S-1'-acetoxyeugenol acetate). According to this study, 1'S-1'-Acetoxychavicol acetate possessed strong anti-colorectal cancer action.

Galangoflavonoid was isolated from the rhizomes of the *Alpinia galanga* by Jaju *et al*. (2009) using column chromatography and eluted with ethyl acetatemethanol (9:1) to obtain a chemical,galangoflavonoid (AG 11). The structure of the substance was clarified by a variety of spectrumtechniques (U.V., I.R., 1H NMR, 13C NMR, and M.S.).

Alpinia galanga and *Alpinia officinarum* were subjected to comparative physicochemicalanalysis by Namdeo and Khale (2015). Additionally, the extractive values of two species were noted as Petroleum ether (3.47 and 0.60), Ethanol (9.04 and 14), Methanol (4.7 and 2.7), and Water (12.5 and 1.6) per cent respectively.

Poonsri *et al.* (2019) have reported the effectiveness of trans-cinnamic acid, which was isolated from *Alpinia galanga* (Zingiberales: Zingiberaceae), for the

management of *Aedes aegypti*, *Anopheles dirus B,* and *Culex quinquefasciatus* (Diptera: Culicidae) (Diptera: Culicidae).According to these findings *A. galanga,* which contains trans-cinnamic acid as an active component, could be a promising good naturally occurring mosquito repellent for all three species.

Eram *et al*. (2019) conducted a study on pharmacological actions, therapeutic qualities, and phytochemical constituents of *Alpinia galanga* leaves and rhizome extract. Results indicatedthat when compared to leaf extract, *Alpinia galanga* rhizome extract contained higher amounts ofphenolic and flavonoid compounds. Further, they also found that the rhizome extract of *Alpinia galanga* had remarkable antibacterial and radical scavenging properties due to its high phenolic and flavonoid content.

Phytochemical analysis of several *Alpinia galanga* components has revealed the presenceof diverse types of chemicals, including phenolic compounds and terpenes (Namdeo and Kale, 2015).

Flavonoids, such as galangin, kaempferol, and kaempferide, were categorized as the phenolic compounds in *Alpinia galanga* (Tungmunnithum *et al*., 2020).

Alpinia species share a lot of morphological similarities between *Alpinia galanga* and *A. officinarum*. While the rhizomes of A. *officinarum* are rich in phenolic compounds such as flavonoids, diarylheptanoids, and essential oil, the rhizomes of *A. galanga* are rich in phenolic compounds such as phenylpropanoids, lignans, flavonoids, and essential oil (Zhou *et al*., 2018).

The presence of phenyl propanoids in *A. galanga* and diaryl hepatanoids in *A. Officinarum* was the primary difference between their chemical compositions (Zhang *et al*., 2016).

The majority of the terpenes in *Alpinia galanga* essential oil are monoterpenes, sesquiterpenes, and diterpenes, namely 1,8-cineole, α-pinene, β-pinene, fenchyl acetate, β-bisabolene, β-caryophyllene, and β -selinene. These substances were utilized extensively in the food and pharmaceutical industries (Chudiwal *et al*., 2010).

Singh *et al*. (2020) examined the various bioactive components using methanolic extracts of *A. galanga* and *A. calcarata's* leaf and rhizomes.

Benzenepropanal (37.35±0.5%), 3-phenyl-2- butanone (20.49±0.6%), and carotol (17.44±0.3%), eucalyptol (13.89±0.2%), 5- hydroxymethylfurfural (11.28±0.3%) were the active phytoconstituents identified in leaves and roots of *Alpinia galanga* respectively.

2.2.2 Flavonoids and phenols

The flavonoids are the reason for the yellow color in the plant tissues and many of whichhave anticancerous activities due to the presence of functional keto (C=O) or aldehyde (–CHO) groups and they are potential sources of antioxidants (Williams *et al*., 2004).

A qualitative phytochemical examination was conducted in *Alpinia galanga* by Subash *etal*. (2012). Phytochemical analysis revealed the presence of a variety of complex compounds, such as phenols and flavonoids, which are responsible for a variety of biological activities.

The study conducted by Nair *et al*. (2013) focused on the radioprotective and free radicalscavenging abilities of *Alpinia galanga.* Results indicate that the number of phenolics, flavonoids,and total antioxidant capacity was higher in the *Alpinia galanga* rhizome extract prepared in acetone than in the extract obtained in ethanol.

Kambar *et al*. (2014) conducted an experiment to assess the antibacterial and radical-scavenging properties of *A. galanga* leaf and rhizome extract. Results indicated that the TFC of *A. galanga* leaf extract ranged from TFC of *A. galanga* leaf extract and rhizome extract rangingfrom 18.06±2.08(µg C.E./mg) and 32.26±3.12(µg C.E./mg) respectively.

The phytochemical screening conducted by Gupta *et al*. (2021) revealed the presence of carbohydrates, flavonoids, tannins, phenol, proteins, and saponins in the *Alpinia galanga* rhizomeextract. An average of 0.856 (mg GAE/100mg) of total phenols and 0.942 (mg Q.E./100mg) totalflavonoid content was obtained in the extract.

Malik *et al*. (2016) conducted an experiment for a comparative assessment of theantibacterial, antioxidant, and antidiabetic potential of *A. galanga* rhizomes. The

total flavonoid content in the methanolic extract of rhizome ranged from 51.76± 1.26 mg /g Q.E.

Aljiobair (2022) reported a significant amount of total phenolic content (53.18 mg GAE/g)and total flavonoid content (14.12 mg CE/g) in *Alpinia galanga* rhizome extract.

The study focused on assessing the antioxidant and free radical scavenging properties of *Alpinia galanga* rhizome extracts prepared in various solvent systems (distilled water, 60% aqueous ethanol, and 60% aqueous methanol) by Devi *et al*. (2018), indicated that compared to other solvents, Methanol (60%) extract showed the best antioxidant activity.

Singh *et al*. (2020) examined the various bioactive components using methanolic extractsof *A. galanga* and *A. calcarata's* leaf and rhizomes. Compared to *A. calcarata* extracts, *A. galanga*leaf extract had the greatest TPC and TFC (77.251.56mg GAE/g of the extract and 64.691.12 mgQuercetin equivalent/g of extract, respectively).

Suresh *et al*. (2015) conducted an experiment using high-performance liquid chromatography, and mass spectroscopy to identify the bio-active phenolic compounds in *Alpiniagalanga* and *A. calcarata*. Gallic acid and Ellagic acid were the two most prevalent phenolic compounds in *A. galanga* (320 and 518 mg/kg each) and *A. calcarata* (75 and 1,200 mg/kg each).

Melanathuru *et al*. (2017) used *Alpinia calcarata* and *Alpinia galanga* rhizomes aqueousextracts for phytochemical screening. The phytochemical screening confirmed the presence of phenols, flavonoids, terpenoids, carbohydrates, and proteins. *Alpinia calcarata* rhizomes had a phenolic concentration of 454.05 mg/g in aqueous extracts, and *Alpinia galanga* had a phenolic value of 457.40 mg/g.

A study by Malik *et al*. (2016) used methanol, ethanol, and water extract of the rhizome of *A. galanga* and they were analyzed to determine the antioxidant potential. Total phenolic content in the methanol, ethanol, and water extract ranged from 37.12±0.39, 49.42±0.38, and 21.77±0.73 mg/g GAE, respectively.

A total of 19 regularly used spices in China were systematically examined for their antioxidant potential and total phenolic content (Lu *et al*., 2011). Galangal had the highest total phenolic content and the highest antioxidant capacity. Galangin was also shown to be the significant phenolic component and the key contributor to Galangal's most excellent antioxidantcapability.

The objective of the research by Ivanovic *et al*. (2021) was to examine and analyze the chemical makeup and antioxidant activity (A.A.) of four distinctive ginger species (cardamom, turmeric, ginger, and galangal). Galangal contained significant amounts of α-terpinyl acetate (40.70%), β-turmerone (25.77%), α -zingiberene (22.69%), and 1,8-cineol (42.71%), respectively. The cardamom water extract had the lowest TPC content (1.04 ±0.29 mg), while the ethanolic extract of galangal had the greatest TPC value (63.01± 1.06 mg).

2.2.3 Starch

Ponmozhi and Kalaiselvi (2011) analyzed the starch, reducing sugars, nonreducing sugars, proteins, and amino acids present in the rhizome extracts. The starch content ranged from11. 5 ± 0.26 mg/g to 93.1 ± 0.11 mg/g.

By conducting a number of tests, Maya and Kumar (2018) reported the histochemical composition of *Alpinia galanga* rhizomes (calcium, starch, callose, chitin, lignin, phenolics, tannins, and total proteins).

The transverse section of the *Alpinia galanga* rhizome showed variation in the size and shape of the starch grains. An analysis described grains as circular, some are muller-shaped, but the majority are long rods with one pointed end and one end that is rounded and broad. There aretiny projections in certain starch grains (Chitra & Thoppil, 2008).

Wijayasiriwardena and Premakumara (2012) undertook the investigation on the plants *Alpinia calcarata Roscoe* and *A. galanga (Linn.)* Willd. using standard pharmacognostic techniques. Compared to *A. galanga*, *A. calcarata* had more simple starch grains in a single parenchyma cell and compound starch grains that were triangular. Only on the powdered rhizomeof *A. galanga* diamond-shaped silica crystals were discovered.

2.2.4 Amino acids

Ponmozhi and Kalaiselvi (2010) estimated the proteins and amino acids present in the rhizome extracts and quantified them using the ethanol and ethyl acetate fractions of the extracts.The rhizome extracts' protein and amino acid contents were calculated, and the results showed that, *Alpinia galanga* had the highest concentrations of both (380.9± 0.56 mg/g, and 12.1± 0.40 mg/g). *Alpinia calcarata* (5.7 ±0.56mg/g) and *Kampferia galanga* (193.2 ±0.07mg/g) had the lowest levels of amino acids and protein, respectively.

Alpinia galangal's aqueous extract was used by Babu *et al*. (2017) for preliminary phytochemical screening, which confirmed the presence of amino acids, flavonoids, and terpenoids. According to the experiment conducted by Namdeo *et al*. (2015), alkaloids, carbohydrates, tannins, amino acids, and saponins were all found in the *Alpinia galanga* rhizomeextract.

2.2.5 Volatile oil profile

Essential oils are a complex mixture of plant volatile compounds. Nowadays, particularlyin the field of medicine, essential oils are used in larger amounts. The composition of oil varies according to location, environmental condition, stage of harvest, and extraction methods (Fokou *et al*., 2020).

Denys *et al*. (1992), identified twelve chemical compounds from the oil extracted from the rhizome and leaves of *Alpinia galanga* Willd, by G.C. and GC/MS; Myrcene was the main primary substance (94.51% in the rhizome and 52.34% in the leaves).

Alpinia galanga rhizome volatile oil was subjected to G.C. and GC/MS analysis (Lakshmi *et al*., 2007). A total of 16 compounds were found in essential oil. The predominant component, at 44.80 per cent, was zerumbone. This is the first description of zerumbone in *A. galanga* and the chemical makeup of Sri Lankan-grown *A. galanga* rhizome oil.

The oil profiling of four different Alpinia species was described by Raj *et al*. (2013). Results showed that all Alpinia species rhizome oils contained 37 to 48 components (96.8 and 100%). 1,8-Cineole was discovered to be abundant in *A. galanga* (52.9%). Potential compounds such as chavibetol acetate (5.6%), 2-(1E)-propenyl phenol (4.7%), and phenol, 4-(2-propenyl)- acetate (1.1%) were found in *A. galanga* rhizome oil, supporting its usage as a flavoring agent.

The objective of the research conducted by Yan *et al*. (2014) was to identify the chemical components and biological effects of the essential oil from the rhizomes of *Alpinia galanga (L.)* Willd. on mature cigarette beetles. Eucalyptol (22.63%), (1S)-(1)-pinene (14.36%),1R-pinene (10.89%), α -terpineol (8.59%), and L(-)-borneol (8.41%) were found to be the primaryconstituents of the essential oil, followed by camphor (4.21%) and camphene (4.14%). This resultwas utilized in the identification of the compounds responsible for pesticidal and repellent activityto control insects in stored grains.

Raina and Abraham (2015) examined the chemical profiles of the essential oils obtained from the rhizomes of two Alpinia species. Major components found in the essential oils of both Alpinia spp. were 1,8-cineole (63.4 and 44.2%), α -terpineol (2.8 and 6.3%), α -pinene (1.9 and 2.0%), and terpinen-4-ol (2.8 and 4.5%), respectively. The major difference in the oil compositionof the two Alpinia were *A. officinarum* oil contained camphor (4.0%) and α -fenchyl acetate (8.9%), whereas *A. galanga* oil contained chavicol (0.9%), ß-farnesene (8.4%), ß- sesquiphellandrene (2.6%), ß -bisabolene (0.3%), and eugenol acetate (3.3%).

Alpinia calcarata and *Alpinia galanga* rhizomes were hydrodistilled to produce essential oils, which were then evaluated by Suresh *et al*. (2016) using gas chromatography-massspectrometry. Cubenol (15.0%), 1,8-cineole (12.1%), and 1,8-fenchyl acetates (12.9 and 9.7%, respectively) were the main constituents in *A. calcarata*, while 1,8-cineole (32.9%), α -terpineol(12.7%), and D- germacrene (6.1%) were the main constituents in *A. galanga.*

Using capillary G.C. and GC/MS, the rhizomes and leaves of *Alpinia galanga* Willd. fromBangalore and Hyderabad in India were examined for their essential oils (Gopal *et al*., 2002). Similar components were detected in the oils of the rhizomes and

leaves from the two locations. Limonene (3.7% and 3.5%), 1,8-cineole (33.0% and 30.2%), camphor (5.0% and 14.0%), α -terpineol (9.3% and 2.3%), α -fenchyl acetate (12.7% and 1.1%, respectively), and (E)-methyl cinnamate (5.3% and 2.6%, respectively) were the main components in the rhizome oils from Bangalore and Hyderabad. The primary components of the leaf oils collected from the same places were 1,8-cineole (34.4% and 30.7%), camphene (5.0% and 5.1%), 1,5-pinene (6.6% and 6.3%,), 1,6-pinene (21.5% and 23.5%), and α -pinene (7.8% and 12.8%, respectively).

Jantan *et al*. (2004) did an investigation on galangal oils extracted from the rhizomes andseeds of Malaysian *Alpinia galanga (L.)* Willd. Investigation showed that 1,8-cineole made about 40.5% of the rhizome oil and the sesquiterpenoids like β-bisabolene (8.4%), (Z, E)-farnesol (3.8%), β -caryophyllene (3.6%), and (E)- β -farnesene (3.2%) were also present in the oil in considerable concentrations. In the seed oil, β-bisabolene (37.6%), β-farnesene (22.7%), β- farnesyl acetate (7.9%), β-farnesol (3.9%), and β-caryophyllene (3.0%) were the major components.

2.2.6 Uses of *Alpinia galanga*

Fresh rhizomes have a refreshing, minty, and aromatic odor with a spicy taste. Fresh anddried rhizomes were commonly used as one of the key spices for curries, soups, and seafood meals throughout countries in South-East Asia (Kubota *et al*., 1999).

The rhizomes possess high carbohydrate content (78.9%), low-fat content (11.14%), and nutritional value (311.7 calories per 100g). Copper (0.485 ppm), chromium (0.283 ppm), zinc (6.038 ppm), manganese (12.44 ppm), nickel (0.328 ppm), iron (17.23 ppm), magnesium (9680 ppm), calcium (348.3 ppm), sodium (31.80 ppm), and potassium (1525 ppm) are the macro- andmicronutrients found in the rhizomes (Indrayan *et al*., 2009).

Studies conducted by Arfa and Ghannam (2022) showed that beef burger patties containing galangal had excellent sensory quality and palatability, particularly those containing (2.5 and 3.5 g) galangal powder and (10% and 15%) galangal extract, even after beingstored for six months in a frozen environment.

Galangal rhizomes are frequently used in Asian countries as spice or ginger substitutes for flavoring dishes (e.g., meat and curries). Galangal extract is used to flavor liquor and also usedto enhance or replace alcohol in both alcoholic and non-alcoholic beverages (Yang *et al*., 1999).

Alpinia galanga Willd. roots and stalks can be utilized as sources of colorants in calico printing, and it is a prominent dye-generating plant in India (Gokhale *et al*., 2004). Galangin is the main chemical constituent in *Alpinia galanga* that acts as a coloring agent (Sukhirun *et al*., 2011).

Galangal acetate was used on hard candies and chewing gum offered a peppery pungent flavor. The addition of 100 ppm galangal acetate to toothpaste or mouthwash makes them warm/hot sensations (Yang *et al*., 1999).

Aljobair (2022) reported notable concentrations of ash (5.38%), carbohydrate (81.13%), protein (5.86%), potassium (159.79 mg/kg), phosphorous (17.36 mg/kg), magnesium (15.57 mg/kg), calcium (25.7 mg/kg), iron (7.2 mg/kg), and manganese (3.82 mg/kg) in *Alpinia galanga* rhizome.

2.2.7 Medicinal and Pharmacological uses of *Alpinia galanga*

Alpinia galanga has an intense anti-inflammatory action. It is used in many Asian countries to treat diarrhea, stomach cancer, fever, dyspepsia, and urinary incontinence (Namsa *et al*., 2009).

The active ingredient 1'S-1'acetoxychavicol acetate, which is present in the galangal rhizome extract, is essential for preventing anti-human immunodeficiency virus type 1 reverse transport replication (Ye and Li., 2006).

A methanolic extract from the galangal rhizome effectively prevented hemoglobin from being glycosylated. The rhizome has a substantial antidiabetic effect by inhibiting α-amylase andα -glucosidase activities in-vitro (Heera *et al*., 2014)**.**

The rhizome was used to treat many illnesses, including bacterial infections, diabetes, throat infections, kidney problems, heart conditions, ulcers, bronchitis,

rheumatism, chronic enteritis, bad breath, whooping cough, and fever (Mahae and Chaiseri, 2009; Pompimon *et al*., 2009).

According to Babu *et al*. (2017), the plant's rhizome possesses medicinal properties thatinclude digestive, anti-emetic, anti-tumor, anti-helminthic, anti-diuretic, anti-ulcerative, and anti- dementia. The rhizome extract exhibits anti-tubercular, hypothermic, bronchial catarrhal, tonic, stomachic, and stimulant properties.

Alpinia galanga is frequently used in cooking as an aromatic stimulant or flavoring agentas well as a traditional remedy for rheumatism, bronchial catarrh, and respiratory conditions, including asthma (Chudiwal *et al*., 2010).

Achuthan and Padikkala (1997) reported that twenty milligrams of *A. galanga*'s ethanolicextract administered to rats every day for four weeks had a substantial effect on their serum levelsof HDL (high density lipoproteins).

The strong antidiabetic efficacy of powdered Alpinia rhizome was demonstrated by Akhtar *et al*. (2002). The administration of powdered rhizome from *Alpinia galanga* resulted ina considerable reduction in the blood glucose levels of normal rabbits.

Hot water soluble polysaccharide extract of *A. galanga* rhizome possesses a marked stimulating effect on the reticuloendothelial system (RES) and increased the number of peritonealexudates cells and spleen cells of mice (Bendjeddou *et al*., 2003).

Sukhiran *et al*. (2010) tested four *Alpinia galanga* Wild Linn. (Zingiberaceae) rhizome extracts against adult flies. They discovered that hexane and ethanol extracts had the highest levels of effectiveness (LC50 4,866 and 6,337 ppm, respectively, after 24 h). They concluded that the detoxification enzymes carboxylesterase (C.E.) and glutathione transferase (GST) enzymes inhibited with plantallelochemicals could be a beneficial alternative strategy for managing the pest in the field.

2.3 ANTIMICROBIAL ACTIVITY

Numerous studies have been conducted to investigate the antimicrobial potential using the rhizomes, leaves, flowers, pseudostems, and seeds of *Alpinia galanga* (Subramanian and Nishan,2015). The 1,8-cineol, a marker compound in the *Alpinia* spp., had a potent biological activity. A wide range of biological effects, including antibacterial, antifungal, anticancer, antiulcer, antiallergic, antioxidant impact, and insecticidal action have been confirmedin *Alpinia galanga.*

A study was conducted by Malik *et al*. (2016) to detect the antibacterial efficacy of galangal rhizomes. The result shows that *Klebsiella pneumoniae* ATCC 10031 strain had the highest inhibition zone, measuring 12.0±0.12 mm, followed by *Yersinia enterocolitica* ATCC 27729, *Micrococcus luteus* ATCC 10240, *Bacillus cereus* ATCC 14579, and *Staphylococcus aureus* ATCC 29737.

In another study (Avci *et al*., 2020), galangal and ginger's antibacterial effects were studied using their ethanol and water (ultra-pure) extracts. The result indicated that ethanol extracts had more potent antibacterial activity than extracts prepared with distilled water. Againsteach of the tested bacterial strains, ethanol extracts showed considerable antibacterial activity (P< 5%). In comparison to the disc diffusion approach, the inhibition zones were found to be greater in the agar diffusion method. Ginger and galangal extracts had a weak antimicrobial effect on *E. coli,* while both plants' ethanolextracts had a moderate level of antimicrobial effect on *S. aureus, E. faecalis,* and *P. aeruginosa*(P<5%)

The main objective of the investigation was to examine the antibacterial activity of *Alpiniagalanga Linn*. against food-borne bacteria and to identify the most potent fraction from these plants (Prakatthagomol *et al*., 2012). Four different strains of *Escherichia coli*, *Corynebacteriumsp.*, and *Staphylococcus aureus* were inhibited by the crude ethanolic extract of *A. galanga*. Results showed that *A. galanga* produced the most effective crude ethanolic extract used against both gram-positive and gram-negative food-borne bacteria.

Kambar *et al*. (2014) evaluated the antibacterial and radical-scavenging properties of *A. galanga* leaf and rhizome extract. Agar well diffusion assay was

followed to measure the antimicrobial activity of extracts against 15 clinical isolates of bacteria (from a burn, dental caries,and urinary tract infection), as well as two fungi (*Candida albicans* and *Cryptococcus neoformans*). High inhibitory action against fungus and clinical isolates of bacteria was discovered in rhizome extract. When compared to isolates from the urinary tract, there was considerable inhibitory action against burn and dental caries isolates. In general, extracts were more effective against gram-positive bacteria, and the potential of rhizome extract was described due to high phenolic and flavonoid content.

D, L-10-acetoxychavicol, was present in several plants in the family Zingiberaceae, particularly galangal (Oonmetta-aree *et al*., 2006). The galangal extract had the most substantialdeterrent impact against *S. aureus* compared to ginger, and turmeric. Some yeast and gram- positive bacteria were sensitive to the extract, particularly those from Staphylococcal species.

Alpinia galanga and *Phyllanthus emblica Linn.* extracts were used to evaluate theantibacterial and antioxidant properties by Mayachiew and Devahastin (2007). The antibacterialproperties of plant extracts against *Staphylococcus aureus* were assessed using two different techniques (disc diffusion and agar dilution procedures). Galangal and Indian gooseberry extracts were found to have minimum biocidal concentrations (MBC) of 13.97 and 2.34 mg/ml, respectively, and minimum inhibitory concentrations (MIC) of 13.97 and 0.78 mg/ml.

Molika *et al*. (2020) evaluated the phytotoxicity on seed germination and seedling growthof pak choi (*Brassica rapa var. Chinensis*) as well as the effectiveness of galangal rhizome ethanolic extract in reducing *Curvularia lunata* and *Fusarium sacchari*. 10 per cent of ethanolic extractin the solution was used to test the antifungal activity. *Fusarium sacchari*'s mycelial growth was greatlyreduced by ethanolic extract 60, 80, and 95 per cent, with an inhibition range of 85.12 to 90.83 per cent. The most effective inhibitor of *C. lunata*, ethanolic extract of 60 per cent, showed 82.12 per cent inhibition.

A study was conducted by Sanit (2016) to investigate the antifungal properties of twenty- four medicinal plants including galangal using crude ethanolic extracts. Extract checked against the *Fusarium spp.* (the pathogen causing filthy panicle disease

in rice) using the approach of poisoned food technique at 0, 1000, 2500, 5000, 7500, and 10,000 ppm. They assessed the inhibition of mycelial growth. All twenty-four crude extracts showed 100 per cent mycelial inhibition against *Fusarium sp.* In contrast, *S. bicolor* and *S. nodiflora* inhibited by 32 per cent and 42 per cent respectively, at 10,000 ppm concentration.

Win *et al*. (2022) conducted an experiment using fifteen plant materials extracted using 60 per cent ethanol through maceration. *Fusarium sacchari* and *Curvularia lunata* were tested against poisoning food techniques. The result showed that 10 per cent concentration of dried So dried *Alpinia galanga* rhizome extract fully inhibited the growth of *F. sacchari:* while a 20 per cent concentration significantly reduced *C. lunata* mycelial growth by about 78.30 per cent. Then *A. galanga powder* extract was proved to be the best alternative for controlling outbreaks of the narrow brownleaf spot disease, grain discoloration (G.D.) diseases, and neck blast disease in field conditions.

Pillai *et al*. (2019) used an ethanolic extract from dried rhizomes (AGEE) and water extracts from Malaysian species of *Alpinia galanga* fresh and dried rhizomes (AGWF and AGWD) to assess their ability to inhibit eight different bacterial isolates, *viz. E. coli, Salmonella typhi,* and *Staphylococcus aureus, Micrococcus luteus, Pseudomonas aeruginosa, Bacillus subtilis*, and *Bacillus thuringiensis, Pseudomonas merobilis* and two fungus isolates, *Candida albicans* as well as *Aspergillus niger*. They revealed a range of activities, from none to weak (12 mm), moderate (12 to 20 mm), and strong (20 mm).

The study done by khodavandi *et al*. (2013) used plant extract from *Alpinia galanga*, which was tested for its ability to inhibit some clinically common species of Candida. The antifungal property of the extract was examined in terms of inhibition zone and minimum inhibitory concentration. Results showed that *A. galangal* was also able to prevent the growth of*Candida tropicalis* and *Candida glabrata* to a lesser extent. The MIC value for *A. galangal* for both Candida tested was 64 µg/ml.

The inhibitory effect of 50 extracts from 35 plants representing 23 different botanical families was examined by Kambar *et al*. (2014). The poisoned food technique was used to test the methanol extracts for antifungal efficacy against *Colletotrichum*

capsici, which was isolated from chilli anthracnose. The effectiveness of each extract in preventing *C. capsici* growth rangedfrom 16 to 74 per cent inhibition.

Handajani and Purwoko (2008) studied the zone of inhibition of filamentous fungi *Fusarium moniliforme*, *Aspergillus flavus*, *Aspergillus terreus*, and *Aspergillus niger* on an ethanol extract of galangal rhizome based on biomass and colony area. *F. moniliforme*, *A. flavus*,and *A. niger* colony areas were significantly ($p<5\%$) inhibited by the galangal rhizome extract. *A. flavus*, *F. moniliforme*, and *A. niger* required extracts with minimum concentrations of 816, 1,682, and 3,366 mg/l, respectively, to suppress their growth.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present investigation entitled "Variability in greater galangal (*Alpinia galanga* (L.) Wild. genotypes for yield and quality'' was carried out at the Department of Plantation, Spices, Medicinal & Aromatic crops, College of Agriculture, Vellanikkara, Kerala Agricultural University, Thrissur, during 2020-2022. This chapter contains detailed information about the materials used, the methodology adopted, observations recorded, and the statistical procedure adopted during the investigation.

The field gene bank of *Alpinia galanga* maintained at the National Bureau of Plant GeneticResources (NBPGR) Regional station, Thrissur (100 36' N latitude and 760 26'E longitudes andthe location situated at an altitude of 40m above MSL was utilized for the study. The phytochemical analysis and anti-microbiological study were carried out at the Department of Plantation, Spices, Medicinal & Aromatic crops and Department of Plant Pathology, College of Agriculture, Vellanikkara, Kerala Agricultural University, Thrissur.

3.1 EXPERIMENTAL MATERIALS

Seventeen *Alpinia galanga* genotypes collected from South Indian states are maintained at the field gene bank of NBPGR Regional Station, Thrissur, and two maintained at the Plantation and Spice farm, College of Agriculture, Vellanikkara, formed the study material (Plate 1).

Table 3.1: List of genotypes and their sources

Sl.No	Accessions	Location of collection
1.	IC265610	Kottayam
2.	IC565492	Thiruvananthapuram
3.	IC582791	Kollam
4.	IC373609	Pathanamthitta
5.	IC565488	Thiruvananthapuram
6.	IC565490	Thiruvananthapuram
7.	IC582808	Pathanamthitta
8.	IC582804	Kollam
9.	IC582817	Kozhikode

Table 3.1 contd.

10.	IC582795	Kollam
11.	IC582766	Ernakulam
12.	IC582780	Alappuzha
13.	IC087883	Idukki
14.	IC557436	Kanyakumari
15.	IC565501	Kollam
16.	IC582782	Thiruvananthapuram
17.	IC402361	Palakkad
18.	PCSAG-1	CoA, Vellanikkara
19.	PCSAG-2	CoA, Vellanikkara

3.2 EXPERIMENTAL LAYOUT

Location: ICAR-NBPGR Regional Station, Vellanikkara

Genotypes: Nineteen

Nineteen *Alpinia galanga* accessions planted during 2009-2012 were selected forthe study. The plants were planted at 1m x1m spacing.

3.2.1 Meteorological data

Weather data of the experimental site was collected from the Department of Agricultural Metrology, College of Agriculture, Vellanikkara, and is attached as appendixI.

3.2.2 Intercultural operations

Necessary cultural operations were carried out during cropping season. Summer irrigation was given at monthly intervals for the regeneration of plants, and need-based irrigation was given during other months. The weeding was done twice a year.

3.2.3 Harvesting

After a period of vegetative growth, the plant showed drying symptoms from February to April. It's the ideal time for harvesting rhizomes. The rhizomes were harvested by manual digging. Later arial pseudo stem was cut and removed and the rhizomes were collected, cleaned,and washed to free off soil and dust.

