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Editorial message

Dear Readers, Authors, and Colleagues,

It is with great pleasure that I welcome you to the third volume of the third issue of the *Journal of Agriculture & Forestry Research*. This issue reflects our ongoing commitment to fostering innovative research and promoting scientific knowledge in the fields of Agriculture and Forestry.

As we continue to explore and promote sustainable solutions, the journal remains dedicated to publishing research that not only advances scientific understanding but also has practical implications for farmers, foresters, and policymakers alike.

We extend our heartfelt gratitude to all the authors, reviewers, and editorial board members who have contributed to the success of this issue. Your dedication and expertise are the backbone of this journal, and we appreciate your commitment to maintaining high standards of scholarly excellence.

Thank you for your continued support, and we look forward to presenting more innovative research in the coming issues.

Sincerely,
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Research Article Open access

Screening of Effective Formulants for *Fusarium* sp. FGCCW#16 Cell free Culture broth in Controlling *Parthenium hysterophorus*

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ABSTRACT
Natural herbicidal compound from *Fusarium* sp. FGCCW#16 cell free culture broth was used as a mycoherbicide product for the control of *Parthenium hysterophorus* a weed that causes considerable problem to the biodiversity, agriculture, economy, and health of livestock and human beings. Formulations containing distilled water or culture filtrate and different adjuvants (different oil like mineral oil, palm oil or soybean oil), surfactant like Glycerol and Tween 80) were evaluated in order to increase the bioherbicidal activity through post-emergence bioassays. The herbicidal activity of culture filtrate was improved using different combinations of adjuvants. The best formulation was 5% (w/v) of mineral oil, 5% (w/v) of surfactant (glycerol and Tween 80) and a hydrophilic-lipophilic balance (HLB), which resulted in a higher herbicidal activity (100%) (complete death of plants). The suitable combination of adjuvants in association with culture filtrate from *Fusarium* sp. FGCCW#16 increased up to 2.5 times the efficiency of bioherbicide for the post-emergence control of *Parthenium hysterophorus* weed.

Keywords: Noxious invasive weed; Parthenium; FGCCW#16; Mycoherbicide; Mass Production; Formulation

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1. INTRODUCTION

Parthenium hysterophorus, enlisted in Global Invasive Species database (Callway et al. 2004) Thus, nowadays it is found infesting almost all parts of the world. Its rapid and extensive spread can be attributed to both human activities during globalization and colonizing potential of the weed plant over wide range of habitats and climatic conditions. Parthenium weed is found in both natural and agroecosystems. It shows many adverse effects on agriculture, biodiversity, and health of animals and human beings. The control of Parthenium sp. is usually accomplished through the use of chemical herbicides such as glyphosate. However, over the years, some species, including Parthenium sp., become resistant to the mechanism of action of certain chemicals (Bracamonate et al. 2016). Such synthetic products, like glyphosate, effectively assist the farmer in achieving high productivity in short-term. However, in the long-term, they have negative results for the society and environment, thus requiring studies for using natural bioproducts (Bonny 2016; Confortin et al. 2019; Todero et al. 2019; Balesteros et al. 2014; Keerthi et al. 2019; Reichert et al. 2019). Taking into account this scenario, the prospection and discovery of new molecules appear as an important tool for the control of resistant weeds (Hassan et al. 2020). Some studies have shown promising results in the weed control

using fermented broth containing the secondary metabolites produced by fungi via submerged fermentation (Brun et al. 2016; de Souza et al. 2015; de Souza et al. 2017; Triolet et al. 2020; Kaur and Aggarwal 2015). Secondary metabolites can damage weeds by penetrating the plant followed by the destruction of the cell wall and induction of necrotic lesions (Kumar et al. 2017; Varejao et al. 2013).

On the other hand, many promising biomolecules are early discarded during the stages of mycoherbicide development because they present low herbicidal activity. In a general way, low efficiency is a consequence of the very low concentration of biomolecules in the fermentation media (Boyette et al. 2016). Therefore, some strategies to concentrate these molecules are essential to obtain an efficient product. The formulation is an important parameter for successful product development. The word formulation is described as a mixture of an active ingredient (a.i.) and the appropriate or compatible inert ingredients. Adjuvants are the most popular inert ingredients commonly found in bioherbicide formulations. Adjuvants are well known to improve the efficacy of bioherbicides by altering their physicochemical properties. Surfactants, emulsifiers, and hydrophilic polymers are examples of adjuvants important for improving the effectiveness of bioherbicides. Mycoherbicide compounds are generally applied to weeds in the form of an emulsion, which can increase weed control stability and effectiveness (Castro et al. 2013). The hydrophilic polymers are readily miscible with water; emulsifiers are employed to blend water (hydrophilic) and oil (hydrophobic) components in a formulation into a stable emulsion. Unfortunately, the cost of bioherbicides sometimes increases because of the high cost of the adjuvants used in the formulation. Additionally, a careful selection of surfactants must be taken into consideration, since certain ingredients used in herbicide formulations can be toxic to humans (Hazrati et al. 2017). The vegetable oil and surfactants increased the adsorption ability of polar molecules, dissolved cuticular fatty acids, and thereby improved the penetration of hydrophilic active substances (Lost and Raetano 2010).

In the meanwhile, it is necessary to use an adequate combination of adjuvants in the formulation to increase the herbicidal activity. Adjuvants are substances present in a formulation with the aim of modifying the biological activity or the application characteristics of the formulation (Mirgorodskaya et al. 2020; De Almedia et al. 2020; Bastos et al. 2017). This is highlighted in a study reported by Bastos et al. (2017), in which the authors increased three times the herbicidal activity of culture filtrate from *Diaphorte* sp. obtained by solid state fermentation using 5 wt % of palm oil , 5 wt % of surfants and HLB 15.0.

The genus *Fusarium* sp. has been widely used in the production of metabolites for weed control (Reveglia et al. 2018). The herbicidal activity of different species (*Fusarium avenaceum, F. acuminatum, F. redolens, F. culmorum*/*F. cerealis* and *F. solani*) was evaluated in different grass species, demonstrating promising results using *F. avenaceum* and *F. acuminatum* (Pearson et al. 2016). Previously, isolated and identified the fungus *Fusarium* sp. FGCCW#16 was obtained from Parthenium weed with herbicidal activity towards target plants (Singh and Pandey, 2019). Based on these aspects, the objective of this study was to evaluate the efficiency of using adjuvants in association with fermented broth produced by the fungus *Fusarium* sp. FGCCW#16 isolated from the Parthenium, for the control of *Parthenium* weeds.

2. MATERIALS AND METHODS

2.1. Materials:

Potato dextrose agar (PDA), (NH4)2SO4, FeSO4.7H2O, MnSO4.H2O, MgSO4, Tween 80, Corn steep liquor (CSL), Palm oil (*Elaeis guineensis*), Soybean oil, Sucrose, Mineral oil and Glycerol were used in experiment.

2.2. Fermentation:

Fusarium sp. FGCCW#16 was previously isolated by Singh and Pandey (2019). The culture was maintained in a petri dish with PDA medium between 4 °C and 6 °C and sub cultured every 15 days. For pre-inoculum cell production was done incubating the culture on PDA in a Petri dish for 8 days at 28 ºC. Afterward, three discs of 6 mm of fungal mycelium were transferred to the fermentation flasks. The fermentation was carried out in Erlenmeyer flasks containing 125 mL of fermentation medium under stirring in an Orbital Incubator Shaker at 31°C and 200 rpm for 7 days. The medium was composed of (g/L): Sucrose (10.0), corn steep liquor (50.0), (NH4)2SO4 (0.5), FeSO4.7H2O (1.0), MnSO4.H2O (1.0) and MgSO4 (0.5). The initial

pH was adjusted to 6.0 (Brun et al. 2016). After the fermentation, the cells were separated from the medium by centrifugation at 4000 rpm for 10 min and the supernatant was filtered using a 0.45µm Polyvinylidene Difluoride (PVDF) membrane. The filtered samples (culture filtrate) were used to formulate the mycoherbicide applied in the control of Parthenium weed.

2.3. Formulation of Mycoherbicide:

Initially, three different oils (palm, soybean, and mineral oil) in association with distilled water or culture filtrate were tested (Table 1). The objective of using oil in the formulation was to increase the adhesion and permanence of the fermented in the foliar area of the plants (Todero et al. 2018).

The formulations were prepared at 25ºC using a homogenizer. Firstly, oil (oil and glycerol) and aqueous (distilled water or culture filtrate and Tween 80) phases were homogenized separately for 1 min at 7000 rpm. Then, the oil phase was slowly added to the aqueous phase and the mixture was homogenized at 7000 rpm for 10 min. The final emulsion volume was 100 mL. The samples were maintained at rest for 1 h before their use in the bioassays.

2.4. Bioassays:

The formulation efficiency in the control of *Parthenium* sp. was evaluated through post-emergence bioassays with young plants. The plants were collected in the experimental area of R. D. University, Jabalpur and transplanted into polyethylene cups containing 200 g of the sterilized soil. They were cultivated in a greenhouse for 15 days before the application of mycoherbicide, which was applied using a hand sprayer. Phytotoxicity assays were also performed with the respective formulations, however replacing the culture filtrate by distilled water. The plant injury was visually estimated 15 days after mycoherbicide application, following the methodology proposed by Frans and Crowley (Frans et al. 1986).

3. RESULTS AND DISCUSSION

The influence of oil used in the formulations is presented in Table 1. Treatments T1 and T2 formulated with palm and soybean oil, respectively, had a slight effect on the aerial part of Conyza sp. plants, whereas the treatment T3, formulated with mineral oil, showed a severe effect. The control treatments T4 and T5 did not present a phytotoxic effect, while the control treatment T6 showed a slight effect on plants. The treatment T7, composed of culture filtrate, had a low effect on plants, with only a slight yellowing at the leaf ends. Among the oils tested in the study, mineral oil presented the best results. Therefore, it was selected for the next steps of processing and analysis.

Table 1: Bioherbicidal activity of culture broth formulations with different oils

Treatment	Formulation (ml)	Herbicidal activity (%)
T1	Culture filtrate (95)+ Palm oil (5)	10
T2	Culture filtrate (95)+ Soyabean oil (5)	10
T3	Culture filtrate (95)+ Mineral oil (5)	90
T4	Distilled water (95) + Palm oil (5)	5
T5	Distilled water (95) + Soyabean oil (5)	5
T6	Distilled water (95) + Mineral oil (5)	10
T7	Culture filtrate (100)	5
T8	Distilled water (100)	0

The obtained results suggest that the mycoherbicide produced by *Fusarium* sp. FGCCW#16 could be a hydrophilic molecule because surfactants with a high HLB increase the cuticle hydration. Consequently, it promotes a better permeability of hydrophilic herbicides into the leaves, increasing their diffusion rate at a constant concentration gradient (Hess and Foy, 2000). The results obtained were similar to those obtained by Bastos et al. (2017), whose authors verified that the efficiency of formulated bioherbicide from *Diaporthe* sp. was higher with HLB 15.0.

The formulation is an important step in the development of a mycoherbicide with metabolites from fungi. Without an adequate combination of adjuvants, many bioproducts with great attractiveness in the market are discharged. In this work, the efficiency of the mycoherbicide was improved approximately 2.5 times when compared with non-formulated products. Bastos et al. (2017) [24] increased 3 times the efficiency of a bioherbicide produced from *Diaphorte* sp. Likewise, Pes et al. (2016) accentuated the suppressive effect on the growth of *Conyza* sp. and *Echinochloa* sp. for a bioherbicide formulated from *Diaphorte* sp.

Aybeke(2017) reported toxic effects of *Fusarium oxysporum* on a root parasitic weed (*Orobanche* spp.). Li et al. (2014) reported that ethyl acetate extract of the fermentation broth of *Fusarium proliferatum* provided selective phytotoxic activity against the radicle growth of *Amaranthus retroflexus*. Nevertheless, there are no reports about metabolites from *Fusarium* sp. in the control of Parthenium sp. up to now. Therefore, this work can be considered the first study related to the herbicidal activity of metabolites from *Fusarium* sp. FGCCW#16 isolated from Jabalpur in an infected Parthenium for the control of this weed.

4. CONCLUSIONS

In this study, the necessity of a correct combination of adjuvants to increase the herbicide activity of culture filtrate was demonstrated. Depending on the adjuvant's combination, the herbicidal activity may reach values insufficient to follow the next steps in the development stage. The better result of herbicidal activity was obtained with a formulation containing 5% (w/v) of mineral oil, 5% (w/v) of surfactant and HLB of 15, a condition in which a complete death of *Parthenium* sp. plants was observed. This promising combination of adjuvants and culture filtrate from *Fusarium* sp. FGCCW#16 increased 2.5 times the efficiency of mycoherbicide for the post-emergence control of *Parthenium* sp.

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Mount Arayat Protected Landscape Protection: Knowledge, Perception and Practices of the Youth

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ABSTRACT

The study aimed to assess the knowledge, perception, and practices of youth of San Agustin Magalang Pampanga towards the conservation and protection of Mt. Arayat Protected Landscape where the target respondents in the study are 265 youth of San Agustin, Magalang, Pampanga. Survey questionnaires were used to gather the data and the information. In summary, it is noticeable that the youth respondents have a high level of knowledge about MAPL as a protected area and the thereafter protocols, activities, and rules with a general weighted mean of 3.32 which implies High awareness and expertise. It also indicated that Youth respondents do highly agree that proper management and protection, cigarettes caused by forest fires, people participation, and youth involvement are beneficial to Mt Arayat Protected Landscapes with an overall mean of 3.41. It is also noticeable that good practices were being implied in the results from the youth respondents. From avoiding littering, paying entrance fees, complying with the Do's and Don'ts's of any Protected landscapes. This implies the correlation with the high knowledge of the respondents on the Mt Arayat Protected landscape.

Keywords: Youth, Knowledge, Perception, Practices, Mt. Arayat and Survey

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INTRODUCTION

The enchanting Mt Arayat is located in the Pampanga towns of Arayat and Magalang, and is one of the simplest parks to reach from Manila. The Pampanga River passes through the provinces of Bulacan, Pampanga, and Nueva Ecija and has a circular volcano crater at its crest. Mt. Arayat, famed for its crystal-clear waterfalls and quiet surrounding, was designated as a Forest Reserve in 1921 before being designated a National Park in 1933. Mt. Arayat is home to endemic and endangered flora and wildlife, as well as geological rock formations with tourist potential. It is also an important supply of water for

domestic and agricultural usage (Eco - exploration, 2022).

The abundance of species on Mt. Arayat, according to the Department of Environment and Natural Resources, is the fundamental reason for its designation as a protected area. Other considerations include Mt. Arayat's irreplaceability or the presence of species with restored ranges and congregations, its vulnerability or the presence of endemic and globally threatened species, its naturalness or the presence of intact natural cover Forest cover, abundance and diversity of flora and fauna, and the uniqueness or outstanding cultural and geological features (Mayuga, 2021).

Young people, on the other hand, can actively contribute to the preservation and enhancement of the environment. They have the power to change their way of life and the impact it has on the environment. They may make their homes, schools, and youth organizations more environmentally friendly by implementing eco-friendly practices, recycling various goods, and conserving resources such as water and electricity. Engaging young people in environmental preservation significantly affects their attitudes and behaviors, as well as their families and even their parents (Acharya, 2013).

According to Simpao et al., (2020), the importance of exploiting natural resources as a driver of development is recognized by experts and practitioners in various areas. Young people who are empowered and ecologically concerned may be the most powerful change agents. For the long-term safety and management of the environment. Pellier et al. (2014) also stated that they routinely practice acting to address environmental challenges, and that practice may necessitate a high level of dedication.

Therefore, this study aims to provide knowledge, perception and to determine regarding their practices on the protection of Mt. Arayat Protected Landscape.

MATERIALS AND METHODS

The study was conducted in Brgy San Agustin Magalang Pampanga. The study used standardized survey questionnaires. Descriptive analysis was used in this study to determine the respondent's knowledge, perception, and practices on the protection of Mt. Arayat Protected Landscape.

A total of 851 (Philippine Statistics Authority, 2019) households are located in San Agustin, Magalang Pampanga separated by four purok. Through the Slovins Formulation Population Sampling, 265 households were randomly surveyed divided proportionally within the four purok. Within each household, only 15 to 30 years old respondents were selected in this study, as stated by the WHO as "Youth".

The elicited quantitative data had undergone checking, scoring, analysis, and interpretation.

RESULTS AND DISCUSSION

Socio-Economic Profile

The highest number of respondents is the age ranging to 15-19 years old with 52.07% out of the total respondents, followed by ages 20-24 with 44.15% and 3.77% from ages 25 to 30.

Half of the respondents are High School graduates accumulating to 54% and 32.07% currently and undergraduate collegiate students.

Knowledge of San Agustin's Youth on the Protection of Mt Arayat Protected Landscape (MAPL)

a. Mt. Arayat is a Protected Area

Respondents are asked if they are aware if Mt Arayat is a protected area. As a result, the respondents are highly aware with a general mean of 3.41.

b.MAPL is home of numerous Wildlife species

The respondents are highly aware that Mt. Arayat Protected landscape is a home of numerous wild animals and other species with a mean of 3.26.

c. MAPL is rich in Natural resources

As shown in table 1 with a mean average of 3.42, are highly knowledgeable that Mt. Arayat Protected landscape is rich in resources such as clean water and food.

d. MAPL is an Ecotourism Site

With a mean of 3.19, the response is interpreted as under "aware" that MAPL is an Ecotourism site.

e. MAPL is a Beautiful Natural feature of Pampanga

It is also visible that the respondents are highly aware that the Mt. Arayat Protected landscape is one of the most beautiful natural features in the Province of Pampanga, as listed in the table with an average of 3.50.

Table 1: Knowledge of Youth on Protection of Mt Arayat

QUESTIONS		HIGHLY AWARE	AWARE	NOT AWARE	HIGHLY NOT AWARE	Total	MEAN
a. Mt. Arayat is a Protected Area	FREQUENCY	125	124	16	0	265	3.41
	PERCENTAGE	47.17	46.79	6.04	0.00		
b. MAPL is home of Wildlife	FREQUENCY	100	135	30	0	265	3.26
	PERCENTAGE	37.74	50.94	11.32	0.00		
c. MAPL is reach in Natural resources	FREQUENCY	126	125	14	0	265	3.42
	PERCENTAGE	47.55	47.17	5.28	0.00		
d. MAPL is a Ecotourism Site	FREQUENCY	100	122	36	7	265	3.19
	PERCENTAGE	37.74	46.04	13.58	2.64		
e. MAPL is a Beautiful Natural feature of Pampanga	FREQUENCY	150	101	10	4	265	3.50
	PERCENTAGE	56.60	38.11	3.77	1.51		
f. Increasing Visitors of MAPL helps Families in their Living	FREQUENCY	95	125	44	1	265	3.18
	PERCENTAGE	35.85	47.17	16.60	0.38		
g. Mt Arayat is a prohibited dump all waste	FREQUENCY	140	100	22	3	265	3.42
	PERCENTAGE	52.83	37.74	8.30	1.13		
h. Landslides in Mt. Arayat occurs as a result of Forest Degradation	FREQUENCY	102	115	45	3	265	3.19
	PERCENTAGE	38.49	43.40	16.98	1.13		
i. Cutting of Trees is prohibited inside MAPL	FREQUENCY	132	100	30	3	265	3.36
	PERCENTAGE	49.81	37.74	11.32	1.13		
j. Collecting and hunting Plants and Animals is Prohibited in MAPL	FREQUENCY	108	117	35	5	265	3.24
	PERCENTAGE	40.75	44.15	13.21	1.89		
GENERAL MEAN: 3.32							

Legend: 1-1.75 = Highly not aware, 1.76-2.5 = Not Aware, 2.26-3.25 = Aware and 3.26-4.00 Highly Aware

f. Increasing Visitors of MAPL helps Families in their Living

All in all, the respondents are aware that the increasing number of visitors to Mt. Arayat Protected landscape can also help the local families who live there make a living with a mean of 3.18 which is under "aware".

g. Mt Arayat is a prohibited dump all waste

In general, 3.42 is the mean weighted average of the respondents who are highly aware that Mt. Arayat is prohibited from dumping all types of waste.

h. Landslides in Mt. Arayat occurs as a result of Forest Degradation

This table also shows that most of the respondents are aware with a mean average of 3.19 that Landslides in MAPL occur as a result of forest degradation and the cutting of trees.

i. Cutting of Trees is prohibited inside MAPL

Results also show with a mean of 3.36, responses are categorized under "highly aware" that Cutting trees is prohibited inside MAPL

j. Collecting and hunting Plants and Animals is Prohibited in MAPL

Also, the respondents are aware that the collection and hunting of plants and animals are also prohibited in Mt.Arayat Protected Landscape with a weighted mean of 3.24.

In summary, it is noticeable that the youth respondents have a high level of knowledge about MAPL as a protected area and the subsequent protocols, activities, and rules with a general weighted mean of 3.32 which implies High awareness and knowledge.

Perceptions of San Agustin's Youth on the Protection of Mt Arayat Protected Landscape (MAPL)

a. MAPL needs to be protected because of its richness and multiple benefits

The respondents are asked if Mt. Arayat needs to be protected; most of the respondents highly agree that Mt. Arayat needs to be protected not only because of the richness of its diverse species but also the

number of benefits that the people gain from it, with a mean of 3.62.

b. The Ecotourism function of MAPL is one reason to preserve and conserve

The Table 2 also shows that the respondents do highly agree that the Ecotourism function of Mt. Arayat Protected Landscape is also one of the reasons why Mt. Arayat needs to be preserved and conserved as the general weighted average of 3.29.

Table 2: Perceptions of Youth on Protection of Mt Arayat

QUESTIONS		HIGHLY AGREE (4)	AGREE (3)	NOT AGREE (2)	HIGHLY NOT AGREE (1)	Total	MEAN
a. MAPL needs to be Protected because of its richness and multiple benefits	FREQUENCY	171	89	4	1	265	3.62
	PERCENTAGE	64.53	33.58	1.51	0.38		
b. The Ecotourism function of MAPL is one reason to preserve and conserve	FREQUENCY	102	142	18	3	265	3.29
	PERCENTAGE	38.49	53.58	6.79	1.13		
c. Negligence might one of the main causes of Damages tin MAPL	FREQUENCY	121	103	38	3	265	3.29
	PERCENTAGE	45.66	38.87	14.34	1.13		
d. Proper managing of forest improve tourism, wind barriers and prevent landslides	FREQUENCY	148	104	11	2	265	3.50
	PERCENTAGE	55.85	39.25	4.15	0.75		
e. one of the causes of Forest Fires is the cigarette lift lit in MAPL	FREQUENCY	91	122	35	17	265	3.08
	PERCENTAGE	34.34	46.04	13.21	6.42		
f. Protecting MAPL is beneficial to next generations	FREQUENCY	164	93	6	2	265	3.58
	PERCENTAGE	61.89	35.09	2.26	0.75		
g. restoring and enhancing MAPL increase Visits from local and foreign and thereby improve livelihood of local communities	FREQUENCY	141	112	10	2	265	3.48
	PERCENTAGE	53.21	42.26	3.77	0.75		
h. People participation aroung MAPL is an important aspect of MAPL Protection	FREQUENCY	127	125	12	1	265	3.43
	PERCENTAGE	47.92	47.17	4.53	0.38		
i. Youth can help in conserving and protecting MAPL	FREQUENCY	124	124	14	3	265	3.39
	PERCENTAGE	46.79	46.79	5.28	1.13		
GENERAL MEAN: 3.41							

Legend: 1-1.75 = Highly not aware, 1.76-2.5 = Not Aware, 2.26-3.25 = Aware and 3.26-4.00 Highly Aware

c. Negligence might be one of the main causes of damage in MAPL

The respondents are asked if negligence is the main cause of damage to MAPL with an average of 3.29, as a result, the respondents are highly agreeing.

d. Proper management of forests improves tourism, and wind barriers and prevents landslide

Overall, the respondents do highly agree that proper management of forest can improve tourism, serve also as a wind barrier, and prevent landslides with a mean of 3.50.

e. One of the causes of forest fires is the cigarette littering

As a result, the respondents agree that one of the causes of forest fires is cigarette littering in MAPL with a mean of 3.08.

f. Protecting MAPL is beneficial to next-generation

In general, the respondents strongly agree that protecting Mt. Arayat Protected Landscape is beneficial to the next generation and so on with a mean of 3.58.

g. Restoring and enhancing MAPL increases visits from local and foreign and thereby improves the livelihood of local communities.

Mostly, the respondents do highly agree that restoring and enhancing Mt. Arayat can help to increase the visitation from both local and foreign

visitors, and therefore can help improve the livelihood of the local community in the area with a mean of 3.48.

h.People participation around MAPL is an important aspect of MAPL Protection

As a result, the respondents do highly agree that people's participation around MAPL is an important aspect of the protection of Mt Arayat with a weighted mean of 3.43.

i. Youth can help in conserving and protecting MAPL

Lastly, the respondents highly agree that the youth can help in conserving and protecting MAPL with a mean of 3.39.

In General Perception, the results of the study imply that Youth respondents do highly agree that proper management and protection, cigarettes caused forest fires, people participation, and youth involvement are beneficial to Mt Arayat Protected Landscapes with an overall mean of 3.41.

Practices of San Agustin's Youth on the Protection of Mt Arayat Protected Landscape

Table 3: Practices of Youth on the Protection of Mt. Arayat

QUESTIONS		ALWAYS (4)	SELDOM (3)	RARELY (2)	NEVER (1)	Total	MEAN
a. Avoid littering within MAPL	FREQUENCY	200	40	25	0	265	3.66
	PERCENTAGE	75.47	15.09	9.43	0.00		
b. Pay appropriate entrance fee	FREQUENCY	156	60	26	23	265	3.32
	PERCENTAGE	58.87	22.64	9.81	8.68		
c. Helps collect garbage within MAPL	FREQUENCY	103	111	30	21	265	3.12
	PERCENTAGE	38.87	41.89	11.32	7.92		
d. Collects fruits and wild plants.	FREQUENCY	21	56	58	130	265	1.88
	PERCENTAGE	7.92	21.13	21.89	49.06		
e. Hunts wild animals ex. Monkeys, alligators	FREQUENCY	14	14	22	215	265	1.35
	PERCENTAGE	5.28	5.28	8.30	81.13		
f. Participate in tree planting activities.	FREQUENCY	60	90	70	45	265	2.62
	PERCENTAGE	22.64	33.96	26.42	16.98		
g. Do kaingin or charcoal making.	FREQUENCY	20	35	25	185	265	1.58
	PERCENTAGE	7.55	13.21	9.43	69.81		
h. Cut trees.	FREQUENCY	6	24	20	215	265	1.32
	PERCENTAGE	2.26	9.06	7.55	81.13		
i. Help disseminate information about the importance of MAPL.	FREQUENCY	105	83	54	23	265	3.02
	PERCENTAGE	39.62	31.32	20.38	8.68		
j. Aid in reporting violators and violations of laws within MAPL.	FREQUENCY	90	66	62	47	265	2.75
	PERCENTAGE	33.96	24.91	23.40	17.74		
GENERAL MEAN: 2.46							

Legend: 1-1.75 = Never, 1.76-2.5 = Rarely, 2.26-3.25 = Seldom and 3.26-4.00 always

(MAPL)

a. Avoid littering within MAPL

As shown in table 3, there are 75.47% of the respondents always avoid littering within MAPL with a general mean of 3.66

b. Pay appropriate entrance fee

With a weighted mean of 3.32, respondents shows that they always pay appropriate entrance fees to all boarders of the MAPL.

c. Helps collect garbage in MAPL

Table 3 also shows seldom of the respondents participated in the garbage collection of MAPL with a weighted mean of 3.12.

d. Collects fruits and wild plants

With the highest responses, never did the respondents collected fruits and wild plants in MAPL with a weighted mean of 1.88.

e. Hunts wild animals

The results in the table 3 also show that 81.13% of the respondents never hunted any wild animals in the MAPL with a weighted mean of 1.35.

f. Participate in the tree planting activities

With a weighted mean of 2.62, Youth respondent's seldomly participated in ant tree planting activities of MAPL.

g. Do kaingin or charcoal making

The results also shown in table 3 that 69.81% of the respondents never do Kaingin or charcoal making. Gaining a general weighted mean of 1.58.

h. Cut trees

It also implies positively that 81.13% of the respondents never had cut trees in MAPL

i. Helps disseminate information about MAPL

This study also resulted to knowing that Youth respondents helps in dissemination of information on the importance of MAPL with 3.02 weighted mean.

j. Aid in reporting violators

Table 3 also shown significant result that 90 of the respondents are do always aid in reporting violators and violators of the laws within MAPL.

In summary, it is noticeable that good practices were being implied in the results from the youth respondents. From avoiding littering, paying entrance fees, complying with the Do's and Don'ts's of any protected landscapes. This implies the correlation with the high knowledge of the respondents on the Mt Arayat Protected landscape.

Willingness to pay for the protection of Forests

Results revealed that 92.08% of the youth respondents are willing to donate and pay for the protection of the forests

Table 4: Willingness to donate

willingness to donate/pay	Frequency	Percentage
YES	244	92.08
NO	21	7.92
TOTAL	265	100

The record also shows that 73.36% of the who said YES is willing to donate the amount ranging of 1 peso to 100 pesos.

Table 5: Amount to be donated

Pesos	Frequency	Percentage
1-100	179	73.36
101-500	45	18.44
501-1000	16	6.56
1001-3000	1	0.41
3001-5000	0	0
5000 and above	3	1.23
total	244	100

Respondents were also asked to which entity they will entrust their donations, surprisingly, they had chosen the Local Government Unit with almost half of the respondents (47.13%).

Table 6: Entity to entrust their donations

AUTHORITY	frequency	Percentage
DENR	92	37.7
PNP	14	5.74
LGU	115	47.13
NGO	23	9.43
TOTAL	244	100

Implications

In summary, it is noticeable that the youth respondents have a high level of knowledge about MAPL as a protected area and the subsequent protocols, activities, and rules with a general weighted mean of 3.32 which implies high awareness and knowledge. It also indicates that Youth respondents do highly agree that proper management and protection, cigarettes caused by forest fires, people participation, and youth involvement are beneficial to Mt Arayat Protected Landscapes with an overall mean of 3.41. It is also noticeable that good practices were being implied in the results from the youth respondents. From

avoiding littering, paying entrance fees, and complying with the Do's and Don'ts's of any protected landscapes. This implies the correlation with the high knowledge of the respondents on the Mt Arayat Protected landscape.

RECOMMENDATIONS

Since high knowledge prevailed in the study, it is highly recommended that Natural resources campaigns and movement be designated to Youth by the Local Government Unit, as the youth's Knowledge, Perceptions, and Practices are commendable. Future efforts must focus on inspiring young people to become involved in community life and to develop an interest in the forestry industry.

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Survey on the Post-Harvest Handling Practices of Some Selected Soup Condiments in Awka, Anambra State and Isolation of Fungi Responsible For Their Spoilage

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ABSTRACT

A survey on the post-harvest handling practices of *Citrullus colocynthis*, *Irvingia gabonensis* and *Brachystegia eurycoma* among traders in Awka was conducted from January to April, 2023 in Eke-Awka, Amaenyi and Ifite markets. A total of 180 respondents (sixty from each market) were randomly selected for the study. The well structured questionnaire captured social demographic information about the respondents while the second section focused on the post-harvest handling practices of soup thickeners. Most of the traders/respondents in the study area are within the Age range 31-35 and 41-45 with percentages of 16.67 and 16 respectively. While the Age groups with the lowest frequency is the age group 26-30 with 3 (1.67%) respondents. Majority of the respondents (80%) purchase these soup thickeners from wholesalers while 21 (11.67%) purchase directly from farmers. Most of the respondents (82.78%) dry soup thickeners, 30 (16.61%) sort them and 1 (0.56%) soak them in water as a post-harvest spoilage control practice. The most observed change in the seeds after 8 months in storage is change in colour (43.89%), the least observed change is the formation of mucor (0.56%) while 9 (5%) of the study population stated that there is no change after 8 months in storage. All the 180 respondents (100%) do not use chemical preservatives for preservation of these soup thickeners. For the microbial study, samples of Egusi, Ogbono and Achi collected from Eke-Awka, Amaenyi and Ifite markets were analyzed using standard mycological techniques. Fungi were isolated and identified based on their morphological and molecular characteristics. The results showed that all the samples were contaminated with fungi, with the highest level of contamination in Egusi seeds from Eke Awka with a mean occurrence of 41.33 ± 8.145. The most common fungal genera identified were *Aspergillus, Fusarium* and *Rhizopus.* A significant proportion of the isolates belonged to toxigenic species, which could potentially produce mycotoxins that pose a health risk to consumers. The findings also underscore the importance of proper handling, storage and processing of these seeds to minimize fungal contamination.

Keywords: Survey, Post-harvest, Fungi, Anambra state, Soup condiments, Markets

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INTRODUCTION

Stored foods travel through different stages before reaching the final consumer. In each of the pre-harvest and post-harvest operations, they encounter various barriers that affect the final quality of the stored food products. The deterioration of stored food quality is chiefly caused by fungal invasion. Stored food materials contaminated by microorganisms tend to develop foul odour, lose their nutritional quality, weight, flavor and color (Gulumbic and Kulik, 2012; Ahmed et al. 2016; Kumar, 2017). These fungal organisms produce highly toxic and carcinogenic metabolites or mycotoxins and also cause spoilage of agricultural produce (Tsehaye et al. 2017).

The major storage fungi of foods in the tropic region are *Aspergillus flavus, Aspergillus niger* and *Mucor* spp. These organisms causes easy deterioration of these seeds making it lose its taste and nutritional values. Some of these fungi also produce mycotoxins which are harmful to the human health when ingested especially in large quantities (Anukwuorji et al. 2012, 2013; Okigbo et al. 2012, 2014).

During storage, melon seeds foods are attacked by different storage fungi, some of which are from the genera *Aspergillus* and *Penicillium.* These fungi affect the quality and quantity of these stored foods causing a change in colour and decrease in their nutritional, economic and market value (Anukwuorji et al. 2020).

In most developing countries, fungi contaminate foods such as cereals, legumes, seeds, nuts, root and tuber crops, etc. The exposure occurs in towns and villages that produce their own food, hence regulatory measures to control exposure are largely ineffective (Wild and Gong, 2010). Unsafe food causes many acute and lifelong diseases ranging from diarrheal to various forms of cancer. The monitoring of contaminants in food provides important information on risks associated with the consumption of contaminated foods and on the efficiency of control measures that are in place. The safety of foods and feeds for human and animal consumption should be of topmost priority with regards to the regulation of agricultural and food industries so the markets are not compromised by the sale of low quality or unsafe food (Conway and Toenniessen, 2003). It is against the backdrop of the above that this research was conducted to ascertain the post harvest handling practices of soup condiments and to isolate the fungi responsible for their spoilage in storage.

Therefore, the purpose of this study is to evaluate the quality of soup condiments sold in open markets in Awka, Anambra, and investigate the fungal species that contaminate the soup thickeners. The objectives of this study were to assess the post-harvest handling practices of ogbono (*Irvingia gabonensis*), egusi (*Citrullus colocynthis*) and achi (*Brachystegia eurycoma*) by local farmers in Awka, Anambra and to determine the level of fungal infestation of ogbono, egusi and achi seeds sold in markets within Awka.

MATERIALS AND METHODS

Study Area

This study was conducted in Awka, Anambra State. Awka is the capital of city of Anambra state It has an area of roughly 523.2km^2 and is located in a fertile tropical valley, however some portions of its rainforest has been lost to farm clearing and urbanization.

Ethno-study

Survey was conducted between December 2022 and March 2023 in three markets in Awka, Anambra State. The markets were Eke-Awka, Amaenyi and Ifite markets. A well-structured questionnaire on post-harvest handling practices of egusi (*Citrullus colocynthis*), ogbono (*Irvingia gabonensis*) and achi seeds (*Brachystegia eurycoma*) was designed and administered randomly to one hundred and eighty (sixty in each market) traders within the study area. The questionnaire was made up of two sections: the first section was made up of information about the respondents while the second section which focused on the post-harvest handling practices of soup thickeners.

Sample collection

The method of Adetunji et al. (2014) was adopted in sampling food materials; the food materials sampled were *I. gabonensis*, *C. colocynthis* and *B. eurycoma*. The objective of the sampling was to obtain a small quantity of food material that represents the whole. Using simple random sampling technique, a total of three (3) markets from Awka were sampled. The specimens were labeled, numbered and annotated with the date of collection and locality.

Sample Preparation

The samples were air-dried and were later weighed with a weighing machine and ground into a powdered form. Each sample was labeled, packaged in a polythene bag, and taken to the laboratory for analysis.

Isolation of Fungi from Stored Soup Thickeners

One gram (1g) from each sample was weighed on a sensitive meter scale. A test-tube plastic rack was arranged with 9ml of sterile test-tubes each containing 9 ml of sterile distilled water (SDW). A tenfold serial dilution (Fasole and Oso, 1988) was carried out by dispersing 1g sample into the first test-tube (10^{-1}) shaken together. One ml was again taken from (10^{-1}) dilution and transferred to the next test tube (10^{-2}). The dilutions continue to (10^{-9}). Each test tube was shaken vigorously before transfer. A pour plate method (Fasole and Oso, 1988; modified by Okigbo *et al.*, 2015) was used in plating all the samples. One mill (1 ml) from dilution (10^{-9}) was dispensed into a sterile Petri dish with a sterile pipette. A molten Potato Dextrose Agar was poured into the plates (about 10 ml). The plates were swirled for easy mix-up of the sample and the media. All plates were allowed solidification on the bench. Each plated sample was duplicated.

Sub culturing and Identification of purified cultures

A flamed surgical blade was used for sub-culturing the mycelia from Potato Dextrose Agar plates (PDA) into a newly prepared PDA plates for purification. All plates were incubated at 25^0C for 3-5days. Macroscopic examination was done by physical characteristics of the mycelia-like structure and color of the mycelia. Microscopic characteristics through the morphological structure according to (Mathur and Kongsdal, 2003) was employed. A wet mount method (Fasole and Oso, 1988) was done before viewing the isolates under ^40 compound microscope. The morphological structures viewed include septate or non-septate mycelia, presence of sporangiosphores, fruiting bodies and special organs like rhizoids. Each morphological structure of each isolates was matched with a mycology atlas for identification.

Determination of percentage of fungal occurrence

This was done to determine the frequency of occurrence of the different fungal isolates. Isolations were made from the three plant materials. The number of occurrence for each of the isolates in each of the samples (plant materials) were recorded and calculated as a ratio of the total number of occurrence and was then expressed as a percentage. It was given by the formula below;

Percentage occurrence= $^{x}/_{n}$ X $^{100}/_{1}$, where x= Total number of each organism in all the samples. n = Total number of the entire organism in all the samples screened.

Statistical Analysis

Descriptive statistics of frequency and percent counts were used to summarize the data collected from the survey. The data collected from the microbial analysis were subjected to analysis of variance (ANOVA) and means were separated using Duncan's Multiple Range Test (1955) at 0.05 probability level to determine significant differences among the samples obtained from the different locations and periods.

RESULTS

Socio-demographic characteristics of the respondents

Most traders/respondents in the study area are within the Age groups 31-35 and 41-45 with the frequency of 30 and 27 and percentages of 16.67 and 16 respectively. At the same time, the Age groups with the lowest frequency is the age group 26-30 with frequency of 3 (1.67%) (Fig. 1 and Table 1). Majority of the respondents were female with a frequency of 168 (93.33%). The result presented in the table also shows that 55.00% of the respondents were married, 27.78% were widowed and 17.22% were single. The highest level of education achieved by the respondents was SSCE, followed by OND/NCE, and FSLC with a frequency of 99, 45 and 21 and percentage of 55.00%, 25.00% and 11.67% respectively. Only 15 (8.33%) of the respondents had obtained a B.Sc./HND degree (Fig. 1 and Table 1).

Point of purchase, storage, and post-harvest spoilage control methods of soup thickeners

From Table 2 below, 144 (80%) respondents purchase these soup thickeners from wholesalers while 21 (11.67%) purchase soup thickeners directly from farmers and 15 (8.33%) purchase them from the farm gate. It was also observed that 123 (68.33%) of the respondents stored these soup thickeners for more

than 8 months before selling to customers, 54 (30.00%) stored them for 8 months before selling and 3 (1.67%) stored them for less than 8 months before selling to a customer. For storage materials, nylon bags were the most common storage materials used by the respondents to store these soup thickeners, with a frequency were 70 (38.89%). Hermetic bags were the least used storage materials, with a frequency of 9 (5.00%).

For post-harvest spoilage control, 149 (82.78%) of the respondents dry these soup thickeners, 30 (16.61%) sort them and 1 (0.56%) soak them in water. According to the respondents, the most observed change in the seeds after 8 months in storage is change in colour, with a frequency of 79 (43.89%), the least observed change is the formation of mucor 1 (0.56%) while 9 (5%) of the study population stated that there is no change. Before being stored, 78(43.33%) of the respondents displayed these soup thickeners on the bare floor, while 64(35.56%) placed them in baskets and 38 (21.11%) displayed them on aluminum tray (Table 2),

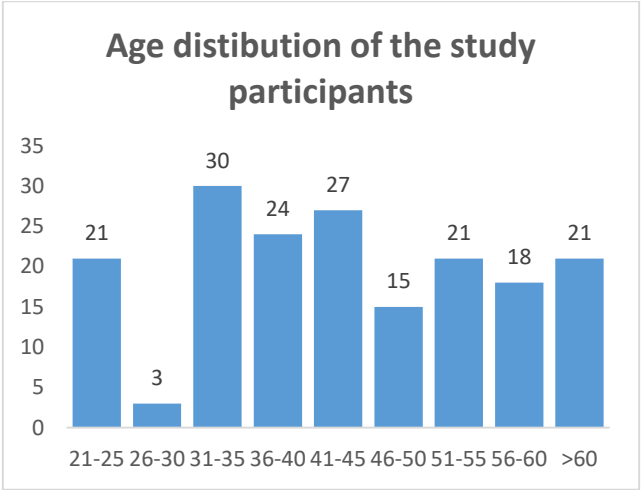

Fig 1: Age distribution of the participants involved in the study.

Traders perception on the post-harvest handling procedure for egusi, ogbono and achi

From table 3 below, all the respondents 20 (13.4%) in Eke-Awka market dry their Ogbono seeds, 5 (16.7%)sort their Achi seeds while none of the respondents soak any of these soup thickeners as a post-harvest handling technique to prevent spoilage. However, only one of the respondents from the three markets soak their Achi seeds in water. In Nkwo-Amaenyi, only 2 (6.7%) of the food handlers sort Egusi seeds. From table 3, it can be deduced that drying is the most common postharvest handling procedure with a frequency of 149 while soaking in water is the least common with a frequency of 1.

Occurrence of Fungi Pathogens on Stored Food Samples from Different Locations in Awka

Based on the growth of the fungi on the cultured soup thickeners, Table 1 reveals the presence of *Aternaria alternata, Rhizopus stolonifer, Fusarium oxysporium, Aspergillus niger* and *Aspergillus flavus*. *Aspergillus niger* was observed in the samples from Achi and Ogbono from Eke Awka and Ifite respectively, *Aspergillus flavus* was present in Ogbono samples from Eke Awka and from Egusi and Ogbono samples from Ifite while *Alternaria alternata* wasseen in Egusi samples from all locations and also from Ogbono sample in Nkwo Amaenyi. However, *Fusarium oxysporium* was isolated in Achi collected from Ifite and Amaenyi, it was also isolated from Ogbono from sampled from Eke Awka. *Rhizopus stolonifer* was seen only in Achi samples from Eke Awka (Table 4).

Identification of Fungi Isolates on Stored Food Samples Analyzed

Table 5 shows the fungal species isolated from the samples. These isolated organisms were characterized and identified based on physical and microscopic observations of their growth, hyphae, fruiting bodies, and the types of resting spores.

Mean Occurrence of Fungi Pathogens on Stored Food Samples from Different Locations in Awka

Result on the mean occurrence of fungi isolates from the cultured stored food samples showed that *Alternaria alternata* was higher in Egusi sampled from Eke Awka market (41.33±8.145) but lower in the Egusi samples purchased from Nkwo Amaenyi market (37.33±5.508) (Table6). The fungi isolates of *Fusarium oxysporium* was higher in Ifite (41.00±8.718) than in Nkwo Amaenyi (36.00±7.937) (Table 7). The mean percentage occurrence of *Aspergillus flavus* was significantly higher ($p<0.05$) in stored Ogbono samples collected in Eke Awka (41.00±4.000) than that of Ifite (33.00±3.606) (Table 8). There was a significant difference in the percentage occurrence of fungi isolates of stored food samples from the different locations ($p<0.05$).