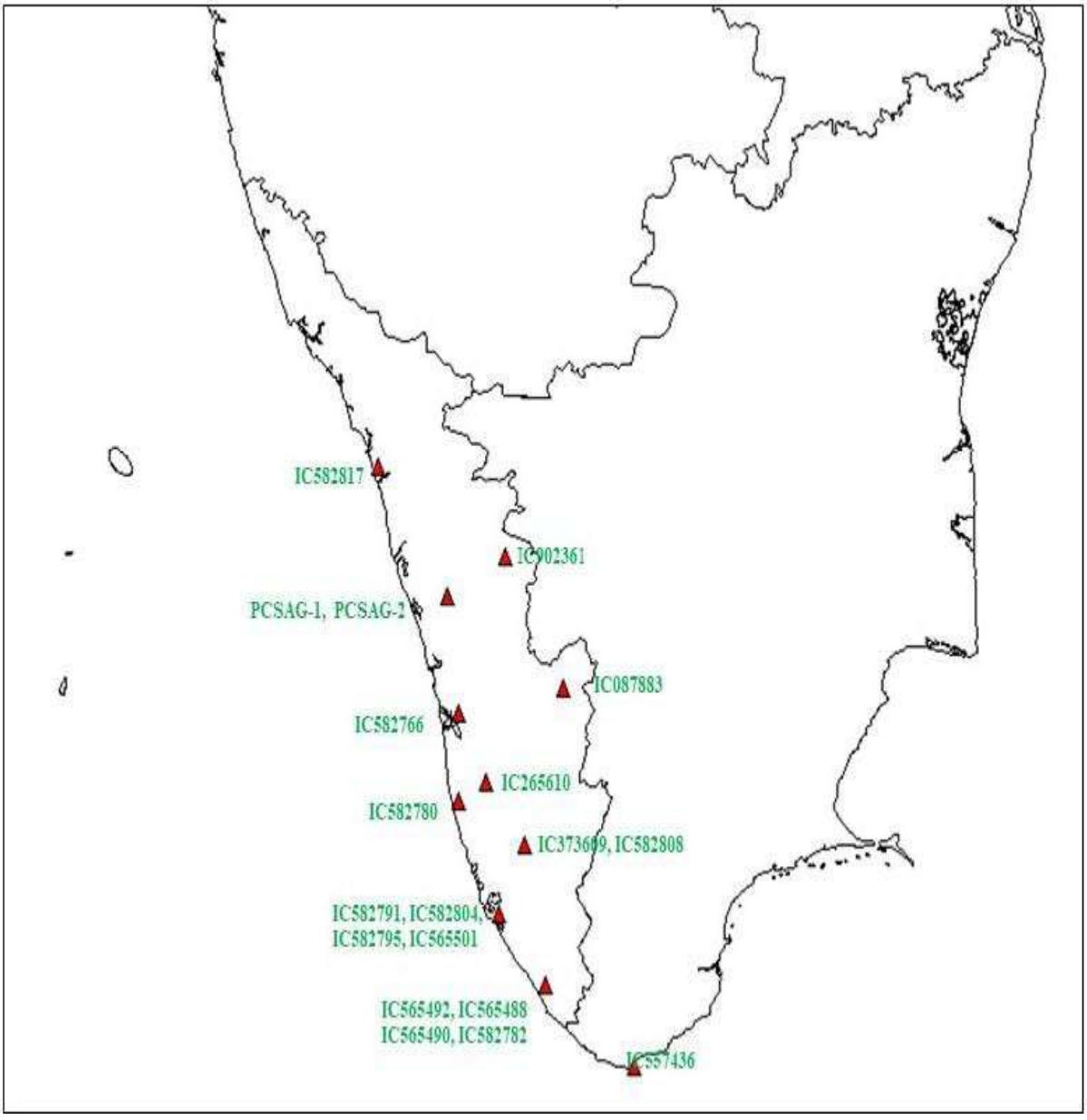


Plate 1: Collection sites of genotypes

3.3 MORPHOLOGICAL EVALUATION

3.3.1 Qualitative characters

The following qualitative parameters were recorded as per the procedure given below,

3.3.1.1 Leaf Shape

Leaf shape was categorized according to the Manual of leaf Architecture (Ash *et al.*,1999).

3.3.1.2 Leaf base

The shape of the leaf base was categorized according to the Manual of leaf Architecture(Ash *et al.,* 1999).

3.3.1.3 Leaf apex

The shape of the leaf apex was categorized according to the Manual of leaf Architecture (Ash *et al.*,1999).

3.3.1.4 Season of flowering

For recording flowering season plants were observed at weekly intervals.

3.3.1.5 Color of petals

The color of the petal was judged according to the Royal Horticulture Society (RHS) color chart.

3.3.1.6 Flowering phenology

Observations were recorded from flower bud initiation to the complete opening of the flower and also from fruitset to ripening on daily basis. The principal growth stages were observed and coded as per the BBCH (*Biologische Bundesanstalt, Bundessortenamt and CHemical industry*) scale.

3.3.1.7 Rhizome shape

Rhizome shape was observed and recorded by visual means.

3.3.1.8 Core color

The core color of the rhizomes were recorded according to the Royal Horticulture Society(RHS) color chart.

3.3.1.9 Skin color

The skin color of the rhizomes were recorded according to the Royal Horticulture Society(RHS) color chart.

3.3.1.10 Seed characters

The seed color was recorded according to the Royal Horticulture Society (RHS) color chart.

3.3.2 Quantitative characters

The following quantitative parameters were recorded at monthly intervals starting from November to February.

3.3.2.1 Tiller length/plant height (cm)

The plant height was measured from ground level to the tip of the topmost leaf of a tiller.Ten healthy tillers were randomly selected to measure height. The average was taken and expressed in centimeters.

3.3.2.2 Number of tillers

The total number of shoots which were arise from the mother plant was recorded on monthly basis.

3.3.2.3 Diameter of tiller(cm)

The diameter was taken from the middle portion of selected tillers and stated in centimeters.

3.3.2.4 Internodal length (cm)

The distance between the two nodes was measured and expressed in centimeters.

3.3.2.5 Number of leaves

Counted the total number of leaves produced in the induvial tiller at monthly intervals.

3.3.2.6 Leaf length

By using a scale, leaf length was measured at monthly intervals. The average was taken from ten tillers and expressed in centimeters.

3.3.2.7 Leaf width

Leaf width was measured from ten selected tillers using a scale at monthly intervals and expressed in centimeters.

3.3.2.8 Leaf area (cm2)

A leaf area meter was used to measure the leaf area of ten leaves at monthly intervals andwas expressed in square centimeters.

3.3.2.9 Petiole length (cm)

Fourth most matured and fully opened leaf from the top was taken for measuring petiole length. Measured the stalk length from the leaf base to the point attached to the shoot and expressed it in centimeters.

3.3.2.10 Inflorescence length (cm)

During flowering, randomly selected three inflorescences were used for analyzing the length, which was worked out as the average and expressed in centimeters.

3.3.2.11 Duration of flowering

The number of days from flower to initiation to fruit set was observed from taggedinflorescence on alternate days.

3.3.2.12 Number of branches / Inflorescence(cm)

The number of branches/inflorescences was observed from three randomly selectedinflorescence and the average was stated in centimeters.

3.3.2.13 Number of flowers

The number of flowers per inflorescence was counted manually from three randomlyselected inflorescences.

3.3.2.14 Length of flower(cm)

Randomly picked three flowers from the inflorescence. The length of the flower wascalculated using the scale and the average was calculated and expressed in centimeters.

3.3.2.15 Width of the flower(cm)

Randomly picked three flowers from the inflorescence. The width of the flower wascalculated using the scale and the average was calculated and expressed in centimeters.

3.3.2.16 Rhizome length(cm)

The rhizome length was measured using vernier caliper and the average length of fiverhizomes was calculated and expressed in centimeters.

3.3.2.17 Rhizome width(cm)

The rhizome width was measured using vernier caliper and the average length of fiverhizomes was calculated and expressed in centimeters.

3.3.2.18 Rhizome weight(g) per tiller

The weight of the rhizomes which were harvested from a single tiller were recorded andthe average weight of rhizome per tiller was taken and expressed in grams.

3.3.2.19 Dry recovery

The fresh weight and dried weight of the rhizomes were recorded and the dryingpercentage was worked out and expressed in per cent.

$$Drying\ percentage = \frac{\text{The dry weight of rhizome (g)} \times 100}{\text{Fresh weight of rhizome(g)}}$$

3.4 SEED DORMANCY STUDY

The mature seeds collected from the fruits were subjected to dormancy breaking treatmentsas given below.

Treatments

a) **One season dry stored seeds**- Fresh seeds have nondeep simple morphological dormancy hence previous season seeds were stored for four months and used for conducting germination tests.

b) **Hot water scarification**- Dropped the seeds into hot water at 75^0C for 5 mins. Both fresh and stored seeds were used.

c) **Treating with growth regulator**- After removing aril, fresh and one season stored seeds were treated with gibberellic acid @ 25 mg/l and 50 mg/l.

d) **Imbibition test**- Fresh and previous season seeds soaked separately in distilled water and kept overnight.

3.5 PHYTO-CONSTITUENTS

Content of oleoresin, volatile oil, starch, flavonoids, phenols, and amino acids of the rhizome of 19 *Alpinia galanga* accessions was estimated by employing the procedures given here. Profiling of the volatile oil was done only in the promising accession (IC878883)

3.5.1 Oleoresin

Oleoresin content in the dried rhizome powder was estimated using the Solvent extractionmethod as per AOAC (1990). Ten grams of sample packed in filter paper was reflexed with 150mlof methanol. The extraction process continued until the solvent in the thimble became colorless.Then the extract was collected to a pre-weighed glass beaker from the round bottom flask and dried the excess solvent using a rotary evaporator; then weighed the recovered oleoresin content, which was expressed in percentage.

$$\text{Oleoresin (\%)} \left(\frac{\text{w}}{\text{w}}\right) = \frac{\text{Weight of the extract(g) x100}}{Weight\ of\ dried\ rhizome\ taken}$$

3.5.2 Volatile oil

The volatile oil was extracted by hydro distillation method using the Clevenger apparatus,Guenther (1972). Coarsely grounded 100-gram dried rhizome sample was distilled for 5 hours. The volatile oil recorded in the Clevenger's tube was collected and the oil content was expressedon a dry weight basis (% v/w).

$$\text{Oil yield (\%V/W)} = \frac{\text{The volume of essential oil (ml)} \times 100}{\text{Weight of rhizome taken (g)}}$$

3.5.3 GCMSMS profiling of volatile oil

This work was carried out at the Pesticide Residue Research lab of the College of Agriculture, Vellanikkara. Triple quadruple GCMSMS (Model TSQ 8000 MSMS) was used to analyze the rhizome oil of *Alpinia galanga*. The stationary phase was TG5M5 column (30 * 0.25 mm, 0.25 m film thicknesses). The oven temperature increased steadily at a rate of 3 °C/min from60 °C to 240 °C. Helium was used as the carrier gas, and the flow rate was 1 mL/min. Finnigan injected a microliter of oil (1: 100 in HPLC grade methanol). Electron impact positive mode at 70 electron volts was used to operate the autoinjector AI3000 with a split ratio of 10:1. By matching mass spectra and retention time indices with the NIST MS Search 2.0 Library, the chemical components were identified. Peak area was shown as a percentage.

3.5.4 Flavonoids

The total flavonoid content was estimated as per the method suggested by Sereena *et al.* (2011). One gram of rhizome powder was homogenized with 10ml of methanol (80%) using a mortar and pestle. The extract was centrifuged and the supernatant was pooled, finally made up the volume to 50ml. Took 1 ml of methanol extract in a test tube, 0.3 ml of 5% $NaNO_2$ solution was added, and waited for two minutes, followed by the addition of 0.3 ml of 10% $AlCl_3$ solutionto the test tube. After two minutes, 3.4ml of NaOH solution was added and kept at room temperature for 10 minutes. The absorbance was taken at 510nm against a blank. A standard calibration curve was drawn using various concentrations. From the standard graph, the total flavonoid content of the sample was determined and expressed in milligrams/gram. Quercetin was used as standard.

3.5.5 Phenols

Total phenolic content was determined by the Folin Ciocalteu method as per Sadasivam and Manickam's (1992) procedure using gallic acid as standard. One mg rhizome powder was weighed and homogenized with 10 ml of 80 per cent ethanol. It was centrifuged at 10000 rpm for 10 minutes to collect the supernatant solution. Again, it was centrifuged with 10ml of 80 per cent ethanol to the residue, pooled supernatant, and discarded the residue. Pipetted 2ml of extract into a test tube and made up the volume to 3ml with water. 0.5ml of Folin Ciocalteau reagent was added and waited for 3min followed by 2ml of 20% Na_2CO_3 solution. Later, that mixture was placed in boiling water bath for one minute, cooled, and measured the absorbance at 650nm against a blank. Prepared a standard graph using different concentrations of catechol. From the standard graph,found out phenol content in the sample and expressed it as mg phenols per gram sample.

3.5.6 Starch

Using the Anthrone method, as per Hedge & Hofreiter's (1962) procedure, starch contentwas estimated. Took 0.1g of weighed dried rhizome powder sample and homogenized it with 80% hot ethanol to remove sugar content in the sample. The content was centrifuged and retainedthe residue, then the residue was washed repeatedly with 80 per cent hot ethanol until the residue did not give color with anthrone reagent. Then the residue was dried in a water bath, followed by adding 5ml water and 6.5ml of 52 per cent perchloric acid to the residue. The content was centrifuged, saved the supernatant, repeated the same procedure with perchloric acid, saved the supernatant, and made the volume up to 100ml using distilled water. Pippeted out 0.1 ml of supernatant and made up to 1 ml with water. Prepared the standards by taking different concentrations from 0.2mlto 1ml of working standard and made up the volume to 1ml with water. For that, 4ml of anthrone reagent was added, heated the tubes for 8mintues in a water bath, cooled the test tubes and read the intensity at 630nm. Found out the glucose content by using a standard graph and then multiplied the amount of glucose content by 0.9 to work out the starch content.

3.5.7 Free Amino acids

The amino acid was estimated as per the procedure given by Sadasivam and Manickam (1992). Took 500mg of weighed sample and homogenized with 10ml of 80% ethanol in mortar and pestle. The content was centrifuged and took 0.1ml of filtered extract mixed with 1ml of ninhydrin reagent. Then made up the volume up to 2ml with distilled water. Boiled the content ina water bath for 20minute followed by adding 5ml dilutant (mixture of equal proportion of propanol and water) and mixing the solution thoroughly. After 15 minutes, read the intensity of the purple color against the blank in a spectrophotometer at 570nm. Drew a standard curve using absorbance against the concentration of leucine. Calculated the total free amino acid in the sampleand expressed milligrams/gram sample.

3.6 *IN VITRO* EVALUATION OF ETHANOLIC EXTRACT OF RHIZOME AGAINST MAJOR FUNGAL PATHOGENS AND PLANT PATHOGENIC BACTERIA

3.6.1 *In vitro* evaluation of ethanolic extract of rhizome against major fungal pathogens

1. Sample preparation

Harvested rhizomes were cleaned thoroughly then fresh rhizomes were sliced and dried in a tray-dryer at 50 ^{0}C for 24 h. For later use, dried rhizomes were blended into a fine powder. Ten grams of rhizome powder were soaked with 100 ml of 80% ethanol and left at room temperature overnight. Whatman No. 1 filter paper was used to filter the content later the extractwas kept at 4 ^{0}C until it was used for the experiment.

2. *In vitro* evaluation of antifungal activity of rhizome ethanolic extract

In vitro evaluation of nineteen *Alpinia galanga* genotypes extract was tested against four major fungal pathogens at three different concentrations *viz,* 2 per cent, 5 per cent, and 10 per cent concentrations by the following protocol of poisoned food technique (Sanit, 2016). According to the standard protocol, three different concentrations of rhizome extract (2%, 5%, and 10%) were measured and added to 100ml sterilized PDA media separately and thoroughly mixed with the medium to get a uniform concentration. Pour 20 ml of the medium into sterile Petri plates; inoculate Petri plates with 8mm mycelial disc of *Colletotrichum* spp. *Fusarium* spp.*Phytophthora* spp. *and Rhizoctonia* spp. taken from the pure culture of each fungus and placed atthe center of plates. Petri plate poured without any extract used as control plate. Observation fromcontrol and treatment plates was recorded until full growth was attained in the control plate, andthe Per cent inhibition of the mycelial growth was calculated by the formula as follows (Yang *et al*., 2005). Fungal pathogens are presented in Pate 2.

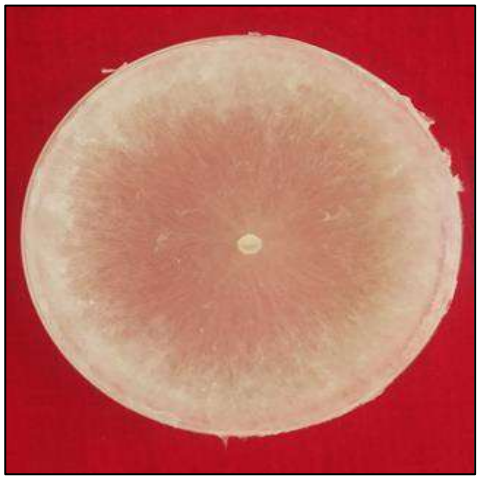
Whereas,

$$\text{Per cent inhibition} = \frac{A-B}{A} \times 100$$

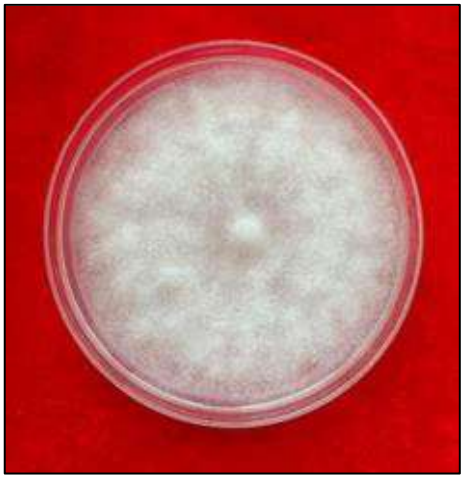
PI- Per cent inhibition of mycelial growth (%)

A- Growth of fungus in control plate(cm)

B-Growth of fungus in treatment plates(cm)



a. Rhizoctonia spp.



b. Phytophthora spp.

c. *Colletotrichum* spp.

e. *Fusarium* spp.

d. Ralstonia solanacearum

Plate 2: Tested fungal pathogens and plant pathogenic bacteria

3.6.2 *In vitro* evaluation of antibacterial activity of ethanolic extract of rhizome against *Ralstonia solanacearum*

1. Sample preparation

Rhizome extract at three different concentrations (2%, 5%, and 10%) from nineteen genotypes were prepared by soaking 0.2g, 0.5g, and 1g separately in 2ml, 5ml and 10ml (80%) ethanol and left at room temperature overnight. Whatman No. 1 filter paper was used to filter the content later the extract was kept at 4 ^{0}C until it was used for the experiment.

2. Bacterial culture preparation

Ralstonia solanacearum gram-negative bacteria was cultured in TZC (Triphenyl tetrazoliumchloride) media for getting pure culture later it was incubated for 18–24 h. The stock culture of the above-mentioned bacteria was prepared by using one loop of virulent colonies of *R.solanacearum* in sterile water and stored at 4 ^{0}C until it was used for the experiment. The bacteriawere streaked by taking a single colony in nutrient agar medium and incubated at 35 ^{0}C for 18–24 hours one day before the experiment.

3. Inhibition zone assay by agar well diffusion method

A virulent single colony from nutrient agar media was transferred into 10 ml sterile watershaken vigorously and used for bacterial lawn preparation. 0.1ml of the bacterial suspension waspipetted out and placed over solidified sterile nutrient agar and spread the bacterial suspension uniformly over solidified medium using a sterilized L rod. A agar well of 8mm diameter was prepared with help of a sterile cork borer and the bacterial suspension was filled with 50µl extractof respective concentrations. Control plates with streptomycin maintained as the positive controland sterile water as the negative control. Inoculated plates were incubated Incubate the plates at room temperature for 24 hours. A clear zone was appeared around agar wells if the extract represented the antibacterial activity. Compare the diameter of clear zone in the control plate andtreatment plate and the percentage of inhibition was calculated using the given formula.

$$Per\ cent\ inhibition = \frac{Growth\ in\ control(C) - Growth\ in\ treatment(T)}{Growth\ in\ control(T)} \times 100$$

3.7 Statistical analysis

The observational data with respect to morphological parameters during the course of researchwork were tabulated and statically analyzed with help of SPSS software using analysis of variance (one-way ANOVA).

Further, the data generated from biochemical parameters were analyzed with the help ofGeneral R- shiny based Analysis Platform Empowered by Statics (KAU-GRAPES 1.0.0., 2020)package developed by Gopinath *et al*. (2021). The experimental design followed was CRD (completely randomized design) wherever, the result found significant, the critical difference (CD) was calculated at one and five per cent level of significance.

The correlation coefficient was attempted to ascertain the relationship between morphological traits with yield parameters and antimicrobial content in phytoconstituentsagainst fungal pathogen in all the possible ways suggested by Johnson *et al*. (1955).

Experimental data generated from the antimicrobial studies were analyzed by using the web Agri stat package (WASP2.0), and data transformations were made as required. Multiple comparison of the treatment means was done using critically difference method.

RESULTS

4. RESULTS

The present study investigated the variability in greater galangal (*Alpinia galanga* L.) Wild.genotypes for yield and quality. Morphological, biochemical and antimicrobial studies were conducted and the data generated were statistically analyzed using standard procedures. Results obtained during the course of the investigation are presented under the following subheadings.

4.1 MORPHOLOGICAL EVALUATION

Morphological evaluation of *Alpinia galanga* genotypes involved 19 accessions. The study comprised of qualitative and quantitative characterization of *Alpinia galanga* genotypes for yieldand quality. Variation among the genotypes was analyzed with the analysis of variance (one-wayANOVA). Further, the Karl Pearson correlation coefficient was attempted for the correlation of rhizome yield with the other morphological characters.

4.1.1 Morphological evaluation of *Alpinia galanga* genotypes

4.1.1.1Qualitative parameters

During the study period, nineteen *Alpinia galanga* genotypes were characterized for qualitative parameters like rhizome color and shape, the shape of leaf, leaf base and leaf apex, and color of flower petals, and seed. The data pertaining to the qualitative parameters of *Alpiniagalanga* used in the study are presented in Table 4.1A, 4.1 B and Plate 3.

Table 4.1A: Qualitative morphological parameters of *Alpinia galanga* genotypes

Parameters	**Observation**
Rhizome color: Skin color Core color	Greyed yellow (RHS color chart) Yellowish white (RHS color chart)
Rhizome shape	Cylindrical
Leaf shape	Lanceolate -oblong
Shape of the leaf apex	Acuminate
Shape of the leaf base	Cuneate
Season of flowering	2 seasons (May-July and August - October)
Color of petal	White with pink markings
Seed color	Dark brown to black (RHS color chart)

Details regarding the season of flowering among the accessions are presented in Table 4.1B.

Table 4.1B: Season of flowering in *Alpinia galanga* genotypes

Accession	Flowering season
IC582791	August - October
IC373609	May - July
IC565488	May - July
IC582766	May - July
IC582804	August - October
IC582817	May – July
IC878883	August – October
IC565501	August – October
IC582782	August – October
IC557436	May- July
IC565490	August – October
IC402361	August – October
IC582780	May – July
IC565492	May – July
IC582795	August – October
IC265610	August – October
IC582808	August - October
PCSAG-1	May- July
PCSAG-2	May - July

There was no significant variation observed for the above mentioned qualitative traits amongthe genotypes except for the season of flowering (Table 2A). Out of 19 accessions, nine accessions (IC373609, IC565488, IC582766, IC582817, IC557436, IC565492, IC582780, PCSAG-1, and PCSAG-2) showed early flowering from May-July. In the rest of the genotypes flowering was late from August to October.

4.1.1.2 Quantitative character

Morphological characteristics like plant height, number of tillers, diameter of the tiller, number of leaves, leaf length, leaf width, petiole length, and leaf area were recorded at monthlyintervals from November to February to observe the growth pattern of the plant. Flower characters were observed during the peak season of flowering and rhizome characters were recorded after the harvest of rhizomes (Plate 3).

4.1.1.2.1 Plant height

Table 4.2 A: Plant height in *Alpinia galanga* genotypes at monthly intervals

	Plant height(cm)			
Accessions	**November**	**December**	**January**	**February**
IC582791	176.53	189.70	194.90	190.30
IC373609	170.32	183.37	191.00	187.50
IC565488	198.67	214.05	221.60	218.00
IC582766	237.85	253.18	257.90	257.90
IC582804	226.60	239.29	242.60	245.40
IC582817	239.90	254.99	259.80	259.90
IC878883	232.70	245.30	247.60	250.30
IC565501	187.40	200.24	203.60	200.90
IC582782	212.90	224.94	227.70	221.20
IC557436	167.20	179.35	185.30	186.30
IC565490	151.50	165.05	170.30	172.30
IC402361	187.68	211.20	216.00	205.50
IC582780	227.20	241.20	226.40	221.90
IC565492	163.52	176.50	182.10	178.70
IC582795	195.37	208.90	213.10	209.20
IC265610	241.00	256.50	251.50	237.00
IC582808	233.25	251.20	254.30	248.40
PCSAG-1	257.60	269.40	277.90	278.60
PCSAG-2	265.00	278.80	280.60	255.20
Mean	209.06	223.32	226.54	222.34
SD	43.52	43.12	44.79	46.04
F value (5%)	**13.486***	**14.431***	**10.256***	**7.762***

Table 4.2B: Monthly variation in plant height in *Alpinia galanga* genotypes

Parameter	**Time of observation**	**Mean**	**Standard deviation**	**Standard error**	**F value (5%)**
Plant height(cm)	November	209.06	34.16	7.84	**1.018***
	December	223.32	34.36	7.88	
	January	226.54	33.08	7.59	
	February	222.34	31.63	7.26	
	Total	**220.32**	**33.34**	**3.82**	

Plant height for nineteen genotypes of *Alpinia galanga* observed for successive months from November to February is presented in Table 4.2A. During the last month of observation, the plantheight ranged from 172.30 to 278.60 cm. PCSAG-1 (278.60cm) was found to be the tallest followed by IC582817 (259.90cm). Accession IC565490 (172.30cm) was found to be the shortestamong all the genotypes.

While comparing the growth pattern of the genotypes during these months, genotypes recorded the highest height during January with a mean value of 226.54 cm depicted in Table 4.2B.

4.1.1.2.2 Tiller diameter

Significant variations were observed among the genotypes for the diameter of the tillers as shown in Table 4.3 A. The diameter recorded among the genotypes ranged between 4.15 cm to 4.67 cm during February. Accession IC565492 and IC582795 (4.67cm) recorded the highest diameter followed by IC878883(4.66cm) whereas the lowest diameter was recorded in the accession IC402361 (4.15cm).

There was a significant increase in the diameter of the tiller during these months. A significantly highest tiller diameter was recorded in February with a mean value of 4.43 cm (Table 4.3B).

Table 4.3A: Tiller diameter in *Alpinia galanga* genotypes at monthly intervals

	Tiller diameter (cm)			
Accessions	**November**	**December**	**January**	**February**
IC582791	3.99	4.26	4.26	4.26
IC373609	4.09	4.23	4.23	4.23
IC565488	4.34	4.48	4.48	4.48
IC582766	4.31	4.37	4.37	4.37
IC582804	4.12	4.36	4.51	4.53
IC582817	4.50	4.62	4.62	4.62
IC878883	4.55	4.64	4.66	4.66
IC565501	4.29	4.39	4.47	4.47
IC582782	3.64	4.00	4.17	4.22
IC557436	3.71	4.02	4.16	4.21
IC565490	3.99	4.24	4.26	4.26
IC402361	3.42	3.78	4.12	4.15

Table 4.2A contd.

IC582780	4.45	4.62	4.62	4.62
IC565492	4.47	4.67	4.67	4.67
IC582795	4.50	4.67	4.67	4.67
IC265610	4.24	4.56	4.60	4.60
IC582808	4.32	4.45	4.45	4.48
PCSAG-1	4.03	4.29	4.29	4.29
PCSAG-2	3.82	4.10	4.28	4.30
Mean	4.15	4.36	4.42	4.43
SD	0.47	0.38	0.30	0.28
F value (5%)	**7.71***	**6.73***	**5.86***	**5.92***

Table 4.3 B: Variation in tiller diameter of *Alpinia galanga* genotypes.

Parameter	**Time of observation**	**Mean**	**Standard deviation**	**Standard error**	**F value(5%)**
Diameter of tillers(cm)	November	4.15	0.32	0.07	**5.382***
	December	4.36	0.25	0.06	
	January	4.42	0.19	0.04	
	February	4.43	0.18	0.04	
	Total	**4.34**	**0.26**	**0.03**	

4.1.1.2.3 Number of tillers

There was significant variation observed for number of tillers among the genotypes during the observed months as seen from the data presented in Table 4.4 A. The average number of tillersrecorded in these months ranged from 16.16 to 21.74. Among the accessions, IC582817 (46, 46,42, 42) and PCSAG-1 (44, 44, 38, 37) maintained a maximum number of tillers during the observed months. Whereas accession IC582804 (11, 11, 8, 3) and IC565501 (9, 9, 3, 5) produceda very less number of tillers throughout the observed months.

During the course of the investigation, the maximum number of tillers was recorded in November and December as evident from the data presented in Table 4.4 B. In the latter months,there was no production of tillers at all. At the same time, there was a reduction in tiller number in January and February months because the plant started drying during the summer months.

Table 4.4 A: Number of tillers per plant in *Alpinia galanga* genotypes at monthly intervals.

	Number of tiller/plants			
Accessions	**November**	**December**	**January**	**February**
IC582791	10	10	7	5
IC373609	30	30	17	17
IC565488	18	18	10	7
IC582766	8	8	8	9
IC582804	11	11	8	3
IC582817	46	46	42	42
IC878883	18	18	18	15
IC565501	9	9	3	5
IC582782	19	19	15	12
IC557436	20	20	20	15
IC565490	23	23	15	20
IC402361	11	11	11	8
IC582780	28	28	28	30
IC565492	18	18	16	8
IC582795	16	16	16	17
IC265610	30	30	25	15
IC582808	39	39	35	32
PCSAG-1	44	44	38	37
PCSAG-2	15	15	9	10
Mean	**21.74**	**21.74**	**17.95**	**16.16**

Table 4.4B: Monthly variation in tiller number in *Alpinia galanga* genotypes

Parameter	**Time of observation**	**Mean**	**Standard deviation**	**Standard error**	**F value (5%)**
Number of tillers	November	21.74	11.57	2.654	**1.15***
	December	21.74	11.57	2.654	
	January	17.95	11.018	2.528	
	February	16.16	11.33	2.599	
	Total	**19.39**	**11.41**	**1.31**	

4.1.1.2.4 Internodal length

The internodal length of tillers recorded among the genotypes varied significantly and it ranged between 6.29 cm to 10.16 cm as depicted in Table 4.5 A. The internodal length of tillersrecorded among the genotypes ranged between 6.29 cm to 10.16 cm. Accession PCSAG-2 (10.16cm) followed by PCSAG-1(10.08cm) recorded maximum internodal length and minimuminternodal length was noticed in accession IC557436 (6.29cm).

Similarly, there was slight increase in the internodal length during the observedmonths and the highest was recorded in the month of February with a mean value of 8.59 cm (Table 4.5 B).

Table 4.5 A: Internodal length in *Alpinia galanga* genotypes at monthly intervals

	Internodal length(cm)			
Accession	**November**	**December**	**January**	**February**
IC582791	7.32	7.43	7.78	7.78
IC373609	8.80	8.84	8.84	8.84
IC565488	8.87	8.87	8.87	8.87
IC582766	8.58	8.82	8.82	8.82
IC582804	8.38	8.40	8.43	8.43
IC582817	8.87	8.87	8.87	8.87
IC878883	7.85	8.03	8.22	8.22
IC565501	8.87	8.87	8.87	8.87
IC582782	6.45	6.91	7.31	7.31
IC557436	5.65	6.09	6.29	6.29
IC565490	7.72	8.04	8.11	8.11
IC402361	8.25	8.35	8.43	8.43
IC582780	8.66	8.66	8.66	8.66
IC565492	8.80	8.88	8.88	8.88
IC582795	8.81	8.81	8.81	8.81
IC265610	8.92	8.95	8.95	8.95
IC582808	8.87	8.87	8.87	8.87
PCSAG-1	9.85	10.08	10.08	10.08
PCSAG-2	10.10	10.16	10.16	10.16
Mean	8.40	8.52	8.59	8.59
SD	1.14	1.01	0.92	0.92
F value (5%)	**43.41***	**49.24***	**46.00***	**46.00***

Table 4.5 B: Monthly variation in internodal length in *Alpinia galanga* genotypes

Parameter	Time of observation	Mean	Standard deviation	Standard error	F value (5%)
Internodal length(cm)	November	8.40	1.06	0.24	**0.176***
	December	8.52	0.96	0.22	
	January	8.59	0.86	0.20	
	February	8.59	0.86	0.20	
	Total	**8.53**	**0.92**	**0.11**	

4.1.1.2.5 Number of leaves

There was significant variation observed for number of leaves among the genotypes during the observed months as presented in the data in Table 4.6 A. The number of leaves pertiller recorded among the accessions ranged between 7.4 to 13.50. Accession IC582782(15.20, 16.20, 15.70, 13.50) and IC373609 (13.10, 14.10, 12.10, 12) recorded highest number of leaves per tiller during observed months, whereas IC565501 (10.60, 11.60, 9.40, 7.40) recorded the lowest number of leaves among all the genotypes.