Table 1: Socio-demographic characteristics of the respondents

Socio-demographic characteristics	Frequency (n=180)	Percentage (%)
Age group (years)		
21-25	21	11.67
26-30	3	1.67
31-35	30	16.67
36-40	24	13.33
41-45	27	15.00
46-50	15	8.33
51-55	21	11.67
56-60	18	10.00
>60	21	11.67
Sex		
Female	168	93.33
Male	12	6.67
Marital status		
Married	99	55.00
Single	31	17.22
Widow	50	27.78
Highest level of education		
B.SC/HND	15	8.33
FSLC	21	11.67
OND/NCE	45	25.00
SSCE	99	55.00
Total	**180**	**100.0**

Table 2: Point of purchase, storage, and control of post-harvest spoilage of soup thickeners

Variables	Frequency (n=180)	Percentage (%)
Point of Purchase		
Farm gate	15	8.33
Farmer	21	11.67
Wholesalers	144	80.00
Average storage time before selling to customers		
Less than 8 months	3	1.67
8 months	54	30.00
More than 8 months	123	68.33
Storage Materials used by the respondents		
Hermetic Bags	9	5.00
Metal Buckets	18	10.00
Moulded plastic boxes	26	14.44
Nylon bags	70	38.89
Synthetic fibre sack	57	31.67
Post-harvest spoilage control		
Drying	149	82.78
Soaking in water	1	0.56
Sorting	30	16.67
Changes in seed after 8 months in storage		
Change in colour	79	43.89

Dusty	31	17.22
Formation of mucor	1	0.56
Moist	19	10.56
Shrinking/reduction in size	41	22.78
None	9	5.00
Mode of display before storage		
Aluminum tray	38	21.11
Bare floor	78	43.33
Basket	64	35.56
Use of synthetic chemical preservatives		
No	180	100.0

Table 3: Post-harvest handling procedure for Egusi, Ogbono and Achị by traders in Eke-Awka, Nkwo-Amaenyi and Ifite Second market

Markets	Post-harvest handling procedure			Total (%)
	Drying (n=149)	Soaking in water (n=1)	Sorting (n=30)	
Eke Awka-Ogbono	20 (13.4)	-	-	20 (11.1)
Eke- Awka –Achi	15 (10.1)	-	5 (16.7)	20 (11.1)
Eke- Awka-Egusi	16 (10.7)	-	4 (13.3)	20 (11.1)
Ifite- Achi	15 (10.1)	1 (100)	4 (13.3)	20 (11.1)
Ifite-Egusi	17 (11.4)	-	3 (10.0)	20 (11.1)
Ifite-Ogbono	14 (9.4)	-	6 (20.0)	20 (11.1)
NkwoAmaenyi –Achi	20 (13.4)	-	-	20 (11.1)
NkwoAmaenyi-Egusi	18 (12.1)	-	2 (6.7)	20 (11.1)
NkwoAmaenyi-Ogbono	14 (9.4)	-	6 (20.0)	20 (11.1)
Total	149 (100.0)	1 (100)	30 (100)	180 (100.0)

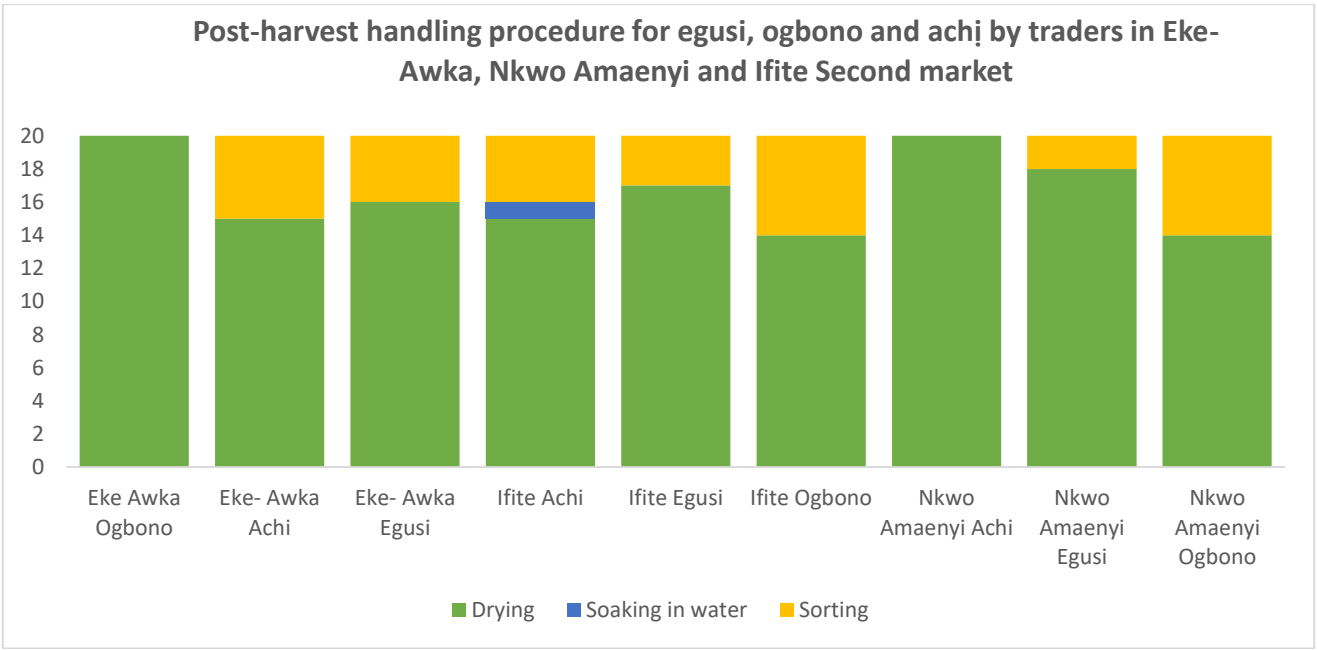

Fig 2: Stacked bar chart showing the Post-harvest handling procedure for egusi, ogbono and achị by traders in Eke-Awka, NkwoAmaenyi, and Ifite Second markets

Table 4: Occurrence of Fungi Pathogens on Stored Food from Different Locations in Awka

	Locations								
	Eke-Awka			Nkwo-Amaenyi			Ifite		
Fungi Isolates	Food Samples			Food Sample			Food Samples		
	Egusi	Achi	Ogbono	Egusi	Achi	Ogbono	Egusi	Achi	Ogbono
Aspergillus niger	-	+	-	-	-	-	-	-	+
Aspergillus flavus	-	-	+	-	-	-	+	-	+
Alternaria alternata	+	-	-	+	-	+	+	-	-
F. oxysporium	-	-	+	-	+	-	-	+	-
R. stolonifer	-	+	-	-	-	-	-	-	-

Table 5: Identification of Fungal Isolates on Stored Food Samples Analyzed

MACROSCOPY	MICROSCOPY	ORGANISM
Black colony, powder with diffused hyphae in media	Smooth-walled stipe, conidiophores radiate and terminate in vesicle.	*A. niger*
Light green and powdery colonies	Rough and coarse aerial hyphae present with simple sporangiophore which are shaped globose.	*A. flavus*
Texture deeply cottony; White becoming gray-brown on surface	Angular, subglobose or ellipsoidal sporangia, aerial erect hyphae.	*R. stolonifer*
Whitish colonies later turn brownish	Brown-black, globose sporangia, rhizoids also present, and zygospores.	*A. alternata*
White cottony colonies and dark-purple undersurface	Mesoconidia, and microconidia arranged in false heads present.Intercalary chlamydospores.	*F. oxysporium*

Table 6: Mean Occurrence of Fungi Pathogens on Stored Egusi Samples from Different Locations

Fungi Isolates	Eke Awka	Nkwo Amaenyi	Ifite
A. niger	-	-	
A. flavus	-	-	41.33±16.073
R. stolonifer	-	-	-
A. alternate	41.33^{a}±8.145	37.33^{a}±5.508	38.00^{b}±3.306
F. oxysporium	-	-	-

Values are mean ± standard deviation of triplicate determination. Means on the same row with different superscripts are significantly different at ($p<0.05$).- = Absent

Table 7: Mean Occurrence of Fungi Pathogens on Stored Achi Samples from Different Locations

Fungi Isolates	Eke Awka	Nkwo Amaenyi	Ifite
A. niger	38.67±3.512	-	-
A. flavus	-	-	-
R. stolonifer	178.33±8.505	-	-
A. alternata	-	-	-
F. oxysporium	-	36.00^{a}±7.937	41.00^{a}±8.718

Values are mean ± standard deviation of triplicate determination. Means on the same row with different superscript are significantly different at ($p<0.05$). - = Absent

Table 8: Mean Occurrence of Fungi Pathogens on Stored Ogbono Samples from Different Locations

Fungi Isolates	Eke Awka	Nkwo Amaenyi	Ifite
A. niger			49.00±7.211
A. flavus	41.00^{a}±4.000	-	33.00^{b}±3.606
R. stolonifer	-	-	-
A. alternate	-	36.00±7.810	-
F. oxysporium	106.00±12.288	-	-

Values are mean ± standard deviation of triplicate determination. Means on the same row with different superscript are significantly different at ($p<0.05$). - = Absent

DISCUSSION

The survey carried out showed that the food handlers/respondents across Awka had fairly uniform ways of handling soup thickeners. In this study, storage of food materials before marketing was reported to last as long as 8 months, such an extended storage period promotes insects/rodents and mold infestation and can lead to discoloration and change in flavor (Kaaya and Eboku, 2010). Bankole et al. (2005) reported that one major problem that besets food materials especially *C. colocynthis* is that it deteriorates quickly in storage due to fungal infestation. Storage of food materials for a long period of time decreases its nutritive value. On the same hand, the results of a study conducted by Kolapo and Sanni (2006) confirmed that prolonged storage negatively affect the overall quality of foodstuffs such as its proximate composition.

This study also confirmed that food handlers in Awka do not use chemical preservatives to prevent spoilage which is likely to be one of the factors that predisposes these soup thickeners to fungal infestation. In Awka, the manner in which food materials are processed and handled increases their vulnerability to contaminants. This is due to the disregard and neglect of several drying and storage factors that could otherwise reduce contaminants in stored food materials. These factors include the need for proper drying of seeds to eliminate moisture, controlling storage insect pests by separating damaged parts from undamaged ones, employing safe and appropriate preservatives, and raising awareness of the risk posed by pests and contaminants in stored foodstuffs (Bankole et al., 2005).

Fungi isolated from these stored soup thickeners are *Aternaria alternata, Rhizopus stolonifer, Fusarium oxysporium, Aspergillus niger* and *Aspergillus flavus*. Most of the organisms isolated from this research work are storage fungi whose spores may have attached to the seeds during storage/processing or handling and have been at various times implicated in the spoilage of fruits and vegetables. This is in tandem with the result of a similar experiment conducted by Bankole abd Adebanjo (2003) but differs with the report of Oyedele and Fatoki (2017) who in addition to *Aspergillus* species also isolated *Mucor racemosus* and *Saccharomyces cerevisiae* from various food materials sold in various markets in Bayelsa state, Nigeria. Members of the fungal group micromycetes which include all the fungi isolated from the present research work have at various times implicated as being responsible for the spoilage of stored fruits of the family Cucurbitaceae to which *C. colocynthis* belongs. The high occurrence of *Aspergillus* species in the food samples agrees with the report of so many workers who reported *Aspergillus* species as one of the most frequent organisms associated with seed rot of melon.

CONCLUSION

Complete elimination of stored fungi detected in the soup condiments is not feasible even through thermal -processing. The stored fungi can cause irreparable damage to these seeds. Therefore, devising a sustainable strategy to prevent contamination and proliferation of fungi in stored foodstuffs as a whole is of utmost importance. It is necessary to control the factors that are responsible for fungi contamination and growth, the most important being moisture content. Insect infestation should be avoided and proper hygienic practices should be maintained throughout the supply chain.

RECOMMENDATION

➢There is need for consistent sensitization of the masses in Awka and awareness campaign on dangers of microbial infestation in food materials sold in the open markets. In addition to this, food handlers should also be sensitized on good food handling/processing procedures with respect to storage and drying techniques/duration.

➢Government should provide good storage facilities to prevent post-harvest losses and contamination of these food materials.

➢Information concerning the level of microbial infestation of foodstuffs sold in the open market should be made available to the masses by relevant agencies such as the Research institutes and the Federal Ministry of Health, this information should be disseminated at all levels of society in the country by extension workers.

➢Government should prioritize research and make available funds for the development of relatively cheaper biological control and plant based agents. There is no doubt that the prospect of Nigeria becoming food secured and is tied to the above recommendations.

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Screening of Secondary Metabolites and Antagonistic Activity of Endophytic Fungi of Selected Medicinal Plants in Western Kenya

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ABSTRACT

Endophytes mimic biochemical pathways of their host plants to synthesize secondary metabolites with antimicrobial properties. Anti-phytopathogenic activities and secondary metabolites of fungal endophytes from some medicinal plants have been reported. However, there is scanty information on the antifungal properties and secondary metabolites of *Carissa edulis, Microglossa pyrifolia* and *Stegonataenia araliacea.* Roots, stems and leaves of the three plants were collected from western Kenya, processed and cultured on potato dextrose agar to recover endophytic fungi. Antagonistic activity of endophytic fungi against *Cercosporae zeae maydis* and *Fusarium verticillioides* was determined by dual culture technique. Secondary metabolites were determined from endophytic fungi ethyl acetate extracts using standard protocols. Fifteen fungal endophytes recovered from the three plants had varied growth inhibition percentage against *C. zeae maydis* and *F. verticillioides.* Mean percentage inhibition of the isolates against the pathogens was not significantly ($p>0.05$) different but the highest mean inhibition percentage against *Cercosporae zeae maydis* and *Fusarium verticillioides* was from DSTS2 (72.67%) and DSRR2 (73%) respectively. Tannins and alkaloids were present in all fungal extracts while saponins were absent. The inhibitory effects of endophytic fungi against *Cercosporae zeae maydis* and *Fusarium verticilloides* are attributed to their ability to synthesis secondary metabolites with antimicrobial properties. Fungal endophytes from these medicinal plants should be exploited for the management of fungal pathogens of maize.

Keywords: Endophytes, Antagonistic, Econdary metabolites, *Fusarium verticillioides*, *Cercosporae zeae maydis*, Medicinal plants

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INTRODUCTION

Endophytes, including bacterial, fungal, and actinomycete species, quietly inhabit healthy plant tissues, both intercellularly and intracellularly, without causing any visible signs of disease (Musyimi et al. 2021; Emitaro et al. 2022). This relationship benefits both the endophytes and their host plants, as they engage in a symbiotic exchange of nutrients (Li et al. 2022; Mejia et al. 2009). Endophytes enhance the plant's nutrient uptake, fostering robust growth and development. They are

widespread, colonizing virtually all plant species and tissues, with their presence observed across a broad spectrum of plants studied to date (Saaikkonen et al. 2010). Their association with plants can be either obligate or facultative, yet they pose no harm to their host plants.

Endophytes from medicinal plants inhibit the growth of plant pathogens a trait mirrored from the host plant due to their ability to synthesize secondary metabolites. Recent studies have delved into the colonization patterns of vegetative tissues by endophytes and their impact on plant growth, showcasing their antagonistic effects against plant pathogens like *Xanthomonas campestris pv. Musacearum* (Emitaro et al. 2021). However, there remains a gap in our understanding regarding the antagonistic potential of endophytic fungi residing within *Carissa edulis, Microglossa pyrifolia*, and *Steganotaenia araliacea* against plant pathogens such as *Cercosporae zeae maydis* and *Fusarium verticillioides,* which are notorious for causing maize loss. These pathogens are transmitted through seeds and pose significant threats, leading to the deterioration of grain quality, decreased germination rates, and diminished vigor (Agrios, 2005). Despite the availability of various natural and synthetic products to combat fungal pathogens, the rise of resistant fungi underscores the necessity of discovering novel sources of antifungal compounds.

Research has demonstrated that the biological functions of endophytes can be attributed to specific secondary metabolites that they produce (Ogbe et al. 2020). These metabolites encompass a wide range of compounds including alkaloids, terpenoids, steroids, quinones, isocoumarin derivatives, flavonoids, peptides, and phenols, as found in fungal extracts (Zhao *et al.* 2010). Such bioactive compounds hold significant value across pharmaceuticals, environmental applications, agriculture, and industries (Egamberdieva *et al.* 2017). However, limited information is available regarding the secondary metabolites produced by endophytic fungi residing within *Carissa edulis, Microglossa pyrifolia*, and *Steganotaenia araliacea* plants. Such knowledge gaps are particularly significant in identifying antagonistic activities against pathogens like *Cercosporae zeae maydis* and *Fusarium verticillioides* which continue to threaten maize yields and compromise food security. Therefore, there is a pressing need for further research to elucidate the secondary metabolites of endophytic fungi associated with these medicinal plants. Therefore, the objective of this study was to investigate the antagonistic abilities of endophytic fungi isolated from the three indigenous Kenyan medicinal plants—*Carissa edulis, Microglossa pyrifolia*, and *Steganotaenia araliacea* and the variety of secondary metabolites production by these endophytes.

MATERIALS AND METHODS

Study site, sampling and processing

Plants material of *Carissa edulis, Microglossa pyrifolia* and *Steganotaenia araliacea* were collected from parts of Kakamega, western Kenya, 0° 17' 3.19'' N and 34° 45' 8.24'' E. Leaves, stems and roots were collected in different cloth bags. Identification of plants was done at Maseno Botanical Garden. Laboratory experiments were carried out at JOOUST microbiology laboratory. Maize for pathogens *Cercosporae zeae maydis* and *Fusarium verticillioides* were isolated from maize fields showing symptoms of the pathogens around Jaramogi Oginga Odinga University of Science and Technology.

Endophytic Fungi Isolation

Isolation of fungal endophytes was carried out according to the procedure developed by Mahadevamurthy et al. (2016). Roots, stems, and leaves of *Carissa edulis, Microglossa pyrifolia,* and *Steganotaenia araliacea* were separately obtained from cloth bags and transported in a cooler box to the laboratory for identification. Roots were washed with running tap water to remove soil attached to them. Stems, roots, and leaves of each plant species were cut separately and dipped in 70% ethanol for 3 minutes then washed with 4% fresh sodium hypochlorite solution for about 5 minutes and finally washed five times with sterilized distilled water. Fungal endophytes were isolated by plating 3-5cm pieces of each plant part separately on Potato dextrose agar plates amended with streptomycin (1.0 g/L) to inhibit bacterial growth. Plates were sealed with parafilm and incubated at 25 ± 2°C for 7 days in a completely randomized design. The endophytic fungi colonies that emerged from the plant host were picked with sterile fine-tip needles and were subcultured on fresh PDA plates without antibiotic to get pure cultures for identification and subsequent procedure.

Isolation of fungal plant pathogens

Diseased maize leaves with specific symptoms of *Cercosporae zeae maydis* and *Fusarium verticillioides* were identified based on field identification manual (CIMMYT 2004), collected and processed according to Khaiyam et al. (2017). Samples were washed thoroughly under running tap water, surface sterilized with 4% NaOCl, rinsed several times with distilled water and blotted to dry. Leaf samples were cut into pieces of approximately 4 cm and placed on sterile moist blotter in a sterile petri dish and incubated at 25°C for 5 days to allow the pathogen to develop and sporulate in growth cabinets under a 12 hour fluorescent light/dark regime. Plant sections were then examined under a binocular microscope Leica DM 500 for the presence of conidia. Conidia were picked with an isolation needle and plated on PDA in triplicates to obtain pure cultures. Plates were incubated at 25°C for 5-7 days and hyphal tips from the advancing colony margins were transferred onto PDA with isolating needle as pure culture and kept at 4°C.

Morphological characterization of *Cercosporae zeae maydis* colonies on PDA; the mycelial colour was grey, light, brown corn silk and white on top and dark grey, brown, corn silk, and grey on the bottom. Morphological characterization of *Fusarium verticillioides*; the colour of the colony varied from white to purple and the type of mycelium from aerial to compact. On PDA, isolates formed aerial mycelia and produced pigmentation that varied from dark violet or dark magenta on PDA media (Leslie and Summerell, 2008).

Pathogenicity* test of *Cercosporae zeae maydis* and *Fusarium verticillioides

This was done according to Shemnkande *et al.* (2023). Five local maize seeds "rachar" were hand sown in nine 30 cm pots containing sterilized soil and kept in greenhouse. Di-ammonium phosphate fertilizer was applied during planting at the rate of 1.5 g/pot. Calcium ammonium nitrate was applied at the second leaf (V2) growth stage at the rate of 2.5 g/pot. Experimental pots were arranged in a randomized complete block design. Fungal isolate was inoculated on fresh PDA and incubated in darkness for 9 days to induce sporulation. Conidial suspension was prepared by adding 5ml of sterile distilled water onto fresh cultures, then straining suspension between two layers of cheesecloth, and conidia concentration was adjusted to 2×10^4 conidia/ml in a spectrophotometer at 460 nm. Four plants were inoculated in triplicates at the sixth leaf (V6) growth stage by spraying the conidia suspension of the pathogens using a hand sprayer until runoff. Inoculated plants were covered with transparent plastic bags for 5 days for maximum humidity.

Growth inhibition potential of endophytic fungal isolates against Cercosporae zeae maydis and Fusarium verticillioides

The growth inhibition activity of isolated endophytic fungi against fungal pathogens was determined using dual culture method (Katoch and Pull, 2017). Discs of isolated endophyte and pathogen measuring 0.5mm were co-cultured at two equidistant opposite ends of PDA plates, sealed with parafilm and incubated at 25±2°C for 7 days. Plates inoculated with fungal pathogen disc at the centre without endophyte served as control. The experiment was replicated thrice with plates arranged in a completely randomized design. Radial growth of pathogenic fungi in the presence and absence of the endophyte was measured after 7 days, and growth inhibition percentage was calculated using the formular

Growth inhibition% = CDC-CDT/CDC *100
Where CDC – represents the colony radial growth of the pathogen in mm on the control plate
CDT- represents the colony radial growth of the pathogen in mm in the treatment plates.

Extraction of Endophytic Fungal Metabolites

Endophytic fungi isolated from all the plant parts were each inoculated in a 500 ml conical flask containing 250 ml of Potato Dextrose broth according to Al-mahi Idris *et al.,* (2013) and incubated for 7 days at 25±2°C. The broth was then filtered and mixed with 250 ml ethyl acetate in a 500 ml conical, shaken to mix and transferred to a separating funnel, and left to stand for 15 minutes. After the solutions were separated, the lower one having PDB was discarded and the upper one was collected and evaporated in a rotor evaporator at 45°C to dryness. The extracts were reconstituted in DMSO for secondary metabolite determination.

Screening endophytic fungal extracts for secondary metabolites

Test for Steroids

2ml of chloroform and 2ml of concentrated H2SO4 was added together with 5ml aqueous fungal crude extract and then heated. The formation of a brown ring indicated the presence of steroids (Setyawati et al. 2019).

Test for Terpenoids

Two milliliters of chloroform was mixed with 5 ml aqueous fungal extract and heated in water bath and then boiled with 3 ml of concentrated H_2SO_4. Formation of grey colour indicated presence of terpenoids (Bhandary et al. 2024).

Test for Saponins

Five milliliters of test solution was mixed with 5ml of water, shaken vigorously, and observed for the formation of foam, which was stable for 15 minutes for a positive result (Gul et al. 2017).

Test for Alkaloids

Presence of alkaloids was determined according to Sheel et al. (2014). Five milliliters of fungal extract was added to Dragendorff's reagent. Appearance of orange red precipitate indicated a positive result.

Test for Flavonoids

An alkaline reagent test was carried out where 2 ml of 2.0% NaOH was mixed with 5 ml aqueous fungal crude extract. A concentrated yellow colour was produced, which became colourless when 2 drops of diluted H2SO4 acid were added. Colourless appearance indicated the presence of flavonoids. (Gul et al. 2017) while persistence of the yellow colour indicated absence of flavonoids.

Test for Tannins

Presence of tannins in the fungal extract was determined by Ferric Chloride Test method as described by Sheel et al. (2014). 50mg of the extract was boiled with 5 ml of 45% solution of ethanol for 5 minutes, cooled and filtered. 1ml of filtrate was diluted with distilled water in a ration of 1:1 and two drops of ferric chloride added. A transient greenish-to-black colour indicated the presence of Tannins.

Data analysis

Data on percentage inhibition of the endophytes was subjected to one way analysis of variance (ANOVA) and means separated by least significant difference at $p \leq 0.05$.

RESULTS

There was no significant difference ($p > 0.05$) in the antagonistic potential of fungal isolates against *Cercosporae zeae maydis* and *Fusarium verticilloides* regardless of the plant species and plant part. Endophytic fungal isolates from Microglossa pyrifolia exhibited varied growth inhibition percentages against the two pathogens (Plate 1 & 2). The highest mean inhibition percentage against *Cercosporae zeae maydis* and *Fusarium verticillioides* was from DENS4 from stem at 67 % and 62 % respectively. The lowest growth inhibition against *Cercosporae zeae maydis* and *Fusarium verticillioides* was produced by isolate DENL2 (40%) and DENR1 (48%) from the leaf and root (Table 1).

Fungal endophytes isolated from stems and leaves of *Steganotaenia aralicea* inhibited the growth of *Cercosporae zeae* maydis and *Fusarium verticilloides* with no significant difference Table 2. Isolate DSTS4 from the stem produced the largest inhibition percentage (76%) against *Cercosporae zeae* maydis followed by isolate DSTS2 (69%) also from the stem. Highest inhibition percentage against *Fusarium verticilloides* was produced by DSTL2 (64.67%) from the leaf followed by DSTS2 (63.3%) from the stem. *Carissa edulis* had only two isolates which also inhibited the growth of the two pathogens without no significant difference. Isolate DSRR2 was more active against *C. zeae maydis* and *F. verticilloides* compared to isolate DSRL3.

Screening of Secondary metabolites of the fungal endophytes extracts

All tested endophytic isolate extracts exhibited positive results for tannins and alkaloids (Plate 3) but negative for saponins regardless of the plant species or plant part from which they were extracted (Plate 2 and Table 3). Terpenoids and flavonoids were detected in extracts from six fungal isolates: DENL1, DENS4, DENR2, DSTL1, DSTS5 and DSRL2. Among these, DENL1, DENS4 and DENR2 were isolated from the leaves, stems and root of *Microglossa pyrifolia*, DSRL2 from *Carissa edulis* leaves while DSTL1 and DSTS5 were from leaf and stem of *S. araliacea* (Table 4). Isolate DENS3 and DSRR2, tested positive for

terpenoids but negative for flavonoids and were isolated from the stem of M. pyrifolia and root of C. edulis. Conversely, DENR1 extract did not exhibit the presence of terpenoids and flavonoids. Ten isolates tested positive for flavonoids were terpenoids (DENL2, DENS1, DENS2, DENR3, DSTR1, DSTS1, DSTS2, DSTS3, DSTS4, and DSRL3).

Table 1: Inhibition % of fungal isolates from *Microglossa pyrifolia* against *Cercosporae zeae maydis* and *Fusarium verticilloides*

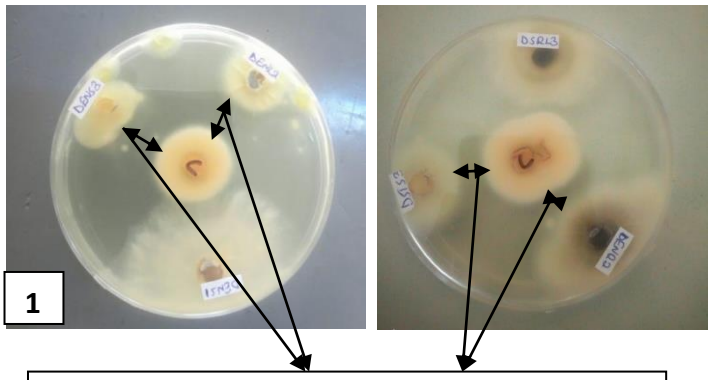
Pathogen	Fungal isolates from *Microglossa pyrifolia*							
	Leaf	Root			Stem			P value
	DENL2	DENR1	DENR2	DENR3	DENS1	DENS3	DENS4	
C. zeae maydis	40[a]	54[a]	40.67[a]	49.67[a]	51[a]	40.33[a]	67[a]	0.75
F. verticilloides	61.67[a]	48[a]	61.33[a]	62[a]	60[a]	49.67[a]	62[a]	0.89

Inhibition % followed by same super script letter is not significantly different

Table 2: Inhibition % of fungal isolates from *Steganotaenia aralicea* and *Carissa edulis* against *Cercosporae zeae maydis* and *Fusarium verticilloides*

Pathogen	Fungal isolates from *Steganotaenia aralicea*							Fungal isolates from *Carissa edulis*		
	Leaf		Stem				P	Leaf	Root	P
	DSTL1	DSTL2	DSTS2	DSTS3	DSTS4	DSTS5	value	DSRL3	DSRR2	value
C. zeae maydis	50[a]	63.33[a]	69[a]	41.67[a]	76[a]	46.67[a]	0.32	51.67[a]	59.33[a]	0.68
F. verticilloides	53.33[a]	64.67[a]	63.33[a]	55.67[a]	59.33[a]	52.33[a]	0.94	59.33[a]	73[a]	0.26

Inhibition % followed by same super script letter is not significantly different



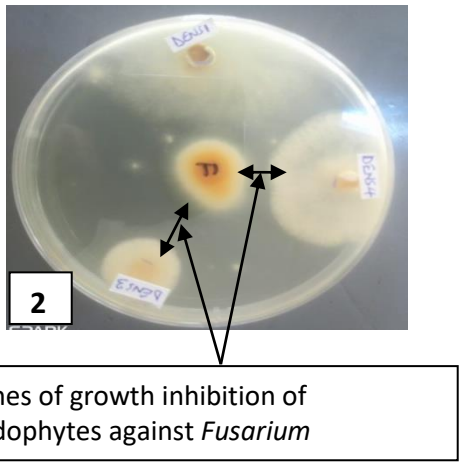


Plate 1 and 2: Zones of inhibition against *C. zeae maydis* and *F. verticilloides*

DISCUSSION

This study findings shows that fungal endophytes isolated from leaves, stems and roots of *Steganotaenia araliacea, Carissa edulis* and *Microglossa pyrifolia* possess a heightened ability to inhibit the growth of *Cercosporae zea maydis* and *Fusarium verticilloides* which are important fungal pathogens of maize. The inhibition of growth could be attributed to their efficient utilization of nutrients in the media, enabling faster synthesis and secretion of antifungal compounds. The selection of endophytes by each plant is influenced by various environmental factors, both abiotic and biotic which also determine the synthesis of potent antifungal chemicals (Hamzah et al., 2018). The prevalence of host medicinal plants like *Steganotaenia araliacea* in environments with low air humidity, such as riverine

and forest edges or open woodlands, affects the diversity of soil microorganisms recruited as endophytes (Opande, 2022) which could have influenced the composition of secondary metabolites synthesised that contains antifungal activities.

Table 4: Secondary metabolites from endophytic extracts

ISOLATE	SAPONNINS	TANNINS	FLAVONOIDS	TERPENOIDS	ALKALOIDS
DSTL1	-	+	+	+	+
DENS3	-	+	-	+	+
DSTS4	-	+	+	-	+
DSTR1	-	+	+	-	+
DSTS2	-	+	+	-	+
DENR3	-	+	+	-	+
DSTS3	-	+	+	-	+
DENS2	-	+	+	-	+
DENL2	-	+	+	-	+
DSRR2	-	+	-	+	+
DENL1	-	+	+	+	+
DENR1	-	+	-	-	+
DENS1	-	+	+	-	+
DSRL3	-	+	+	-	+
DSTS1	-	+	+	-	+
DSRL2	-	+	+	+	+
DENR2	-	+	+	+	+
DSTS5	-	+	+	+	+
DSTL2	-	+	+	+	+
DENS4	-	+	+	+	+

Key: DSR- Carissa edulis DEN- Microglossa pyrifolia DST- Steganotaenea aralicea L- Leaf, S- Stem and R-Root

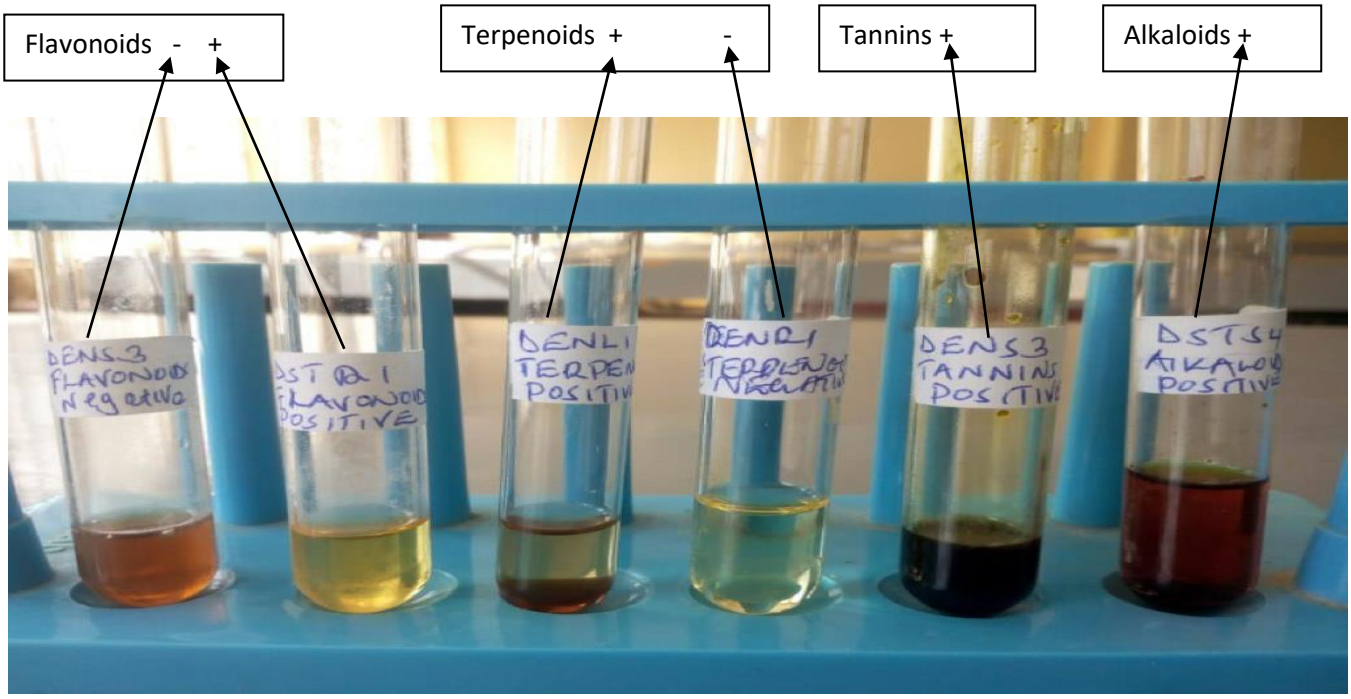


Plate 3: Tubes showing secondary metabolites from endophytic extracts

The inhibitory effects of endophytic fungi against *Cercosporae zeae maydis* and *Fusarium verticilloides* are attributed to their ability to synthesize extracellular enzymes and antifungal compounds in the growth media. These results are in agreement with Grabka et al. (2022) who investigated their role in pests and pathogens management. These compounds, such as volatile oil, n-butanol, and ethyl acetate, inhibit pathogen growth by various mechanisms, including hydrolyzing pathogen proteins and presence of biocontrol genes in some endophytic fungi (Adeleke et al., 2022), which could have been the same mechanisms used in this case.

Notably, endophytic fungi isolated from stems exhibited the highest growth inhibition potential against *Cercosporae zeae maydis*, possibly due to the storage of different secondary metabolites with antifungal properties in the stem. Stems, being prone to attack by feeding insects that may introduce pathogens, harbour more endophytes capable of synthesizing protective chemicals against fungal pathogens (Zaynab et al. 2018). In contrast, leaves contained fewer endophytic fungal isolates with growth-inhibiting abilities. This could be attributed to the fact that endophytes in leaves often prioritize synthesizing chemicals to deter herbivory rather than microbial attacks. The bioprospecting of fungal endophytes associated with medicinal plants holds significant promise in agriculture, primarily due to the presence of secondary metabolites in their crude fungal extracts (Raghav *et al.* 2022; Toppo *et al.* 2023).

The secondary metabolites screening from fungal endophytic extracts revealed presence of Tannins, alkaloids, flavonoids, terpenoids and absence of saponins in the endophytic fungi. The presence of most secondary metabolites could be attributed to the medicinal values of the host plants which are mimicked by the endophytic fungi. Similarly the presence of these secondary metabolites could be because host plants are prone to attack by insects, microbial pathogens and other animals of which they have evolved mechanisms of protecting the host plant against such. These results are in agreement with the report by Milugo et al. (2013) that presence of alkaloids and terpenoids in the absence of saponins confer strongest pathogen inhibition. These results were contrary to those reported by Dawa et al. (2021) in *Carissa edulis* fungal extracts contain saponins. Difference in the secondary metabolites detected in *C. edulis* could be attributed to difference in solvents used in extraction as he used hexane while in this study, ethyl acetate was used. Also, the difference could be because these plants occupied different ecological zones.

Our findings corroborate these previous studies (Chakraborty et al. 2021; Wang et al. 2023; Dawa et al. 2021), highlighting the consistent presence of alkaloids, flavonoids, phenols, terpenoids, and sterols in various fungal extracts.

Some of the fungal extracts tested positive for all the secondary metabolites and negative for saponins. This could be probably because the endophytic fungi and their host medicinal plants could be having a wide range of microbial pathogens, therefore the need to confer protection to the plants. These could also be attributed to other ecological functions of the metabolites such as symbiotic interactions with their host plants and endophytes, competition by other microbes and defense against predation. These results are in agreement with reports from Clemensen et al. (2020) that secondary metabolites have ecological implications that enhance agricultural sustainability. According to reports by Zaynab et al. (2018), secondary metabolites prominently function in protecting plants against microbes and insect attacks and therefore applicable in controlling fungal pathogens and insects. Similar reports have been recorded by Demain and Fang (2000) that secondary metabolites function as competitive weapons against pathogens and agents of symbiotic interactions between microbes, plants, and other organisms.

The presence of alkaloids and the absence of saponins could be attributed to the use of these plants for medicinal purposes which have been exhibited in endophytes to inhibit the growth of *Cercosporae zeae maydis* in this study. These results are in agreement with reports by Milugo et al. (2013) that alkaloid-saponin interaction significantly reduced antagonistic activities as recorded in the antagonistic activity in extracts from *Rauvolfia caffra* but the presence of alkaloids in the absence of saponin had higher inhibition activity. Presence of alkaloids in the extracts indicates that these fungi synthesize bio compounds with diverse activities such as pharmacological characteristics which include antimicrobial and anti-inflammatory effects, which suggests their applications in agriculture, medicine and other industrial uses (Behar et al. 2024).

The presence of flavonoids in some endophytic extracts could also have contributed to their antifungal properties. Flavonoids can crosslink microbial enzymes, and inhibit secretion of cellulases and other microbial enzymes which may act as a physical barrier during pathogen attack (Das et al. 2024). Detection of terpenoids in the extracts could be attributed to pathogenic attacks on host plants which activated the recruitment of endophytes with the ability to synthesize terpenoids by the host medicinal plant. Several terpenoids have their roles in plant defense against biotic and abiotic stresses or they are treated as signal molecules to attract the insects of pollination (Singh and Sharma, 2015).

CONCLUSION

The present study establishes a significant platform for exploring the potential of endophytic fungi derived from *Carissa edulis, Microglossa pyrifolia* and *Steganotaenia araliacea* plants. These endophytes exhibit promising traits such as the production of bioactive secondary metabolites, facilitation of plant growth, and the secretion of extracellular enzymes. The secondary metabolites screening from fungal endophytic extracts revealed presence of Tannins, alkaloids, flavonoids, terpenoids and the absence of saponins. The presence of most secondary metabolites was attributed to their antagonistic activities against plant pathogens since most secondary metabolites are known to possess antagonistic characteristics. Leveraging these fungal endophytes offers a sustainable and environmentally friendly approach to supporting crop development, particularly crucial in the context of contemporary challenges posed by climate change.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

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Floristic Composition, Structure and Diversity of Urban Reserve Forests: An Implication for Biodiversity Conservation and Forest Management

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ABSTRACT

The establishment of forested areas adjacent to urban centers, known as reserve forests (RFs), is increasing and gaining importance in India. However, there is limited understanding of their role in supporting biodiversity. To address this, a study using phytosociological techniques was conducted in three RFs near Tezpur city in Assam, India, to evaluate their contribution to biodiversity. A total of 204 plant species from 180 genera and 64 families were identified. The cumulative curve showed irregularities with asymptotic trends. Species with high importance value indices (IVI) included *Cynodon dactylon* and *Cymbopogon nardus* for grasses, *Clitoria ternatea* and *Chromolaen*a among forbs, *Mikania micrantha* and *Piper betle* among climbers, *Lantana camara* and *Clerodendrum viscosum* among shrubs, and *Tectona grandis* and *Shorea robusta* among trees. The basal area was lowest in grasses (0.04-0.13 m^2 ha^{-1}) and highest in trees (29.18-63.61 m^2 ha^{-1}). Diversity indices ranged from 2.06 to 3.34 (Shannon), 0.04 to 0.17 (Simpson), 0.72 to 0.94 (Pielou's), and 1.3 to 14.62 (Margalef). The Whitford index indicated a contagious distribution pattern. Sørensen similarity was highest between Bhomoraguri and Balipara for grasses (60.87%), shrubs (81.97%), and trees (54.79%), and between Bhomoraguri and Sengelimari for forbs (37.93%) and climbers (54.55%). The floristic composition recorded in RFs suggests a viable strategy for biodiversity conservation in these areas.

Keywords: Biodiversity conservation, Floristic composition, Forest management, Diversity indices, Reserve forests, Urban greening.

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1. INTRODUCTION

India is experiencing a growing trend in establishing forests adjacent to urban centers, known as reserve forests (RFs) or urban forests. Understanding species composition, structure, and diversity is crucial for managing these urban forests (Yan and Yang 2017). Indian forests, including those in Assam, are recognized for their rich biodiversity (Barbhuiya et al. 2013; Dibaba et al. 2019). Reserve forests (RFs) serve as critical habitats for species diversity (Kanagaraj et al. 2017; Nohro and Jayakumar 2020) and help offset CO_2 emissions through carbon sequestration (Enríquez-de-Salamanca 2020; Malunguja et al. 2020; Caviedes and Ibarra 2017). They are also effective in protecting species from extinction (Deori and Talukdar 2015). Like other protected areas, RFs host native species and local endemics, providing various ecosystem goods and services (Giri et al. 2019; Gogoi and Sahoo 2018; Paudel et al. 2022). Reserve forests are designated for specific purposes where human activities are highly restricted. Studies highlight the role of forests, whether urban or rural, in biodiversity conservation (Dri et al. 2024; Baul et al. 2022; Borah et al. 2015; Caviedes and Ibarra 2017; Kanagaraj et al. 2017). Ecological monitoring of forest ecosystems is crucial to estimate their contribution to species sustainability (Flores-Galicia et al. 2024; Anitha et al. 2010; Buragohain and Swargiari 2016; Echeverría et al. 2007; Hubálek 2000; Matuszkiewicz et al. 2013; Malunguja et al. 2020; Naveenkumar et al. 2017; Paudel et al. 2022; Sala et al. 2000; Wade et al. 2003).

In India, reserve forests support species biodiversity and sustainability, as addressed in the Constitution of India, Act 1976 (Kumar et al. 2020). There is a continuous effort to develop a scientific basis for sustainable forest management through forest management plans (Kumar et al. 2020). These plans aim to increase forested areas, particularly reserve forests, adjacent to developing cities as part of an urban greening strategy. Currently, approximately 33.42% of India's total land area is covered by reserve forests (FSI 2019; Gandhi and Sundarapandian 2017). Despite their extensive coverage, there is a lack of studies elucidating the role of reserve forests in biodiversity. This lack of information hinders the evaluation of their ecological well-being. Existing studies on RFs are often specific to certain topics (Bhattacharjee et al. 2014; Borah et al. 2020; Dutta and Devi, 2013; Malunguja and Devi 2022) and focus on other forest categories like biosphere reserves, national parks, and wildlife sanctuaries (Behera et al. 2017; Bora et al. 2017; Dar and Sundarapandian 2015; Deori and Talukdar 2015; Gogoi and Sahoo 2018; Gogoi et al. 2017; Duchok et al. 2005; Giri et al. 2019; Kar et al. 2019; Kalita and Kalita 2014; Sharma et al. 2010; Sumita et al. 2015). Consequently, these data do not provide a complete ecological characterization of reserve forests. A detailed phytosociological study was conducted to create a floristic baseline for better forest management. The study aimed to (i) conduct a floristic inventory of plant species and (ii) assess plant community structure and diversity. Beyond urban greening, the findings of this study will help policymakers, ecologists, and environmentalists develop effective conservation strategies to enhance biodiversity conservation.