Variations in leaf per tiller in *Alpinia galanga* genotypes ranged between 7.4 to 13.5 andthe data are depicted in Table 4.6 B. While comparing the growth pattern in all the accessionsduring these months, the plant maintained the highest number of leaves in the month of December followed by November with the mean value of 14.52 and 13.52 respectively.

Table 4.6 A: Number of leaves in *Alpinia galanga* genotypes at monthly intervals

	Number of leaves/tiller			
Accessions	**November**	**December**	**January**	**February**
IC582791	13.30	14.30	10.50	9.10
IC373609	13.10	14.10	12.10	12.30
IC565488	13.00	14.00	10.90	10.10
IC582766	14.90	15.90	13.80	11.30
IC582804	13.50	14.50	10.60	10.30

Table 4.6 A contd.

IC582817	14.70	15.70	11.90	10.60
IC878883	14.20	15.20	11.30	11.40
IC565501	10.60	11.60	9.40	7.40
IC582782	15.20	16.20	15.70	13.50
IC557436	10.60	11.60	10.20	8.50
IC565490	11.90	12.90	12.00	9.90
IC402361	12.30	13.30	12.50	10.50
IC582780	14.00	15.00	13.90	11.60
IC565492	13.50	14.50	10.70	8.90
IC582795	12.90	13.90	13.20	11.50
IC265610	14.10	15.10	12.40	10.50
IC582808	15.10	16.10	10.40	9.50
PCSAG-1	14.10	15.10	12.80	10.60
PCSAG-2	15.80	16.80	11.20	9.60
Mean	13.52	14.52	11.86	10.37
SD	2.38	2.38	2.34	2.51
F value (5%)	**5.001***	**5.001***	**3.86***	**4.15***

Table 4.6 B: Monthly variation in number of leaf in *Alpinia galanga* genotypes

Parameter	**Time of observation**	**Mean**	**Standard deviation**	**Standard error**	**F value(5%)**
Number of leaves	November	13.52	1.44	0.33	**33.94***
	December	14.52	1.44	0.33	
	January	11.58	1.29	0.3	
	February	10.37	1.42	0.32	
	Total	**12.5**	**2.13**	**0.24**	

4.1.1.2.6 Leaf length

Data on the leaf length of the genotypes are presented in Table 4.7 A. Leaf length varied from 36.32 cm to 54.81 cm. Accessions namely IC265610 (54.81cm), PCSAG-1 (54.22cm), and IC582766 (54.14cm), recorded the highest leaf length and lowest leaf length was noted in the accession IC565490(39.75cm).

Data given in Table 4.7 B shows that leaf length remained the same during observed monthswith the mean value of 48.86 cm.

Table 4.7A: Leaf length in *Alpinia galanga* genotypes at monthly intervals

	Leaf length(cm)			
Accession	**November**	**December**	**January**	**February**
IC582791	46.38	46.38	46.38	46.38
IC373609	46.37	46.37	46.37	46.37
IC565488	50.16	50.16	50.16	50.16
IC582766	54.14	54.14	54.14	54.14
IC582804	51.09	51.09	51.09	51.09
IC582817	54.09	54.09	54.09	54.09
IC878883	52.22	52.22	52.22	52.22
IC565501	53.72	53.72	53.72	53.72
IC582782	50.42	50.42	50.42	50.42
IC557436	36.32	36.32	36.32	36.32
IC565490	37.75	37.75	37.75	37.75
IC402361	41.35	41.35	41.35	41.35
IC582780	42.16	42.16	42.16	42.16
IC565492	46.38	46.38	46.38	46.38
IC582795	50.46	50.46	50.46	50.46
IC265610	54.81	54.81	54.81	54.81
IC582808	52.51	52.51	52.51	52.51
PCSAG-1	54.22	54.22	54.22	54.22
PCSAG-2	53.75	53.75	53.75	53.75
Mean	48.85	48.85	48.85	48.85
SD	6.83	6.83	6.83	6.834
F value (5%)	**20.54***	**20.54***	**20.54***	**20.54***

Table 4.7 B: Monthly variation in leaf length of *Alpinia galanga genotypes*

Parameter	Time of observation	Mean	Standard deviation	Standard error	F value (5%)
Leaf length(cm)	November	48.86	5.79	1.33	0.000
	December	48.86	5.79	1.33	
	January	48.86	5.79	1.33	
	February	48.86	5.79	1.33	
	Total	**48.86**	**5.67**	**0.65**	

4.1.1.2.7 Leaf width

Data on leaf width recorded in the *Alpinia galanga* at monthly intervals as presented in Table 4.8 A. At the end of February month, the leaf width ranged from 6.75 cm to10.35 cm. Leaf width recorded the highest value in the accession IC565501(10.35cm) followed by IC878883(10cm) whereas, accession IC557436(6.75cm) recorded lowest leaf width in the observed months.

In the monthly growth pattern studies, there was no significant variation observed forleaf width in all the observed months (Table 4.8 B).

Table 4.8 A: Leaf width in *Alpinia galanga* genotypes at monthly intervals

	Leaf width (cm)			
Accession	**November**	**December**	**January**	**February**
IC582791	8.06	8.06	8.06	8.06
IC373609	8.24	8.24	8.24	8.24
IC565488	9.32	9.32	9.32	9.32
IC582766	9.93	9.93	9.93	9.93
IC582804	9.55	9.55	9.55	9.55
IC582817	9.78	9.78	9.78	9.78
IC878883	10.00	10.00	10.00	10.00
IC565501	10.35	10.35	10.35	10.35
IC582782	8.28	8.28	8.28	8.28

Table 4.8 A contd.

IC557436	6.75	6.75	6.75	6.75
IC565490	7.15	7.15	7.15	7.15
IC402361	7.59	7.59	7.59	7.59
IC582780	9.86	9.86	9.86	9.86
IC565492	8.06	8.06	8.06	8.06
IC582795	9.30	9.30	9.30	9.30
IC265610	9.84	9.84	9.84	9.84
IC582808	9.02	9.02	9.02	9.02
PCSAG-1	9.74	9.74	9.74	9.74
PCSAG-2	9.60	9.60	9.60	9.60
Mean	8.96	8.96	8.96	8.96
SD	1.43	1.43	1.43	1.43
F value (5%)	**10.51***	**10.51***	**10.51***	**10.51***

Table 4.8 B: Monthly variation in leaf width of *Alpinia galanga* genotypes

Parameter	**Time of observation**	**Mean**	**Standard deviation**	**Standard error**	**F value (5%)**
Leaf width(cm)	November	8.97	1.07	0.24	0.000
	December	8.97	1.07	0.24	
	January	8.97	1.07	0.24	
	February	8.97	1.07	0.24	
	Total	**8.97**	**1.04**	**0.12**	

4.1.1.2.8 Leaf area

The data on leaf area of *Alpinia galanga* genotypes are presented in Table 4.9 A. Leaf areasignificantly varied among the genotypes and ranged from 172.78 m^2 to 391.02 m^2 with amean of 311.31 m^2 in the last month of observation. The highest leaf

area was recorded in theaccession IC565501 (391.02 m^2) followed by IC265610 (378.64 m^2) whereas, the lowest leafarea was recorded in IC557436 (172.78 m^2).

There was no significant increase in the leaf area during the observed months in all the accessions (Table 4.9 B).

Table 4.9 A: Leaf area in *Alpinia galanga* genotypes at monthly intervals

	Leaf area (cm^2)			
Accession	**November**	**December**	**January**	**February**
IC582791	262.56	262.56	262.56	262.56
IC373609	268.39	268.39	268.39	268.39
IC565488	328.89	328.89	328.89	328.89
IC582766	377.78	377.78	377.78	377.78
IC582804	343.39	343.39	343.39	343.39
IC582817	372.38	372.38	372.38	372.38
IC878883	367.48	367.48	367.48	367.48
IC565501	391.02	391.02	391.02	391.02
IC582782	293.29	293.29	293.29	293.29
IC557436	172.78	172.78	172.78	172.78
IC565490	189.23	189.23	189.23	189.23
IC402361	219.97	219.97	219.97	219.97
IC582780	291.70	291.70	291.70	291.70
IC565492	262.56	262.56	262.56	262.56
IC582795	330.48	330.48	330.48	330.48
IC265610	378.64	378.64	378.64	378.64
IC582808	332.63	332.63	332.63	332.63
PCSAG-1	369.97	369.97	369.97	369.97
PCSAG-2	361.80	361.80	361.80	361.80
Mean	311.31	311.31	311.31	311.31
SD	82.09	82.09	82.09	82.09
F value (5%)	**16.09***	**16.09***	**16.09***	**16.09***

Table 4.9 B: Monthly variation in leaf area in *Alpinia galanga* genotypes

Parameter	Time of observation	Mean	Standard deviation	Standar derror	F value (5%)
Leaf area (cm^2)	November	311.31	66.70	15.30	0.000
	December	311.31	66.70	15.30	
	January	311.31	66.70	15.30	
	February	311.31	66.70	15.30	
	Total	**311.31**	**65.35**	**7.50**	

4.1.1.2.9 Petiole length

Petiole length recorded among the accessions ranged between 0.66 to 0.82 cm as seen fromthe data depicted in Table 4.10 A. Accessions namely IC878883 (0.82 cm) followed byIC373609 (0.80 cm) IC565488(0.80 cm) and IC565501(0.80 cm) were found to possess the highest petiole length and lowest petiole length was recorded in IC582808 (0.66 cm).

Similarly, there was no significant difference in the petiole length during the observed months in all the genotypes (Table 4.10 B).

Table 4.10 A: Petiole length in *Alpinia galanga* genotypes at monthly intervals

	Petiole length (cm)			
Accession	**November**	**December**	**January**	**February**
IC582791	0.71	0.71	0.71	0.71
IC373609	0.80	0.80	0.80	0.80
IC565488	0.80	0.80	0.80	0.80
IC582766	0.67	0.67	0.67	0.67
IC582804	0.79	0.79	0.79	0.79
IC582817	0.75	0.75	0.75	0.75
IC878883	0.82	0.82	0.82	0.82
IC565501	0.80	0.80	0.80	0.80
IC582782	0.72	0.72	0.72	0.72
IC557436	0.73	0.73	0.73	0.73

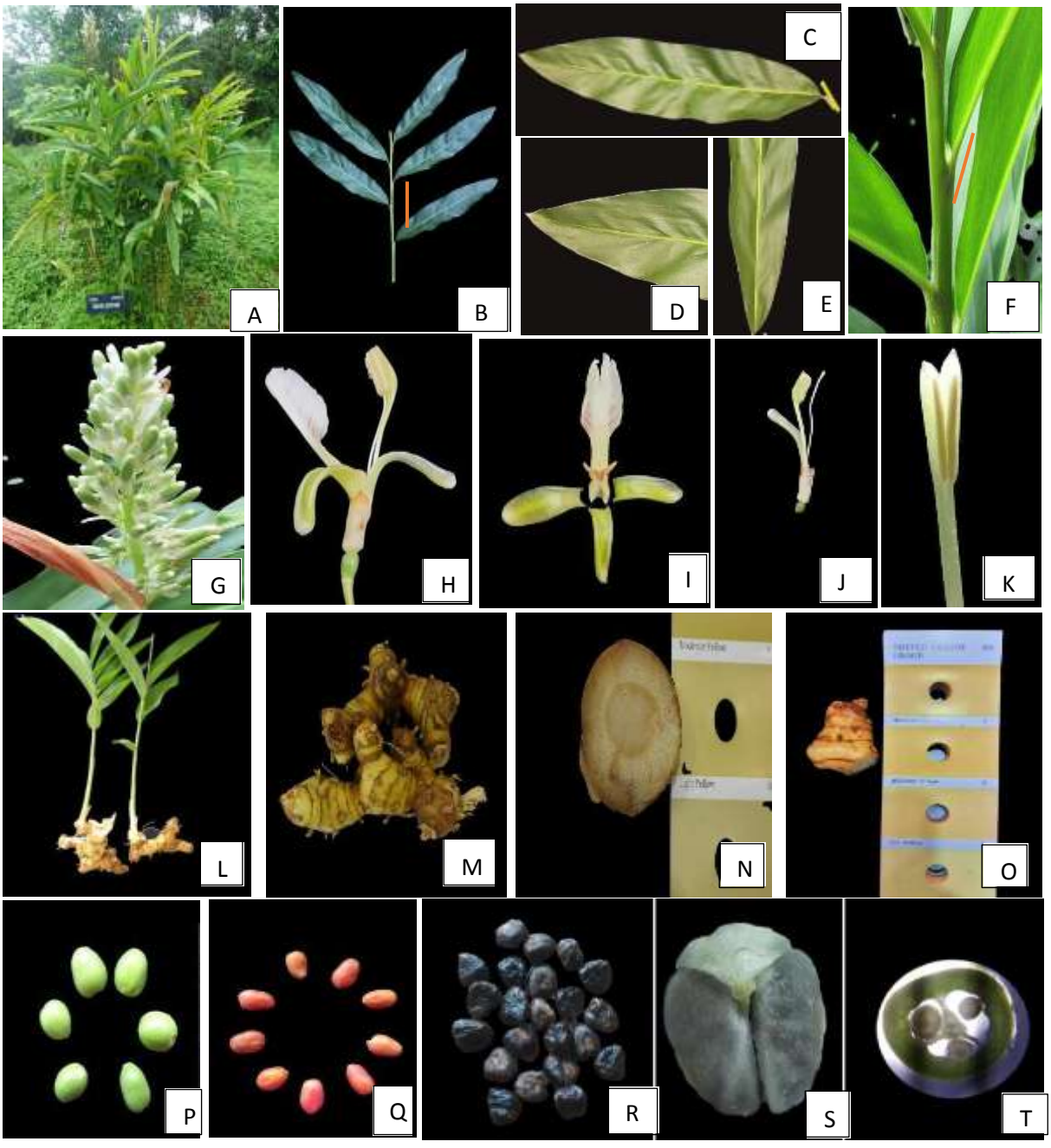


Plate 3: **Plant morphological characteristics of *Alpinia galanga*** A) Clump, B) Internodeal distance, C) Leaf shape, D) Leaf apex, E)Leaf base, F) Leaf petiole, G) Inflorescence, H I J K) Flower parts, L M) Rhizome, N O) Core and skin color, P) Young fruits, Q)Ripen fruits, R) Seeds S) Trilocular ovary, T) Parietal placentation.

Table 4.10 A contd.

IC565490	0.72	0.72	0.72	0.72
IC402361	0.71	0.71	0.71	0.71
IC582780	0.72	0.72	0.72	0.72
IC565492	0.71	0.71	0.71	0.71
IC582795	0.79	0.79	0.79	0.79
IC265610	0.73	0.73	0.73	0.73
IC582808	0.66	0.66	0.66	0.66
PCSAG-1	0.67	0.67	0.67	0.67
PCSAG-2	0.73	0.73	0.73	0.73
Mean	0.74	0.74	0.74	0.74
SD	0.09	0.09	0.09	0.09
F value (5%)	**3.06***	**3.06***	**3.06***	**3.06***

Table 4.10 B: Monthly variation in petiole length of *Alpinia galanga* genotypes

Parameter	**Time of observation**	**Mean**	**Standard deviation**	**Standard error**	**F value (5%)**
Petiole length (cm)	November	0.74	0.05	0.01	0. 000
	December	0.74	0.05	0.01	
	January	0.74	0.05	0.01	
	February	0.74	0.05	0.01	
	Total	**0.74**	**0.05**	**0.01**	

4.1.1.2.10 Flower characters

Floral parameters of *Alpinia galanga* recorded in the experiment are given in Table 4.11A.

Table 4.11 A: Floral parameters of *Alpinia galanga* genotypes

Parameters	Description
Inflorescence type	Panicle
Color of flower	Greenish white
Color of petal	White with pink markings in the midrib
No. of days for flower bud emergence to maturation	12-15 days
Duration of flowering	20-25 days
Number of flowers that open per day	6
Time of anthesis	Early morning (6 to 6.30)
Flower longevity	1 day
Color of stamen	White
Color of anther	Yellowish white

4.1.1.2.10.1 Floral morphology

The inflorescence is a terminal, erect, dense flowered panicle that bears greenish-white fragrant flowers. Peduncle pubescent; flowers 3-4 cm long; sepals 3 in number, green color; corolla tube 1.2 cm long greenish white in color; labellum 2 cm long, white with pink color markings on the upper surface along the midrib, wavy margins; Anthers are yellowish white; stamen are white; style creamy white; ovary usually trilocular; parietal placentation.

4.1.1.2.10.2 Floral morphs

The studied population of *A. galanga* contained two floral morphs that exhibit different flowering behaviors. One group expressed cataflexistyly and the other expressed anaflexistyly. In the cataflexistylous morph, the stigma is positioned above the dehiscent anther at the time ofanthesis in the early morning and becomes curved underneath the anther in the noon time; but in the case of anaflexistyly form, the receptive stigma decurved under the indehiscent anther first and then moved into a

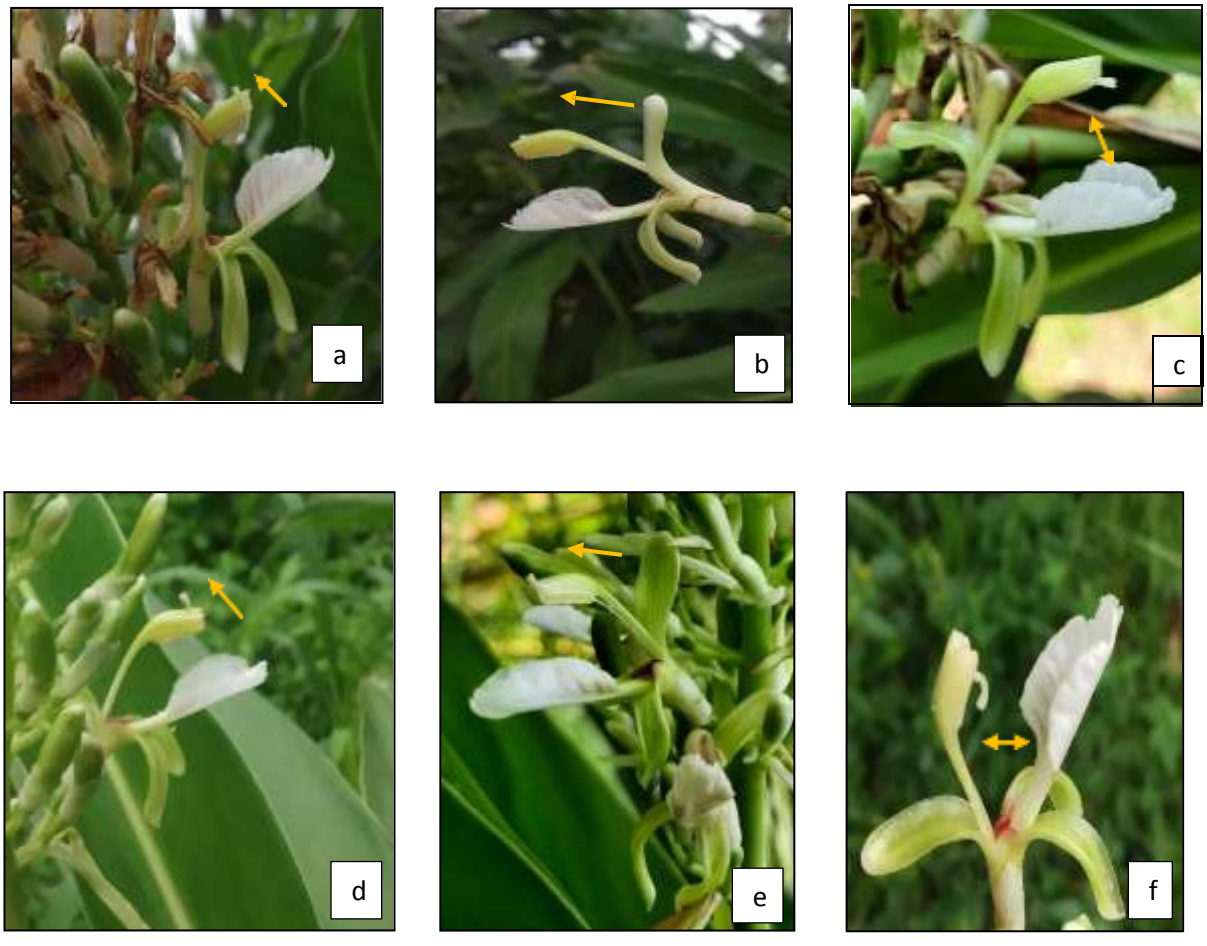


Plate 4: Flowering behavior of the two floral morphs in *Alpinia galanga*.

a) anaflexistyly flower before noon, in which the stigma is below the undehisced anther

c) anaflexistyly flower afternoon, with the stigma now erect above the anther

d) cataflexistyly flower before noon, in which the stigma is above the dehisced anther

f) cataflexistyly flower afternoon, with the stigma below the anther lobe,

b and e) intermediate stages in anaflexistyly and cataflexystyly flower.

reflexed superior position above the anther as it started to shed pollen around midday (Plate 4).

4.1.1.2.10.3 Flowering phenology of *Alpinia galanga*

Along with aromatic rhizomes, *Alpinia galanga* got vibrant green foliage and attractive flowers with great ornamental value. There is no clearly distinguished data on the developmentalstages of the inflorescence on this crop. The present study also attempted on identifyingthe flowering phenology of *Alpinia galanga* inflorescence and described the phenological stages as per the BBCH (*Biologische Bundesanstalt, Bundessortenamt and CHemical industry*) scale.

The extended BBCH scale is an approach for the universal coding of penologically significant growth stages of all mono and dicotyledonous plant species. The entire crop developmental cycle (germination to senescence) is divided into ten clearly visible phases whichare termed as principal growth stages and they are coded from 0 to 9. Secondary and meso stages are used to define the advanced stages in between the principal growth stages which are coded with two-digit and three-digit numbers respectively. In the present study, there are four principal growth stages observed in the *Alpinia galanga* plant from flower bud emergence to seed maturity. The principal growth stages are coded from 5 to 8 in anextended BBCH scale as given below (Plate 5).

Principle growth stage 5: Inflorescence emergence

Flower bud emergence was seen in the terminal position of newly formed matured tillers.The emergence of flower buds began during the rainy season, it started from the 2nd week of Mayto the last week of July in the first season then the 3rd week of August up to the last week of October in the second season. From flower bud emergence to maturity, it took 12-15 days. The secondary stages are coded with two-digit numbers and meso stages are coded with three-digit numbers are shown below.

50. The emergence of reproductive bud

500. Terminal inflorescence bud visible- emergence of light yellowish green colored reproductive bud on the leaf shoot.

501. Inflorescence bud had grown to 10% of its full size.

505. The terminal inflorescence bud was half its maximum size

509. The inflorescence bud had grown to 90% of its maximum size.

51. The inflorescence bud begins to swell.

53. Slight opening of bracts

55. Form a pyramidal shape

56. The inflorescence bud keeps expanding

59. 90% of inflorescence burst open and is clearly visible

Principal growth stage 6: Flowering

The flower buds in a panicle take 20-25 days to complete opening after panicle emergence.A single branch has flower buds of three different maturity stages. The flower in the branches opens one by one in sequential order. The panicle has 5–6 open flowers every day, and each blossom lasts for 24 hours. Anthesis of the flower occurs in the early morning hours. Generally, *Alpinia galanga* flowers are pollinatedby bees and wasps. The secondary growth stages under the flowering stages are explained below.

60. Bract separation and the complete opening of the panicle

61. 10% of the flowers in the panicle had finished blooming and started to wilt.

65. 50% of the flowers in the panicle had finished blooming and started to wilt.

68. 80% of the flower had completed blooming and dried off.

69. All flowers in the panicle had finished blooming and dried off.

Principal growth stage 7: Fruit development

Fruit development in *Alpinia galanga* took 90-100 days from fruit set to ripening

70. Swelling of ovary: the swelling of ovary takes place after successful pollination.

71. Fruit grew to 10% of its full size: corolla dries off after 15 days but calyx remains on the fruit even after maturity

72. Fruit at 20% of its ultimate size

75. The fruit had grown to 50% of its full size.

79. Fruit reaches its full size. shows dark green color

Principal growth stage 8: Fruit maturity

It took 80-85 days for the complete ripening of fruits

80. Fruits had grown to their full size, the fruit turned into dark green color

50 500 501 505 509

51 53 55 56 59

60 61 62 68 69

70 71 72 75 79

80 81 85 89

Plate 5: Phenological growth stages of *Alpinia galanga* according to the extended BBCH scale

81. Fruit ripening started - 53 days after final fruit maturity, fruit wall color changes to yellowish-orange

85. Advanced level of ripening: color changes from orange to red in 32 to 35 days

89. Fruits were completely ripe, fruit color changed to a dark

Table 4.11 B: Description of phenological growth stages of *Alpinia galanga* according to theextended BBCH scale

BBCH code	Description
Principle growth stage 5:	**Inflorescence emergence**
50	Emergence of reproductive bud
500	Visible terminal inflorescence bud
501	10% of its full length.
505	50% of its full length
509	90% of its full length
51	Initiation of reproductive bud swell
53	Slight bracts opening
55	Form pyramidal shape
56	Expanding of reproductive bud
59	90% of inflorescence burst open and clearly visible
Principal growth stage 6:	**Flowering**
60	Bract separation and complete opening of the panicle
61	10% flowers finished blooming and started to wilt
62	50% flowers finished blooming and started to wilt.
68	80% flower finished blooming and dried off.
69	All flowers finished blooming and dried off.
Principal growth stage 7:	**Fruit development**
70	Swelling of ovary
71	Fruit at 10% of its full size
72	20% of its full size
75	50% of its full size
79	90% of its full size
Principal growth stage 8:	**Fruit maturity**
80	Fruits reached it full size
81	Fruit wall color changes to yellowish-orange
85	Color changes from orange to red
89	Color changed to a dark red

4.1.1.2.10.4 Inflorescence characters

Significant variation was observed among the genotypes for inflorescence characters like panicle/inflorescence length, number of branches per inflorescence, number of flowers per inflorescence, and length and width of the flower.

Table 4.11 C: Inflorescence characters of *Alpinia galanga* genotypes

Accession	Panicle length (cm)	No. of branches	No. of flowers	Length of flower (cm)	Width of flower(cm)
IC582791	12.67	20.67	62	3.20	2.30
IC373609	18.83	30.00	90	3.50	2.93
IC565488	22.27	37.67	113	4.00	3.30
IC582766	16.60	24.33	73	3.33	2.63
IC582804	16.17	32.33	97	3.50	2.93
IC582817	23.63	35.67	107	4.10	3.33
IC878883	19.00	36.00	108	3.17	2.90
IC565501	16.83	24.00	72	3.10	2.37
IC582782	20.17	29.33	88	3.17	2.60
IC557436	11.17	19.00	57	3.30	2.87
IC565490	13.50	22.33	67	3.40	3.17
IC402361	26.60	34.00	102	3.30	2.47
IC582780	23.33	29.00	87	3.97	2.90
IC565492	12.17	20.67	62	3.80	3.17
IC582795	18.50	32.00	96	3.40	2.83
IC265610	18.17	29.67	89	3.96	3.27
IC582808	17.77	32.00	96	3.93	2.83
PCSAG-1	23.33	35.33	103	3.93	3.27
PCSAG-2	18.67	34.33	106	3.97	3.10
Mean	18.39	29.39	88.15	3.58	2.91
SD	4.27	6.19	18.58	0.42	0.37
F value(5%)	**34.48***	**13.57***	**13.57***	**3.99***	**5.62***

The data with respect to panicle length, number of branches per panicle, number of flowers per panicle, flower length and width are depicted in Table 4.11 C. Inflorescence length recordedhighest value in the accession IC402361 (26.60 cm) followed by IC565488 (22.27 cm) and shortest inflorescence length was recorded in the accession IC557436 (11.17 cm).

Similarly, the highest number of branches and flowers per inflorescence was recorded in IC565488 (37.67, 113) and IC582817 (35.67, 107) respectively, whereas the lowest number ofbranches and flowers per inflorescence were found in IC565492 (20.67, 62) and IC582791 (20.67, 62) respectively.

Significant variation was observed in the length of the flower which ranged from 3.10 to4.10 cm. Accession IC582817 (4.10 cm) recorded the highest flower length followed byIC565488 (4.00 cm). The shortest flower length was recorded in IC565501 (3.10 cm).

Flower width varied significantly among the genotypes and ranged between 2.30 cm to3.33 cm. Among the genotypes, IC582817 recorded the highest value (3.33cm) followed byIC565488 (3.30 cm) and the shortest flower width was recorded in IC582791 (2.30 cm).

4.1.1.2.11 Rhizome characters

Fresh rhizome weight, length, width and drying recovery of *Alpinia galanga* genotypes are presented in Table 4.12. Wide variability was noticed among the genotypes for rhizome characters like rhizome weight, dry recovery and length and width of the rhizome. Significant variation was found among the accessions for rhizome weight, ranging from 43.00g to 170.00g. IC582817 (170.00g) recorded highest rhizome weight followed by IC878883(156.67g) and IC582766 (156.67g) whereas, IC5655490 (43.00g) recorded the lowest rhizomesweight.

Table 4.12: Rhizome characters of *Alpinia galanga* genotypes

Accession	Fresh rhizome weight/tiller (g)	Rhizome length (cm)	Rhizome width (cm)	Drying recovery (%)
IC582791	63.33	5.87	3.37	20
IC373609	60.00	6.40	3.63	20
IC565488	83.33	6.03	4.93	23.25
IC582766	133.33	5.63	3.63	20.11
IC582804	120.00	5.90	4.50	20.69

Table 4.12 contd.

IC582817	170.00	7.87	6.10	25.20
IC878883	156.67	5.83	4.07	22.99
IC565501	43.33	7.00	4.17	23.05
IC582782	73.33	6.23	5.00	19.80
IC557436	45.00	6.86	2.97	20.00
IC565490	43.00	6.10	4.00	24.44
IC402361	73.33	6.37	4.00	23.04
IC582780	110.00	6.73	4.93	25.43
IC565492	65.00	6.00	3.73	23.00
IC582795	120.00	6.50	5.00	23.21
IC265610	120.00	7.10	3.80	24.81
IC582808	130.00	5.37	2.57	24.96
PCSAG-1	135.00	6.27	3.13	23.28
PCSAG-2	138.00	5.90	3.63	25.38
Mean	99.08	6.31	4.06	22.77
SD	43.62	0.87	1.05	
F value (5%)	**10.18***	**1.677***	**3.566***	

Wide variability was noticed among the accessions with respect to the length of the rhizomeand it ranged between 5.37cm to 7.87cm. IC582817 (7.87cm) recorded highest length and the shortest was recorded by IC582808 (5.37cm). Similarly highest rhizome width was recorded in the accession IC582817(6.10cm) followed by IC582795 and IC582782(5cm). The lowestrhizome width was recorded in IC582808 (2.57cm) (Plate 6).

The dry rhizome recovery of the genotypes ranged from 20 to 25.43 per cent. The dry recovery was more for the accession IC582780 (25.43%) followed by PCSAG-2 (25.38%) and the lowest was recorded in accessions namely, IC582782 (19.8%) IC582791 (20%), IC373609 (20%) and IC657436 (20%).

Plate 6: Rhizomes of *Alpinia galanga* genotypes

While considering overall yield parameters (fresh weight of rhizome /tiller and dry recovery)IC582817 (170g, 25.2%) recorded the highest value followed by IC582780 (110g, 25.43%) respectively.

Even though *Alpinia galanga* is well known for its usage in traditional medicine as well as cooking spice, sometimes people are confused the plant with other species of the Zingiberaceae family. The findings of this study are giving more comprehensive knowledge on *Alpinia galanga*for all morphological features for better identification. Further, the results show significant variations for all morphological characters among the genotypes thus may help in conservation as well as in crop improvement programs for selecting desired traits.

4.1.1.2.13 Seed dormancy

To increase the quality of plants by cross breeding, seed propagation is highly preferred. The hard seed coat (Testa) and less endosperm prevent seeds from germinating in *Alpinia galanga.* Therefore, the current study also focused on breaking seed dormancy in *Alpinia galanga* by conducting basic dormancy-breaking tests.

Table 4.13: Germination percentage of *Alpinia galanga* seeds after dormancy breakingtest results

Treatments	**Germination percentage (%)**
4 months dry storage seeds	10
Hot water treatment	0
Gibberellic acid treatment (50mg/l and 25mg/l)	10
Overnight soaking	0

Ten seeds were used for each treatment. The germination percentage of each treatment are presented the Table 4.13. As per the result, the previous season dry stored seeds and seeds treated with 25mg/l and 50mg/l showed only 10% germination. Other methods had zero percentgermination. No basic test gave satisfactory results for good germination. Hence, advanced dormancy breaking methods and suitable growing media are required for getting the same.

4.2 PHYTOCONSTITUENTS

Rhizome powder of all 19 *Alpinia galanga* accessions was subjected to biochemical analysis for estimation of volatile oil, oleoresin, flavonoids, phenols, amino acid, and starch.GCMSMS analysis of volatile oil was carried out to know the major and minor compounds in rhizome oil (Plate 7).

4.2.1 Volatile oil and oleoresin content in *Alpinia galanga* genotypes

Table 4.14A: Volatile oil and oleoresin recovery in *Alpinia galanga* genotypes

Accession	Oil (%)	Oleoresin (%)
IC582791	0.21^{ef}	3.05^{g}
IC373609	0.21^{ef}	3.15^{fg}
IC565488	0.40^{ab}	4.35^{ab}
IC582766	0.27^{de}	3.35^{efg}
IC582804	0.21^{ef}	3.05^{g}
IC582817	0.29^{d}	4.00^{bc}
IC878883	0.41^{a}	3.95^{bcd}
IC565501	0.31^{bc}	3.65^{cde}
IC582782	0.22^{ef}	3.35^{efg}
IC557436	0.31^{bc}	3.55^{def}
IC565490	0.22^{ef}	3.20^{fg}
IC402361	0.31^{bc}	3.55^{def}
IC582780	0.27^{de}	4.45^{a}
IC565492	0.31^{bc}	3.40^{efg}
IC582795	0.21^{ef}	3.90^{cd}
IC265610	0.27^{de}	3.65^{cde}
IC582808	0.22^{ef}	4.45^{a}
PCSAG-1	0.33^{b}	4.00^{bc}
PCSAG-2	0.32^{b}	4.06^{abc}
CD **(0.05)**	5.76	5.66
CV	**0.033***	**0.43***

Table 4.14 depicts the volatile oil content and oleoresin content (methanol) of *Alpinia galanga* genotypes. Significant difference was observed among the genotypes for volatile oil content and it ranged from 0.21 to 0.41 per cent. Accession IC878883

recorded the highest value (0.41%) followed by IC565488 (0.40%). Accession IC582791, IC582795, IC373609, and IC582804 recorded lowest (0.21%) oil content.

Recovery of oleoresin varied significantly between 3.05 to 4.45 per cent in the accessions studied. The highest oleoresin recovery (4.45%) was registered in the accessions IC582808 and IC582780. The lowest oleoresin recovery (3.05%) was recorded in accessions IC582791 and IC582804.

4.2.1.1 Oleoresin recovery in different solvents

Recovery of oleoresin was estimated using different solvents and their characteristics like color and consistency are analyzed during the course of the experiment and the results are presented in table 4.14B.

Table 4.14 B: Oleoresin extractability percentage using different solvents in *Alpinia galanga*

Sl.No	Solvent used	Recovery (%)	Color of extract	Consistency of extract
1	Petroleum ether	3.60	Brown	Oily
2	Benzene	4.32	Light brown	Sticky
3	Acetone	9.72	Yellowish brown	Oily& sticky
4	Methanol (90%)	4.80	Yellowish brown	Semi-solid

Out of four solvents, maximum oleoresin recovery was observed in acetone (9.72 %) followed by methanol and petroleum benzene with values 4.8 per cent and 4.3 per cent respectively. The color and consistency of oleoresin in acetone are yellowish-brown and oily sticky form respectively.

4.2.1.2 GCMSMS profile of *Alpinia galanga* volatile oil

GCMSMS profile with respect to major components of *Alpinia galanga* accessionIC878883 volatile oil is presented in table 4.14C.

Table 4.14C: GCMSMS profile of *Alpinia galanga* volatile oil

Sl. No.	Compound	RT (min)	Molecular weight (g/mol)	Area (%)
1	à-Pinene	5.55	136	2.42
2	á-Phellandrene	6.16	136	0.32
3	á-Myrcene	6.39	136	0.51
4	Eucalyptol	7.09	250	44.45
5	ç-Terpinene	7.50	136	0.49
6	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)- (+)-2-Bornanone	8.91	153	2.50
7	Camphor	8.91	152	2.50
8	endo-Borneol	9.13	154	1.22
9	Terpinen-4-ol	9.45	154	1.53
10	à-Terpineol	9.68	154	0.79
11	2-Cyclohexen-1-ol, 2-methyl- 5-(1-methylethenyl)-, cis trans-Carveol	9.85	152	0.24
12	Fenchyl acetate	10.18	196	0.24
13	Bornyl acetate	11.41	196	0.67
14	Phenol, 4-(2-propenyl)-, acetate	12.55	176	0.23
15	cis-p-mentha-1(7),8-dien-2-ol	12.62	152	0.32
16	2-Cyclohexen-1-ol, 2-methyl- 5-(1-methylethenyl)-, acetate,	12.89	153	0.58
17	trans-Carveyl acetate	12.89	194	0.58
18	Geranyl acetate	13.24	196	2.09
19	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate (Z)- Nerol acetate	13.24	196	2.09

Table 4.14C contd.

20	Methyl eugenol	13.73	178	0.55
21	2-Allyl-1,4-dimethoxybenzene	13.73	178	0.55
22	Caryophyllene	14.24	204	0.39
23	Bicyclo [3.1.1]hept-2-ene,	14.24	94	0.39
24	trans-à-Bergamotene	14.47	204	0.35
25	cis-á-Farnesene	14.84	204	22.89
26	Humulene	14.96	204	1.38
27	cis-à-Bisabolene	14.96	204	1.38
28	Germacrene D	15.54	204	0.43
29	Pentadecane	15.71	212	3.44
30	à-Farnesene	15.93	204	0.76
31	Isocaryophyllene	16.01	204	0.77
32	trans-Isoeugenol	16.34	164	3.77
33	à-ylangene	16.52	204	0.37
34	Asarone	18.37	208	0.63
35	Cyclohexene, 1,5,5-trimethyl- 6-acetylmethyl-	19.06	236	0.34
36	5-Hexadecyne	19.37	226	0.49
37	8-Heptadecene	19.52	238	0.51
38	Farnesol, acetate	22.92	264	2.49

There were 38 chemical components identified from the rhizome volatile oil of *Alpinia galanga*.Major compounds were Eucalyptol and cis-á- Farnesene (44.45 and 22.81 per cent respectively followed by trans-Isoeugenol(3.77%) Pentadecane (3.44%),Bicyclo [2.2.1] heptan-2-one, 1,7,7- trimethyl-, (1S)- (+)-2-Bornanone (2.50%), Camphor (2.50%), Farnesol, acetate(2.49%), à- Pinene(2.42%), Geranyl acetate(2.09%), 2,6-Octadien-1-ol, 3,7- dimethyl-, acetate (Z)- Nerol acetate(2.09%) and some minor compounds like Terpinen-4- ol(1.53), Humulene(1.38%), cis- à-Bisabolene(1.38%), endo-Borneol(1.22%) and other components present in trace amounts Table (4.14C).

4.2.2 Biochemical parameters of *Alpinia galanga* genotypes

The data on flavonoids, phenols, amino acids and starch content in the rhizomesof *Alpinia galanga* genotypes are presented in Table 4.15.

Table 4.15: Quality attributes of rhizomes of *Alpinia galanga* genotypes

Accession	Flavonoids (mg/g)	Phenols (mg/g)	Amino acid (mg/g)	Starch (mg/g)
IC582791	25.66^{j}	33.95^{ij}	4.09^{f}	80.83^{l}
IC373609	38.86^{f}	34.90^{hi}	4.01^{f}	84.28^{i}
IC565488	43.78^{de}	41.44^{abc}	5.13^{def}	88.16^{cde}
IC582766	36.96^{gh}	38.94^{de}	5.84^{bcde}	84.98^{hi}
IC582804	42.85^{e}	30.04^{k}	5.00^{def}	82.17^{k}
IC582817	48.88^{c}	43.16^{a}	7.38^{ab}	90.47^{b}
IC878883	48.93^{c}	40.07^{cd}	6.43^{bcd}	92.00^{a}
IC565501	35.93^{h}	34.49^{hi}	5.36^{cdef}	85.27^{ghi}
IC582782	38.72^{fg}	37.43^{efg}	4.85^{def}	87.83^{de}
IC557436	33.15^{i}	36.23^{fgh}	6.22^{bcde}	83.04^{jk}
IC565490	53.78^{ab}	39.95^{cd}	5.89^{bcde}	89.35^{bc}
IC402361	43.17^{de}	36.16^{gh}	5.30^{cdef}	82.89^{k}
IC582780	51.95^{b}	42.49^{ab}	7.21^{ab}	88.85^{cd}
IC565492	45.06^{d}	35.50^{hi}	4.76^{ef}	85.95^{fgh}
IC582795	38.88^{f}	32.67^{j}	7.37^{ab}	86.33^{fg}
IC265610	49.67^{c}	41.18^{bc}	5.76^{bcde}	88.62^{cd}
IC582808	54.99^{a}	42.86^{ab}	8.45^{a}	92.89^{a}
PCSAG-1	43.71^{de}	37.88^{efg}	5.28^{cdef}	87.08^{ef}
PCSAG-2	43.94^{de}	37.94^{ef}	6.93^{abc}	84.11^{ij}
CD **(0.05)**	2.10	2.24	13.53	0.657
CV	**1.89***	**1.77***	**1.65***	**1.19***

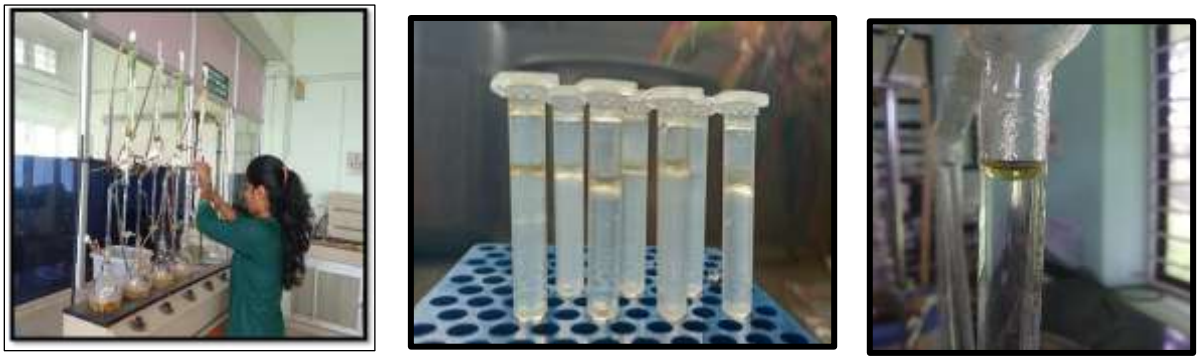


Plate 7 A: Volatile oil of *Alpinia galanga* genotypes

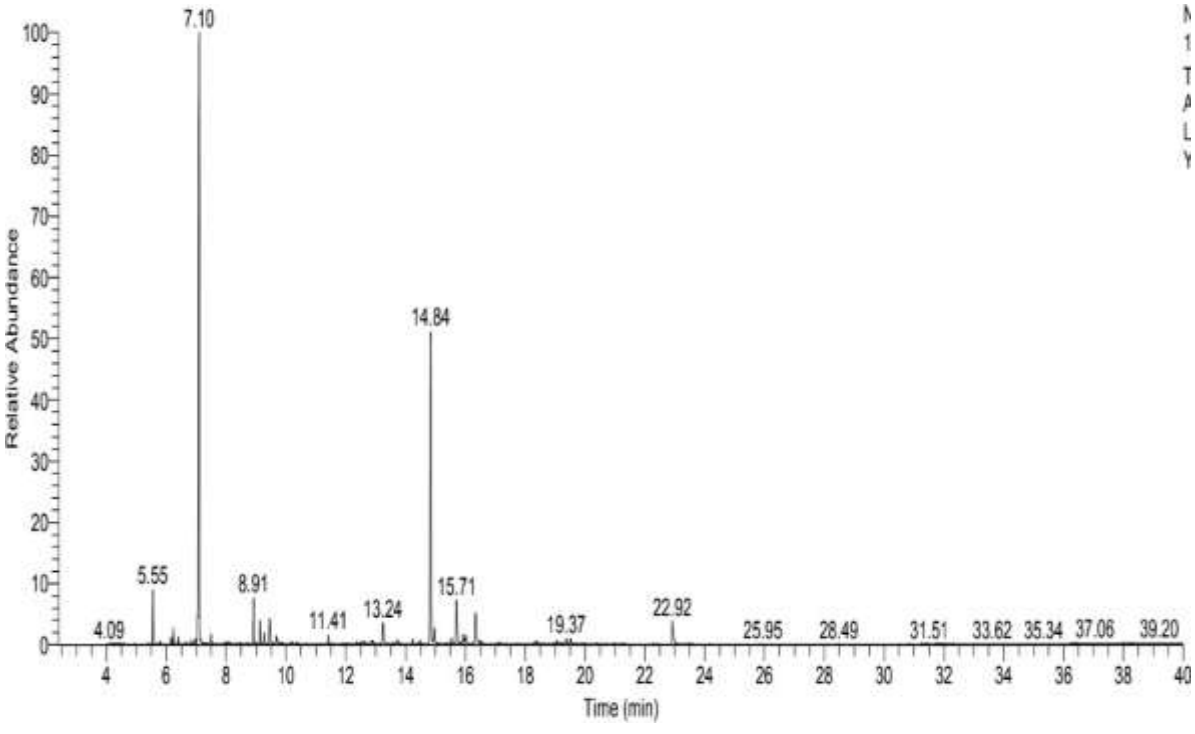


Plate 7B: Chromatogram of GCMSMS profiling for volatile oil of *Alpinia galanga*

A significant difference in flavonoid content was observed among the genotypes which ranged from 25.66 mg/g to 54.99 mg/g. The highest flavonoid content was recorded in the accession IC582808 (54.99mg/g) followed by IC565490 (53.78mg/g) whereas, the lowest flavonoid content was recorded in accession IC582791 (25.66mg/g).

Phenol content varied significantly among the nineteen accessions and ranged from 30.04 mg/g to 43.16 mg/g. The phenol content was found to be highest in accession IC582817 (43.16mg/g) followed by IC582808 (42.86 mg/g) and the lowest phenol content was found in IC582804(30.04 mg/g).

Amino acid content varied significantly among the genotypes and ranged from 4.01 mg/g to 8.45 mg/g. The highest amino acid content was found in IC582808 (8.45 mg/g) followed by IC582817 (7.38 mg/g) whereas, the lowest amino acid content was found in IC373609 (4.01mg/g).

The starch content of nineteen accessions ranged from 80.83 mg/g to 92.89 mg/g. IC582808 (92.89 mg/g) recorded the highest starch content followed by IC878883(92 mg/g) and the lowestst arch content was recorded in IC582791(80.83 mg/g).

As per the experimental results, accession IC582817, IC582808, IC878883 showed comparatively highest value for all biochemical parameters compared to other genotypes.

4.3 *INVITRO* ANTIMICROBIAL ACTIVITY OF GREATER GALANGAL EXTRACT

4.3.1 *In vitro* evaluation of *Alpinia galanga* rhizome extract against major plant pathogens

In the current investigation, ethanolic extract of *Alpinia galanga* rhizomes was evaluatedagainst four major fungal pathogens to know their effectiveness in controlling the mycelial growth of plant pathogens.

Major fungal pathogens selected for *in vitro* analysis were,

a) *Rhizoctonia* spp.

b) *Colletotrichum* spp.

c) *Phytophthora* spp.

d) *Fusarium* spp.

A completely randomized design (CRD) was utilized in the study, with nineteen treatments and three replications. Ethanolic extract at three different concentrations (2%, 5%, and 10%) wasevaluated in the current experiment. The inhibitory effect of extract was tested against *Rhizoctonia* spp. *Colletotrichum* spp. *Phytophthora* spp. *and Fusarium* spp. by using poison foodtechnique.

4.3.1.1 *In vitro* evaluation of ethanolic extract of *Alpinia galanga* rhizome against *Rhizoctonia* spp.

Extract of nineteen *Alpinia galanga* genotypes showed inhibition on the mycelial growth of *Rhizoctonia* spp. at three different concentrations (2, %, 5%, and 10%) and the results are presented in Table 4.16 and Plate 8.

At 2 per cent concentration, Accession IC582780 (44.17%) showed the highest inhibition with a mean colony diameter of 4.47cm followed by IC582808(43.33%). No inhibition was recorded in the accessions IC582791, IC582766, and IC582782 at 2 per cent concentration.

At 5 per cent concentration, per cent inhibition and mean colony diameter ranged from 39.17per cent to 76.25 per cent and 1.09 to 4.87 cm respectively. Accession IC582808 (76.25 %) showed the highest inhibition followed by IC265610 (69.17%), whereas, IC582804 (39.17 %) recorded the lowest per cent of mycelial inhibition.

Out of 19 accessions, 12 accessions showed complete inhibition of mycelial growth and IC582782 showed 86.67 per cent that recorded the lowest per cent inhibition with colony diameter of 1.07cm at 10 per cent concentration.

Accession IC582808 recorded the highest per cent (43.33 %, 76.25 %, and100% respectively) inhibition at all three different concentrations, which was on par with IC265610 (42.08 %, 69.17%, and 88.33%) respectively.

For *Rhizoctonia* spp. 10 per cent of ethanolic rhizome extract was found to be the best concentration for getting 100 per cent inhibition. Hence this can be taken as the best concentration for making botanical formulation against *Rhizoctonia* spp.

4.3.1.2 *In vitro* evaluation of ethanolic extract of *Alpinia galanga* rhizome against *Colletotrichum* spp.

The data presented in Table 4.17 showed that, ethanolic extract at the concentration of 10 per cent had significantly highest per cent of inhibition compared to the other two concentrationsare presented in Table 4.17 and Plate 9.

At 2 per cent concentration, accession IC582808 (23.33 %) recorded the highest per cent inhibition followed by IC582780 (22.08 %), with mean colony diameters of 6.57 and 6.53 cm respectively, whereas, the lowest per cent of inhibition was recorded in the accession IC557436(2.92 %).

At 5 per cent concentration, per cent of inhibition and mean colony diameter ranged from 19.58 per cent to 45.42 per cent and 4.27 to 5.83cm. Accession IC582780 (45.42%) showed the highest inhibition followed by IC878883 (42.50%), whereas, IC582766 (19.58 %) recordedthe lowest per cent of mycelial inhibition.

At 10 per cent concentration, accession IC582808 showed (65.42 %) the highest per cent inhibition followed by IC582780 (65.00 %). The lowest per cent of inhibition was observed in IC582791 (40.83 %) with a 3.60 cm colony diameter.

Accession IC582780 (22.08 %, 45.42 %, and 65.00 %) showed the highest per cent of inhibitionat 2 per cent, 5 per cent, and 10 per cent concentrations respectively and IC582808 (23.33%, 41.67 %, and 65.42 %) was on par with IC582780.

Table 4.16 : *In vitro* evaluation of ethanolic extract of *Alpinia galanga* rhizome against *Rhizoctonia* spp.

Accession	Mean colony diameter(cm)			Inhibition (%)			Transformed value ✿		
	2%	5%	10%	2%	5%	10%	2%	5%	10%
IC582791	8.00	3.83	0.00	0.00	52.08	100	0.00	46.19	90.00
IC373609	7.83	3.33	0.00	2.08	58.33	100	8.21	49.80	90.00
IC565488	7.53	3.13	0.00	5.83	60.83	100	13.96	51.26	90.00
IC582766	8.00	4.27	0.00	0.00	46.67	100	0.00	43.09	90.00
IC582804	7.83	4.87	0.00	2.08	39.17	100	8.21	38.74	90.00
IC582817	6.47	2.63	0.00	19.17	67.08	100	25.96	55.00	90.00
IC878883	7.8	3.3	0.00	2.50	58.33	100	9.10	49.80	90.00
IC565501	7.83	3.37	0.93	2.08	57.92	88.33	8.21	49.56	83.10
IC582782	8.00	4.03	1.07	0.00	49.58	86.67	0.00	44.76	68.59
IC557436	6.60	4.03	1.03	17.50	49.58	87.08	24.70	44.76	75.49
IC565490	5.60	2.50	0.93	30.00	68.75	88.33	33.20	56.01	83.10
IC402361	7.80	3.13	0.93	2.50	60.83	88.33	9.10	51.26	83.10
IC582780	4.47	2.57	0.00	44.17	67.92	100	41.65	55.50	90.00
IC565492	7.60	2.67	0.00	5.00	66.67	100	12.69	54.74	90.00
IC582795	7.93	3.63	0.00	0.83	54.58	100	3.03	47.63	90.00
IC265610	4.63	2.47	0.93	42.08	69.17	88.33	40.44	56.29	83.10
IC582808	4.53	1.90	0.00	43.33	76.25	100	41.16	60.84	90.00
PCSAG-1	8.00	3.80	0.93	0.83	52.50	88.33	0.00	46.43	83.10
PCSAG-2	6.00	2.90	0.00	27.50	63.75	100	30.00	52.98	90.00
Control	8.00	8.00	8.00	0.00	0.00	0.00	0.00	0.00	0.00
CD (0.05)							**6.54**		
CV							**8.02***		

✿ Angular transformed values *Significant at 5 per cent

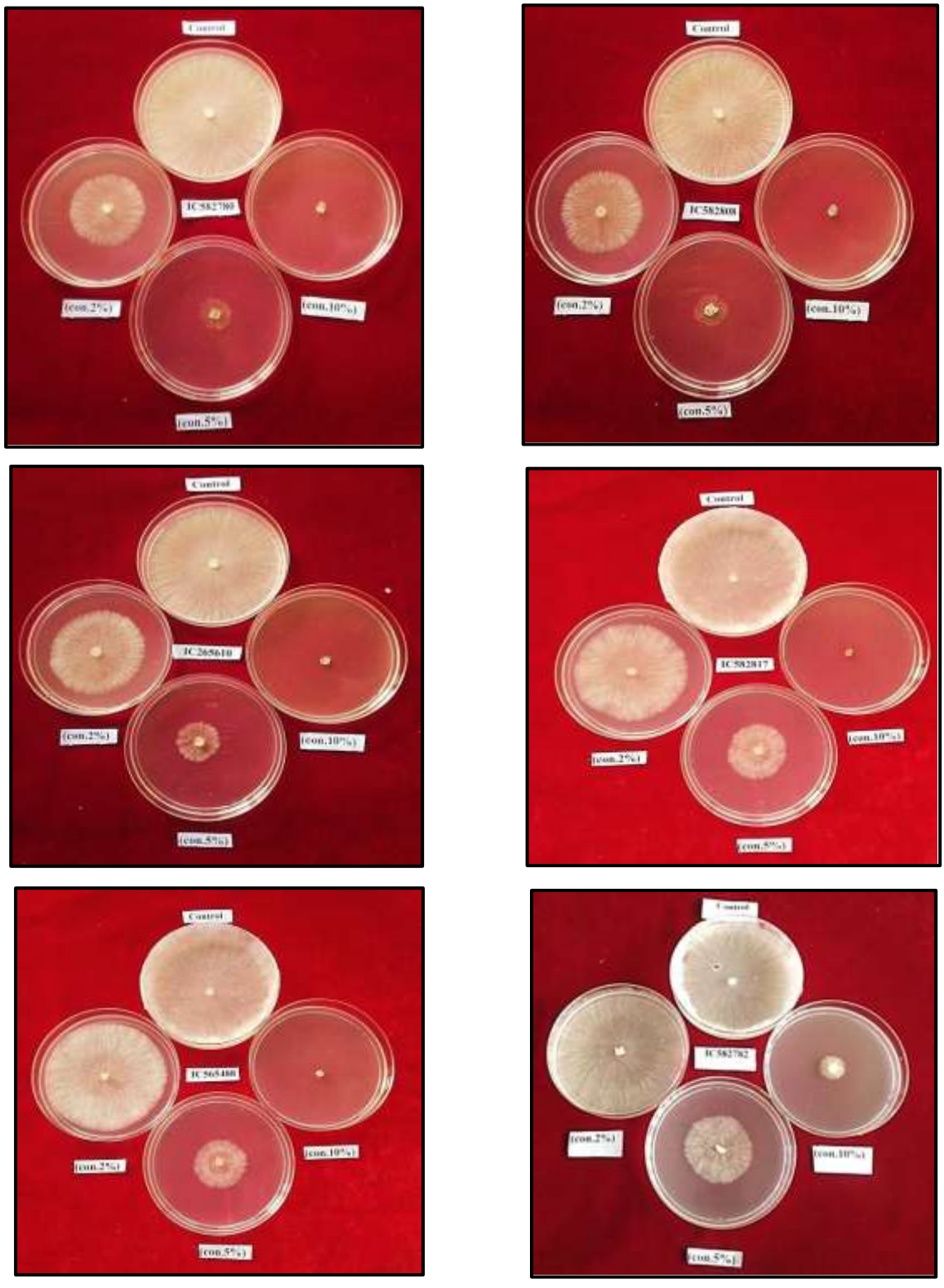


Plate 8: Per cent inhibition of *Rhizoctonia* spp. at three different concentrations

Plate 9: Per cent inhibition of *Colletotrichum spp*. at three different concentrations

Table 4.17*: In vitro* evaluation of ethanolic extract of *Alpinia galanga* rhizome against *Colletotrichum* spp.

Accession	Mean colony diameter(cm)			Inhibition (%)			Transformed value ✿		
	2%	5%	10%	2%	5%	10%	2%	5%	10%
IC582791	7.73	5.77	3.60	3.33	25.83	40.83	10.48	30.55	39.71
IC373609	6.77	5.00	3.73	19.58	34.58	68.33	26.26	36.02	55.76
IC565488	6.03	4.47	2.43	14.58	32.50	60.00	22.45	34.76	50.77
IC582766	6.90	5.47	3.87	10.83	19.58	57.50	19.19	26.26	49.31
IC582804	6.73	5.57	3.97	6.67	20.42	43.75	14.95	26.85	41.41
IC582817	6.53	4.57	2.80	10.83	35.42	70.00	19.21	36.52	56.79
IC878883	7.13	4.23	2.57	20.83	42.50	61.25	27.16	40.68	51.51
IC565501	6.63	4.27	2.93	6.67	35.00	57.92	14.95	36.27	49.56
IC582782	6.37	4.67	2.50	10.83	27.08	56.67	19.21	31.36	48.83
IC557436	6.87	5.83	3.20	2.92	25.83	62.92	9.79	30.55	52.49
IC565490	6.50	4.87	2.97	5.83	28.33	57.08	13.96	32.16	49.07
IC402361	6.50	5.40	3.10	18.75	34.17	57.92	25.66	35.76	49.56
IC582780	6.53	5.77	3.03	22.08	45.42	65.00	28.03	42.37	53.73
IC565492	7.63	4.47	2.50	18.33	30.42	58.75	25.35	33.47	50.04
IC582795	6.83	4.83	2.93	14.17	29.58	60.00	22.11	32.94	50.77
IC265610	5.63	4.47	2.43	19.58	40.42	54.58	26.26	39.48	47.63
IC582808	6.57	4.17	2.67	23.33	41.67	65.42	28.88	40.20	53.98
PCSAG-1	5.80	4.53	2.73	18.33	27.92	59.17	25.35	31.89	50.28
PCSAG-2	6.37	5.00	3.40	11.67	31.67	45.42	19.97	34.24	42.37
Control	8.00	8.00	8.00	0.00	0.00	0.00	0.00	0.00	0.00
CD (0.05)							**1.207**		
CV							**2.155***		

✿Angular transformed value * significance at 5 per cent

4.3.1.3 *In vitro* evaluation of ethanolic extract of *Alpinia galanga* rhizome against *Phytophthora* spp.

Per cent inhibition for the ethanolic extract of *Alpinia galanga* rhizome at three different concentrations (2%, 5%, and 10%) against *Phytophthora* spp. as presented in Table 4.18 and Plate10.

At 2 per cent concentration, accession IC265610 (29.58 %) recorded the highest per cent inhibition followed by PCSAG-1 (27.50 %), whereas, the lowest per cent of inhibition was recorded in the accession IC582791(3.33 %). The mean colony diameter at this concentration ranged from 5.63 to 7.73 cm.

At 5 per cent concentration, per cent of inhibition and colony diameter ranged from 27.08 per cent to 47.92 per cent and 4.17 to 5.83 cm respectively. Accession IC582808 (47.92 %) showed the highest inhibition followed by IC878883 (47.08 %), whereas, IC557436 (27.08 %)recorded the lowest per cent mycelial inhibition.

At 10 per cent concentration, accession IC265610 and IC565488 showed 69.58 per cent highest per cent inhibition with colony diameter of 2.43 cm followed by accession IC582780 (65.00 %). The lowest per cent of inhibition was observed in IC582804 (50.42 %).

Accession IC265610 (29.58 %, 44.17 %, and 69.58 %) showed maximum mycelial inhibitionat all three concentrations (2 %, 5 %, and 10 %). Accession IC565488 (24.58 %, 44.17 %, 69.58%) showed on par results with that of IC265610.

4.3.1.4 *In vitro* evaluation of ethanolic extract of *Alpinia galanga* rhizome against *Fusarium* spp.

Per cent inhibition for the ethanolic extract of *Alpinia galanga* rhizome on *Fusarium* spp. atthree different concentrations with their mean colony diameter is presented in Table 4.19 and (Plate 11).

The data shows that at 2 per cent concentration accession IC582808(15.83%) was recorded as the highest per cent inhibition followed by IC582780(15.00%), whereas, lowest per cent of inhibition was recorded in the accession PCSAG-2(2.92%).

At 5 per cent concentration per cent of inhibition ranges from 16.67 per cent to 47.08 per cent. Accession IC582808 (47.08 %) showed the highest inhibition followed by IC265610 (44.58%), while, IC565490 (16.67 %) recorded the lowest per cent mycelial inhibition.

At ten per cent of extract concentration accession IC582808 and IC265610 showed 61.67 %and 59.17 % inhibition respectively, the lowest per cent of inhibition was observed in IC582766(24.58 %).

Accession IC582808 (15.83 %, 47.08 %, and 61.67 %) showed the highest per cent mycelialinhibition at all three concentrations (2 %, 5 %, and 10 %) respectively and IC265610 (4.58 %,44.58 %, and 59.17 % respectively) showed similar results with IC582808.

4.3.1.5 *In vitro* evaluation of *Alpinia galanga* rhizome extract against *Ralstonia solanacearum.*

Ralstonia solanacearum was used to evaluate the antibacterial activity of the extracts ofdifferent *A. galanga* genotypes. The ethanolic extract of *Alpinia galanga* rhizome was used atthree different concentrations namely 2 per cent, 5 per cent, and 10 per cent concentrations weretested against the *Ralstonia solanacearum* by agar well diffusion method. The antibioticstreptomycin sulphate was used as a positive control and distilled water as a negative control.The results clearly indicated that the control plate with streptomycin showed 30 per centinhibition but there was no inhibition in the ethanolic extract at all three different concentrations
used (Plate 12).