2. MATERIALS AND METHODS

2.1 Study area

The current study was conducted in three reserve forests (RFs) in the Sonitpur district of Assam, a state in northeast India. The RFs—Balipara, Bhomoraguri, and Sengelimari—were chosen due to their proximity to Tezpur City, an urban area experiencing rapid industrialization and urbanization. These forests are also connected to one of the busiest National Highways (NH-15), which operates year-round. The district is situated between 92° 16' E and 93° 43' E longitudes and 26° 30' N to 27° N latitudes (Nath et al. 2013). It has a subtropical climate, with seasonal temperatures ranging from a minimum of 7°C to a maximum of 36°C (Saxena et al. 2014). A map showing the locations of the three studied RFs is provided in Fig. 1.

2.2 Vegetation sampling

A total of 105 circular plots with a 15 m radius (covering an area of 707.14 m²) were systematically established along transects (45 in Balipara RF, 30 in Bhomoraguri RF, and 30 in Sengelimari RF). Within each plot, two sub-plots with a 10 m radius (area of 314.29 m²) and a 5 m radius (area of 78.57 m²) were established to record shrubs and herbaceous plants, respectively, following a modified method from Zahabu (2008). The general layout of transect lines and plots is illustrated in Fig. 2. Woody individuals with a diameter at breast height (dbh) of less than 5 cm, characterized by single or multiple stem branches at ground level were recorded as shrubs. Those with a

dbh of 5 cm or more were recorded as trees. The frequency of herbaceous species (%) was determined using the quadrat method (Pieper 1988; Rubanza et al. 2006). Four metal frames of 0.5 m × 0.5 m (area of 0.25 m^2) were placed within the 5 m radius sub-plot to record herbaceous composition. If the frame landed on a previously sampled point or in a dense shrub layer, it was re-thrown to avoid these factors (Czapiewska and Dyderski 2019). Plant species were identified based on their local and botanical names with the help of local botanists and relevant floras. For species difficult to identify in the field, specimens were collected and herbarium samples were prepared for further identification at the Department of Environmental Sciences, Ecology Laboratory, Tezpur University, India.

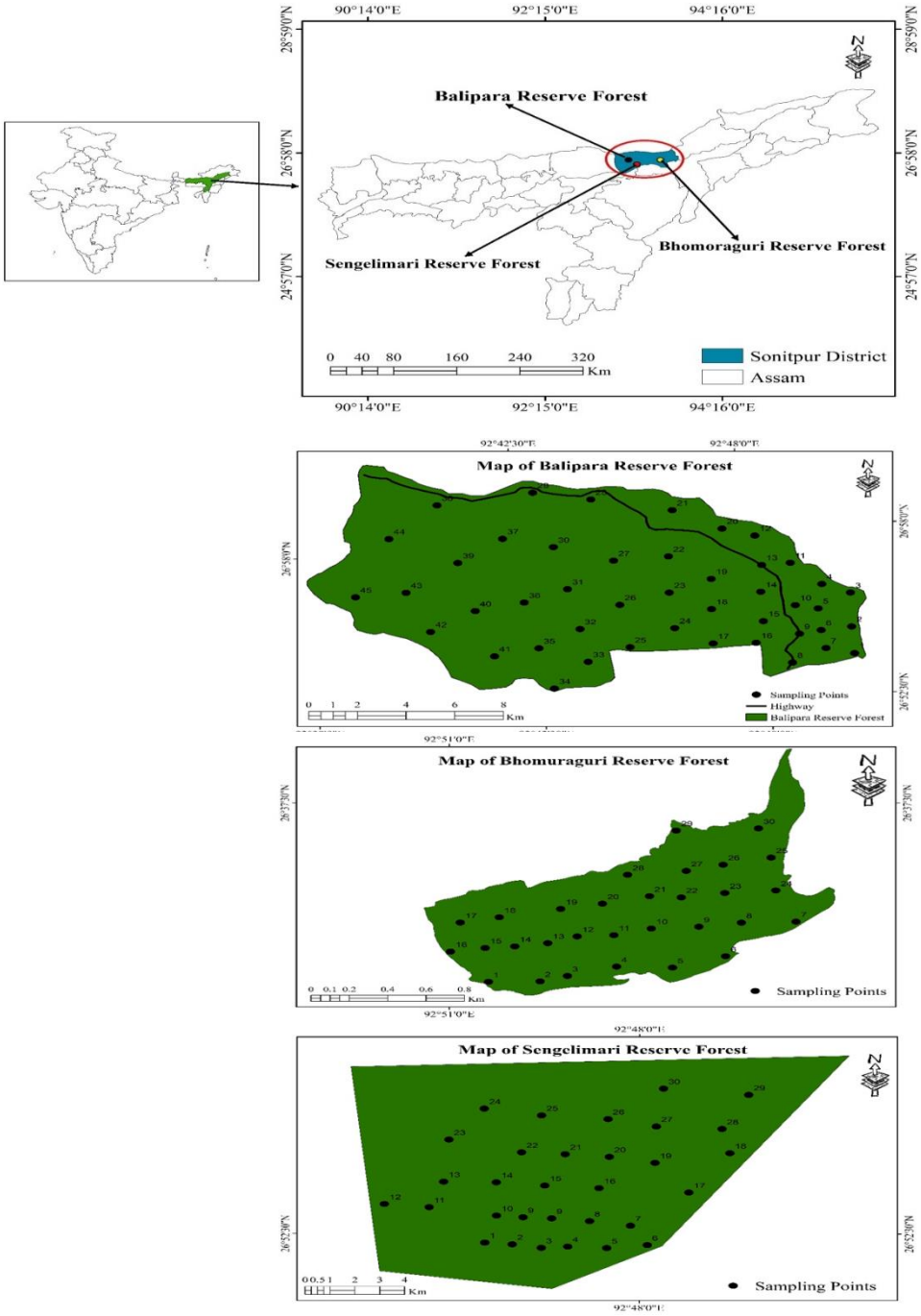


Figure 1: Map showing the location and sampling points of the studied RFs.

2.3 Floristic composition and diversity

Quantitative community attributes and phytosociological characteristics, including species density, abundance, relative frequency, relative

density, and relative dominance, were calculated according to Curtis and McIntosh (1950). The basal area was determined using the allometric method (Kanagaraj et al. 2017). The importance value index (IVI) of individual species was calculated following Misra (1989), which involves summing the relative frequency, relative density, and relative dominance. Plant diversity indices such as the Shannon-Wiener index (Shannon and Weaver 1949), Simpson's dominance index (Simpson 1949), Pielou's evenness index (Pielou 1966), Margalef's index (Margalef 1969), and Sørensen similarity index (1948) were used to quantify phytosociological attributes. The plant distribution pattern was analyzed based on the abundance to frequency ratio (Whitford 1949).

2.4 Statistical analysis

Multivariate analysis was used to determine significant differences in plant diversity both across and within forests. The Shapiro-Wilk and Levene tests were employed for parametric tests to ensure that data fit normal distribution conditions and was homogeneous, respectively. The Fisher's Least Significant Difference (LSD) test was used to determine whether the means were statistically different at $P \leq 0.05$. The Pearson correlation coefficient was employed to examine the relationships between plant life forms (grasses, forbs, climbers, shrubs, and trees). Statistical analyses were performed using the SPSS software package (version 20, Chicago, IL). Additionally, graphs were created using the Origin Pro software package (trial version 8.5) and Microsoft Excel.

3. RESULTS

3.1. Plant species recorded in RFs

The present investigation identified 204 plant species (i.e., 24 grasses, 62 forbs, 19 climbers, 40 shrubs and 59 trees) belonging to 180 genera and 64 families in the three studied reserve forests (RFs). Table 1 enlisted all the plant species enumerated in this study based on their life forms. The results showed that, some species were recorded in all the studied forests like *Cynodon dactylon*, and *Brachiaria reptans* for grasses; *Amaranthus spinosus* and *Centella asiatica* for forbs; *Argyreia speciosa* and *Cissus rotundifolia* for climbers; *Gloriosa superba and Alangium chinense* for shrubs; *Eugenia orbiculata* and *Bombax ceiba* for trees. Species that were only found in one of the studied sites are *Aristida adscensionis* (Sengelimari RF) and *Arundo donax* (Bhomoraguri RF) for grasses; *Achyranthes aspera* and *Chamaecrista rotundifolia* (Bhomoraguri RF) for forbs; *Cissus quadrangularis* (Sengelimari RF) and *Hedyotis scandens* (Balipara RF) for climbers; *Lantana camara* and *Solanum spinosum* (Bhomoraguri RF) for shrubs; and *Acacia retinodes* (Sengelimari RF), and *Caesalpinia pulcherrima* (Bhomoraguri RF) for trees.

Additionally, the present study recorded a variability of plant life forms between the reserve forests. For instance, grasses recorded 24 species from 22 genera and 2 families in three studies reserve forests. Forbs enumerated 63 species from 59 genera and 25 families. Climber recorded 19 species from 17 genera and 11 families, while shrub recorded 40 species from 37 genera and 21 families. Furthermore, trees enumerated 59 species from 53 genera and 29 families. As stated previously, some species were recorded in all three studied reserve forests, some in two or in only one. Such phenomenons lead into variability of individual species in the studied forests. For such reasons, Bhomoraguri RF recorded 123 plant species (13 grasses, 39 forbs, 9 climbers, 31 shrubs, and 31 trees). While, Balipara RF recorded 121 plant species (10 grasses, 29 forbs, 10 climbers, 30 shrubs, and 42 trees). Then, Sengelimari RF recorded 83 plant species (12 grasses, 19 forbs, 12 climbers, 17 shrubs, and 23 trees).

3.2. Community structure analysis

The sampling effort made for studying community structure analysis helps in understanding the rate of accumulation of new species over the increasing sampling units. The species cumulative curve portrayed an irregular along with asymptotic notations to all plant life forms (Fig. 3). The number of shrub and tree species increased along with the increase in number of sampling plots for all studied forests. Such observation suggests presence of diverse species in the community for forbs, shrubs and trees. Furthermore, it was found that in Bhomoraguri RF, grass species only 15 studied plots were enough to record their richness, while, new species was accumulated up to 35 of sampling units in Balipara RF. Similar trend was also observed among forb species for all studied forests. Climber species, on the other hand, were recorded mostly within 15 plots in Sengelimari RF. Such results suggest that, species richness (number of different species), specifically, for grasses and climbers was small as compared to other plant life forms (forbs, shrubs and trees) (Fig. 3).

Table 1: List of plant species recorded in Bhomoraguri RF (1), Balipara RF (2), and Sengelimari RF (3) RFs

Sl. No.	Scientific name	Family	Life form	1	2	3
1	*Aristida adscensionis* L.	Poaceae	Grass	x	x	✓
2	*Arundo donax* L.	Poaceae	Grass	✓	x	x
3	*Axonopus compressus* (Sw.) P.Beauv.	Poaceae	Grass	✓	x	x
4	*Brachiaria reptans* (L.) C.A. Gardner.	Poaceae	Grass	✓	✓	✓
5	*Centotheca lappacea* (L.) Desv.	Poaceae	Grass	✓	x	x
6	*Cymbopogon nardus* (L.) Rendle	Poaceae	Grass	x	✓	x
7	*Cymbopogon schoenanthus* (L.) Spreng	Poaceae	Grass	x	x	✓
8	*Cynodon dactylon* (L.) Pers.	Poaceae	Grass	✓	✓	✓
9	*Cyperus rotundus* L.	Cyperaceae	Grass	✓	✓	x
10	*Cyrtococcum patens* (L.) A. Camus	Poaceae	Grass	x	✓	x
11	*Digitaria ciliaris* (Retz.) Koeler	Poaceae	Grass	✓	✓	x
12	*Eleusine indica* (L.) Gaertn.	Poaceae	Grass	x	x	✓
13	*Eragrostis amabilis* (L.) Wight	Poaceae	Grass	✓	x	x
14	*Eragrostis atrovirens* (Desf.) Trin. ex Steud.	Poaceae	Grass	x	x	✓
15	*Fimbristylis miliacea* (L.) Vahl	Cyperaceae	Grass	✓	✓	x
16	*Hemarthria compressa* (L.f.) R.Br.	Poaceae	Grass	✓	x	✓
17	*Imperata cylindrica* (Linn.) Beauv.	Poaceae	Grass	✓	✓	x
18	*Leersia hexandra* Sw.	Poaceae	Grass	✓	✓	x
19	*Lipocarpa chinensis* (Osbeck) J.Kern	Cyperaceae	Grass	x	x	✓
20	*Lophatherum gracile* Brongn.	Poaceae	Grass	x	x	✓
21	*Paspalum conjugatum* P.J. Bergius	Poaceae	Grass	x	x	✓
22	*Poa angustifolia* L.	Poaceae	Grass	✓	x	x
23	*Thysanolaena latifolia* (Roxb. ex Hornem.)	Poaceae	Grass	x	✓	✓
24	*Tripogon bromoides* Roth	Poaceae	Grass	x	x	✓
Total	No. of species = 24, genus = 22	Family = 2		13	10	12
25	*Abelmoschus moschatus* Medik.	Malvaceae	Forb	✓	x	x
26	*Achyranthes aspera* L.	Amaranthaceae	Forb	✓	x	x
27	*Acilepis saligna* (DC.) H. Robinson	Asteraceae	Forb	x	x	✓
28	*Adiantum capillus-veneris* L.	Pteridaceae	Forb	x	✓	x
29	*Aerva lanata* (L.) Schult.	Amaranthaceae	Forb	x	✓	x
30	*Ageratum conyzoides* L.	Asteraceae	Forb	✓	x	✓
31	*Ajuga decumbens* Thunb.	Lamiaceae	Forb	✓	x	x
32	*Alternanthera sessilis* (L.) R.Br. ex DC.	Amaranthaceae	Forb	✓	x	x
33	*Amaranthus spinosus* L.	Amaranthaceae	Forb	✓	✓	✓
34	*Arum maculatum* L.	Araceae	Forb	✓	x	x
35	*Bidens pilosa* L.	Asteraceae	Forb	✓	x	x
36	*Blumea holosericea* DC.	Asteraceae	Forb	x	✓	x
37	*Cardiospermum halicacabum* L.	Sapindaceae	Forb	x	✓	x
38	*Cassia absus* L.	Fabaceae	Forb	✓	x	x
39	*Centella asiatica* (L.) Urban	Apiaceae	Forb	✓	✓	✓

40	*Chamaecrista rotundifolia* (Pers.) Greene	Fabaceae	Forb	✓	x	x
41	*Chenopodium album* L.	Amaranthaceae	Forb	x	✓	x
42	*Chlorophytum tuberosum* (Roxb.) Baker	Asparagaceae	Forb	x	✓	x
43	*Chromolaena odorata* (L.) R.M.King.	Asteraceae	Forb	✓	✓	✓
44	*Cicuta virosa* L.	Apiaceae	Forb	✓	x	x
45	*Colocasia esculenta* (L.) Schott	Araceae	Forb	✓	✓	✓
46	*Commelina benghalensis* L.	Commelinaceae	Forb	✓	x	x
47	*Corchorus olitorius* L.	Malvaceae	Forb	✓	x	x
48	*Coriandrum sativum* L.	Apiaceae	Forb	✓	x	x
49	*Cyathea cooperi* (Hook. ex F. Muell.)	Cyatheaceae	Forb	✓	✓	✓
50	*Datura stramonium* L.	Solanaceae	Forb	✓	x	x
51	*Diplazium esculentum* (Retz.) Sw	Athyriaceae	Forb	✓	✓	x
52	*Drymaria cordata* (L.) Willd. ex Schult.	Caryophyllaceae	Forb	x	✓	x
53	*Drynaria quercifolia* (L.) J. Sm.	Polypodiaceae	Forb	x	✓	x
54	*Dysphania ambrosioides* (L.) Mosyakin & Clemants	Amaranthaceae	Forb	x	x	✓
55	*Eclipta prostrata* L.	Asteraceae	Forb	x	✓	x
56	*Elsholtzia griffithii* Hookf	Lamiaceae	Forb	x	x	✓
57	*Enydra fluctuans* Lour.	Asteraceae	Forb	✓	x	x
58	*Euphorbia hirta* L.	Euphorbiaceae	Forb	x	✓	x
59	*Galinsoga parviflora* Cav.	Asteraceae	Forb	x	✓	x
60	*Grona triflora* (L.) H.Ohashi & K.Ohashi	Fabaceae	Forb	✓	x	x
61	*Heliotropium indicum* L.	Boraginaceae	Forb	x	✓	✓
62	*Hydrocotyle sibthorpioides* Lam.	Araliaceae	Forb	✓	✓	✓
63	*Jussiaea suffruticosa* L.	Onagraceae	Forb	x	✓	x
64	*Lagenaria siceraria* Hook.f.	Cucurbitaceae	Forb	✓	x	x
65	*Leucas aspera* (Willd.) Link	Lamiaceae	Forb	✓	✓	✓
66	*Leucas zeylanica* (L.) R.Br.	Lamiaceae	Forb	✓	x	x
67	*Lippia geminata* H. B. & K.	Verbenaceae	Forb	x	x	✓
68	*Malva sylvestris* L.	Malvaceae	Forb	✓	x	x
69	*Mirabilis jalapa* L.	Nyctaginaceae	Forb	x	x	✓
70	*Ocimum basilicum* L.	Lamiaceae	Forb	x	✓	✓
71	*Ocimum gratissimum* L.	Lamiaceae	Forb	✓	x	x
72	*Ocimum tenuiflorum* L.	Lamiaceae	Forb	✓	x	x
73	*Oxalis articulata* Savign	Oxalidaceae	Forb	✓	x	x
74	*Oxalis corniculata* L.	Oxalidaceae	Forb	✓	x	x
75	*Persicaria strigosa* (R.Br.) Nakai	Polygonaceae	Forb	x	✓	✓
76	*Physalis peruviana* L.	Solanaceae	Forb	✓	✓	x
77	*Ranunculus multifidus* Forssk.	Ranunculaceae	forb	✓	x	x
78	*Rumex acetosa* L.	Polygonaceae	Forb	✓	x	x
79	*Scoparia dulcis* L.	Plantaginaceae	Forb	✓	✓	✓
80	*Sida acuta* Burm. f	Malvaceae	Forb	x	✓	x
81	*Sphaeranthus indicus* L.	Asteraceae	Forb	✓	x	x

82	*Sphagneticola calendulacea* (L.) Pruski	Asteraceae	Forb	✓	✓	x
83	*Stachytarpheta indica* (L.) Vahl	Verbenaceae	Forb	x	✓	x
84	*Stellaria Apetala* Ucria ex Roem.	Caryophyllaceae	Forb	x	x	✓
85	*Tragia involucrata* L.	Euphorbiaceae	Forb	✓	x	x
86	*Urtica dioica* L.	Urticaceae	Forb	✓	x	✓
Total	No. of species = 62, genus = 59	Family= 25		39	29	19
87	*Argyreia argentea* (Roxb.) Arn. ex Choisy	Convolvulaceae	Climber	✓	✓	✓
88	*Argyreia speciosa* (L.f.) Sweet	Convolvulaceae	Climber	✓	✓	✓
89	*Centrosema brasilianum* (L.) Benth.	Fabaceae	Climber	x	✓	x
90	*Cissampelos pareira* L.	Menispermaceae	Climber	x	✓	x
91	*Cissus quadrangularis* L.	Vitaceae	Climber	x	x	✓
92	*Cissus rotundifolia* Vahl	Vitaceae	Climber	✓	✓	✓
93	*Cucumis anguria* L.	Cucurbitaceae	Climber	✓	x	✓
94	*Dioscorea hoffa* Cordem.	Dioscoreaceae	Climber	x	x	✓
95	*Clitoria ternatea* L.	Fabaceae	Climber	x	✓	x
96	*Hedyotis scandens* Roxb.	Rubiaceae	Climber	x	✓	x
97	*Ipomoea cheirophylla* O'Donell,	Convolvulaceae	Climber	x	✓	✓
98	*Merremia umbellata* (L.) Hallier f.	Convolvulaceae	Climber	✓	x	x
99	*Mikania micrantha* Kunth	Asteraceae	Climber	x	✓	✓
100	*Coronilla varia* L.	Fabaceae	Climber	x	x	✓
101	*Paederia foetida* L.	Rubiaceae	Climber	✓	x	✓
102	*Piper betle* L.	Piperaceae	Climber	✓	x	✓
103	*Smilax ovalifolia* Roxb. ex D.Don	Smilacaceae	Climber	x	✓	x
104	*Thunbergia grandiflora* (Roxb. ex Rottler) Roxb	Acanthaceae	Climber	x	✓	✓
105	*Tinospora sinensis* (Lour.) Merr.	Menispermaceae	Climber	✓	x	x
Total	No. of species = 19, genus = 17	Family =11		9	10	12
106	*Abelmoschus manihot* (L.) Medik	Malvaceae	Shrub	✓	✓	✓
107	*Abrus maculatus* Noronha	Fabaceae	Shrub	x	x	✓
108	*Abutilon indicum* L.	Malvaceae	Shrub	✓	x	x
109	*Alangium chinense* (Lour.) Harms	Cornaceae	Shrub	✓	✓	✓
110	*Antidesma acidum* Retz.	Phyllanthaceae	Shrub	✓	✓	✓
111	*Baliospermum solanifolium* (Burman) Suresh	Euphorbiaceae	Shrub	✓	✓	✓
112	*Capparis spinosa* L.	Capparaceae	Shrub	✓	✓	x
113	*Clerodendrum indicum* (L.) Kuntze	Lamiaceae	Shrub	✓	✓	x
114	*Clerodendrum infortunatum* L.	Lamiaceae	Shrub	✓	✓	x
115	*Clerodendrum viscosum* Vent	Lamiaceae	Shrub	✓	✓	✓
116	*Coffea benghalensis* B.Heyne ex Schult.	Rubiaceae	Shrub	✓	✓	x
117	*Crotalaria albida* Roth	Fabaceae	Shrub	✓	✓	x
118	*Crotalaria sessiliflora* L.	Fabaceae	Shrub	✓	✓	x
119	*Croton caudatus* Geiseler	Euphorbiaceae	Shrub	x	✓	x
120	*Datura metel* L.	Solanaceae	Shrub	✓	x	x
121	*Deeringia amaranthoides* (Lam.) Merr.	Amaranthaceae	Shrub	x	x	✓