Table 4.18: *In vitro* evaluation of ethanolic extract of *Alpinia galanga* rhizome against *Phytophthora* spp.

Accession	Mean colony diameter(cm)			Inhibition (%)			Transformed value		
	2%	5%	10%	2%	5%	10%	2%	5%	10%
IC582791	7.73	5.77	3.60	3.33	27.92	55.00	10.48	31.89	47.87
IC373609	6.77	5.00	3.73	15.42	37.50	53.33	23.12	37.76	46.91
IC565488	6.03	4.47	2.43	24.58	44.17	69.58	29.72	41.65	56.53
IC582766	6.90	5.47	3.87	13.75	31.67	51.67	21.75	34.22	45.96
IC582804	6.73	5.57	3.97	15.83	30.42	50.42	23.42	33.47	45.24
IC582817	6.53	4.57	2.80	18.33	42.92	65.00	25.35	40.93	53.74
IC878883	7.13	4.23	2.57	10.83	47.08	67.92	19.21	43.33	55.50
IC565501	6.63	4.27	2.93	17.08	46.67	63.33	24.39	43.09	52.74
IC582782	6.37	4.67	2.50	20.42	41.67	68.75	26.85	40.20	56.01
IC557436	6.87	5.83	3.20	14.17	27.08	60.00	22.11	31.36	50.78
IC565490	6.50	4.87	2.97	18.75	39.17	62.92	25.66	38.74	52.49
IC402361	6.50	5.40	3.10	18.75	32.50	61.25	25.64	34.75	51.51
IC582780	6.53	5.77	3.03	18.33	27.92	62.08	25.35	31.89	51.99
IC565492	7.63	4.47	2.50	4.58	44.17	68.75	12.27	41.65	56.02
IC582795	6.83	4.83	2.93	14.58	39.58	63.33	22.45	38.98	52.74
IC265610	5.63	4.47	2.43	29.58	44.17	69.58	32.95	41.65	56.53
IC582808	6.57	4.17	2.67	17.92	47.92	66.67	25.04	43.81	54.74
PCSAG-1	5.80	4.53	2.73	27.50	43.33	65.83	31.63	41.17	54.25
PCSAG-2	6.37	5.00	3.40	20.42	37.50	57.50	26.86	37.76	49.32
Control	8.00	8.00	8.00	0.00	0.00	0.00	0.00	0.00	0.00
CD (0.05)							**1.621**		
CV							**2.656***		

Angular transformed values *Significant at 5 per cent

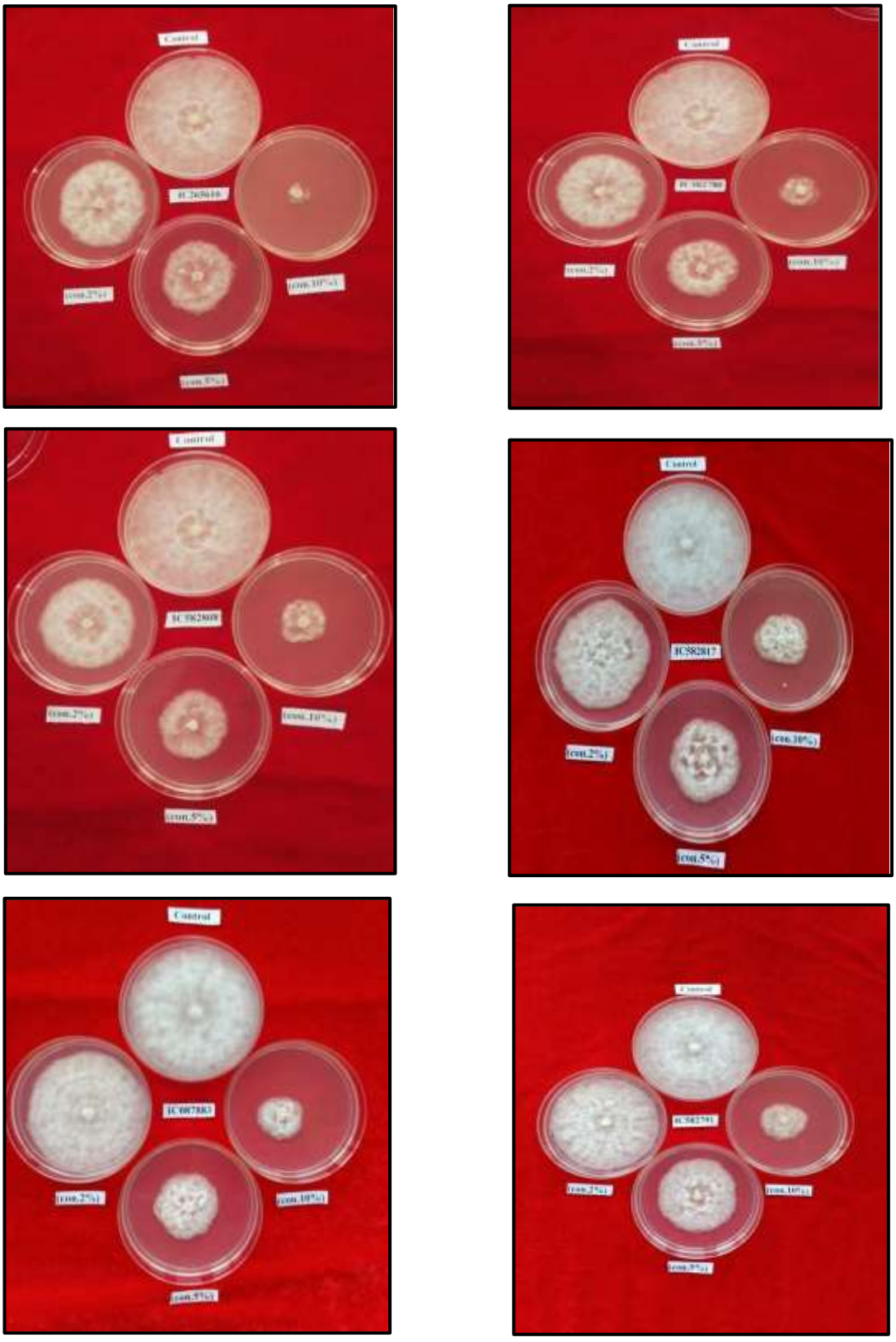


Plate 10: Per cent inhibition of *Phytophthora* spp. at three different concentrations

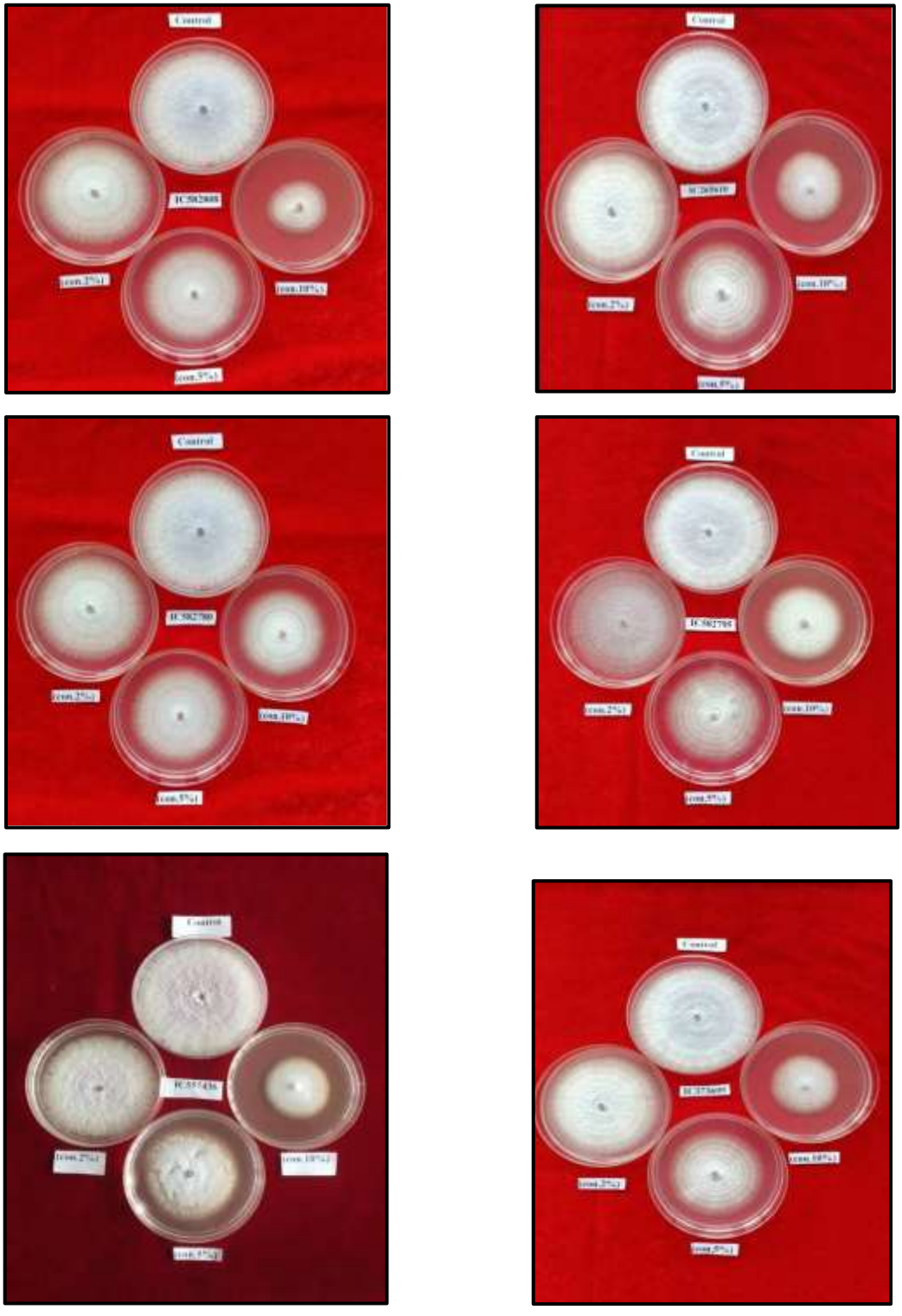


Plate 11: Per cent inhibition of *Fusarium* spp. at three different concentrations

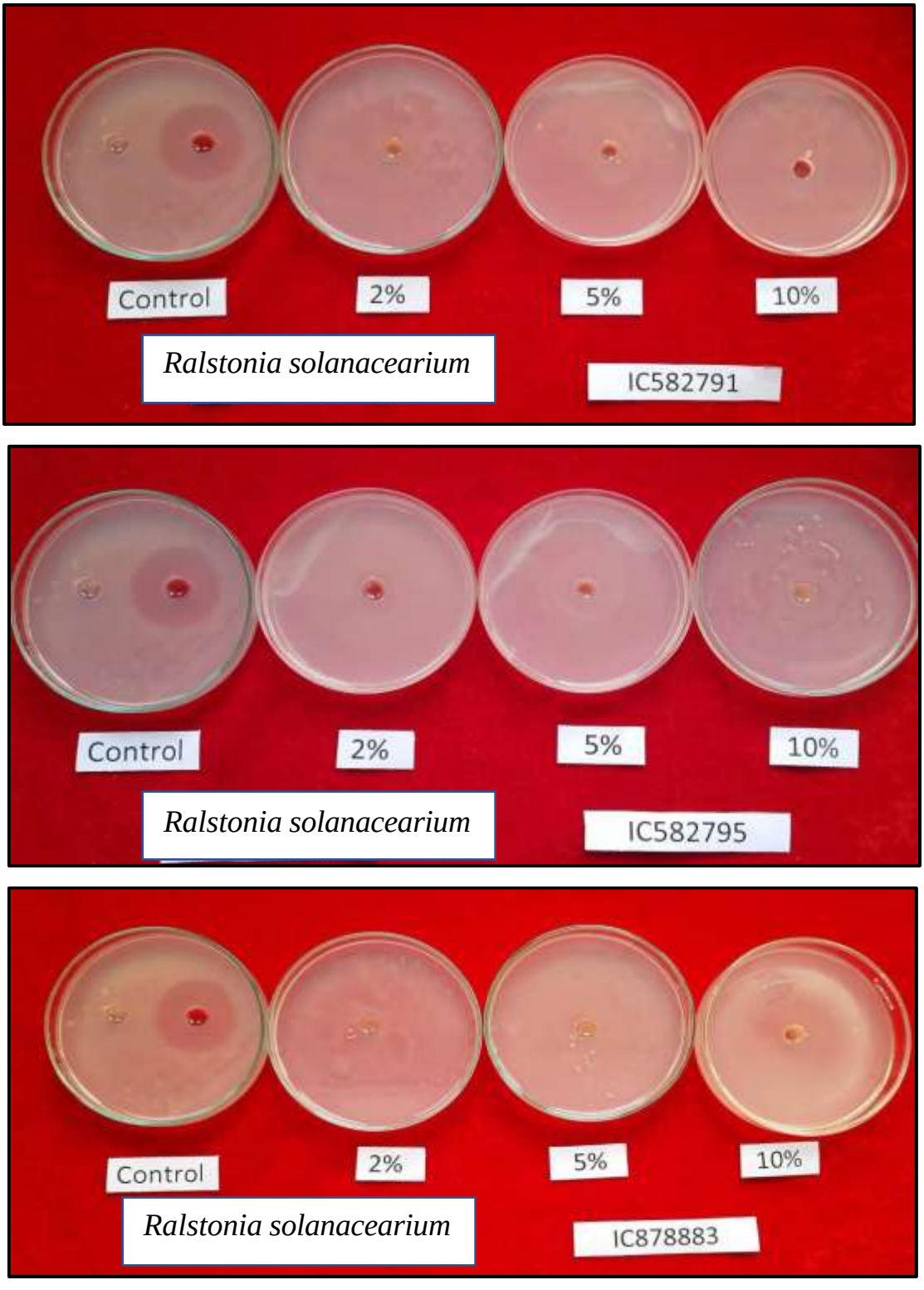


Plate 12: Per cent inhibition of *Ralstonia solanacearum* at three different concentrations

Table 4.19: *Invitro* evaluation of ethanolic extract of *Alpinia galanga* rhizome against *Fusarium* spp.

Accession	Mean colony diameter(cm)			Inhibition (%)			Transformed value ✹		
	2%	5%	10%	2%	5%	10%	2%	5%	10%
IC582791	7.37	6.47	4.40	7.92	19.17	45.00	16.30	25.96	42.13
IC373609	7.53	6.57	4.83	5.83	17.92	39.58	13.96	25.03	38.99
IC565488	7.73	6.57	4.23	3.33	17.92	47.08	10.48	25.03	43.33
IC582766	7.47	6.63	6.03	6.67	17.08	24.58	14.90	24.40	29.72
IC582804	7.47	6.60	4.60	6.67	17.50	42.50	14.95	24.70	40.68
IC582817	7.30	6.17	3.70	8.75	22.92	53.75	17.21	28.59	47.15
IC878883	7.07	5.57	3.87	11.67	30.42	51.67	19.97	33.47	45.96
IC565501	7.43	6.37	4.57	7.08	20.42	42.92	15.42	26.85	40.93
IC582782	7.63	6.27	4.63	4.58	21.67	42.08	12.34	27.74	40.45
IC557436	7.43	6.20	4.03	7.08	22.50	49.58	15.42	28.31	44.76
IC565490	7.63	6.67	4.93	4.58	16.67	38.33	12.34	24.08	38.25
IC402361	7.47	6.27	4.23	6.67	21.67	47.08	14.95	27.74	43.33
IC582780	6.80	5.43	4.53	15.00	32.08	43.33	22.78	34.50	41.17
IC565492	7.17	5.93	4.83	10.42	25.83	39.58	18.82	30.55	38.99
IC582795	7.53	6.63	4.67	5.83	17.08	41.67	13.96	24.41	40.20
IC265610	7.63	4.43	3.27	4.58	44.58	59.17	12.34	41.89	50.28
IC582808	6.73	4.23	3.07	15.83	47.08	61.67	23.44	43.33	51.75
PCSAG-1	7.23	6.43	3.83	9.58	19.58	52.08	18.03	26.26	46.19
PCSAG-2	7.77	6.50	4.27	2.92	18.75	46.67	9.79	25.66	43.09
Control	8.00	8.00	8.00	0.00	0.00	0.00	0.00	0.00	0.00
CD (0.05)							**1.438**		
CV							**3.097***		

✹ Angular transformed values *Significant at 5 per cent

4.4. Correlation studies

Correlation coefficients were calculated for eighteen morphological characters' in order to determine the positive or negative correlation between the morphological trait. This may help toknow the extent to which each traits contributed to rhizome yield and their morphological association with other traits. Before knowing the direct and indirect influence of these parameterson yield selection for yield traits may not be efficient. Correlation for different morphological traits with yield component in *Alpinia galanga* genotypes are presented in the Table 4.20A.

a) Plant height

Plant height was positively and significantly correlated with internodal length (0.517*), number of leaves (0.749***) leaf length (0.731***), leaf width (0.749***), leaf area (0.772***), inflorescence length (0.488*), number of branches (0.632**) and number of flowers per panicle (0.634**), length of flower (0.487*) and fresh rhizome yield/tiller (0.876***).

b) Tiller diameter

Tiller diameter is significantly and positively correlated with leaf width (0.61**), leaf area(0.531*), and fresh rhizome yield/ tiller (0.477*).

c) Number of tiller/plants

There was a positive and significant correlation between number of tillers per plant with length of flower (0.662**), width of flower (0.611**), and dry recovery of rhizomes (0.598*).

d) Internodal length

Internodal length was positively and significantly correlated with plant height (0.517*), leaf length (0.591**), leaf width (0.644**), leaf area (0.633**) number of branches per panicle(0.52*) and the number of flowers per panicle (0.523*), length of flower (0.627**), fresh rhizome yield per tiller (0.496*), and drying recovery of rhizome (0.598**).

e) Number of leaves per tiller

Leaf number per tiller was significantly and positively correlated with plant height(0.749***), leaf length (0.588**), leaf area (0.506*), No. of branches per panicle (0.47*), No.of flowers per panicle (0.481*), length of flower (0.466*) and rhizome yield per tiller (721***).

f) Leaf length

A significant and positive correlation was observed between leaf length with plant height (0.731***), internodal length (0.591**), number of leaves per tiller (0.588**), leaf width (0.828***), leaf area (0.955***), number of branches per panicle(0.505*), number of flowersper panicle (0.505*) and fresh rhizome yield per tiller (0.705***).

g) Leaf width

Leaf width was positively and significantly correlated with plant height (0.749***), tiller diameter (0.61**), internodal length (0.644**), leaf length (0.828***), leaf area (0.955***), number of branches per panicle (0.513*), number of flowers per panicle (0.512*), and fresh rhizome yield per tiller (0.735***).

h) Leaf area

The positive and significant correlation of leaf area was recorded with plant height (0.772***), tiller diameter (0.531*), internodal length(0.633**), number of leaves (0.506*), leaf length (0.955***), leaf width (0.953***), number of branches per panicle (0.52*), number of flowers per panicle (0.519*) and fresh yield per tiller (0.755).

i) Inflorescence length

A significant and positive correlation was observed between inflorescence length with plant height (0.488*), number of branches per panicle (0.805***), and number of flowers perpanicle (0.794***).

j) Number of branches per panicle

The number of branches per panicle was positively and significantly correlated with plant height (0.632**), internodal length (0.523*), number of leaves per tiller (0.47*), leaf length (0.505*), leaf width (513*), leaf area (0.52*), panicle length

(0.805***), number of flowers per panicle (0.998***) and fresh rhizome yield per tiller (0.605**).

k) Number of flowers per panicle

There was a positive and significant correlation between number of flowers per panicle with plant height (0.634**), internodal length (0.523*), number of leaves per panicle (0.481*),leaf length (0.505*), leaf width (0.512*), leaf area (0.519*), panicle length (0.794***), numberof branches per panicle (0.998***) and fresh yield per tiller (0.606**).

l) Length of flower

Length of flower positively and significantly correlated with plant height (0.487*), numberof tillers per plant (0.662**), internodal length (0.627**), number of leaves per tiller (0.466*),width of flower (0.771***), and dry recovery of rhizome (0.625**).

m) Width of flower

The positive and significant correlation of flower width was recorded with number of tillersper plant (0.611**), length of the flower (0.771***) and dry recovery of rhizome (0.511*).

n) Fresh rhizome yield per tiller

Fresh rhizome yield per tiller was positively and significantly correlated with plant height(0.876***), tiller diameter (0.477*), internodal length (0.496*), number of leaves per tiller (0.721***), leaf length (0.705***), leaf width (0.735***), leaf area (0.755***), number of branches per panicle (0.605**), and number of flowers per panicle (0.606**).

o) Rhizome width

The positive and significant correlation of rhizome width was recorded with petiole length(0.471*) and rhizome length (0.521*).

p) Dry recovery

A significant and positive correlation was observed between dry recovery of rhizome withnumber of tillers per plant (0.457*), internodal length (0.598**) length of flower (0.675**) and width of flower (0.511*).

4.4.1 Correlation studies for antimicrobial activity against fungal pathogens

Correlation studies for antimicrobial activity against fungal pathogens are presented in the Table 4.20B.

a) Oleoresin content

Oleoresin content was positively and significantly correlated with flavonoid content (0.523*),phenol content (0.658**), amino acid content (0.72**), starch content (0.622**), per cent inhibition *Rhizoctonia* spp. (0.534*), percent inhibition *Colletotrichum* spp. (0.65**), and percent inhibition of *Fusarium* spp. (0.522*).

b) Total flavonoid content

There was a significant and positive correlation between total flavonoid content with oleoresincontent (0.523*), total phenol content (0.662**), amino acid content (0.532*), starch content (0.786***) and percentage inhibition *Rhizoctonia* spp. (0.705***), percent inhibition of*Colletotrichum* spp. (0.6**) and per cent inhibition of *Fusarium* spp. (0.468*).

c) Total phenol content

Total phenol content is significantly and positively correlated with oleoresin content (0.658**),flavonoid content (0.662**), amino acid content (0.532*), starch content (0.786***), per cent inhibition of Rhizoctonia spp. (0.689**), per cent inhibition of *Colletotrichum* spp. (0.598**)and per cent inhibition of *Fusarium* spp. (0.526*).

d) Amino acid content

Amino acid content is positively and significantly correlated with oleoresin content(0.72***), flavonoid content (0.559*), phenol content (0.532*), starch content (0.599**) and per cent inhibition *Rhizoctonia* spp. (0.483*).

e) Starch content

There was a positive and significant correlation between the starch content with the oleoresin content (0.622**), flavonoid content (0.786***), phenol content (0.785**), amino acid content (0.599**), percent inhibition of *Rhizoctonia* spp. (0.632**), percent inhibition of *Colletotrichum* spp. (0.64**), percent inhibition of *Phytophthora* spp. (0.684**), and percent inhibition of *Fusarium* spp. (0.694***).

Table 4.20 A: Correlation analysis for morphological traits

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14	X15	X16	X17	X18
X1	1																	
X2	0.186	1																
X3	0.34	0.233	1															
X4	0.517 *	0.398	0.279	1														
X5	0.749 ***	0.174	0.312	0.386	1													
X6	0.731 ***	0.417	0.166	0.591 **	0.588 **	1												
X7	0.749 ***	0.61**	0.128	0.644 **	0.432	0.828 ***	1											
X8	0.772 ***	0.531 *	0.152	0.633 **	0.506 *	0.955 ***	0.953 ***	1										
X9	-0.199	0.291	-0.262	-0.015	-0.339	0.081	0.221	0.163	1									
X10	0.488 *	-0.039	0.355	0.422	0.316	0.269	0.397	0.329	0.037	1								
X11	0.632 **	0.135	0.353	0.52*	0.47*	0.505 *	0.513 *	0.52**	0.271	0.805 ***	1							
X12	0.634 **	0.129	0.33	0.523 *	0.481 *	0.505 *	0.512 *	0.519 *	0.283	0.794 ***	0.998 ***	1						
X13	0.487 *	0.33	0.662 **	0.627 **	0.466 *	0.276	0.324	0.3	-0.234	0.36	0.442	0.443	1					

X14	0.274	0.332	0.611**	0.401	0.253	0.137	0.144	0.154	0.047	0.145	0.376	0.371	0.771***	1			
X15	0.876***	0.477*	0.345	0.496*	0.721***	0.705***	0.735***	0.755***	-0.085	0.394	0.605**	0.606**	0.434	0.326	1		
X16	-0.019	0.086	0.361	-0.032	-0.298	-0.031	0.111	0.061	0.25	0.263	0.021	0.015	0.182	0.212	-0.016	1	
X17	0.085	0.295	0.034	0.042	0.096	0.141	0.303	0.22	0.471*	0.455	0.371	0.377	0.125	0.181	0.207	0.521*	1
X18	0.378	0.356	0.457*	0.598**	0.227	0.178	0.369	0.285	-0.091	0.387	0.405	0.414	0.675**	0.511*	0.367	0.243	0.194	1

*** Correlation is significant at 0.001 level (two tailed)

** Correlation is significant at 0.01 level (two tailed)

* Correlation is significant at 5% level (two tailed)

X1 - Plant height
X2 - Tiller diameter
X3 - Number of tillers/plants
X4 - Internodal length
X5 - Number of leaves per tiller
X6 - Leaf length
X7 - Leaf width
X8 - Leaf area
X9 - Petiole length
X10- Inflorescence length
X11 - Number of branches per panicle
X12 - Number of flowers per panicle
X13 - Length of flower
X14 – Width of flower
X15 – Fresh rhizome yield / tiller
X16 – Length of rhizome
X17 – Width of rhizome
X18 – Dry recovery of rhizome

Table 4.20B: Correlation analysis for quality parameters

	X1	**X2**	**X3**	**X4**	**X5**	**X6**	**X7**	**X8**	**X9**
Oleoresin (X1)	1								
Flavonoid content(X2)	0.523*	1							
Phenol content (X3)	0.658**	0.662**	1						
Amino acid content(X4)	0.72***	0.559*	0.532*	1					
Starch content (X5)	0.622**	0.786***	0.785***	0.599**	1				
Percent inhibition of *Rhizoctonia spp.* (X6)	0.534*	0.705***	0.689**	0.483*	0.632**	1			
Percent inhibition of C*olletotrichum spp.* (X7)	0.65**	0.6**	0.598**	0.454	0.64**	0.761***	1		
Percent inhibition *of Phytophthora spp*. (X8)	0.373	0.452	0.35	0.187	0.684**	0.469*	0.421	1	
Percent inhibition of F*usarium spp.*(X9)	0.522*	0.468*	0.526*	0.244	0.694***	0.548*	0.532*	0.726***	1

*** Correlation is significant at 0.001 level (two tailed)

** Correlation is significant at 0.01 level (two tailed)

* Correlation is significant at 5% level (two tailed)

DISCUSSION

5. DISCUSSION

Alpinia galanga has recently gained tremendous industrial importance due to its numerousapplications in the medicinal field. At the same time, it is considered an endangered and threatened medicinal crop because of improper harvesting methods and over exploitation. In order to maintain the balance, conservation, as well as rapid multiplication of this plant has tobe taken up urgently.

The present study entitled "Variability in greater galangal (*Alpinia galanga L.*) Wild. genotypes for yield and quality" was carried out by utilizing seventeen *Alpinia galanga* genotypes maintained at the NBPGR field gene bank and two genotypes maintained at the Plantation Crops and Spice Farm, College of Agriculture, Vellanikkara, which were evaluatedfor morphological, biochemical, and antimicrobial activities. The results of the experiments are discussed in this chapter.

5.1 MORPHOLOGICAL EVALUATION

The mean performance for morphological characters of nineteen accessions was calculatedusing the standard procedures. Significant differences were observed among the accessions forthe tiller, leaf, flower, and rhizome characters. The result indicates significant variation for allthe studied characters among the accessions. This variation may help in identifying the majortraits in the selection procedure, because variability acts as a prerequisite for all crop improvement programs.

5.1.1 Qualitative characters

All the genotypes evaluated in the present study showed similar qualitative characteristics.The skin color of the rhizome was observed to be greyed yellow and yellowish white in the core region (Table 4.1). The shape of the leaves was lanceolate to oblong with a cuneate baseand acuminate apex.

As per the observations recorded during the course of the study, there are two flowering seasons in *Alpinia galanga*, from May to July and August to October. The

color of the petal was judged to be white with pink markings in the center. The seed color was observed as darkbrown to black.

The morphological characteristics of plants can be employed as diagnostic or key factors when describing, classifying, and resolving taxonomic issues (Iroka *et al*., 2015). Trimanto *et al*. (2021) reported the key taxonomic morphological features of *Alpinia galanga* and also described similar results of qualitative characteristics like flower color, leaf shape, and seed color in their studies. Ponmozhi and Kalaiselvi (2010) also observed wide variation in different*Alpinia galanga* genotypes for morphological characters.

5.1.2 Quantitative characters

5.1.2.1 Plant height

Nineteen *Alpinia galanga* genotypes were evaluated during the course of the experimentation. The plant height in *Alpinia galanga* followed an upward trend up to Januarythere after height reduces gradually due to withering of top most leaves (Fig 5.1B). The result also revealed that there was significant difference among the accessions for plant height as shown in Figure 5.1A. The plant reached its maximum height in February when it ranged from172.30 to 278.60 cm. The tallest plant observed was PCSAG-1, followed by IC582817 and PCSAG-2, and the shortest was IC565490. According to Susetyarini *et al*. (2020), even amongmembers of the same species, morphological characteristics might vary in form and structure. However, Parida *et al*. (2011) reported the height of conventionally propagated *Alpinia galanga* plants was 73 ±2.54 cm. Tonwitowat (2008) reported varying heights of yellow *Alpinia galanga* cultivars ranged from 121.4 to 168.7 cm with a mean value of 140.5 cm. Similar range of result of plant height has been reported by Ponmozhi and Kalaiselvi (2010) and Zahara, M. (2020) in *Alpinia galanga*.

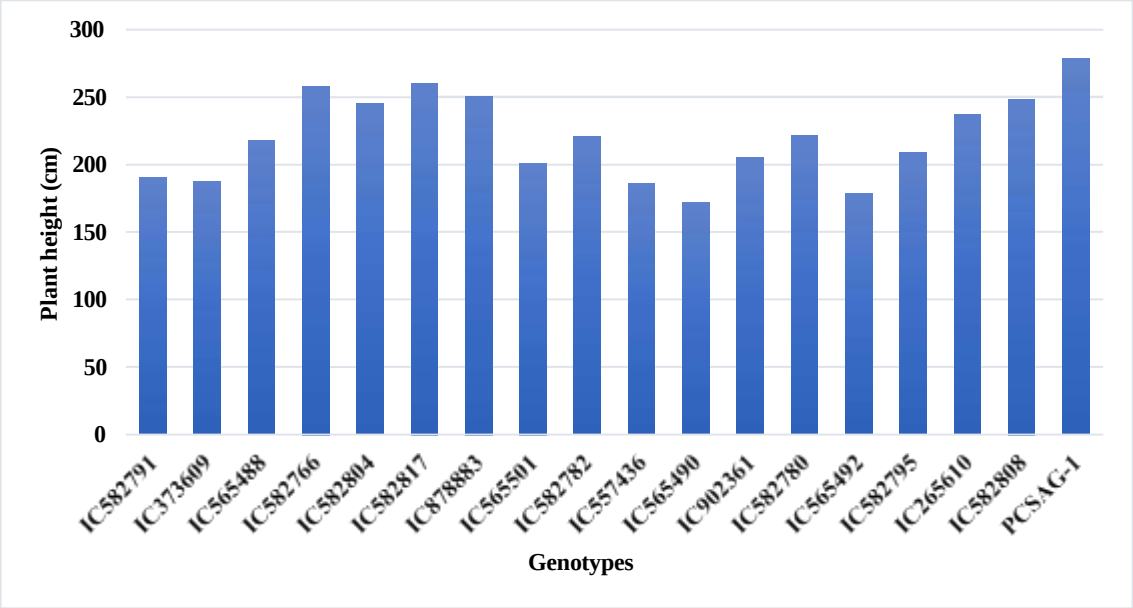


Fig. 5.1A: Plant height in *Alpinia galanga* genotypes

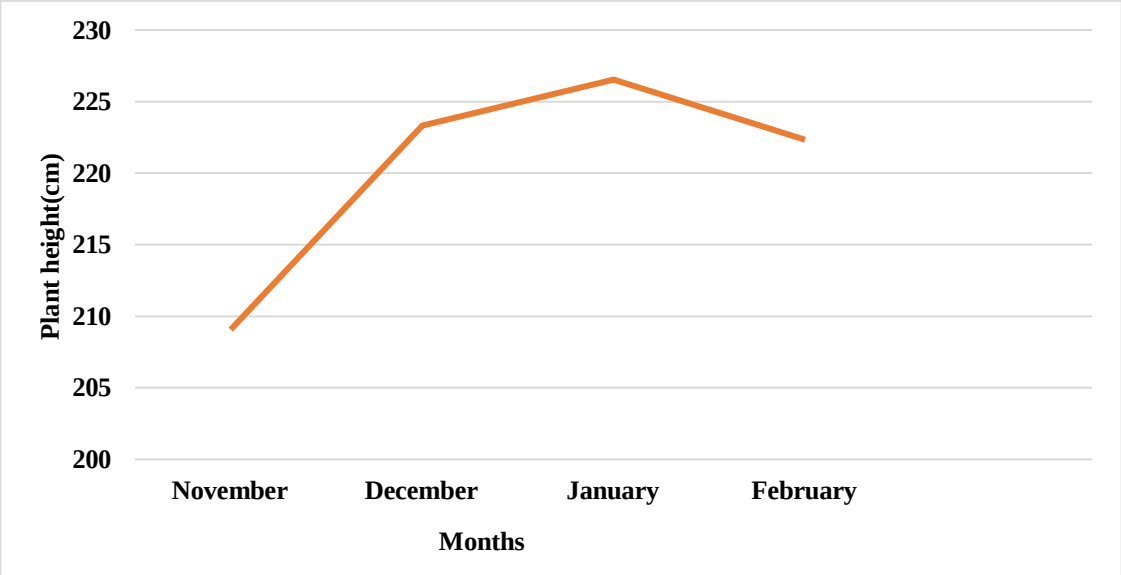


Fig. 5.1B: Monthly variation in plant height in *Alpinia galanga* genotypes

5.1.2.2 Tiller diameter

Tiller diameter was observed to be significant across genotypes (Fig 5.2A). However, the tiller diameter exhibited an increasing trend that peaked in January before switching on to a stable phase as depicted in Figure 5.2B. The tiller diameter ranged from 4.15 cm to 4.67 cm among the genotypes. Accession IC565492 and IC582795 (4.67cm) recorded the highest diameter whereas, the lowest diameter was recorded in the accession IC402361 (4.15cm). Variations in the tiller diameter ranged from 1.5 to 2 cm, as observed by Trimanto *et al*. (2021)in *Alpinia galanga.* Windarsih *et al*. (2021), and Lianah *et al*. (2020) also discussed the same on various Zingibers in their studies.

5.1.2.3 Number of tillers per plant

Significant variation was recorded for tiller production among *Alpinia galanga* genotypes.During the rainy season, *Alpinia galanga* plants show active vegetative growth along with maximum tiller production. This active vegetative growth period ended in December, and in later months there was not much variation in the growth pattern. Lianah *et al*. (2020) stated that rainfall has a significant impact on Zingiberaceae plant growth, and they require a constantwater supply to reach their full potential.

During the observed months, the plant produced the highest number of tillers up to December, which ranged from 8 to 46 per plant among the genotypes (Fig 5.3A). The averagenumber of tillers recorded in the observed months ranged from 16.16 to 21.74 (Fig 5.3B). Accession IC582817 and PCSAG-1 maintained the highest number of tillers in all the observed months, whereas IC582804, and IC565501 showed the lowest number of tillers. Variations in the tillers among the *Alpinia galanga* genotypes were also reported by Tonwitowat (2008) it ranged from 22 to 45 per plant. Similarly, variation in the number of tillers in *Kaempferia galanga* was reported by Divya (2008) and Devi *et al*. (2018).

5.1.2.4 Internodal length

The internodal length was observed for four consecutive months from November to February. The internodal length in *Alpinia galanga* followed an upward trend from Novemberto January, after which there was not much variation from January to February (Fig 5.4B). Butthere was significant variation observed for internodal length among the genotypes, which ranged from 6.29 cm to 10.16 cm, and the highest internodal length was recorded in PCSAG- 2, followed by PCSAG-1(Fig 5.4A), whereas the lowest internodal length was observed in IC557436. Windarsih *et al*. (2021) found that the internodal length of *Alpinia purpurata* rangedfrom 10.7 to 12.3 cm. Variations in the internodal length were reported by Pushpa *et al*. (2017)and Momina *et al*. (2011) in ginger.

5.1.2.5 Number of leaves

In the current experiment, the number of leaves produced per tiller was recorded on monthly basis as shown in Figure 5.5A. It was noted that the number of leaves produced per tiller varied significantly throughout the studied months, with a mean of 12.5. The leaf production increased gradually, peaked in December, and then started to decline. The numberof leaves per plant tiller ranged from 7.4 to 13.50 among the genotypes (Fig 5.5B). The highestnumber of leaves per tiller was recorded in the accession numbers IC582782 and IC373609 while IC565501 had the fewest. In red *Alpinia galanga* cultivars, the total number of leaves per plant ranged from 176 to 406 (Tonwitowat, 2008). Similar results for the number of leavesper tiller in *Alpinia galanga* were reported by Joy *et al*. (2001). According to Nybe (1978), variation in the number of leaves can be attributed to the indirect effects of the plant's height on the number of tillers.

5.1.2.6 Leaf length, width, and leaf area

The genotypes differ significantly in terms of leaf length, width, and area. However, theseparameters were measured from fully opened mature leaves, so there was no discernible increase in leaf characteristics during the studied months as shown in Figure 5.6B. Among thegenotypes, IC265610 (54.81 cm) and IC565490 (39.75 cm) recorded the highest and lowest leaf lengths, respectively. Accession IC565501 had the

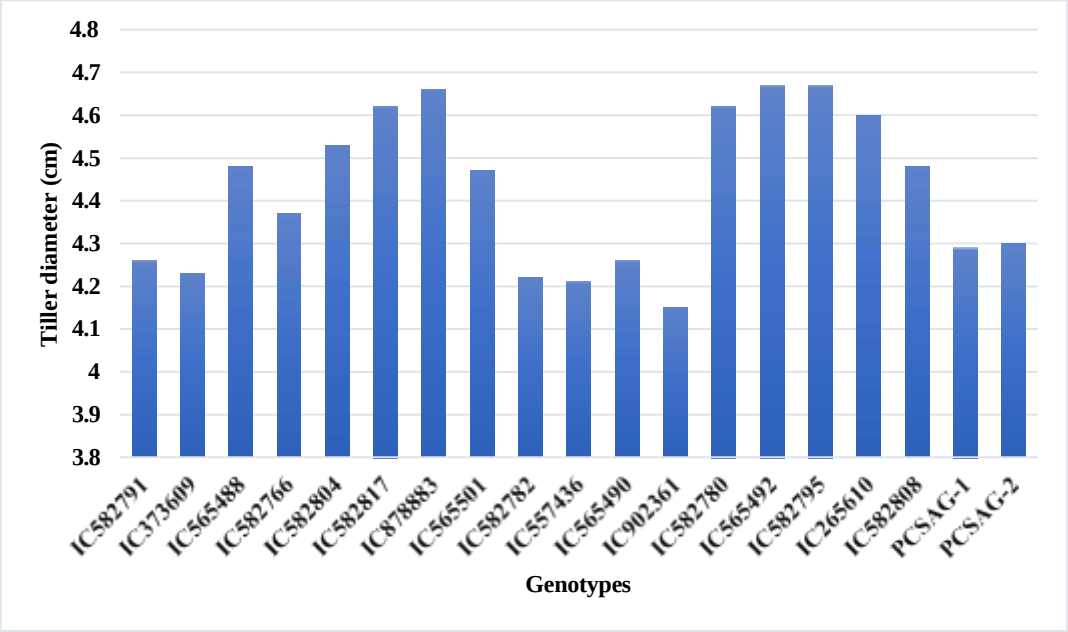


Fig. 5.2A: Tiller diameter in *Alpinia galanga* genotypes

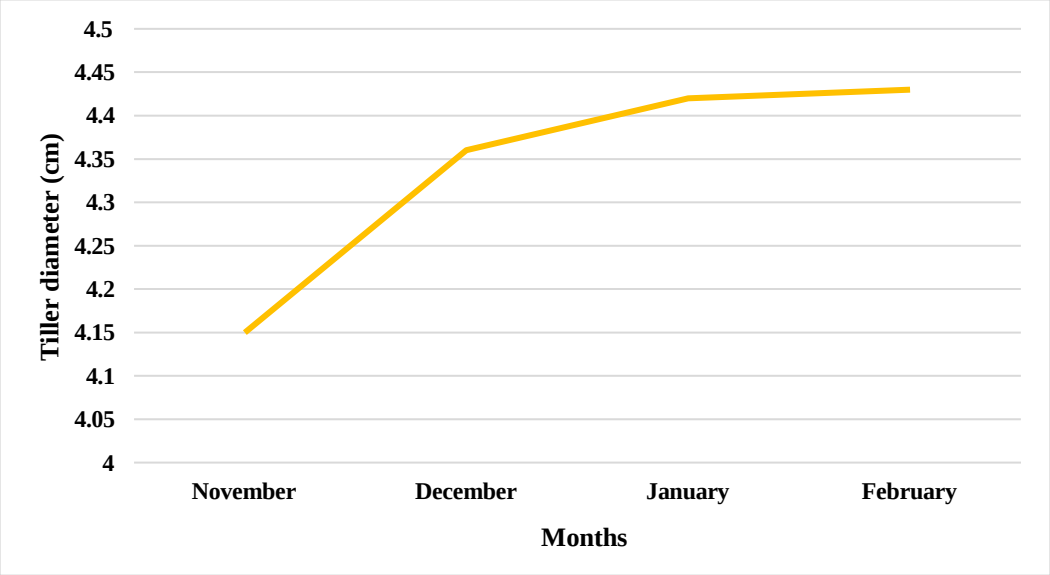


Fig. 5.2B: Monthly variation in tiller diameter in *Alpinia galanga*

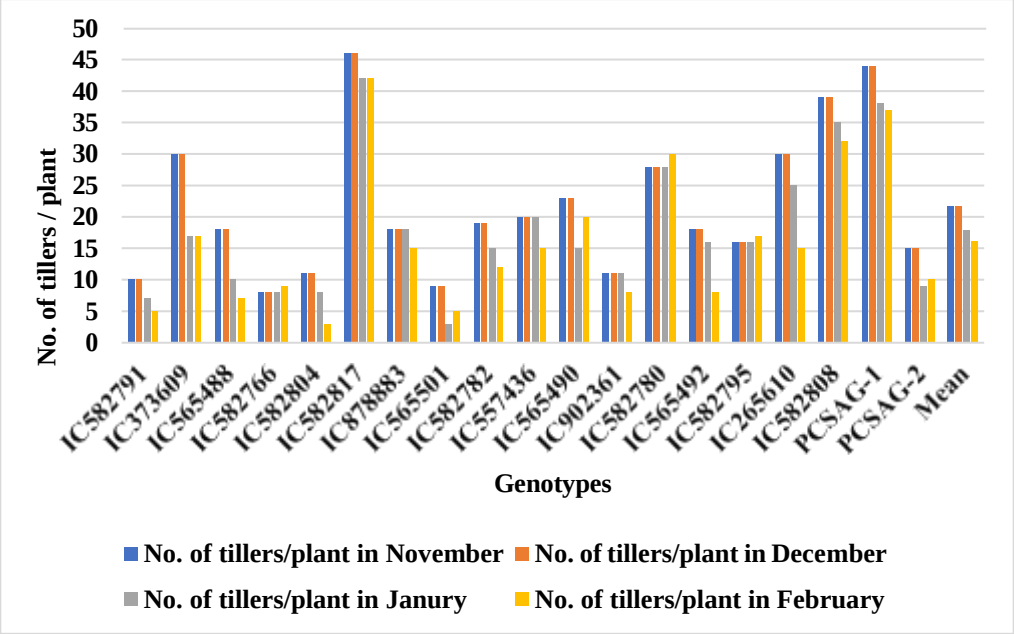


Fig. 5.3A: Number of tillers per plant in *Alpinia galanga* genotypes

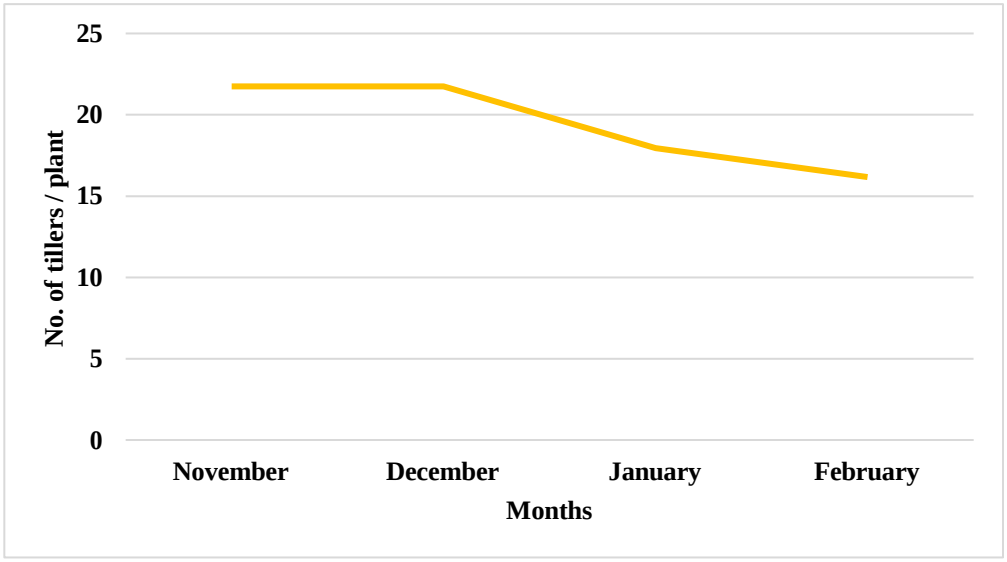


Fig. 5.3B: Monthly variation in tiller number in *Alpinia galanga*

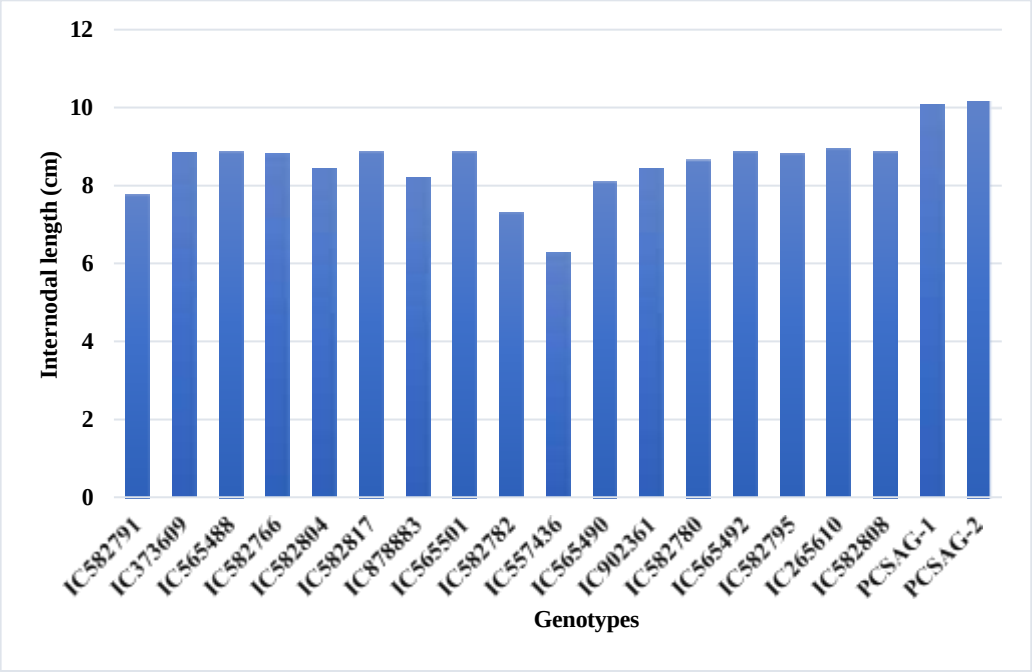


Fig. 5.4A: Internodal length in *Alpinia galanga* genotypes

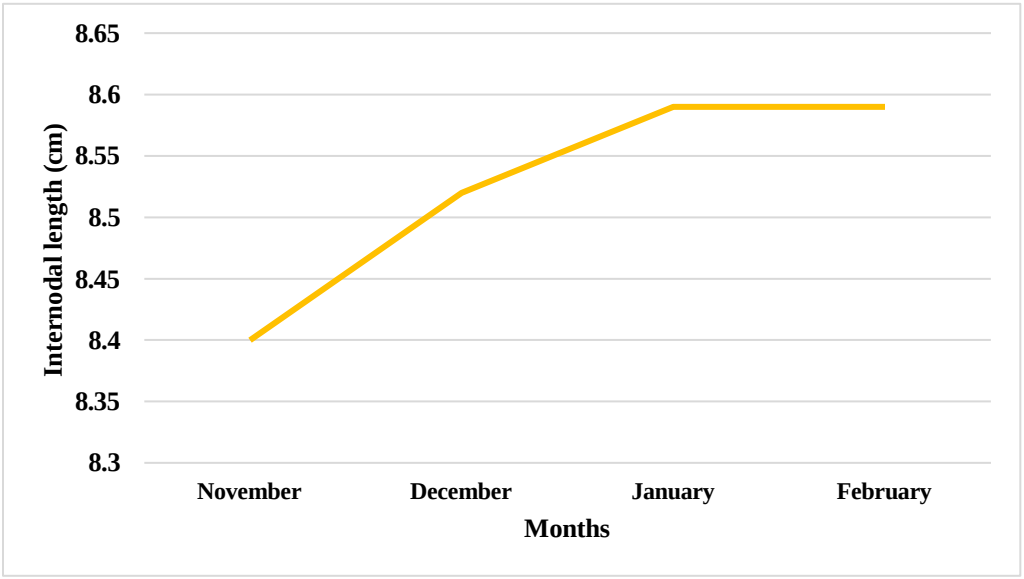


Fig. 5.4B: Monthly variation in internodal length in *Alpinia galanga*

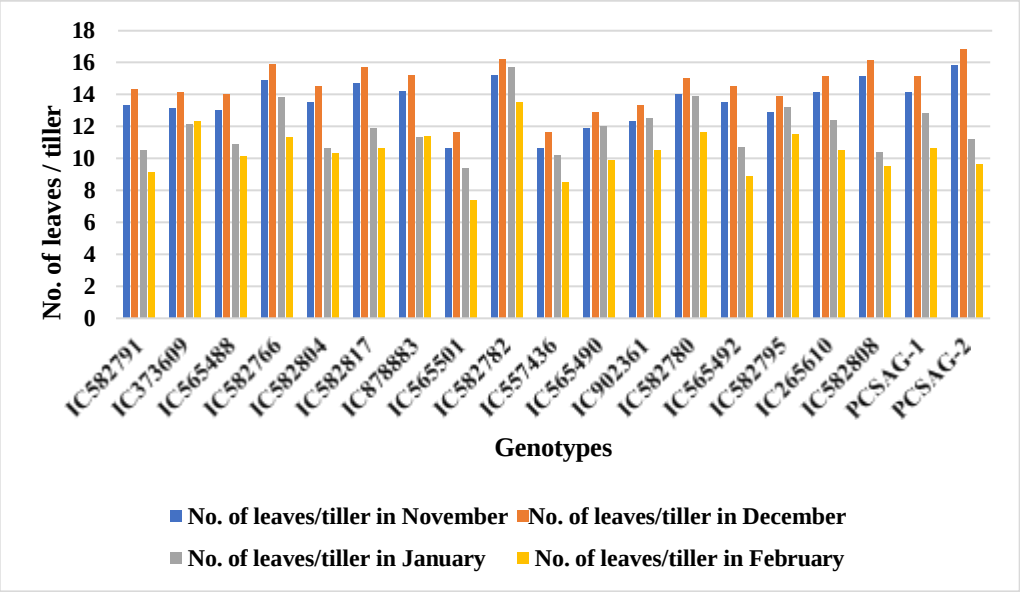


Fig. 5.5A: No. of leaves/tiller in *Alpinia galanga* genotypes

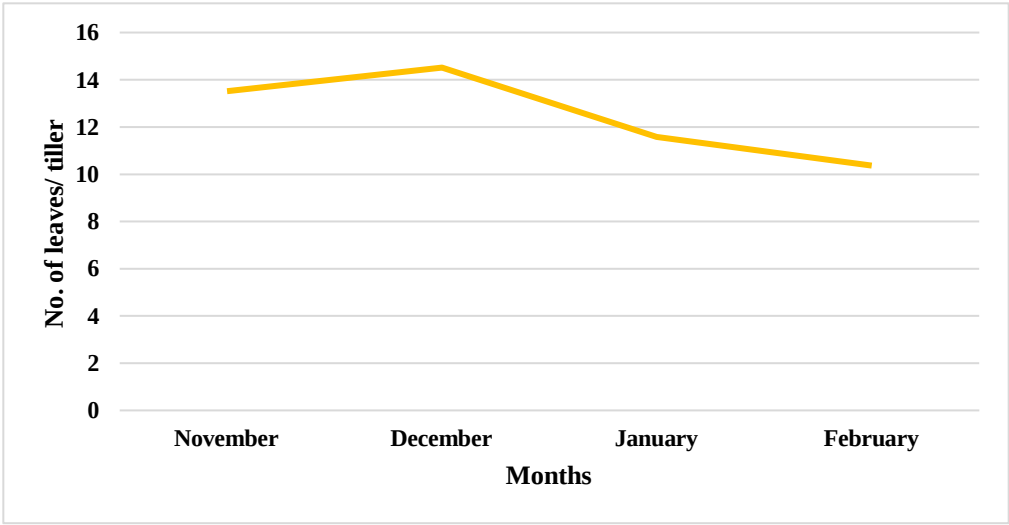


Fig. 5.5B: Monthly variation in leaf number in *Alpinia galanga*

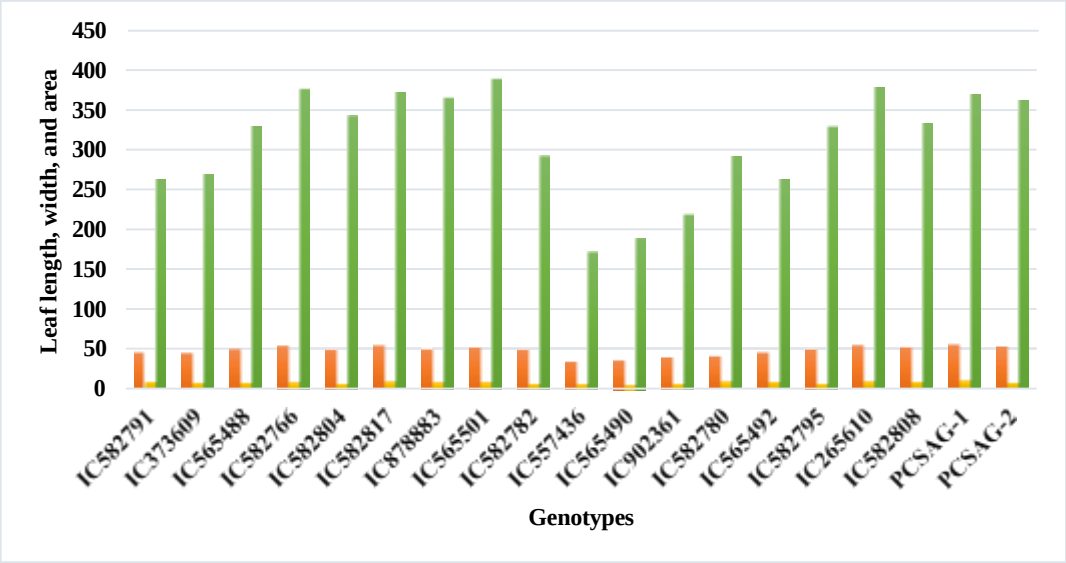


Fig. 5.6A: Leaf length, width, and area in *Alpinia galanga* genotypes

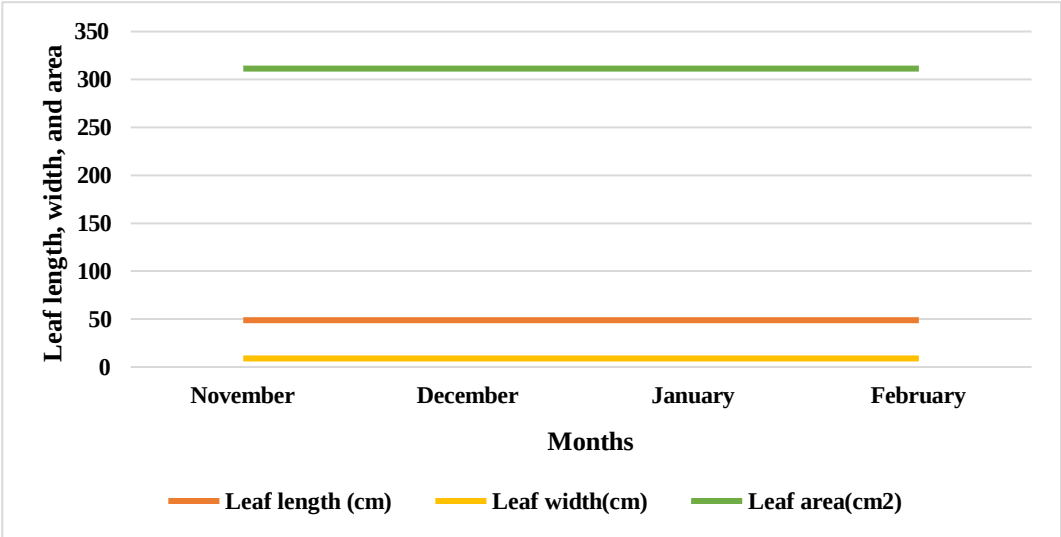


Fig. 5.6B: Monthly variation in leaf length, width, and area in *Alpinia galanga* genotypes

greatest leaf width and leaf area (10.35cm and 391.02 m^2 respectively). Accession IC557436 (6.75 cm and 172.78 m^2) recorded lowest leaf width and area in all observed months (Fig 5.6A). Pooja *et al*. (2020) also reportedsimilar results with respect to leaf length, width, and area among *Alpinia galanga* genotypes. A study conducted on 13 Zingiberaceae species collected from Indonesia by Windarsih *et al*. (2021) revealed variations in leaf length and width. Similar variations with respect to leaf length, width, and area have been reported in *Kaempferia galanga* by Latha (1994) and Devi and Raj (2020).

5.1.2.7 Petiole length

The observations for petiole length were recorded at monthly intervals from November to February. The petiole length varies significantly among accessions, although it does not alter significantly over the course of the observational months (Fig 5.7B). In *Alpinia galanga*, petiole length varies between 0.66 and 0.82 cm. The longest and shortest petiole lengths were recorded in accession IC878883, and IC582808. respectively. (Fig 5.7A). Similar variation inthe petiole length among the *Alpinia galanga* genotypes was reported by Pooja *et al*. (2020) and Trimanto *et al*. (2021) in *Alpinia warburgi.*

5.1.2.8 Flower characters

5.1.2.8.1 The floral biology of *Alpinia galanga*

As per the observations of the present study, *Alpinia galanga* exhibited two flowering seasons. In the first season, flowering was seen from the second week of May to the end of July, whereas in the second season, it began in the third week of August and continued up to the last week of October.

According to earlier studies, wide variations are observed for the flowering season in *Alpinia galanga*. Environmental, positional, and juvenile factors are responsible for variationsin the morphological characters of zingibers (Iroka *et al*. 2015). According to Kumar (2019), flowering takes place between May and June, while fruiting happens between August and September. In accordance with the study of Kasarkar and Kulkarni (2011) studies, *Alpinia galanga* produces enormous flowers

throughout the year. According to Sabu's (2006) report, *Alpinia galanga* shows flowering and fruiting from April to December.

The inflorescence is a terminal, upright panicle with many, fragrant greenish-white flowers. Anthers are yellowish white, stamens are white, the style is creamy white, the ovary is trilocular, and parietal placentation. Peduncle pubescent; flowers 3–4 cm long; sepals threein number; green color; corolla tube 1.2 cm long; greenish white color; labellum 2 cm long; white with pink color markings on the upper surface along the midrib; wavy margins. Similarresults for the flower character in *Alpinia galanga* were reported by Gupta (2010), Roy *et al*.(2012), and Mangily and Sabu (1992).

Based on the stigmatic movements, there were two floral morphs observed in the studied population of *Alpinia galanga* each of which displays a different flowering behavior. One group expressed cataflexistyly, and the other expressed anaflexistyly. Similar floral forms wereereported in *A. kwangsiensis* by Li *et al*. (2002), Zhang *et al*. (2003) in *Alpinia blepharocalyx*, and also by Aswani and Sabu (2014) in *Alpinia mutica Roxb*.

5.1.2.8.2 Flowering phenology of *Alpinia galanga*

Clear understanding on the reproductive biology of flowering plants is necessary in order to determine difficulties in seed and fruit set for conservation, pollination, and breeding systems that control the genetic makeup of populations (Tandon *et al*. 2003). For observing flowering phenology in *Alpinia galanga*, observations were made from flower bud initiation to complete flower opening, as well as from fruit setting to ripening. Results indicated that there are four principal growth stages from flower bud emergence to seed maturity, which werecoded according to the BBCH scale.