122	*Desmodium griffithianum* Benth.	Fabaceae	Shrub	✓	✓	x
123	*Gloriosa superba* L.	Colchicaceae	Shrub	✓	✓	✓
124	*Glycosmis pentaphylla* (Retz.) DC.	Rutaceae	Shrub	✓	✓	x
125	*Grewia eriocarpa* Juss.	Malvaceae	Shrub	✓	✓	x
126	*Holarrhena pubescens* Wall. ex G.Don	Apocynaceae	Shrub	x	✓	✓
127	*Holmskioldia sanguinea* Retz.	Lamiaceae	Shrub	x	x	✓
128	*Justicia adhatoda* L.	Acanthaceae	Shrub	x	✓	x
129	*Lantana camara* L.	Verbenaceae	Shrub	✓	x	x
130	*Lawsonia inermis* L.	Lythraceae	Shrub	✓	✓	✓
131	*Ludwigia hyssopifolia* (G. Don) A.W. Exell	Onagraceae	Shrub	x	✓	x
132	*Maesa indica* (Roxb.) A. DC.	Primulaceae	Shrub	✓	✓	x
133	*Melastoma malabathricum* L.	Melastomataceae	Shrub	✓	✓	✓
134	*Meyna laxiflora* Robyns	Rubiaceae	Shrub	✓	✓	x
135	*Millettia pachycarpa* Bentham	Fabaceae	Shrub	✓	✓	x
136	*Mimosa pudica* L.	Fabaceae	Shrub	✓	✓	x
137	*Mucuna pruriens* (L.) DC.	Fabaceae	Shrub	x	✓	x
138	*Murraya koenigii* (L.) Sprengel	Rutaceae	Shrub	x	✓	✓
139	*Mussaenda roxburghii* Hook.f	Rubiaceae	Shrub	✓	✓	✓
140	*Nerium oleander* L.	Apocynaceae	Shrub	✓	✓	✓
141	*Phlogacanthus thyrsiflorus* Nees	Acanthaceae	Shrub	✓	✓	✓
142	*Rauvolfia serpentina* (L.) Benth. ex Kurz	Apocynaceae	Shrub	✓	✓	x
143	*Schefflera venulosa* (Wight & Arn.) Harms	Araliaceae	Shrub	✓	x	x
144	*Solanum spinosum* L.	Solanaceae	Shrub	✓	x	x
145	*Tamarix dioica* Roxburgh ex Roth	Tamaricaceae	Shrub	✓	x	x
Total	No. of species = 40, genus = 37	Family = 21		31	30	17
146	*Acacia retinodes* Schltdl	Fabaceae	Tree	x	x	✓
147	*Aegle marmelos* (L.) Corrêa	Rutaceae	Tree	✓	✓	x
148	*Albizia lucidor* (Steud.) I.C. Nielsen	Fabaceae	Tree	✓	x	x
149	*Alstonia scholaris* (L.) R.Br.	Apocynaceae	Tree	✓	✓	x
150	*Altingia excelsa* Noronha	Altingiaceae	Tree	x	✓	✓
151	*Annona reticulata* L.	Annonaceae	Tree	✓	x	x
152	*Anthocephalus cadamba* (Roxb.) Miq.	Rubiaceae	Tree	✓	✓	✓
153	*Artocarpus heterophyllus* Lam.	Moraceae	Tree	✓	x	✓
154	*Averrhoa carambola* L.	Oxalidaceae	Tree	x	x	✓
155	*Baccaurea ramiflora* Lour.	Phyllanthaceae	Tree	x	✓	✓
156	*Bombax ceiba* L.	Malvaceae	Tree	✓	✓	✓
157	*Bridelia retusa* (L.) A.Juss.	Phyllanthaceae	Tree	x	✓	✓
158	*Caesalpinia pulcherrima* (L.) Sw.	Fabaceae	Tree	✓	x	x
159	*Careya arborea* Roxb.	Lecythidaceae	Tree	x	✓	x
160	*Cassia fistula* L.	Fabaceae	Tree	x	✓	x
161	*Castanopsis indica* (Roxburgh ex Lindl.) A. DC.	Fagaceae	Tree	x	✓	x
162	*Cedrela sinensis* Juss.	Meliaceae	Tree	✓	✓	x

163	*Cryptocarya amygdalina* Nees	Lauraceae	Tree	✓	✓	✓
164	*Dalbergia sissoo* Roxb.	Fabaceae	Tree	✓	x	x
165	*Dillenia indica* L.	Dilleniaceae	Tree	✓	✓	✓
166	*Duabanga grandiflora* (Roxb. Ex DC.) Walpers	Lythraceae	Tree	✓	x	x
167	*Elaeocarpus serratus* L.f.	Elaeocarpaceae	Tree	x	x	✓
168	*Eugenia orbiculata* Lam.	Myrtaceae	Tree	✓	✓	✓
169	*Ficus elastica* Roxb. ex Hornem	Moraceae	Tree	x	✓	x
170	*Ficus hirta* Vahl	Moraceae	Tree	✓	✓	x
171	*Ficus nervosa* B. Heyne ex Roth	Moraceae	Tree	✓	x	x
172	*Ficus religiosa* L.	Moraceae	Tree	x	✓	x
173	*Garcinia lanceifolia* Roxb.	Clusiaceae	Tree	x	x	✓
174	*Garcinia pedunculata* Roxb. ex Buch. Ham.	Clusiaceae	Tree	x	✓	x
175	*Gmelina arborea* Roxb.	Lamiaceae	Tree	✓	✓	x
176	*Kayea floribunda* Wall.	Clusiaceae	Tree	✓	✓	✓
177	*Lagerstroemia speciosa* (L.) Pers.	Lythraceae	Tree	✓	✓	✓
178	*Macaranga denticulata* (Blume) Müll.Arg.	Euphorbiaceae	Tree	x	✓	x
179	*Melia azedarach* L.	Meliaceae	Tree	✓	x	x
180	*Mesua ferrea* L.	Clusiaceae	Tree	x	✓	x
181	*Michelia champaca* L.	Magnoliaceae	Tree	✓	✓	x
182	*Millettia pinnata* (L.) Panigrahi	Fabaceae	Tree	x	✓	x
183	*Mimusops elengi* L.	Sapotaceae	Tree	x	✓	x
184	*Moringa oleifera* Lam.	Moringaceae	Tree	✓	✓	✓
185	*Morus laevigata* Wall.	Moraceae	Tree	✓	✓	x
186	*Oroxylum indicum* (L.) Benth. ex Kurz	Bignoniaceae	Tree	x	✓	x
187	*Phyllanthus distichus* Müll.Arg.	Phyllanthaceae	Tree	x	✓	x
188	*Psidium guajava* L.	Myrtaceae	Tree	x	x	✓
189	*Pterospermum acerifolium* (L.) Willd.	Malvaceae	Tree	✓	✓	✓
190	*Pterospermum lanceifolium* Roxb.	Malvaceae	Tree	✓	x	x
191	*Pyrus pyrifolia* (Burm.) Nak.	Rosaceae	Tree	x	✓	x
192	*Sapindus mukorossi* Gaertn.	Sapindaceae	Tree	x	x	✓
193	*Senna siamea* (Lam.) Irwin et Barneby	Fabaceae	Tree	✓	✓	✓
194	*Shorea robusta* Roth	Dipterocarpaceae	Tree	✓	✓	✓
195	*Spondias pinnata* (L.f.) Kurz	Anacardiaceae	Tree	x	✓	x
196	*Sterculia villosa* Roxb. ex Sm.	Malvaceae	Tree	x	✓	x
197	*Stereospermum chelonoides* DC.	Bignoniaceae	Tree	x	✓	x
198	*Syzygium cumini* (L.) Skeels.	Myrtaceae	Tree	x	✓	✓
199	*Tectona grandis* L.f.	Verbenaceae	Tree	✓	x	x
200	*Terminalia arjuna* (Roxb.) Wight & Arn.	Combretaceae	Tree	✓	✓	✓
201	*Terminalia chebula* Retz.	Combretaceae	Tree	x	✓	x
202	*Trewia nudiflora* (Linn.)	Euphorbiaceae	Tree	✓	✓	x
203	*Zanthoxylum oxyphyllum* Edgew.	Rutaceae	Tree	x	✓	x
204	*Ziziphus mauritiana* Lam.	Rhamnaceae	Tree	✓	x	x

Total	No. of species = 59, genus = 53	Family = 29	31	42	23
Note: ✓ indicate presence of a species, x absence of a species					

Table 2: Phytosociological attributes of plants inventoried in Bhomoraguri RF, Balipara RF, and Sengelimari RFs

Life form	Community parameters	Reserve forests			Statistics (ANOVA)	
		Bhomo-raguri	Balipara	Senge-limari	F ratio	P value
Grasses	No. of species	13	10	12	-	-
	No. of genus	13	10	12	-	-
	No. of family	2	2	2	-	-
	Margalef index (R)	1.81	1.30	1.6	1.141	0.332
	Density (individual ha^{-1})	265618	368594	351147	0.193	0.826
	Basal area ($m^2 ha^{-1}$)	0.15	0.04	0.13	6.131	0.006*
	Shannon Weiner index (H')	2.34	2.19	2.18	0.816	0.451
	Simpson index (C_D)	0.12	0.13	0.14	0.117	0.89
	Pielou evenness index (J)	0.91	0.94	0.83	2.155	0.132
	Whitford index (WI)	0.84	0.89	1.58	1.283	0.291
Forbs	No. of species	39	29	19	-	-
	No. of genus	36	28	19	-	-
	No. of family	19	22	13	-	-
	Margalef index (R)	4.77	3.64	2.71	51.847	0.000
	Density (individual ha^{-1})	375157	283183	410547	0.87	0.423
	Basal area ($m^2 ha^{-1}$)	0.35	2.28	0.66	12.255	0.000*
	Shannon Weiner index (H')	3.34	3.13	2.79	8.255	0.001*
	Simpson index (C_D)	0.04	0.06	0.07	1.564	0.215*
	Pielou evenness index (J)	0.88	0.83	0.92	19.678	0.000*
	Whitford index (WI)	3.58	2.05	1.4	1.837	0.166
Climbers	No. of species	9	10	12	-	-
	No. of genus	8	9	10	-	-
	No. of family	7	8	9	-	-
	Margalef index (R)	1.25	1.38	1.66	12.53	0*
	Density (individual ha^{-1})	115732	95813	116870	0.039	0.962
	Basal area ($m^2 ha^{-1}$)	2.04	3.62	1.34	4.643	0.018*
	Shannon Weiner index (H')	2.06	2.11	2.34	0.594	0.559
	Simpson index (C_D)	0.14	0.14	0.11	0.29	0.751
	Pielou evenness index (J)	0.93	0.92	0.94	1.94	0.163
	Whitford index (WI)	0.83	1.1	0.84	1.31	0.286
Shrubs	No. of species	31	30	17	-	-
	No. of genus	29	26	17	-	-
	No. of family	18	16	14	-	-
	Margalef index (R)	4.22	4.66	3.09	3.334	0.041*
	Density (individual ha^{-1})	1608	1747	1565	3.80	0.027*
	Basal area ($m^2 ha^{-1}$)	2.88	2.79	3.23	0.75	0.476
	Shannon Weiner index (H')	2.65	2.72	2.44	3.336	0.041*
	Simpson index (C_D)	0.12	0.1	0.14	0.374	0.689
	Pielou evenness index (J)	0.78	0.77	0.86	6.84	0.002*
	Whitford index (WI)	9.76	14.62	3.77	6.579	0.002*
Trees	No. of species	31	42	23	-	-
	No. of genus	29	39	23	-	-

	No. of family	20	26	17	-	-
	Margalef index (R)	5.02	7.02	4.44	10.158	0.000*
	Density (individual ha^{-1})	588	464	313	2.299	0.042*
	Basal area ($m^2 ha^{-1}$)	52.21	63.61	29.18	5.677	0.005*
	Shannon Weiner index (H')	2.49	2.71	2.7	4.08	0.02*
	Simpson index (C_D)	0.17	0.16	0.1	0.039	0.962
	Pielou evenness index (J)	0.72	0.72	0.85	8.149	0.001*
	Whitford index (WI)	7.1	14.07	6.18	2.737	0.07*
Note: * indicates significant at p ≤ 0.05						

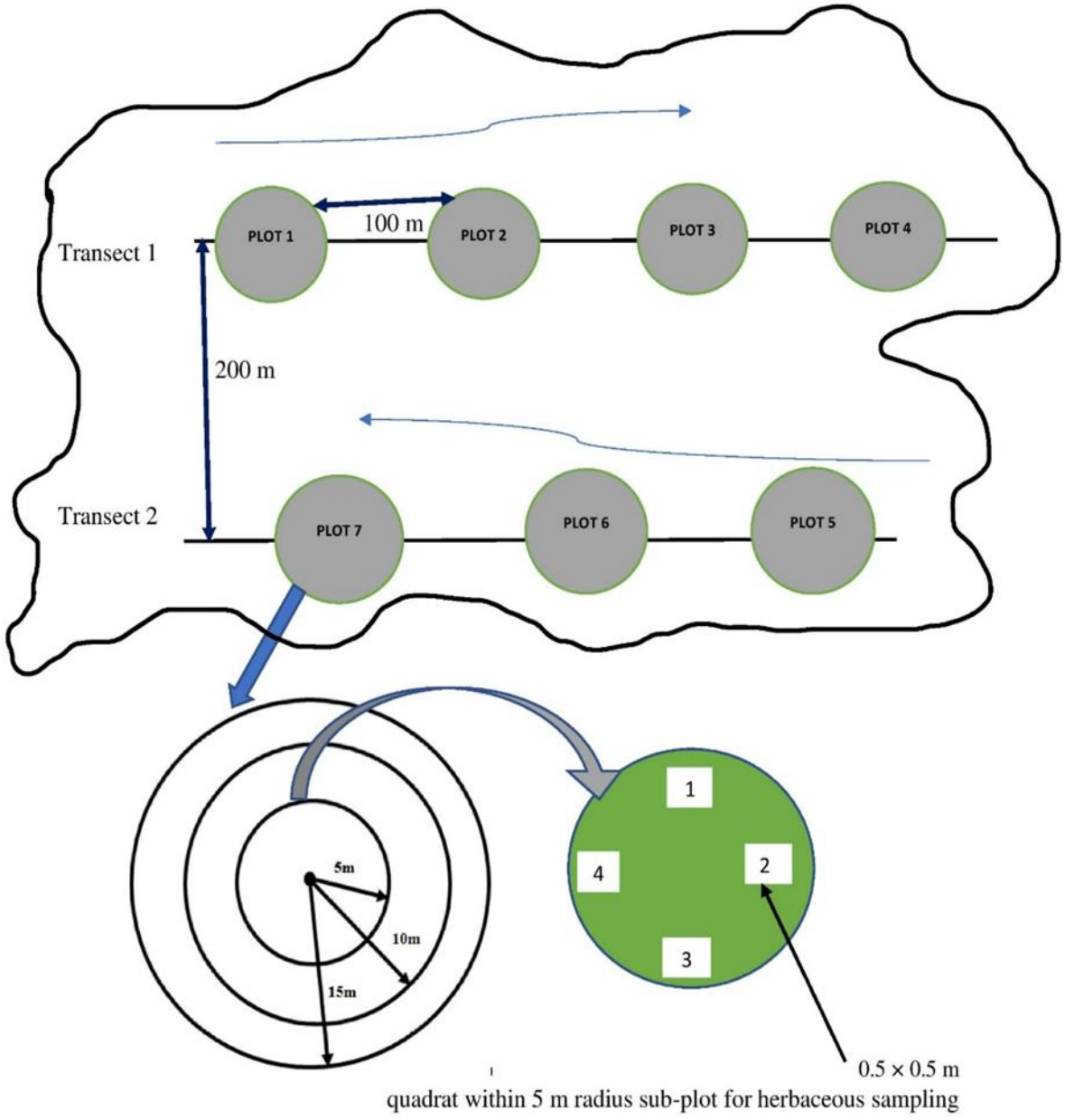


Figure 2: Diagrammatic representation of plots layout and line transects

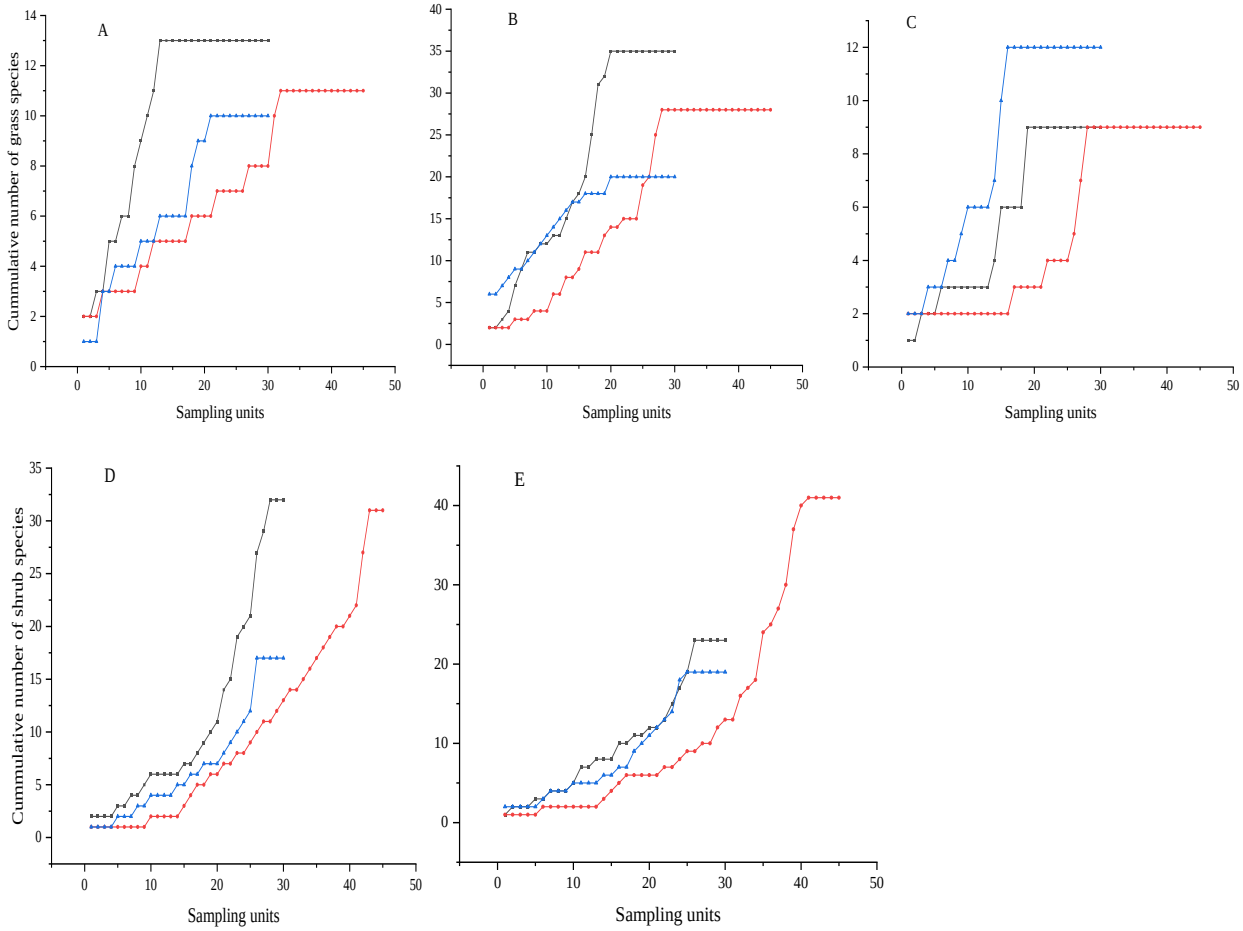


Figure 3: Species-accumulative curve of grass (A), forbs (B), climber (C), shrub (D) and tree (E) species recorded in Bhomoraguri RF ( ), Balipara RF ( ) and Sengelimari RF ( ).

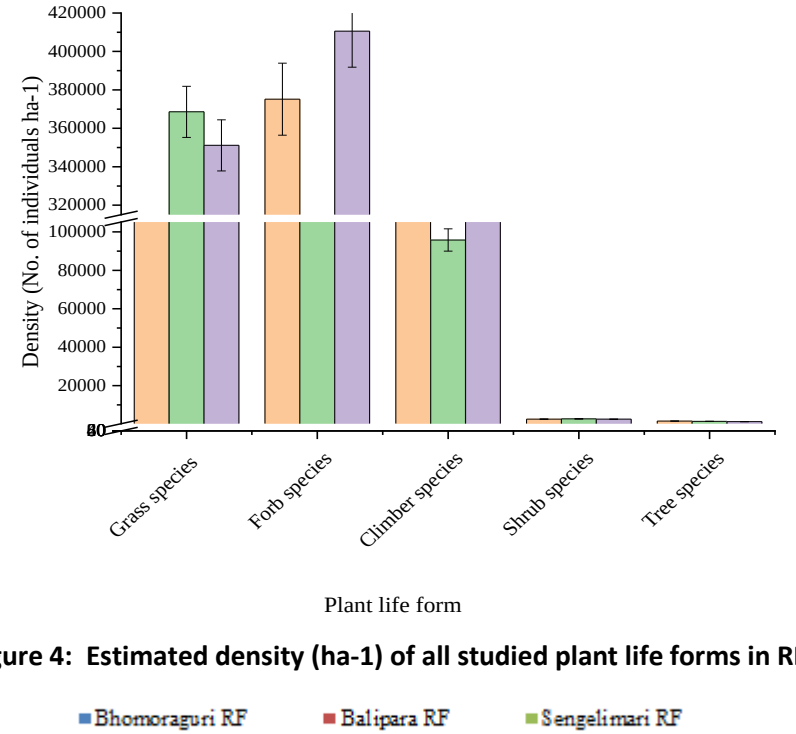


Figure 4: Estimated density (ha-1) of all studied plant life forms in RFs

3.3 Density (individual's ha^{-1}) and basal area (m^2 ha^1) in RFs

Density and basal area are the two important quantitative data which largely determine the dominance of species in a particular forest ecosystem. The overall number of individuals per hectare (individual's ha^{-1}) differed significantly ($p < 0.05$), between forests and plant life forms (grasses, forbs, climbers, shrubs and trees). Analysis of variance (ANOVA) revealed that individual ha^{-1} in the studied reserve forests was significant for trees ($F = 2.299$, $p = 0.042$), and shrub species ($F = 3.802$, $p = 0.027$), but insignificant for grasses ($F = 30.193$, $p = 0.8.26$), forbs ($F = 0.87$, $p = 0.423$), and climbers ($F = 0.039$, $p = 0.962$). The phytosociological attributes of plants inventoried in Bhomoraguri RF, Balipara RF, and Sengelimari RF is summarized in Table 2. Among the herbs, highest density was recorded in forb species than other plant life forms in all the forests. A total of 375157 individual ha^{-1} for forbs were recorded in Bhomoraguri RF, 283183 in Balipara RF, and 410547 individuals' ha^{-1} in Sengelimari RF. Meanwhile, the lowest individual's ha^{-1} was recorded in tree species. For tree species, Bhomoraguri RF recorded 588 stem ha^{-1} followed by Balipara RF (464 stem ha^{-1}) and the lowest stem was recorded in Sengelimari RF having 313 ha^{-1} (Fig. 4.). For shrub and tree species, Sengelimari RF contributed the least number of individuals ha^{-1} and highest was recorded in Balipara RF for shrubs (1747 ha^{-1}) and Bhomoraguri RF for trees (588 ha^{-1}).