The principal growth stage 5 (reproductive bud development) lasted 12-15 days, the principal growth stage 6 (flowering) lasted 20–25 days, and the principal growth stage 7 (fruit development) took 90-100 days from fruit set to maturation. Principal growth stage 8: From maturation to complete ripening, it took 80–85 days. The similar duration from the initiation of the flower bud to the final seed setting in *Alpinia galanga* was reported by Kasarkar and Kulkarni (2011). In the same line phenological phases in

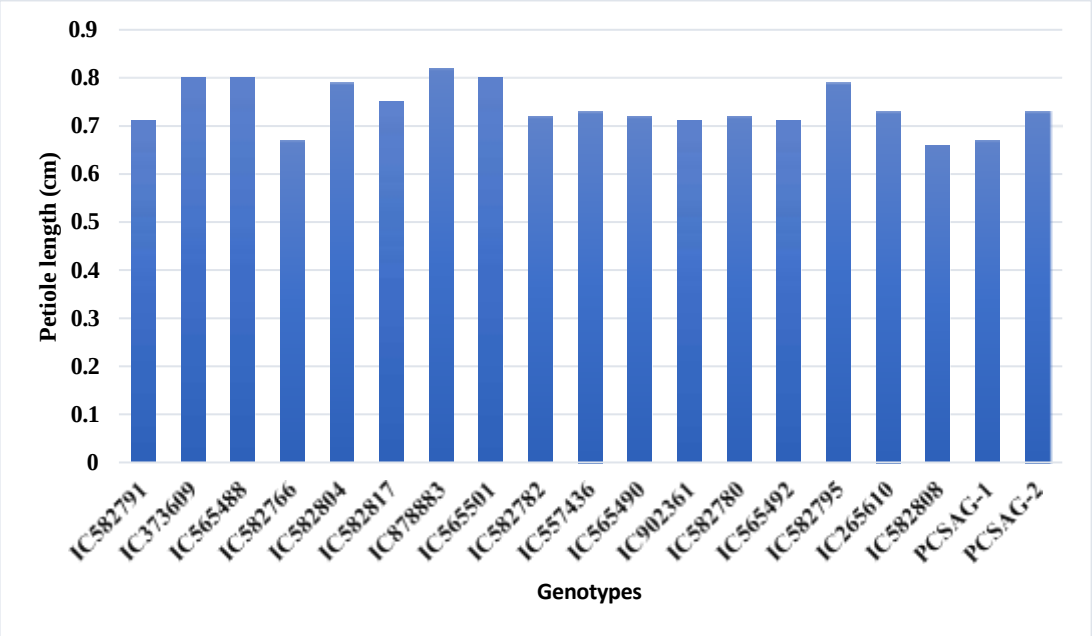


Fig. 5.7A: Petiole length in *Alpinia galanga* genotypes

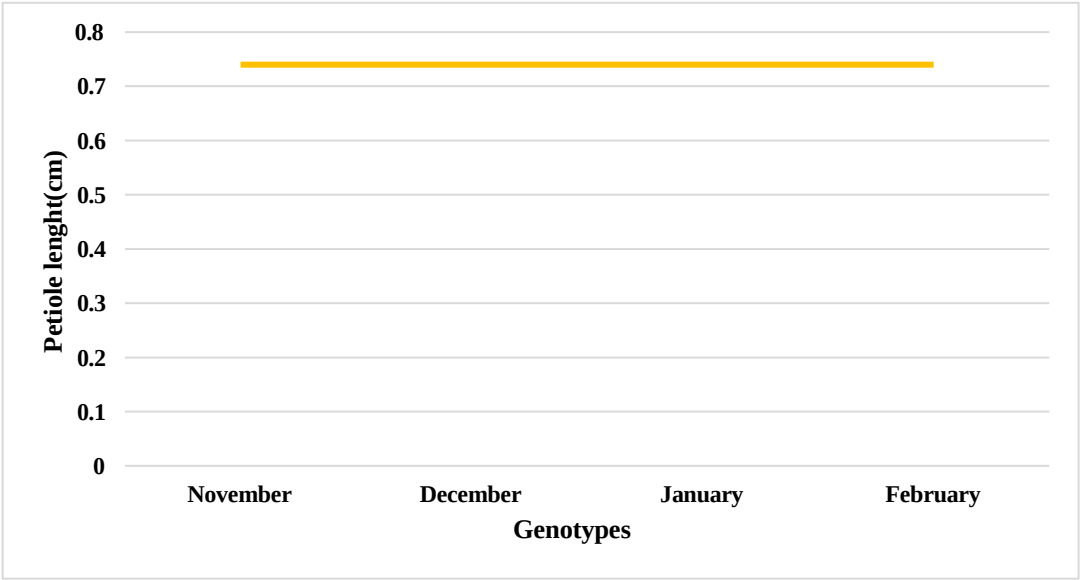


Fig. 5.7B: Monthly variation in petiole length in *Alpinia galanga* genotypes

torch ginger has been reported by Choon *et al*. (2016) and Khairlani *et al*. (2020) in *Thaumatococcus daniellii*. Aswani and Sabu (2015) also reported similar phenological stages of flowering in *Alpinia mutica Roxb.*

5.1.2.8.3 Panicle /inflorescence length, number of branches, and flowers per panicle

Flower characters were observed during the flowering season. A significant variation wasobserved for flower characters in all nineteen accessions shown in Figure 5.8A. The highest panicle length was recorded in IC402361 (26.60 cm), and the shortest panicle length was recorded in accession IC557436 (11.17 cm). Similar results for panicle length variation in *Alpinia galanga* has been reported by Pooja *et al*. (2020) and Tungmunnithum (2020). The number of branches and flowers per panicle is directly related to the length of the panicle. Thenumber of branches per panicle varies from 19 to 37.67 and the highest number of branches was recorded in IC565488, whereas the lowest was in IC582791. A similar observation was made by Sam (2017) in *Alpinia newmanii sp. nov.* (Zingiberaceae), and also by Liu & Wang (2009) on *Alpinia* × *ilanensis* (Zingiberaceae).

Significant variation existed for the length and width of the *Alpinia galanga* flower (Fig 5.8B). The highest flower length was recorded in accession IC582817 (4.10 cm) and the lowestwas recorded in IC565501 (3.10 cm). Similar values for flower length were recorded by Pooja*et al*. (2020) in *Alpinia galanga* and by Trimanto and Hapsari (2014) in *Alpinia warburgii K. Schum*. For flower width, accession IC582817 (3.33 cm) recorded the highest value and the lowest flower width was recorded in IC582791 (2.30 cm). Variations in the length and width of flowers in the genus *Hedychium J. Koen* (Zingiberaceae) were reported by Vanchhawng and Lalramnghinglova (2016).

5.1.2.9 Rhizome characters

Rhizome is the economic part in *Alpinia galanga*. The data with respect to rhizome characters like fresh weight, length, width, and dry recovery of *Alpinia galanga* rhizomes wererecorded after harvesting the rhizomes. Significant differences were noticed for rhizome characters among the genotypes. The fresh rhizome weight per tiller ranged from 43.00 g to 170.00 g (Fig 5.9A). Accession IC582817 (170.00 g)

recorded the highest rhizome weight, whereas, IC5655490 (43.00g) yielded lowest rhizomes weight. Pooja *et al*. (2020), Tonwitowat (2008), Parid *et al*. (2011), and Ponmozhi and Kalaiselvi (2010) all have reported variability in fresh rhizome weight per tiller in *Alpinia galanga.*

Significant difference was observed in rhizome length and width among the genotypes. The mean values of rhizome length and width are 6.31 and 4.06 cm, respectively. IC582817 shows the highest rhizome length and width, wherein IC582808 recorded the lowest rhizome length and width (Fig 5.9B). Variation in length and width was recorded by Chitra and Thoppil(2008) in *Alpinia galanga* rhizome and also by Asaf *et al.* (2018) in ginger. Dry recovery is avery important parameter in the rhizomatous crop for commercial cultivation. Significant variation was observed among the genotypes for dry recovery of the rhizome (Fig 5.9C). The maximum dry recovery per cent was recorded in accession IC582780 (25.43%), followed by PCSAG-2 (25.38%), and the lowest was recorded in accession, IC582782 (19.8%). Variationin the dry recovery percent of ginger rhizomes ranged from 16–25%, as reported by Sasikumar*et al*. (2008), in turmeric it ranges from 15- 30 % by Peter (2007), and in *kaempheria galanga*cultivars 32.78 and 34.48 per cent by (Preetha *et al*., 2016).

5.2 PHYTOCONSTITUENTS

Among the genotypes, wide variation was noticed with respect to quality parameters like volatile oil, oleoresin, flavonoids, phenolic amino acids, and starch content.

5.2.1 Volatile oil and oleoresin content of *Alpinia galanga*

Data on volatile oil and oleoresin content are presented in Figure 5.10A. Significant variation was noticed for volatile oil content among the genotypes, ranging from 0.21 to 0.41 per cent. Accession IC878883 recorded the highest volatile oil, and IC582791, IC582795, IC373609, and IC582804 recorded the lowest (0.21%) oil content. A wide variation in volatileoil content has been reported in *Alpinia galanga* germplasm, ranging from 0.21 to 0.41 per cent, by Raina *et al.* (2017). According to Suresh *et al*. (2016), the oil content in *A. calcarata* and *A. galanga* rhizomes was 0.93 per cent and 0.48 per cent respectively. Similar studies on volatile oil content were

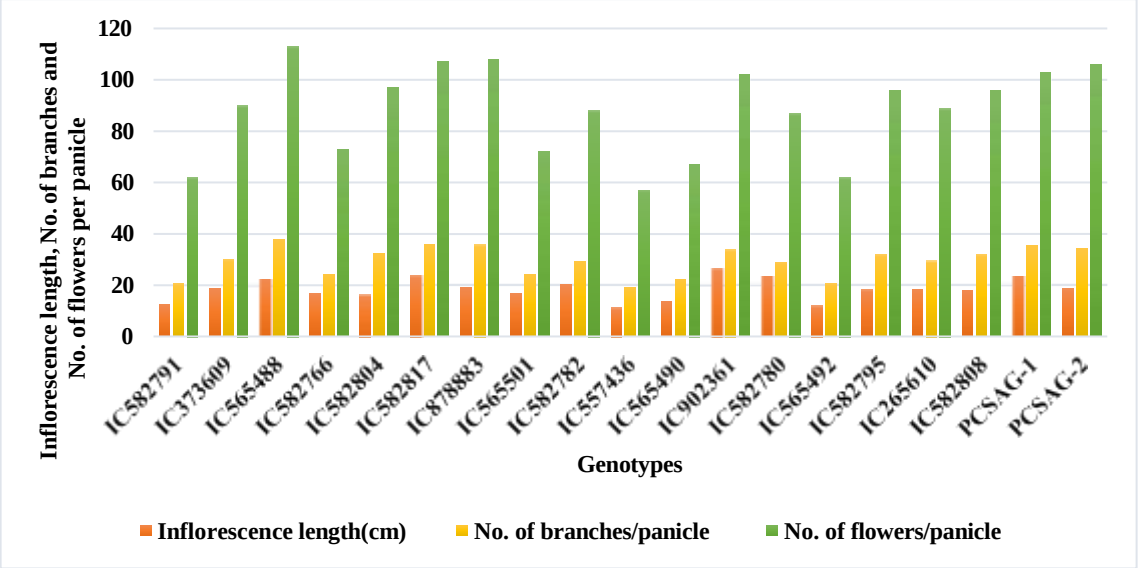


Fig. 5.8A: Inflorescence length, No. of branches and No. of flowers per panicle in *Alpinia galanga*

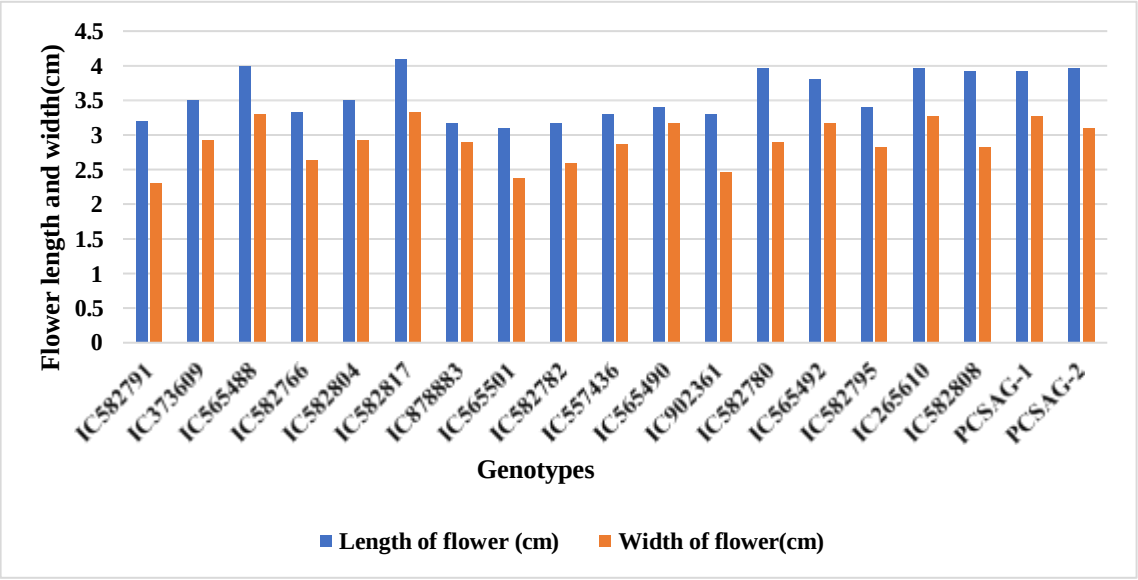


Fig. 5.8B: Length and width of *Alpinia galanga* flower

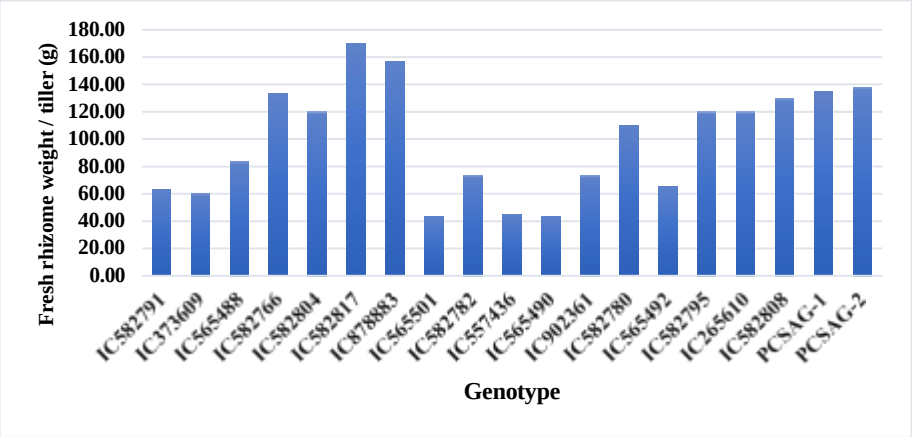


Fig. 5.9A: Fresh rhizome weight/ tiller in *Alpinia galanga* genotypes

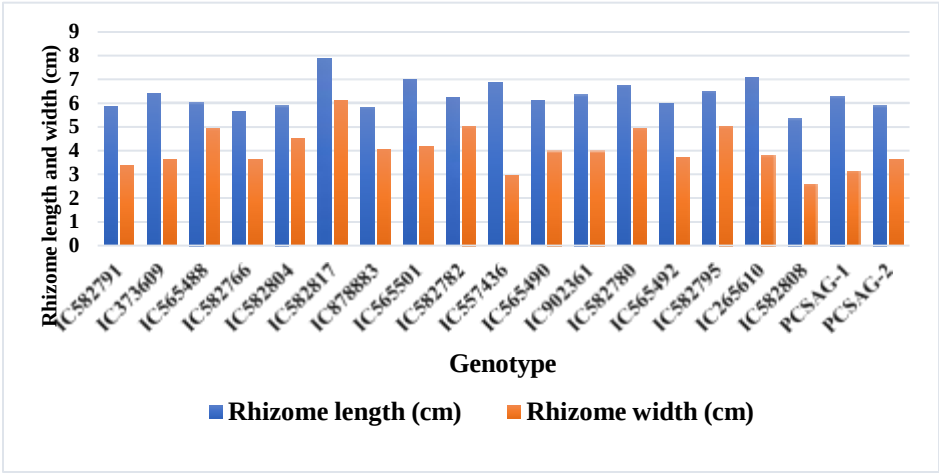


Fig. 5.9B: Length and width of the rhizome

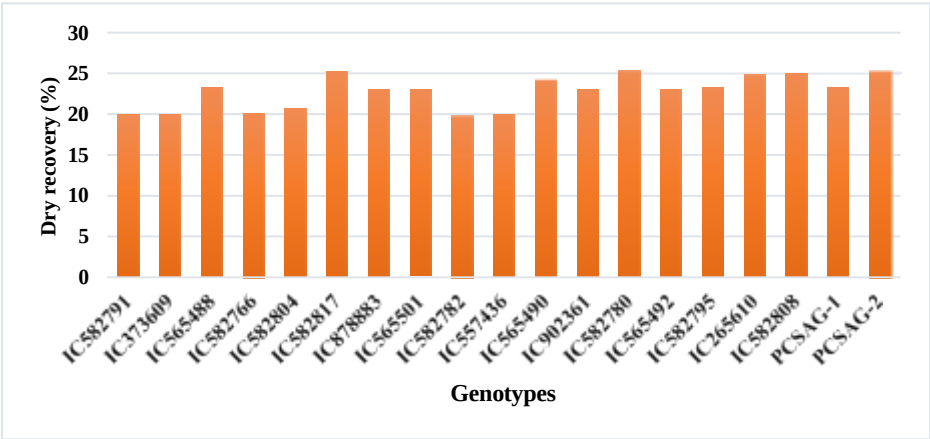


Fig. 5.9C: Dry recovery of *Alpinia galanga* rhizome

reported in *Alpinia officinarum Hance.* by Zang *et al*. (2016). A significant variation was observed for oleoresin content among the genotypes, ranging from 3.05 to 4.45 per cent. The highest oleoresin recovery using methanol as solvent was registeredin accession numbers IC582808 and IC582780. The lowest oleoresin recovery was recorded in accession numbers IC582791 and IC582804. According to Kavitha and Menon (2013), *K. galanga* has an oleoresin concentration ranging from 2.07 to 3.07 per cent. Similarly, oleoresin content in ginger genotypes was recorded as 8.55 per cent by Akshitha *et al*. (2020).

5.2.1.1 Oleoresin recovery in different solvents

The recovery percentage varied greatly among solvents like petroleum ether, benzene, methanol, and acetone. According to the findings, the percent recovery of oleoresin for petroleum ether, petroleum benzene, acetone, and methanol were 3.6, 4.32, 9.72, and 4.80 per cent respectively.

5.2.1.2 GCMSMS profile of *Alpinia galanga* volatile oil

A total of 38 compounds were identified in the volatile oil of *Alpinia galanga*. The most abundant compounds in the volatile oil were eucalyptol (44.45%), cis-á-farnesene (22.81%), trans-isoeugenol (3.77%), pentadecane (3.44%), camphor (2.50%), 1S)- (+)-2-boranone(2.50%), farnesol acetate (2.42%), and à-pinene (2.42%).

Lakshmi *et al*. (2011) reported 16 compounds in the volatile oil with a high concentrationof zerumbone (44.8%) in the Sri Lankan-grown *Alpinia galanga* rhizome. Yang *et al*. (2005)reported eucalyptol (22.63%), (1S)-(1)-pinene (14.36%), α -terpineol (8.59%), and L(-)-borneol (8.41%) as the major compounds in the rhizome oil of *Alpinia galanga.*

Suresh *et al*. (2016) identified major constituents in *Alpinia calcarata* rhizome oil using gas chromatography-mass spectrometry. Major compounds were cubenol (15.0%), 1,8-cineole(12.1%), and 1,8-fenchyl acetates (9.7%).

5.2.2 Biochemical parameters of *Alpinia galanga* genotypes

a. Flavonoids

Because of their antioxidant, anti-aging, and numerous other potential biologicalactions, flavonoids are frequently recognized as interesting substances that can be used in theproduction of cosmetic or cosmeceutical products (Tungmunnithum *et al*., 2020). The flavonoid content in the methanolic extract of *Alpinia galanga* rhizomes ranged from 25.66 mg/g to 54.99 mg/g. The highest flavonoid content was recorded in accession IC582808, whereas, the lowest flavonoid content was recorded in accession IC582791(Fig 5.11A). According to Malik *et al*. (2016), the total flavonoid content in the methanolic extract of rhizomes is 51.76 ± 1.26. The total flavonoid content in *Alpinia galanga* rhizome extract was32.26± 3.12(µg C.E./mg), as reported by Kambar *et al*. (2014).

b. Phenols

Phenolics are one of the abundant classes of secondary metabolites found in plants. These classes of substances have the potential to act as natural antioxidants because of their capacity to scavenge free radicals (Singh *et al*., 2020). The phenol content among theaccessions ranged from 30.04 mg/g to 43.16 mg/g. IC582817 (43.16 mg/g) contains the highestphenol content, and the lowest phenol content was found in IC582804 (30.04mg/g) presented in Figure 5.11A. Malik *et al*. (2016) reported the total phenol content in the methanol, ethanol,and water extracts ranged from 37.12 ± 0.39, 49.42 ± 0.38, and 21.77 ± 0.73 mg/g GAE, respectively. Aljiobair (2022) found significant amount of total phenolic content (53.18 mg GAE/g) in *Alpinia galanga* rhizome extract.

c. Amino acids

Several amino acids can function as building blocks for the synthesis of signaling molecules and secondary metabolites in plants. Significant variation was observed among thegenotypes for amino acid content, which ranged from 4.01 mg/g to 8.45 mg/g. The highest amino acid content was found in IC582808, and the lowest

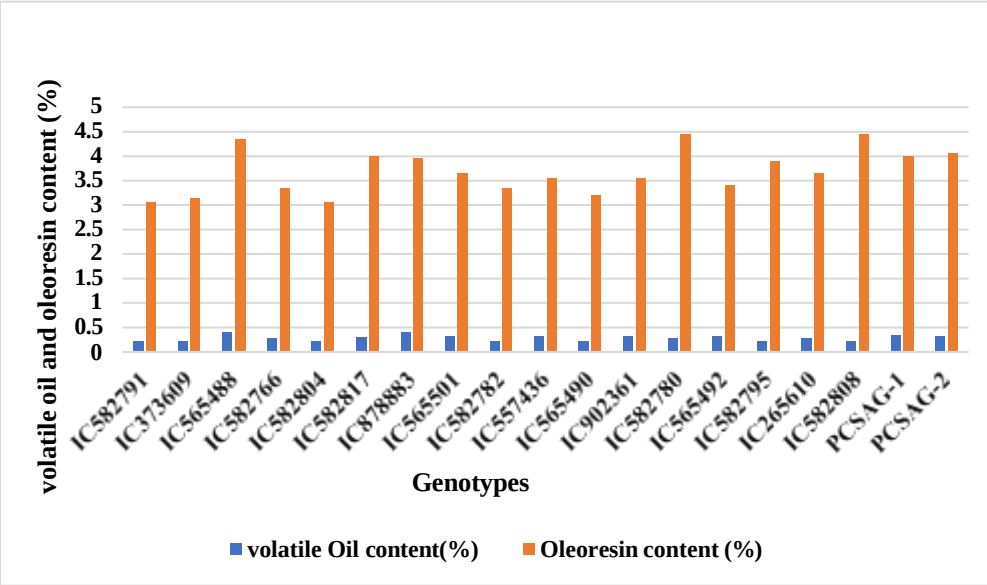


Fig. 5.10A: Volatile oil and oleoresin recovery in *Alpinia galanga* genotypes

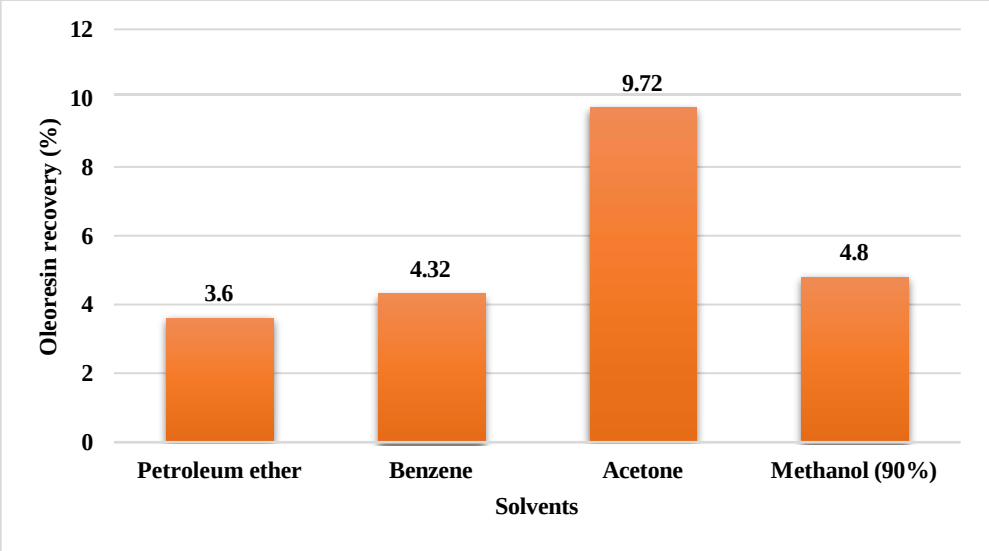


Fig. 5.10B: Oleoresin extractability percentage using different solvents

amino acid content was found in IC37360 (Fig 5.11B). According to Ponmozhi and Kalaiselvi's (2011) report, *Alpinia galanga* had the highest concentrations of protein and amino acids (380.9 ± 0.56 mg/g, 12.1 ± 0.40 mg/g, respectively).

d. Starch

The primary component of rhizomes is starch. The starch content in *Alpinia galanga* rhizomes ranged from 80.83 mg/g to 92.89 mg/g. IC582808 recorded the highest starch content, followed by IC878883, and the lowest starch content was recorded in IC582791(Fig 5.11B). According to Ponmozhi and Kalaiselvi (2011), the starch content ranged from 11. 5 ±0.26 mg/g, to 93.1 ± 0.11 mg/g, in the rhizomes. Tejavathi *et al*. (2020) reported the 76.4 ± 0.3per cent starch in *Curcuma karnatakensis.*

5.3 INVITRO ANTIMICROBIAL STUDIES IN *ALPINIA GALANGA*

Various fungicides are being used for controlling most of the fungal pathogens in order tomanage diseases, it may result in the residual effect of those chemicals on harvested products.To avoid this residual effect, natural products like plant extracts have been extensively studiedto know the inhibitory of the plant extract on mycelial growth. Earlier studies showed the inhibitory activity of ethanolic extract of *Alpinia galanga* rhizome on various food pathogens. Because the rhizome extract is a source of secondary metabolites like phenolics, polyphenols, flavonoids, saponins, phenylpropanoids, glycosides, diarylheptanoids, sesquiterpenes, and diterpenes. These secondary metabolites with fungicidal properties showed biological activityagainst phytopathogenic fungi, which makes them an effective control measure. Since they biodegrade to non-toxic compounds, they could be used as biopesticides in integrated pest management programs. In the current experiment, ethanolic extract of different accession of *Alpinia galanga* rhizome was tested against major fungal pathogens to determine the percent inhibition of mycelial growth. As per the results, the ethanolic extract at different concentrations significantly controlled the mycelium growth of major fungi.

5.3.1 *In vitro* evaluation of an ethanolic extract of *Alpinia galanga* rhizome against *Rhizoctonia* spp.

In the experiment, rhizome extract from 19 genotypes considerably inhibited the mycelialgrowth in PDA media that had been poisoned with ethanolic extract of rhizome at three different concentrations (2%, 5%, and 10%) (Fig 5.12).

At 2 per cent concentration, IC582780 showed the highest inhibition of 44.17 per cent. No inhibition was recorded in the accessions IC582791, IC582766, and IC582782 at 2 per cent concentration. At 5 per cent concentration, per cent of inhibition ranges from 39.17 per centto 76.25 per cent. Accession IC582808 showed the highest inhibition IC582804 had the lowest percentage of mycelial inhibition. Out of 19 accessions, 12 showed complete reduction in mycelial growth at a 10 per cent concentration, and IC582782 showed an 86.67 per cent inhibition, which was the lowest percentage of inhibition. At all three concentrations,accession IC582808 showed the highest percentage of inhibition (43.33%, 76.25%, and 100%). Followed by IC265610 (42.08 %, 69.17 %, and 88.33 %) showed an on par result with IC582808. Similar results with respect to per cent inhibition of *Rhizoctonia solani* by using ethanolic extracts of different medicinally important crops were reported by Al-Baldawy *et al*. (2021), San and Matsumoto (2011), and Sifat & Monjil (2017). Al-Askar and Rashad (2010) observed complete inhibition of *Rhizoctonia solani* when treated with 1 per cent, 2per cent, and 4 per cent ethanolic extracts of clove.

5.3.2 *In vitro* evaluation of an ethanolic extract of *Alpinia galanga* rhizome against *Colletotrichum* spp.