Basal area varied significantly between forests which ranged from 0.04 m2 ha^{-1} (as minimum in grasses) to 63.61 m2 ha-1 (as maximum in trees) (Fig. 5.). The analysis results indicated significant for grasses ($F = 6.131$, $p = 0.006$), forbs ($F = 12.26$, $p = 0.000$), climbers ($F = 4.643$, $p = 0.018$) and for trees ($F = 5.677$, $p = 0.005$); but insignificant for shrubs ($F = 0.749$, $p = 0.476$). The highest basal area of 63.61 m^2 ha^{-1} for tree was recorded in Balipara RF, followed by 52.21 m^2 ha^{-1} in Bhomoraguri RF, and the lowest was recorded in Sengelimari RF (28.87 m^2 ha^{-1}) (Fig. 5.). Among the herbs, the lowest basal area was recorded in grass species for all the studied forests (0.15, 0.04, and 0.13 m2 ha^{-1} for Bhomoraguri RF, Balipara RF, and Sengelimari RF, respectively.

3.4 Dominant plant species in RFs

Species exhibiting high important value index (IVI) values does contribute high values either for density or basal area, or both in the community. The dominant plant species varied from one forest to another ($p < 0.05$). The top most dominant grasses, forbs, climbers, shrubs and trees species based on greater IVI values in Bhomoraguri RF, Balipara RF and Sengelimari RF are presented in Fig. 6a, Fig. 6b, Fig. 6c, Fig. 6d and Fig. 6e, respectively. For Bhomoraguri RF, species with great IVI for grasses were *Cynodon dactylon* (40.28), *Imperata cylindrica* (35.82) and *Brachiaria reptans* (33.56); while *Datura stramonium* (22.21) and *Chromolaena odorata* (14.93) were dominant forbs. *Clitoria ternatea* (52.22), and *Piper betle* (51.28) recorded as dominant climber species. *Lantana camara* (43.48) and *Melastoma malabathricum* (23.88) were dominant shrub species. Nevertheless, tree species such as *Tectona grandis* (58.34%) and *Ficus hirta* (25.09) recorded the high IVI values indicating dominant tree species in Bhomoraguri RF.

Similarly, for Balipara RF, the dominant grass species were *Cymbopogon nardus* (49.08), *Cynodon dactylon* (41.72) and *Cyperus rotundus* (37.81). While *Chromolaena odorata* (19.36), *Colocasia esculenta* (23.78), and *Drymaria cordata* (19.28) were the dominant forbs. *Hedyotis scandens* (46.26) and *Mikania micrantha* (50.27) recorded high IVI values for climbers. Moreover, shrubs species like *Clerodendrum viscosum* (45.09), *Justicia adhatoda* (24.09) and *Melastoma malabathricum* (23.94) were dominant in Balipara RF; while tree species such as *Syzygium cumini* (19.26), *Ficus hirta* (19.63) and *Shorea robusta* (60.71) dominated Balipara RF.

On the other hand, species that recorded high IVI values for Sengelimari RF were *Cynodon dactylon* (44.38), *Lophatherum gracile* (33.96) and *Paspalum conjugatum* (33.25) for grasses. *Chromolaena odorata* (22.33) and *Colocasia esculenta* (38.97) for forbs. Species such as *Piper betle* (36.12), *Paederia foetida* (35.45) and *Argyreia speciosa* (30.63) were dominant climbers. For shrubs, *Antidesma acidum* (23.69) and *Melastoma malabathricum* (49.67) were dominant species and *Artocarpus heterophyllus* (50.22) and *Eugenia orbiculata* (38.69) recorded dominated tree species in Sengelimari RF.

3.5 Diversity indices

3.5.1 Shannon-Wiener diversity and Simpson index

Results on Shannon-Wiener index (H') and Simpson index (C_D) recorded in the three studied reserve forests of Assam differed significantly between species ($p < 0.05$). Comparative analysis of species diversity index (Shannon-

Wiener index) between plant life forms (i.e., grasses, forbs, climbers, shrubs and trees) revealed variables results for each plant life form (Table 3). A significant different was recorded between species of different life forms in Bhomaraguri RF (F =13.90, p < 0.001). Furthermore, significant values of F =18.95, p < 0.001, and F = 3.67, p = 0.009 for plant life forms was recorded for Balipara RF and Sengelimari RF, respectively. On the other hand, analysis between forests indicated no significant different for grass species (F = 0.79, p = 0.46), and climbers (F = 0.59, p = 0.559). However, forbs, shrubs and trees differed significantly between forests (F = 8.255, p = 0.001 for forbs; F = 3.336, p = 0.041 for shrub; and F = 4.08, p = 0.022 for trees).

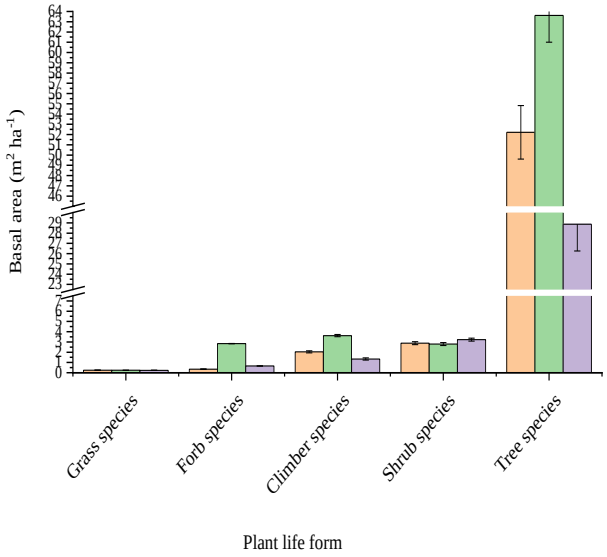


Figure 5: Estimated basal area (m2ha-1) of all studied plant life forms in RFs

For Bhomoraguri RF, forb species recorded the highest diversity (H' = 3.34) and lowest diversity was recorded in climber species (H' = 2.06). A similar trend was also observed in Balipara RF, having greatest diversity in forb species (H'= 3.13); whereby Simpson index ranged from 0.06-0.16, being highest in trees and lowest in forbs (Fig. 7.). Furthermore, forb species recorded great diversity in Sengelimari RF (H' = 2.79; CD = 0.07) followed by tree species (H' = 2.70; CD = 0.11) and the least was recorded by grasses (H' = 2.18; CD = 0.14). Among the studied reserve forests, Bhomoraguri recorded higher diversity for grass, forb and shrub species than Balipara and Sengelimari RFs. Similarly, Balipara RF recorded higher diversity for shrub and tree species than Bhomoraguri and Sengelimari RFs. However, climber species was recorded more diverse in Sengelimari RF than Bhomoraguri and Balipara RFs.

3.5.2 Whitford, Pielous' evenness, Margalef richness indices and Similarity index

The spatial distribution pattern of the species based on abundance and frequency ratio (Whitford index) depicts contagious distribution. Species distribution patterns range from 0.84 to 9.76 (0.84, 3.58, 0.83, 9.76 and 7.10 for grasses, forbs, climbers, shrubs and trees, respectively) in Bhomoraguri RF (Fig. 8). Similar pattern is found in Balipara RF and Sengelimari RF therein it ranges from 0.89 to 14.62 (0.89, 2.05, 1.10, 14.62, and 14.07 for grasses, forbs, climbers, shrubs and trees, respectively) in Balipara RF (8and from 0.84 to 6.18 (1.58, 1.40, 0.84, 3.77, and 6.18 for grasses, forbs, climbers, shrubs and trees, respectively) in Sengelimari RF (Fig. 8). The calculated values fall within the contagious categories of species distribution pattern, suggesting that the studied forests exhibit contagious patterns for all plant life forms.

Pielous' evenness index (J) portrayed a range of 0.72 to 0.93 (i.e., 0.91, 0.88, 0.93, 0.78 and 0.72 for grasses, forbs, climbers, shrubs and trees, respectively) for all plant life forms in Bhomoraguri RF (Fig. 8). Balipara RF, on the other hand, recorded 0.94 for grasses, 0.83 for forbs, 0.92 for climbers, 0.72 for shrubs and 072 for trees; which ranged from 0.72 to 0.94 (Fig. 8). Moreover, a range of 0.83 to 0.94 (0.83, 0.92, 0.94, 0.86, and 0.85 for grasses, forbs, climbers, shrubs and trees, respectively) was recorded in Sengelimari RF (Fig. 8). The Margalef species richness index (R) justifies effective number of species in a particular habitat. Relatively high values are recorded in forbs followed by shrubs and trees. In Bhomoraguri RF, the values ranged from 1.25 to 4.7; 1.3 to 7.2 in Balipara RF, and 1.6 to 4.44 in Sengelimari RF. Forbs recorded 4.77 in Bhomoraguri RF followed by shrubs (R = 4.22) and trees (R = 5.02). Similarly, in Balipara RF, Margalef species richness index values were 3.64 for forbs, 4.66 for shrubs and 7.02 for trees (Fig. 8). And lowest value of forbs is recorded in Sengelimari RF with R = 2.71 followed by shrubs R = 3.09 and trees R= 4.44 (Fig. 8).

The SØrensen similarity index, which is used to measure the degree to which species composition is alike; the higher the value the greater similarity. The result index indicated a maximum similarity between Bhomoraguri RF and Balipara RF (60.87, 81.97 and 54.79% for grasses, shrubs and trees, respectivelly), and between Bhomoraguri RF and Sengelimari RF (37.93 and 54.55% for forbs and climbers) (Table 4). The maximum similarity for grasses (60.87%) was

recorded between Bhomoraguri and Balipara RFs, and the least (24.00%) was recorded between Bhomoraguri and Sengelimari RFs. For forbs, the maximum (37.93%) was recorded between Balipara and Sengelimari RFs, while the lowest (28.21%) was between Bhomoraguri and Balipara RFs. On the other hand, Sengelimari and Bhomoraguri RFs, recorded the maximum (57.14%) for climbers and lowest (31.58%) was between Bhomoraguri and Balipara RFs. Trees and shrubs recorded maximum between Bhomoraguri and Balipara, while the least was between Sengelimari and Bhomoraguri RFs (Table 4). Multivariance analysis (MANOVA) revealed variables results between forests. For instance, Pielous' evenness index was significant for grasses (F =23.038, p = 0.000), forbs (F =19.078, p = 0.000), shrubs (F = 6.84, p = 0.002), and tree (F =8.149, p = 0.001), but insignificant for climbers (F =1.94, p = 0.163).

Table 3: Comparative analysis of diversity index (Shannon-Wiener diversity) between plant life forms and across forests

		Multiple Comparisons (LSD) values					ANOVA between plants
Reserved forests		Grasses	Forbs	Climbers	Shrubs	Trees	
		Sig.	Sig.	Sig.	Sig.	Sig.	
Bhomoraguri RF	Balipara RF	0.236	0.213	0.583	0.957	0.353	F = 13.9, *p* = 0.000
	Sengelimari RF	0.828	0.000	0.271	0.012	0.068	
Balipara RF	Bhomoraguri RF	0.236	0.213	0.583	0.957	0.353	F = 18.95, *p* = 0.000
	Sengelimari RF	0.346	0.002	0.579	0.013	0.006	
Sengelimari RF	Bhomoraguri RF	0.828	0.000	0.271	0.012	0.068	F = 3.67, *p* = 0.009
	Balipara RF	0.346	0.002	0.579	0.013	0.006	
ANOVA between forests		F = 0.816, *p* = 0.451	F = 8.255, *p* < 0.001	F = 0.594, *p* = 0.559	F = 3.336, *p* = 0.041	F = 4.08, *p* = 0.022	

***. The mean difference is significant at the 0.05 level.**

Table 4: Similarity indices (%) of grass, forb, climber, shrub and tree species in RFs

	Grasses			Forbs			Climbers			Shrubs			Tree		
	Bhomoraguri RF	Balipara RF	Sengelimari RF	Bhomoraguri RF	Balipara RF	Sengelimari RF	Bhomoraguri RF	Balipara RF	Sengelimari RF	Bhomoraguri RF	Balipara RF	Sengelimari RF	Bhomoraguri RF	Balipara RF	Sengelimari RF
Bhomoraguri RF	-	-	24.00	-	-	34.48	-	-	57.14	-	-	45.83		-	48.15
Balipara RF	60.87	-	-	28.21	-	-	31.58	-	-	81.97	-	-	54.79	-	-
Sengelimari RF	-	27.27	-	-	37.93	-	-	54.55	-	-	55.32	-	-	49.23	-

3.6 Correlation analysis

Relationship between plant diversity from different plant categories (i.e., grasses, forbs, climbers, shrubs and tree) were determined using Pearson's correlation analysis. The degree of correlation ranged from weak positive and negative to relatively strong (Table 5). The results showed that diversity of forb species was negatively associated to tree species diversity for all the studies RFs (r = -50% for Sengelimari RF; r = -8% for Balipara RF; and r = -5% for Bhomoraguri RF). Similar trend was observed in the relationship between shrub species and tree species diversity (r = -22% for Sengelimari RF; r = -13% for Balipara RF; and, r = -18% for Bhomoraguri RF). These indicate that the tree species are inversely related to forbs and shrub and have a significant negative impact on their diversity. Thus, any increase in number of tree species lowers forbs and shrubs diversity. Furthermore, climber species diversity showed a negative correlation with grass species diversity (r = -15%; r = -7%). On the other hand, grass species diversity exhibited a positive correlation with both

shrubs and tree diversity, with the coefficient value of 20%, 13% and 9% for tree diversity, and 35%, 47%, and 48% for shrubs, suggesting that grass species diversity was affected with other factors rather than the diversity of tree or shrub species.

Table 5: Pearson correlation analysis for different life form of plant diversity recorded in RFs

Bhomoraguri RF					
	Grass diversity	Forb diversity	Climber diversity	Shrub diversity	Tree diversity
Grass diversity	1	0.351 (p = 0.239)	-0.158 (p = 0.684)	0.35 (p = 0.240)	0.199 (p = 0.515)
Forb diversity		1	0.028 (p = 0.942)	0.065 (p = 0.720)	-0.048 (p = 0.797)
Climber diversity			1	-0.245 (p = 0.525)	0.158 (p = 0.685)
Shrub diversity				1	-0.18 (p = 0.333)
Tree diversity					1
Balipara RF					
Grass diversity	1				
Forb diversity	-0.14 (p = 0.699)	1			
Climber diversity	-0.068 (p =0.853)	0.354 (p = 0.315)	1		
Shrub diversity	0.471 (p = 0.169)	-0.054 (p = 0.765)	0.113 (p = 0.756)	1	
Tree diversity	0.129 (p = 0.723)	-0.08 (p = 0.623)	-0.292 (p = 0.413)	-0.132 (p = 0.463)	1
Sengelimari RF					
Grass diversity	1				
Forb diversity	0.162 (p = 0.634)	1			
Climber diversity	-0.392 (p = 0.234)	-0.189 (p = 0.556)	1		
Shrub diversity	0.474 (p = 0.141)	0.286 (p = 0.266)	0.344 (p = 0.273)	1	
Tree diversity	0.094 (p = 0.784)	-0.498* (p = 0.025)	0.233 (p = 0.466)	-0.227 (p = 0.381)	1

4. DISCUSSION

4.1 Floristic composition in reserve forests

The 204 plant species recorded in this study suggest that the RFs under investigation are potential habitats for rich biodiversity. However, an ever-increasing population and rapid urbanization are driving the demand for forest products, leading to biodiversity loss. The presence of grass species from the genus Aristida and Cenchrus, despite their low frequency and abundance, indicates that the ecosystem of the studied reserve forests is disturbed (Rubanza et al. 2006). Similarly, species like Ipomoea spp. and Sida spp. clearly indicate disturbed habitats, which may be caused by specific types of land degradation (Plate 1). In addition to ecological factors, the study observed several human-induced disturbances, such as agriculture, grazing, fuel-wood collection, medicinal herb harvesting, and forest fires within the reserve forests (Plate 2). These activities have contributed to significant vegetation clearance and forest degradation. The high incidence of forest encroachment observed during field visits, particularly in Sengelimari RF, may have led to lower diversity, although this was not investigated in detail. Such activities likely contributed to the vegetation status documented in the studied forests. The investigation also found that valuable timber tree species have been harvested illegally to the extent that it was rare to encounter mature stems, especially in Sengelimari RF. Tree species like Shorea robusta Roth, Tectona grandis, Dillenia indica, and Bombax ceiba are under

threat, as evidenced by the presence of stumps, indicating illegal harvesting of stems.

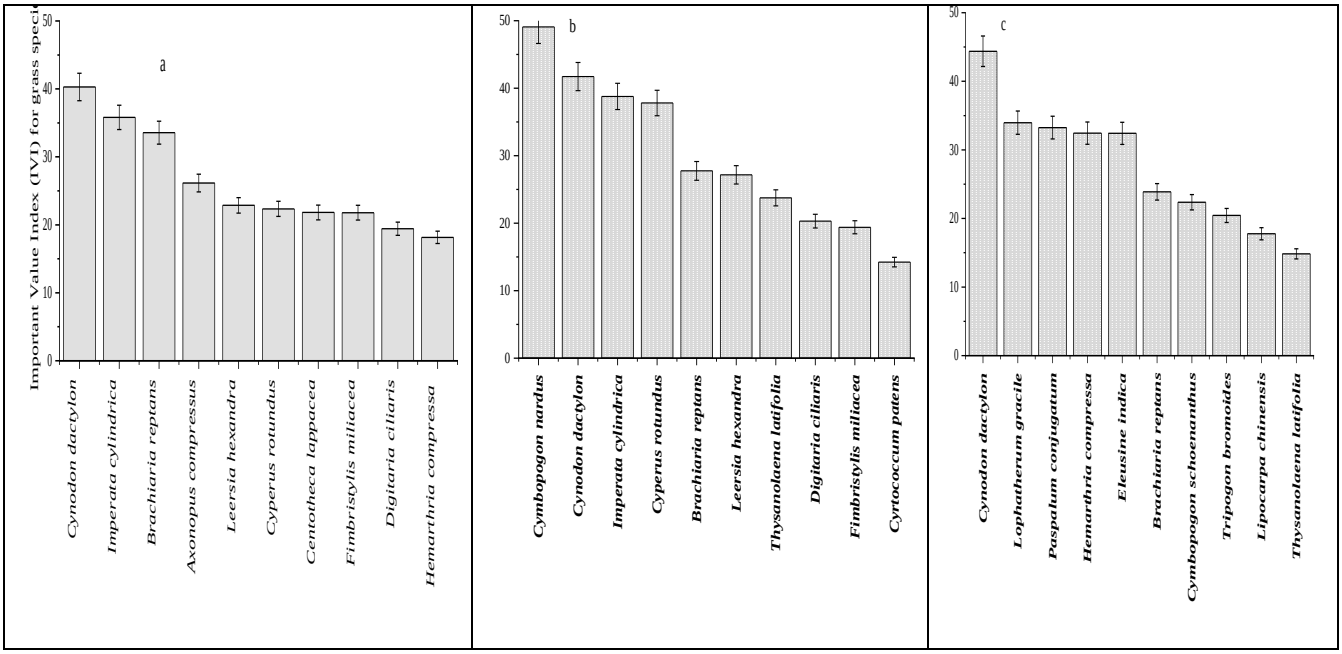


Figure 6a: Top ten dominant grass species based on IVI values in Bhomoraguri RF (a), Balipara RF (b), and Sengelimari RF(c).