Significant difference was observed for per cent inhibition of mycelial growth by an ethanolic extract of *Alpinia galanga* genotypes at three different concentrations (Fig 5.13). Thehighest percent of inhibition was found in IC582808 (23.33%) at 2 per cent concentration. Whereas, the accession IC557436 (2.92%) showed lowest per cent inhibition. Inhibition at 5 per cent concentration ranged from 19.58 to 45.42 per cent. The highest level of inhibition wasseen in accession IC582780. Accession IC582766 had the lowest percentage of mycelial inhibition. Whereas IC582808 showed the maximum per cent inhibition at 10 per cent concentration, measuring about 65.42 per

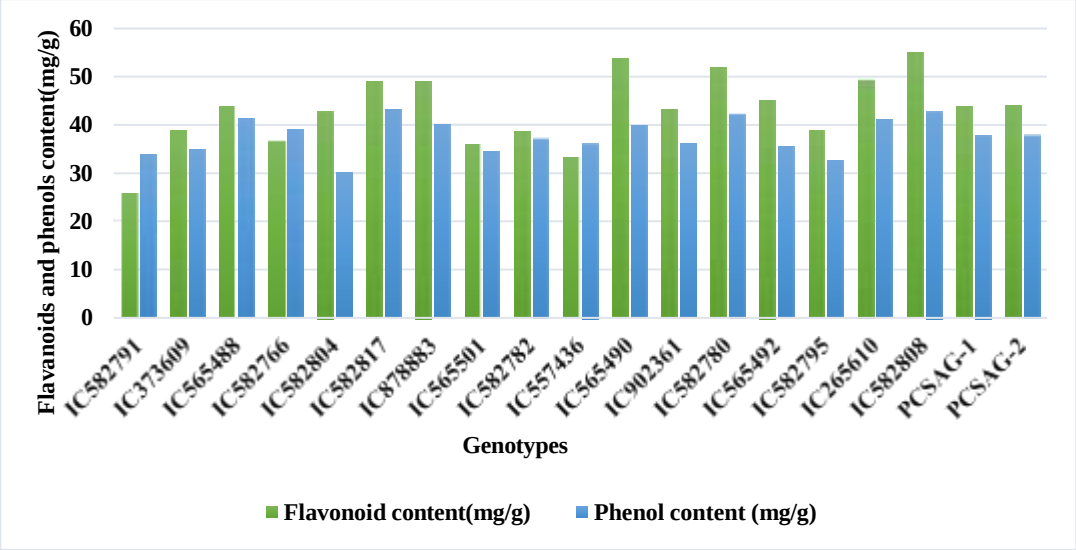


Fig. 5.11A: Flavonoid and phenol content in *Alpina galanga* genotypes

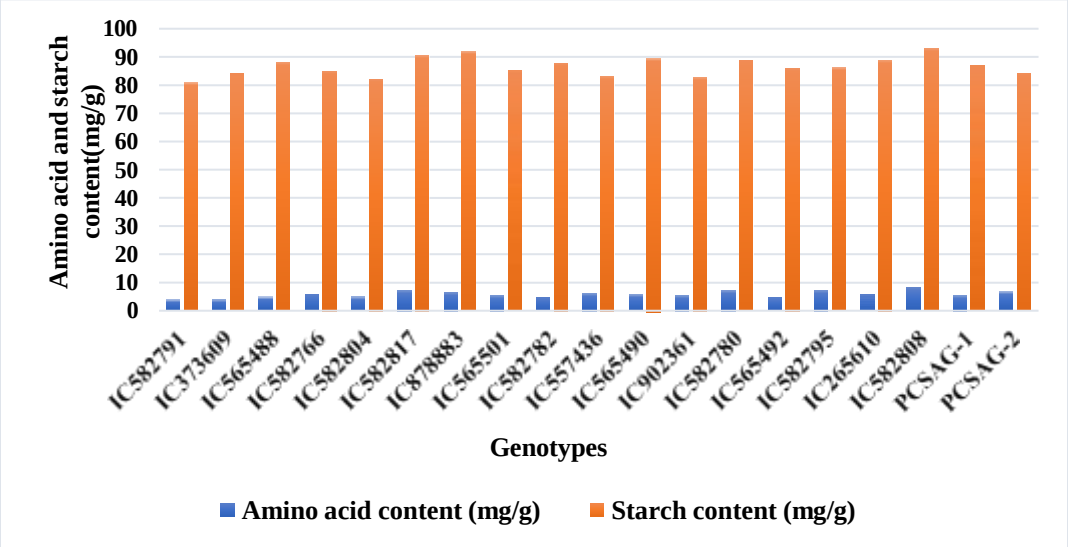


Fig. 5.11B: Amino acid and starch content in *Alpina galanga* genotypes

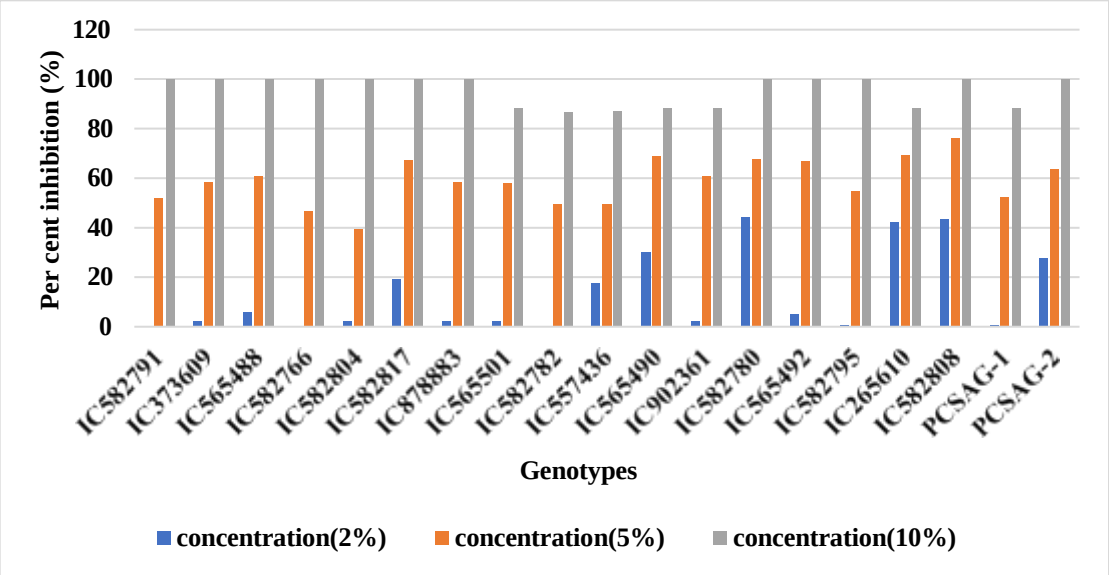


Fig. 5.12: Per cent inhibition of *Rhizoctonia spp*. by ethanolic extract of *Alpinia galanga* genotypes

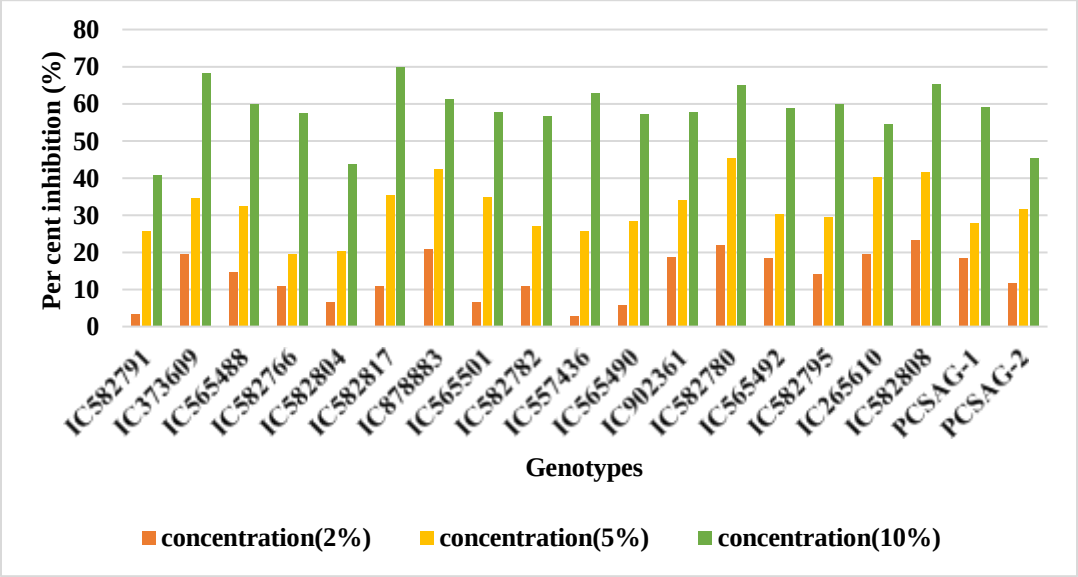


Fig. 5.13: Per cent inhibition of *Colletotrichum spp*. by ethanolic extract of *Alpinia galanga* genotypes

cent. Accession IC582791 showed the least inhibition (40.83%). As per the results at 2, 5, and 10 per cent concentrations, accession IC582780 (22.08%, 45.42%, and 65.00%) showed the highest percentage of inhibition, while IC582808 (23.33%, 41.67%, and 65.42%) exhibited a result that was comparable to IC582780. Similar results with respect to the significant inhibition of *Colletotrichum* spp. by ethanolic extract of galangal rhizomes were reported by Sanit (2020). A study by Hayeeyusoh *et al*. (2015) also showed results congruence with the effectiveness of an ethanol extract of *Alpinia galanga* rhizome against *Colletotrichum* spp.

5.2.3 *In vitro* evaluation of an ethanolic extract of *Alpinia galanga* rhizome against *Phytophthora* spp.

Results of the present study indicated that, the highest per cent of mycelial inhibition was reported in accession IC265610 (29.58%) at 2 per cent concentration. Whereas, the accessionIC582791(3.33%) showed lowest per cent inhibition. Similarly, inhibition percentages at 5 per cent concentration ranged from 27.08 to 47.92 per cent. The highest inhibition of mycelial was reported by accession IC582808, while the lowest percentage was recorded in IC557436. Accession IC265610 and IC565488 showed 69.58 per cent at 10 per cent concentration which was the highest percent inhibition, IC582804 showed the least per cent of inhibition (50.42%). By considering overall results, accession IC265610 (29.58%, 44.17%, 69.58%) exhibited the highest percentage of mycelial inhibition at all three tested concentrations (2%, 5%, and 10%), and IC565488 (24.58%, 44.17%, and 69.58%) showed values comparable to those of IC265610 (Fig 5.14). Per cent inhibition was noticed in all the concentrations of ethanolic extract of *Alpinia galanga,* the results were in agreement with the observations of Suprapta *et al*. (2006). According to Mugao *et al*. (2020), the ethanolic extract of ginger showed a similar result for *Phytophthora* spp. inhibition at different concentrations.

5.3.4 *In vitro* evaluation of an ethanolic extract of *Alpinia galanga* rhizome against *Fusarium* spp.

In vitro evaluation of ethanolic extracts of *Alpinia galanga* rhizomes showed a significantreduction in mycelial growth against *Fusarium* spp. (Fig 5.15). Accession

IC582808 (15.83%)was found to have the highest percent inhibition at 2 per cent concentration. PCSAG-2 (2.92%), on the other hand, showed lowest percentage of inhibition. Inhibition percentages at5 per cent concentration ranged from 16.67 to 47.08 per cent. The greatest inhibition was recorded in accessions IC582808, and IC565490 (16.67%) had the lowest percentage of mycelial inhibition. In addition, the percentage of inhibition at 10 per cent concentration rangedfrom 8.33 to 61.67 per cent. The maximum percentage of inhibition was seen in accession IC582808, and the lowest percentage of inhibition was seen in IC565490. These observationsare in agreement with the observations of Sanit (2016) and Molika *et al*. (2020). Win *et al*. (2022). Reported that among the tested concentration, complete inhibition of the growth of *Fusarium* spp. was observed at 10 per cent concentration.

5.3.5 *In vitro* evaluation *Alpinia galanga rhizome* extract against *Ralstonia solanacearum*

To assess the antibacterial activity of *Alpinia galanga*, the extract was evaluated against *Ralstonia solanacearum* at three distinct concentrations, namely 2, 5, and 10 per cent (lower, recommended, and higher concentrations). As per the observation, no clear zone of inhibition was observed in all the above-mentioned concentrations. Previous research has also revealed minimum inhibition percentages of ethanolic extract of rhizome for various gram-positive andgram-negative bacteria. According to Avci *et al*. (2020), the galangal extracts had a weak antimicrobial effect on *E. coli.* Mayachiew and Devahastin (2007) also stated that galangal extract was found to have minimum biocidal concentrations (MBC) of 2.34 mg/ml and minimum inhibitory concentrations (MIC) of 0.78 mg/ml on *Staphylococcus aureus.*

5.4 Correlation analysis

Morphological parameters of *Alpinia galanga* genotypes were subjected to correlation analysis. The correlation coefficient was examined for the correlation of rhizome yield with the other morphological characters. As yield is a complex character that is linked with numerous yield-contributing characters. The results revealed that yield per tiller had significantly positive correlation with number of tillers and leaves per plant, leaf length, plantgirth and height, length, core diameter, and weight of mother,

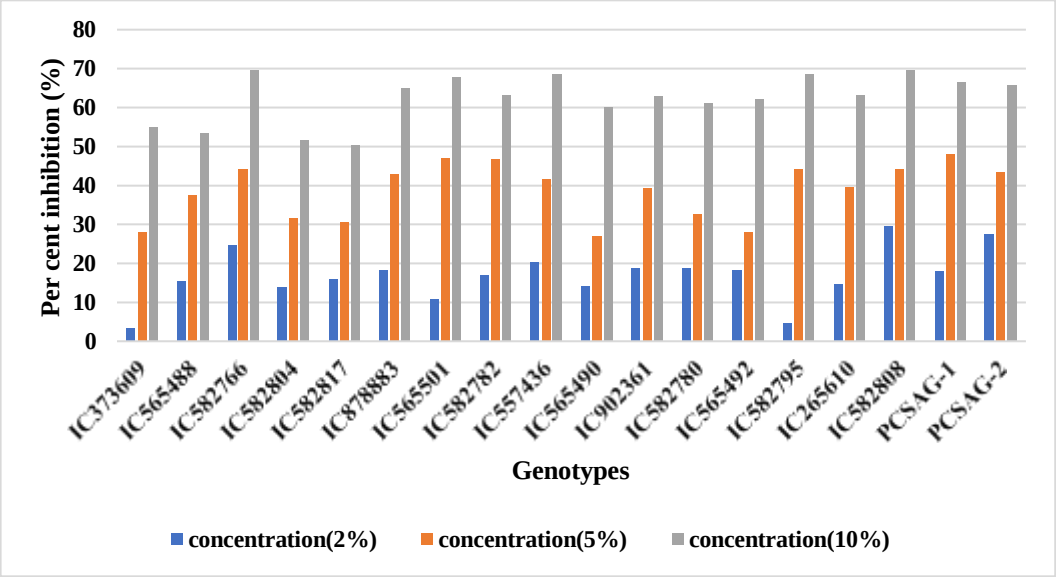


Fig. 5.14: Per cent inhibition of *Phytophthora* spp. by ethanolic extract of *Alpinia galanga* genotypes

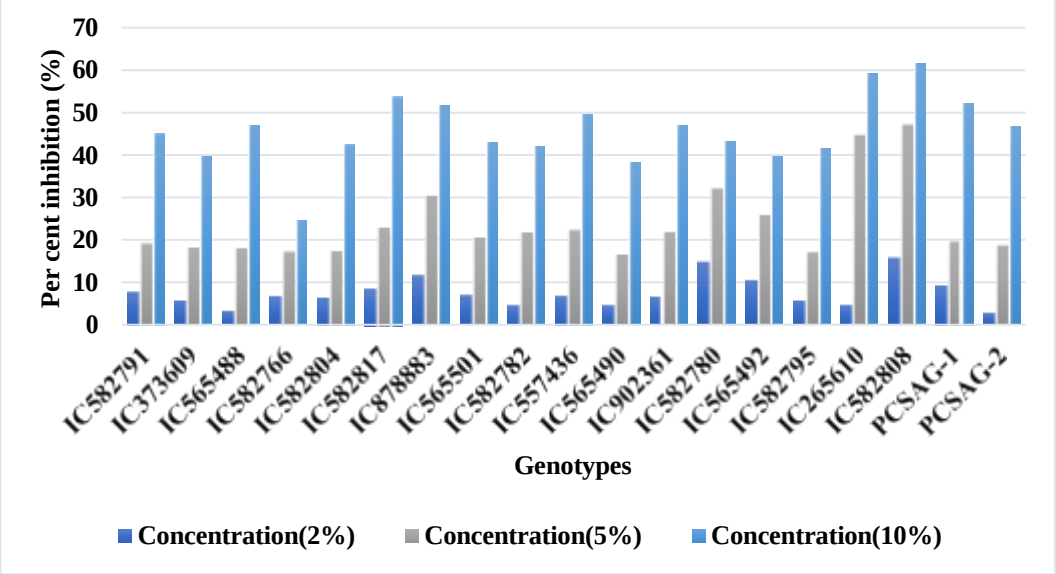


Fig. 5.15: Per cent inhibition of *Fusarium* spp. by ethanolic extract of *Alpinia galanga* genotypes

primary and secondary rhizomes. Fresh rhizome yield per tiller was positively and significantly correlated with plant height, tiller diameter, internodal length, number of leaves per tiller, leaf length, leaf width, leaf area,number of branches per panicle, and number of flowers per panicle. Also significant and positive correlation was observed between dry recovery of rhizome with the number of tillersper plant, internodal length, length of flower, and width of the flower. These characters directlyinfluenced fresh rhizome yield per tiller and dry recovery of the rhizome. As a result, direct selection for these characters would be more effective in improving rhizome yield per hectare. Ravi *et al*. (2017) reported a significant positive correlation of fresh rhizome yield with plant height, number of tillers per plant, leaf area, stem girth, and number of leaves per plant, in ginger genotypes. These results are in agreement with the findings of Prajapati *et al*., (2014), and Rajyalakshmi *et al*., (2013) on turmeric. Similar associations of yield parameters with morphological traits were reported by Basak *et al*. (2019), and also by Dev and Sharma (2020)in ginger.

Meanwhile, to unravel the direct and indirect effects of phytoconstituents with the antifungal property, the correlation coefficient was examined for biochemical parameters like oleoresin, flavonoid, phenol, amino acid, and starch content with percentage inhibition of mycelial growth of *Rhizoctonia* spp., *Colletotrichum* spp., *Phytophthora* spp., and *Fusarium* spp. The results show the content of all phytoconstituents are significantly and positively correlated with each other. All phytoconstituent had significant and positive correlation with the per cent inhibition of *Rhizoctonia* spp., *Colletotrichum* spp., and *Fusarium* spp. High phenolics and flavonoids are responsible for antifungal activity in *Alpinia galanga* (Sharma *etal*., 2015). Various bioactive compounds in oleoresin are responsible for antimicrobial activityby Figueroa-Lopez (2018). According to recent findings, medicinal plants are rich in bioactivesecondary metabolites such as saponins, alkaloids, and terpenoids, all of which have antifungalproperties Sukrasno (2017). The bioactive compounds present in the extract of rhizome mustbe responsible for antimicrobial activity in *Alpinia galanga.*

SUMMARY

6. SUMMARY

The present investigation entitled "Variability in greater galangal (*Alpinia galanga* L.) Wild. genotypes for yield and quality" was carried out at the Department of Plantation, Spices,Medicinal & Aromatic crops, College of Agriculture, Vellanikkara, Kerala AgriculturalUniversity, Thrissur during 2020-2022. The antimicrobial studies were carried out at the Department of Plant Pathology, College of Agriculture, Vellanikkara, Kerala Agricultural University, Thrissur. The study aimed to evaluate the genotypes of greater galangal for yield,quality, and anti-microbial properties. The key findings of the study are summarized here.

6.1 **Morphological evaluation of *Alpinia galanga* genotypes**

Seventeen *Alpinia galanga* genotypes maintained at the field gene bank of NBPGR Regional Station, Thrissur, and two maintained at the Plantation and Spice farm, College of Agriculture, Vellanikkara, were evaluated in the study.

The qualitative traits observed in the current investigation were similar in all nineteen genotypes. The rhizome's skin color was found to be greyed yellow and yellowish white in the core region. The leaves were lanceolate to oblong in form, with a cuneate base and acuminate tip. The plant flowers in two seasons from May to July and August to October. Thepetal color was determined to be white with pink marking near midrib. The seed color was noted to be dark brown to black.

Wide variability was observed for the quantitative morphological characteristics among the genotypes. The plant height in *Alpinia galanga* followed an upward trend from vegetativeto reproductive stage with a mean of 220.32 cm. Significantly highest plant height was recorded in accession PCSAG-1 (278.60cm) followed by IC582817(259.90cm), and theshortest was IC565490 (172.30cm).

There was a significant increase in the tiller diameter during the observed months with a mean of 4.43 cm. Accession IC565492, IC582780, and IC582795 (4.67 cm) recorded the highest tiller diameter whereas the lowest diameter was recorded in the accession IC402361 (4.15 cm).

The tiller production was highest during November and December in all the genotypes. Accession IC582817 produced a significantly greater number of tillers (46) which was on parwith PCSAG-1 (44).

Significant variation observed for internodal length among the genotypes, the highest internodal length was recorded in PCSAG-2, followed by PCSAG-1.

The number of leaves per tiller ranged from 7.4 to 13.50 among the genotypes The highestnumber of leaves per tiller was recorded in the accession numbers IC582782 and IC373609 while IC565501 had the fewest.

Among the genotypes, IC265610 (54.81 cm) and IC565490 (39.75 cm) recorded the highest and lowest leaf lengths. Accession IC565501 had the greatest leaf width and leaf area(10.35 cm and 391.02 m2, respectively). Accession IC557436 (6.75 cm and 172.78 m2) recorded lowest leaf width and area in all observed months.

The petiole length varied significantly among accessions, although it does not altersignificantly over the course of the observational months. The longest and shortest petiole lengths were recorded in accession IC878883 (0.82 cm), and IC582808 (0.66 cm) respectively.

While studying the flowering phenology, using an expanded BBCH (*Biologische Bundesanstalt, Bundessortenamt, and CHemiscle Industry)* scale, four major growth stages were found and described in *Alpinia galanga*. The principal growth stage 5 (formation of reproductive buds) lasted for 10–12 days, the principal growth stage 6 (flowering) for 20–25 days, and the principal growth stage 7 (development of fruits) took 90–100 days from the timeof fruit set to maturity. From maturation to full ripening, primary growth stage 8 took 80–85 days.

A significant variation was observed for flower characters in all nineteen accessions. The highest panicle length was recorded in IC402361 (26.60 cm), and the shortest was recorded inaccession IC557436 (11.17 cm). The number of branches and flowers per panicle was recordedhighest in IC565488, whereas the lowest was recorded in IC582791. Accession IC582817 (4.10 cm) and IC565501 (3.10 cm) recorded the highest and lowest flower length and widths.

Significant differences were noticed for rhizome characters among the genotypes. Accession IC582817 (170.00 g) recorded the highest rhizome weight, whereas, IC5655490 (43.00g) yielded the lowest rhizome weight. The means of rhizome length and width are 6.31and 4.06 cm, respectively. IC582817 shows the highest rhizome length and width, wherein IC582808 recorded the lowest rhizome length and width. The dry rhizome recovery of the genotypes ranged from 20 to 25.43 per cent. The maximum dry recovery per cent was recordedin accession IC582780 and the lowest was recorded in accession, IC582782 (19.8%).

6.2 BIOCHEMICAL EVALUATION

Significant variation was noticed for volatile oil content among the genotypes, ranging from 0.21 to 0.41 per cent. Accession IC878883 recorded the highest volatile oil, andIC582791, IC582795, IC373609, and IC582804 recorded the lowest (0.21%) oil content. Oleoresin recovery was found to be more in acetone compared to other solvents. The highestoleoresin recovery was registered in accession numbers IC582808 and IC582780(4.45%.), whereas IC582791 and IC582804 (3.05%) recorded the lowest. The oil profiling through GCMSMS, detected 38 major and minor compounds in rhizome oil. The major compounds inthe volatile oil were eucalyptol, cis-á-farnesene, trans-isoeugenol, pentadecane, camphor, 1S)-(+)-2-boranone, farnesol acetate, and à-pinene.

The flavonoid content in the methanolic extract of *Alpinia galanga* rhizomes ranged from 25.66 mg/g to 54.99 mg/g. The highest flavonoid content was recorded in accession IC582808, whereas, the lowest flavonoid content was recorded in accession IC582791. Accession IC582817 (43.16 mg/g) contains the highest phenol content, and the lowest phenol content wasfound in IC582804 (30.04 mg/g). A significant variation was observed among the genotypes foramino acid content, which ranged from 4.01 mg/g to 8.45 mg/g. The highest amino acid contentwas found in IC582808, and the lowest amino acid content was found in IC37360. AccessionIC582808 (92.89 mg/g) recorded the highest starch content and the lowest starch content wasrecorded in IC582791(80.83 mg/g).

6.3 IN VITRO ANTIMICROBIAL ACTIVITY IN *ALPINIA GALANGA*

The ethanolic extract of the rhizome at three different concentrations (2%, 5%, and 10%) was screened against *Ralstonia solanaceraum, Rhizoctonia* spp., *Phytophthora* spp., *Colletotrichum* spp. and *Fusarium* spp. under *in vitro* conditions.

6.3.1 *In vitro* evaluation of ethanolic extract of *Alpinia galanga* rhizome against *Rhizoctonia* spp.

At 2 per cent concentration, Accession IC582780 (44.17%) showed the highest inhibitionand no inhibition was recorded in the accessions IC582791, IC582766, and IC582782 at 2 percent concentration. At 5 per cent concentration accession IC582808 (76.25 %) showed the highest inhibition, whereas IC582804 (39.17 %) recorded the lowest per cent of mycelial inhibition. Out of 19 accessions, 12 accessions showed complete inhibition of mycelial growth and IC582782 showed 86.67 per cent that recorded the lowest per cent inhibition at 10 per centconcentration

6.3.2 *In vitro* evaluation of ethanolic extract of *Alpinia galanga* rhizome against *Colletotrichum* spp.

At 2 per cent concentration, accession IC582808 (23.33 %) recorded the highest per cent inhibition, whereas the lowest per cent of inhibition was recorded in the accession IC557436 (2.92 %). At 5 per cent concentration, per cent of inhibition ranged from 19.58 per cent to 45.42per cent. Accession IC582780 (45.42 %) showed the highest inhibition, whereas IC582766 (19.58 %) recorded the lowest per cent of mycelial inhibition. At 10 per cent concentration, accession IC582808 showed (65.42 %) the highest per cent inhibition followed by IC582780 (65.00 %). The lowest per cent of inhibition was observed in IC582791 (40.83 %).

6.3.3 *In vitro* evaluation of an ethanolic extract of *Alpinia galanga* rhizome against *Phytophthora* spp.

At 2 per cent concentration, accession IC265610 (29.58 %) recorded the highest per cent inhibition, whereas the lowest per cent of inhibition was recorded in the accession IC582791(3.33 %). At 5 per cent concentration, per cent of inhibition ranged from 27.08 per cent to 47.92 per cent.

Accession IC582808 showed the highest inhibition, whereas IC557436 recorded the lowest per cent mycelial inhibition. At 10 per cent concentration, accession IC265610 and IC565488 showed 69.58 per cent highest per cent inhibition. The lowest per cent of inhibitionwas observed in IC582804 (50.42 %).

6.3.4 *In vitro* evaluation of ethanolic extract of *Alpinia galanga* rhizome against *Fusarium* spp.

The data indicated that at 2 per cent concentration accession IC582808(15.83%) was recorded as the highest per cent inhibition, whereas lowest per cent of inhibition was recordedin the accession PCSAG-2(2.92%). At 5 per cent concentration, per cent of inhibition ranges from 16.67 per cent to 47.08 per cent. Accession IC582808 showed the highest inhibition and,IC565490 recorded the lowest per cent mycelial inhibition. At ten per cent of extract concentration accession, IC582808 showed 61.67 % inhibition. the lowest per cent of inhibition was observed in IC582766 (24.58 %).

6.3.5 *In vitro* evaluation *Alpinia galanga* rhizome extract against *Ralstonia solanacearum*

The antibacterial activity of *Alpinia galanga*, the extract was evaluated against *Ralstonia solanacearum* at three distinct concentrations, namely 2, 5, and 10 per cent (lower, recommended, and higher concentrations). As per the observation, no clear zone of inhibitionwas observed in all the above-mentioned concentrations.

6.4 Correlation analysis for yield and quality parameters

Fresh rhizome yield per tiller had a positive and significant correlation with plant height, tiller diameter, internodal length, number of leaves per tiller, leaf length, leaf width, leaf area, number of branches per panicle, and number of flowers per panicle. In the same way,phytoconstituent content was significantly and positively correlated with antimicrobial activity.

The selection parameters for *Alpinia galanga* genotypes was worked out by consideringyield parameters, quality parameters and antimicrobial activity of rhizome extract. The accessions IC878883, IC582780, IC582817, IC265610, IC582808 and PCSAG-1 were identified as the best genotypes for undertaking advanced trail.

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APPENDIX

APPENDIX 1

Meteorological data during the period of observation from January 2022 to September 2022

2022	Max Temperature (^{0}C)	Highest Max Temperature (^{0}C)	Min Temperature (^{0}C)	Lowest Max Temperature (^{0}C)	Mean RH %	Rainfall (mm)	Rainy days	Total evaporation (mm)	Mean evaporation (mm/day)	Total sunshine (hrs)	Mean sunshine (hrs/day)
January	33.3	35	22.6	18.9	64	0.0	0	132.2	4.3	281.2	9.1
February	34.8	36.2	23.3	19.5	58	0.0	0	141.5	5.1	231.2	8.3
March	36.1	38.6	24.7	22.4	74	1.7	0	155.7	5.0	215.4	6.9
April	34.2	36.9	25.1	20.5	77	84.3	7	96.1	3.2	175.5	5.9
May	31.1	35.2	24	21.7	85	422.0	23	84.0	2.7	91.8	3.0
June	31.3	32.8	23.6	22.0	84	391.8	19	94.7	3.2	136.1	4.5
July	29.3	33.5	23.5	21.5	88	628.8	21	69.1	2.2	54.6	1.8
August	29.9	32.6	23.6	22.2	84	563.7	15	89.2	2.9	128.9	4.3
September	31.1	33.3	23.7	22.9	81	167.5	12	93.3	3.1	161.8	5.4
October	32.0	33.8	23.6	21.6	77	69.6	9	96.6	3.1	184.4	5.9

VARIABILITY IN GREATER GALANGAL (*ALPINIA GALANGA* (L.) WILD. GENOTYPES FOR YIELD AND QUALITY

By

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ABSTRACT

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ABSTRACT

Greater galangal *(Alpinia galanga* (L.) Wild, popularly known as *Kulanjan* in India belongs to the family Zingiberaceae. The genus Alpinia contains 230 to 250 species spread across tropical and subtropical regions and commonly found in Egypt, Malaysia, India, Sri Lanka, Indonesia, China, and the Gulf. In South India it is sparsely distributed in western ghats. Galangal is an important rhizomatousplant broadly used as condiment for flavoring and in traditional system of medicine for stomachache, cold, inflammation, as carminative and for treating diarrhea. Currently, the plant is categorized as an endangered and threatened medicinal crop due to overexploitation and improper harvesting methods. Kerela is a state having no largescale cultivation of *Alpinia galanga*. It is essential to evaluate the accessions for yield, quality that would result in selecting suitable genotypes for commercial cultivation.In this context, the present study was undertaken with the objective of evaluating genotypes of greater galangal *(Alpinia galanga* (L.) Wild. for yield, quality, and anti-microbial properties. The experiment was carried out in the Department of Plantation, Spices, Medicinal & Aromatic crops, College of Agriculture, Vellanikkara. Seventeen *Alpinia galanga* genotypes maintained at the field gene bank of NBPGR Regional Station, Thrissur, and two genotypes at Plantation and Spice farm, College of Agriculture, Vellanikkara, were utilized for the study.

The morphological evaluation was done from November to February, for quantitative parameters. Flower and rhizome parameters were observed during the peak season of flowering and after harvesting of rhizomes respectively. While evaluating morphological characters, no significant variation was observed for the qualitative traits like rhizome color, shape, leaf shape, and flower petalcolor, among the genotypes, except for the season of flowering. Out of 19 accessions, nine accessions(IC373609, IC565488, IC582766, IC582817, IC557436, IC565492, IC582780, PCSAG-1, and PCSAG-2) showed early flowering from May-July. In the rest of the genotypes flowering was late from August to October. Wide variability was observed for the quantitative morphological characteristics among the genotypes. Accessions like PCSAG-1, PCSAG-2, IC582817, IC878883, IC582766, IC565488, and IC265610

recorded comparatively higher values for plant length, tiller diameter, number of tillers per plant, number of leaves per tiller, leaf length and width and inflorescence characters.

Significant variation was observed among the accessions for rhizome weight and drying recovery ranging from 43.00g to 170.00g and 20 to 25.43 per cent respectively. For yield parameters, accessions IC878883, IC582766, IC582780, IC582817, IC265610 performed well and these can be carried forward for advanced yield trials.

For studying the relation between rhizome yield with other morphological characters correlation coefficient was estimated. Fresh rhizome yield per tiller had a positive and significant correlation with plant height, tiller diameter, internodal length, number of leaves per tiller, leaf length,leaf width, leaf area, number of branches per panicle, and number of flowers per panicle.

While studying the flowering phenology, four principal growth stages were identified and described using an extended BBCH (*Biologische Bundesanstalt, Bundessortenamt, and CHemiscle Industry)* scale. The principal growth stage 5 (reproductive bud development) lasted for 10–12 days, the principal growth stage 6 (flowering) lasted for 20–25 days, and the principal growth stage 7 (fruit development) took 90-100 days from fruit set to ripening. Principal growth stage 8 (from maturation to complete ripening) took 80–85 days. Floral morphology along with two distinct floral morphs that exhibited different flowering behaviors (Anaflexystyly and Cataflexystyly) in *Alpinia galanga* were also described in the study.

In biochemical studies, the volatile oil content of the rhizome ranged from 0.21 to 0.41 per cent.The oil profiling through GCMSMS, detected 38 major and minor compounds in rhizome oil. Major compounds identified were Eucalyptol (44.45 %) and cis-á-Farnesene (22.89 %). The oleoresin contentwas to the tune of 3.05 to 4.45 per cent in methanol solvent and highest oleoresin recovery was obtainedwith acetone compared to other solvents. The accession IC582817, IC878883, IC565490, IC582780, IC265610 and IC582808 recorded higher values for the content of flavonoids, phenols, amino acids and starch.

To study the antimicrobial activity, ethanolic extract of the rhizome of 19 accessions at three different concentrations (2%, 5% and 10%) was screened against *Ralstonia solanaceraum*, *Rhizoctonia*spp., *Phytophthora* spp., *Colletotrichum* spp. and *Fusarium* spp. under *in vitro* conditions. Theethanolic rhizome extract of *Alpinia galanga* exhibited potent antifungal activity on *Rhizoctonia* spp. Out of 19 accessions, 12 accessions showed complete inhibition (100%) of mycelial growth at 10 per cent concentration. Appreciable antifungal activity also noticed on *Colletotrichum* spp. (22.08 %, 45.42%, 65.00 %), *Phytophthora* spp. (29.58 %, 44.17 %, and 69.58%), and *Fusarium* spp. (15.83 %, 47.08%, and 61.67 %) at all three concentrations. Accessions IC565488, IC582808, IC265610, and IC582780 showed the highest per cent of inhibition on the mycelial growth of major fungi. Antibacterial activitywas found to be less in ethanolic extract of rhizome at the studied concentrations for *Ralstonia solanaceraum.*

The selection parameters for *Alpinia galanga* genotypes was worked out by considering yield parameters, quality parameters and antimicrobial activity of rhizome extract. The accessions IC878883, IC582780, IC582817, IC265610, IC582808 and PCSAG-1 were identified as the best genotypes for undertaking advanced trail.