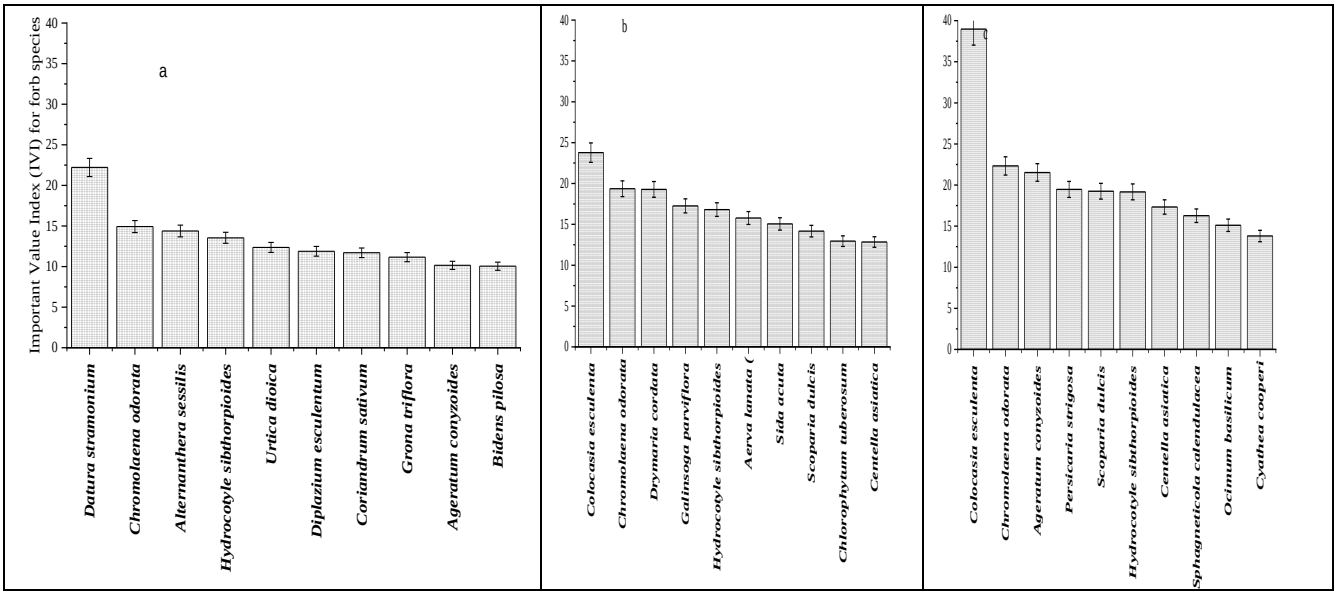


Figure 6b: Top ten dominant forb species based on IVI values in Bhomoraguri RF (a), Balipara RF (b), and Sengelimari RF(c

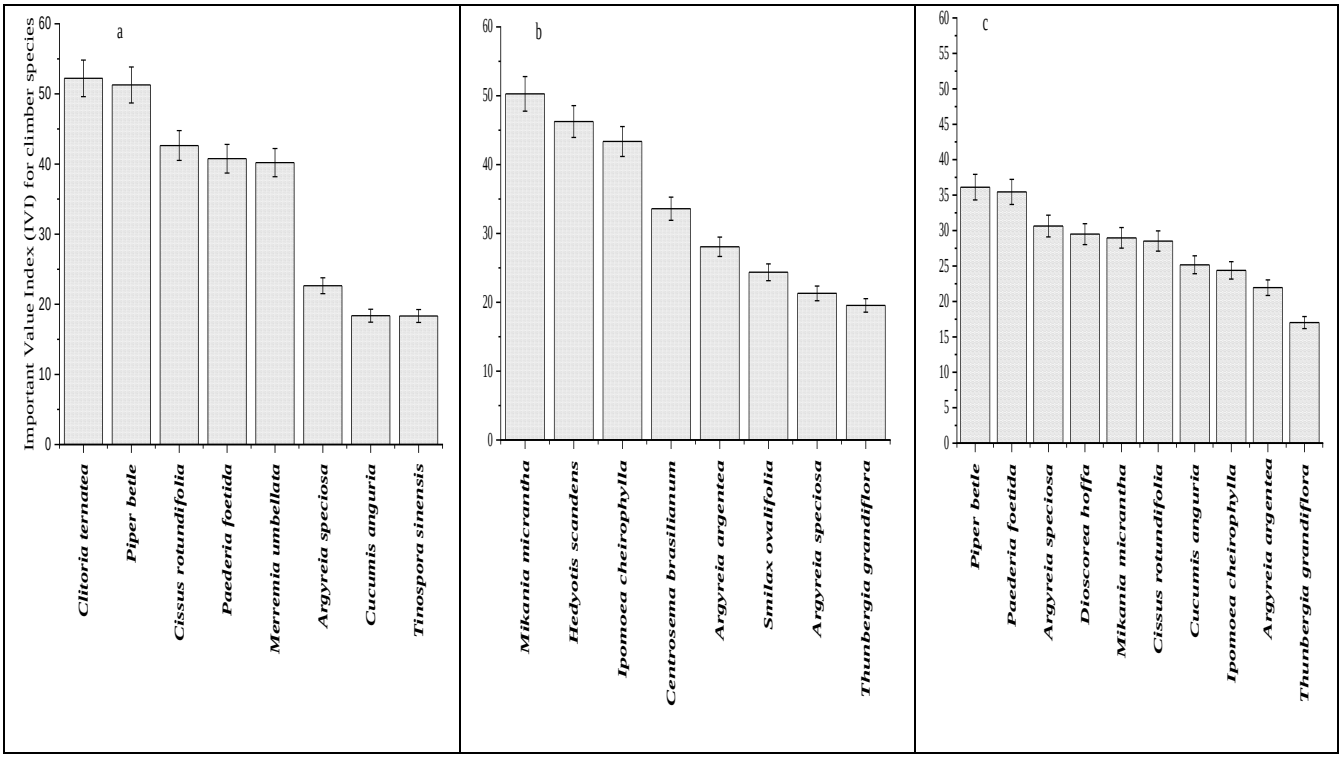


Figure 6c: Top dominant climber species based on IVI values in Bhomoraguri RF (a), Balipara RF (b), and Sengelimari RF(c).

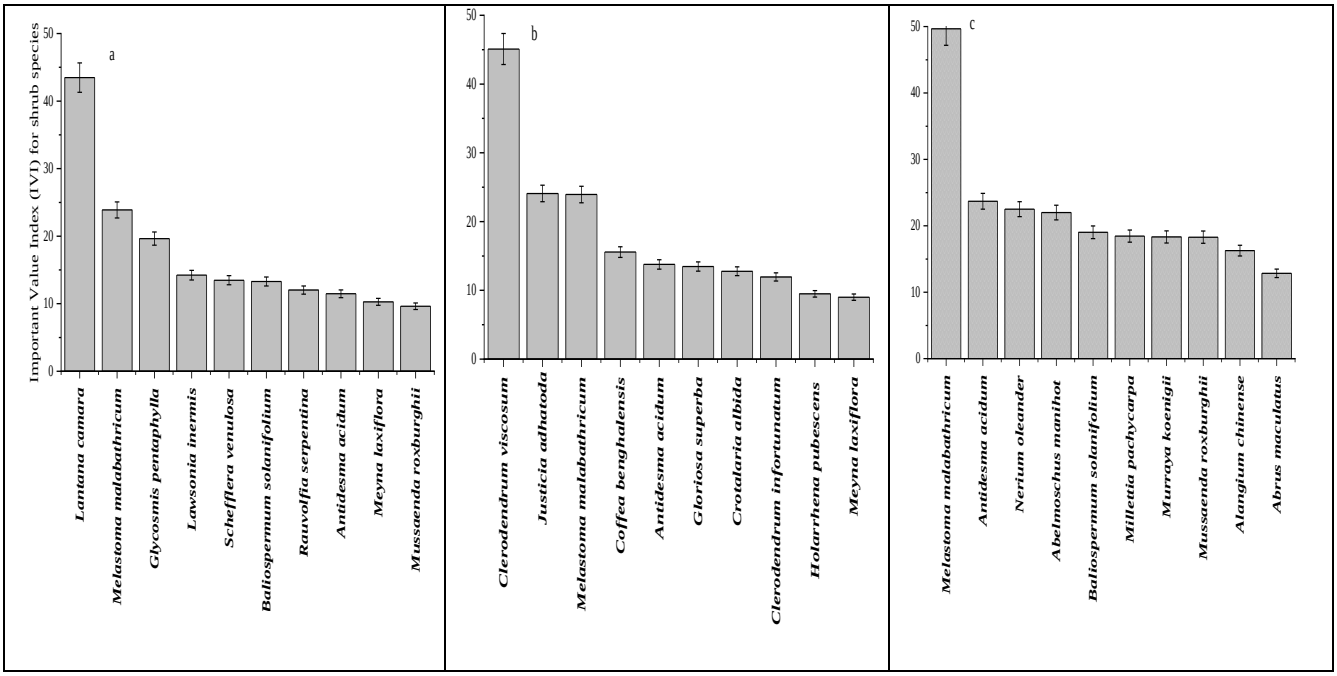


Figure 6d: Top ten dominant shrub species based on IVI values in Bhomoraguri RF (a), Balipara RF (b), and Sengelimari RF(c).

The presence of species like *Chromolaena odorata*, a rapidly growing perennial herb that often acts as a creeper on other vegetation, may have contributed to the poor diversity of grasses and climbers in Bhomoraguri RF. Additionally, the dominance of herbaceous species such as *Hydrocotyle sibthorpioides* and *Cynodon dactylon* reflects their water-loving nature, as these forests frequently experience flooding, favoring the colonization and thriving of such species. Differences in floristic

composition from other reported data could be linked to various forms of anthropogenic activities. For instance, Gogoi and Sahoo (2018) reported that the growing population led to extensive exploitation of natural resources in the tropical rainforest of the eastern Himalayas (India), putting forest biodiversity under severe anthropogenic stress. Thus, proper policies should be implemented. Several scholars have recorded a similar number of species in different forest types. Deori and Talukdar (2015) recorded 159 species (37 climbers, 63 shrubs, and 49 trees) in Laokhowa Wildlife Sanctuary of Assam (India). Kar et al. (2019) recorded 144 species (21 grasses, 35 forbs, 34 climbers, 19 shrubs, and 35 trees) in Borail Wildlife Sanctuary of Assam (India). Gogoi and Sahoo (2018) recorded 129 species (24 forbs, 33 shrubs, and 72 trees) in Jeypore Forest of Assam (India). Borah (2020) recorded 37 herb species in Behali Forest, Assam. Bora et al. (2017) recorded 147 species (35 grasses and 112 trees) in Barail Wildlife Sanctuary. Dutta and Devi (2013) recorded 137 species (19 grasses, 34 shrubs, and 84 trees) in disturbed tropical forests (Doboka RF) of Assam, India. Sarkar and Devi (2014) recorded 98 species (23 shrubs and 75 trees) in Gibbon Wildlife Sanctuary, Assam (India). Sarmah and Borthakur (2009) recorded 602 species (51 grasses, 337 forbs, 21 climbers, 97 shrubs, and 96 trees) in Manas National Park of Assam, India. Similar observations were also reported by Kushwaha and Hazarika (2004).

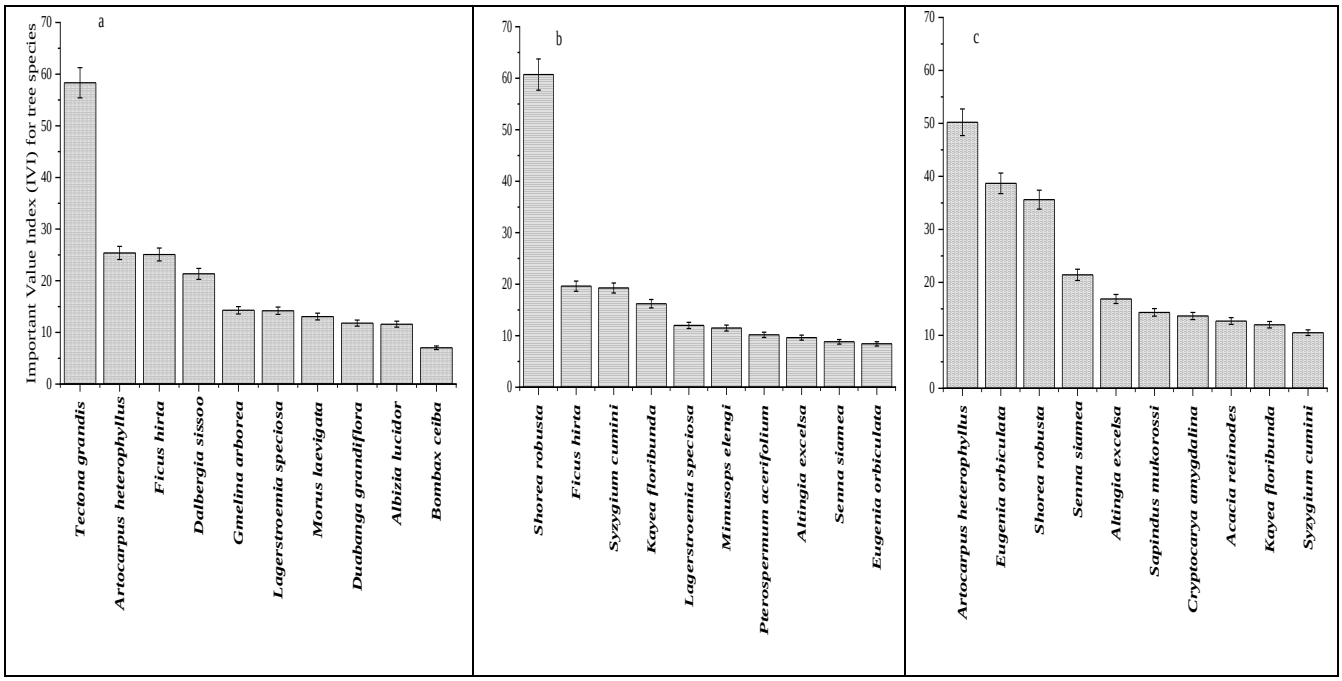


Figure 6e: Top ten dominant tree species based on IVI in Bhomoraguri RF (a), Balipara RF (b), and Sengelimari RF(c).

Furthermore, Kushwaha and Nandy (2012) recorded 477 (230 forb, 113 shrub and 134 tree) species in Moist Sal forests of West Bengal (India). Furthermore, Nath et al. (2005) recorded a range of 14 to 27 and 18 to 50 for shrub and tree species, respectivelly in Tropical wet evergreen forests, Arunachal Pradesh, India, and Parthasarathy, (1999) recorded 114 tree species in Tropical wet evergreen forest of Western Ghat of India. Such studies have reported most of the plant species which are encountered in the present study like, Argyreia spp., Cynodon dactylon, and Brachiaria reptans as dominant, with relatively high density and frequency. The dominat species are Cynodon dactylon., Imperata cylindrica, Brachiaria reptans, and Axonopus compressus for grasses; Datura stramonium, Chromolaena odorata, Alternanthera sessilis and Hydrocotyle sibthorpioides for forbs; Argyreia spp., Cissus rotundifolia and Clitoria ternatea for climbers; Lantana camara, Melastoma malabathricum, Glycosmis pentaphylla and Lawsonia inermis for shrubs; and Artocarpus heterophyllus, Bombax ceiba, Dalbergia sissoo, Duabanga grandiflora, Ficus hirta and Shorea robusta for trees. All these species have contributed to density in the present study.

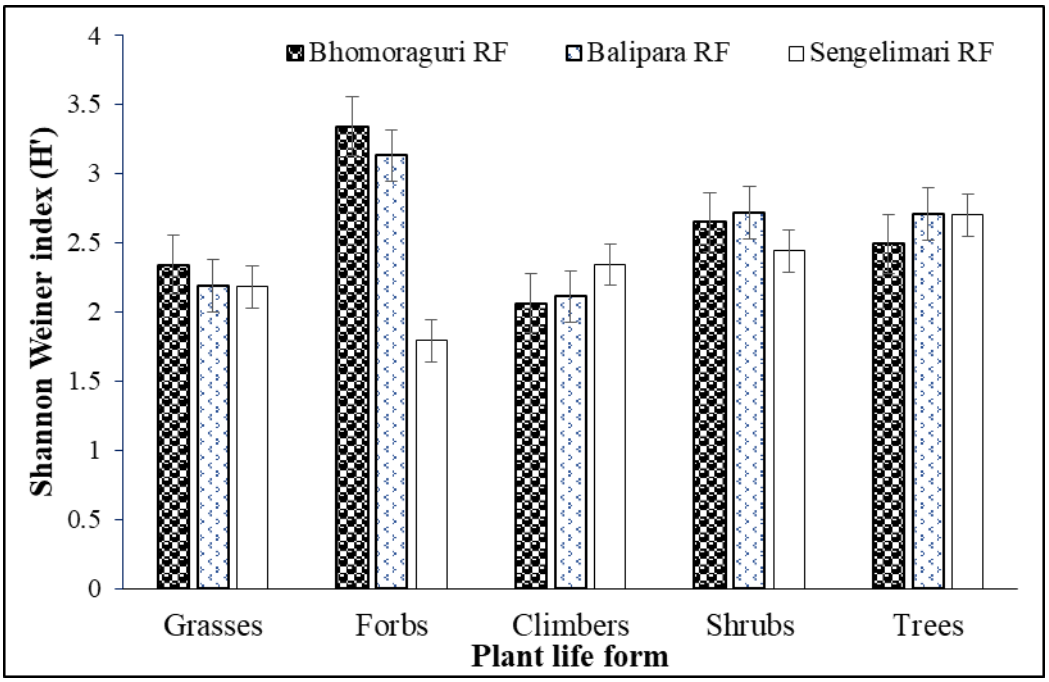


Figure 7: Variability of Shannon-Wiener index (H') in Bhomoraguri RF, Balipara RF, and Sengelimari RF.

Based on the important value index (IVI), studies have reported dominant species that reflected the present study findings. Dutta and Devi (2013) recorded species such as Cynodon dactylon, Ophiuros megaphyllus, Maranta arundinacea, Curcuma amada, Cyperus rotundus, Commelina benghalensis, had relatively high IVI values in Doboka Reserve Forest. Shameem et al. 2010, on the other hand, reported species like Bothriochloa pertusa, C. dactylon, and Stipa sibirica as dominant grasses; while Salvia moorcroftiana, Fragaria nubicola., Galinsoga parviflora and Viola indica as dominant forbs. Furthermore, herbaceous species like Stellaria media, Cynodon dactylon, Persicaria strigosa, Thunbergia grandiflora, Ipomoea cheirophylla, Argyreia nervosa and Ipomoea purpurea were recorded by Kar et al. (2019) in Borail Wildlife sanctuary. The high IVI values and dominant species in Balipara RF such as Dillenia indica (7.94), Sterculia villosa (8.11), Eugenia orbiculate (8.42), Senna siamea (8.83), Altingia excelsa (10.63), Mimusops elengi (11.49), Pterospermum acerifolium (11.78), Kayea floribunda (16.22), Syzygium cumini (19.26), Ficus hirta (19.63), Shorea robusta (60.71) and Lagerstroemia speciosa (12) indicate that these species have more competitive ability to survive and out compete with other tree species. It might also be because of more structural quality through which they can suppress others. Additionally, such species may have better ability to uptake the soil nutrients than others. On the other hand, species like Smilax ovalifolia, Lantana camara and Piper betle are highly resistant and exhibited weed characteristics which increased their dominance and frequency (Naveenkumar et al. 2017). Such species have established themselves as a renowned plant that suppresses the establishment and development of other understory species. Furthermore, Lantana camara is a frequent noxious species in the dry and damp forests (Gogoi and Sahoo 2018; Naveenkumar et al. 2017). It is an aggressive colonizer, especially near forest borders and disturbed ecosystems.

4.2 Density and basal area

The great number of individual ha-1 in the present study was recorded highest in herbs than others plant life forms. Among the herbs; forbs recorded the highest density in Bhomoraguri RF, Balipara RF, and Sengelimari RF. Results on basal area on the other hand, varied significantly for studies plants. For instance, the great trees density and basal area recorded in Bhomoraguri and Balipara RF as compared to Sengelimari RF, could be ascribed by presence of forest security point in Bhomoraguri RF and Balipara RF. This vigilance of forest staff could have reduced people's movement in and out of these forests

compared to Sengelimari RF. The domination of some plant species like Tectona grandis and Shorea robusta, is linked to strategic conservation management by forest department in these RFs, which involves special plantation. The findings from the present study are also comparable to data reported by other scholars from different forest types of India. For instance, Dutta and Devi (2013) recorded a range of 130500 to 237100 individual ha-1 for forbs, 3168 to 5928 individual ha-1 for shrubs, and 138 to 736 individual ha-1 for trees, in Doboka Forest, Gogoi and Sahoo (2018) in Jeypore (36500-16600, 2680-8680 and 235-645 ha-1 for herbs, shrubs and trees, respectively. Other reported data with similar trend includes that of Nohro and Jayakumar (2020) (552.8 ha-1 for trees) in Wetland Forest (India); Nath et al. (2005) (69600-254333, 3080-13280 and 34-610 density ha-1 for herbs, shrubs and trees, respectively) in Tropical wet evergreen forests, Arunachal Pradesh (India); Kushwaha and Nandy (2012) (438 density ha-1 for tree); Parthasarathy (1999) (575-855 density ha-1 for trees) in Tropical wet evergreen forest of Western Ghat, India.

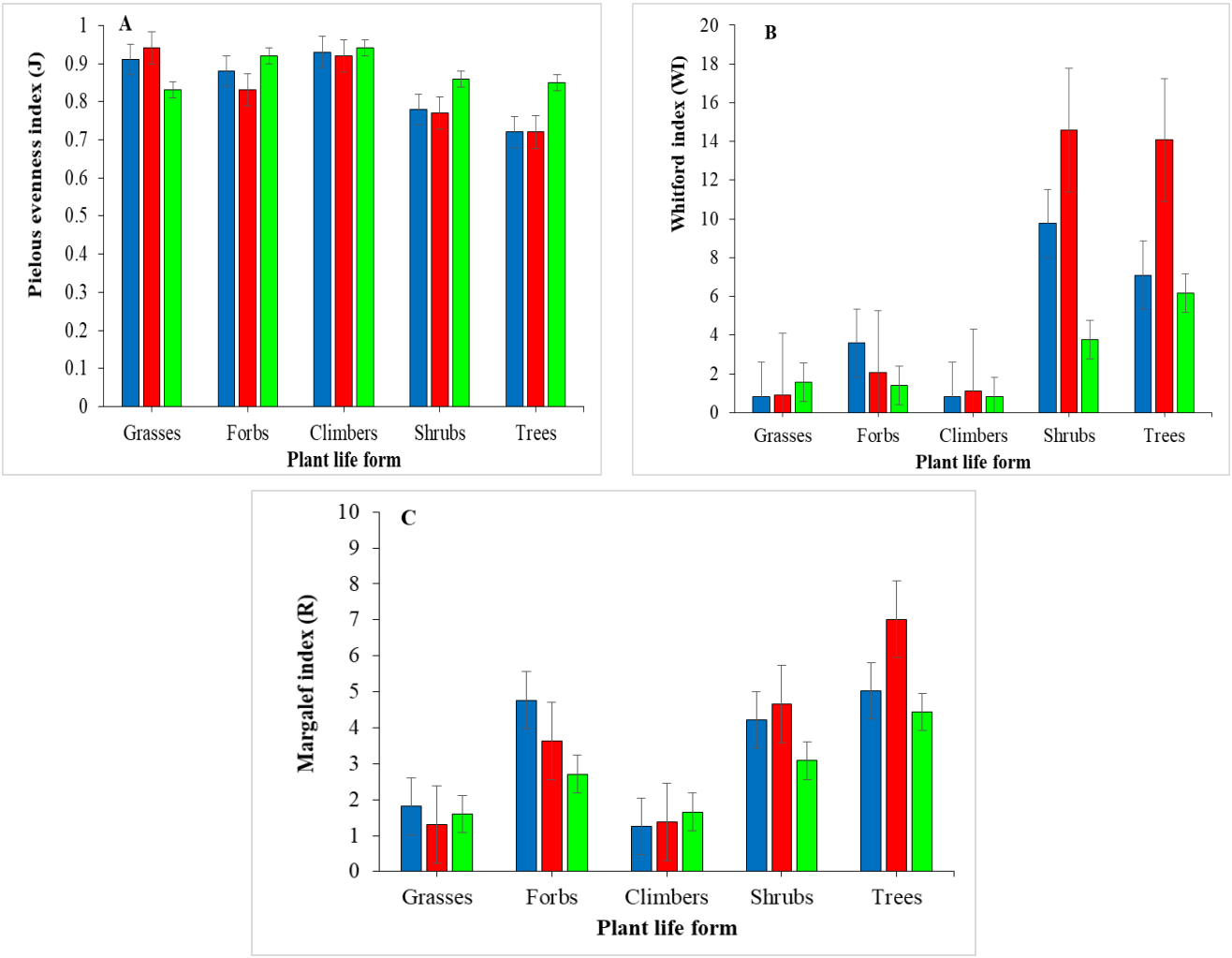
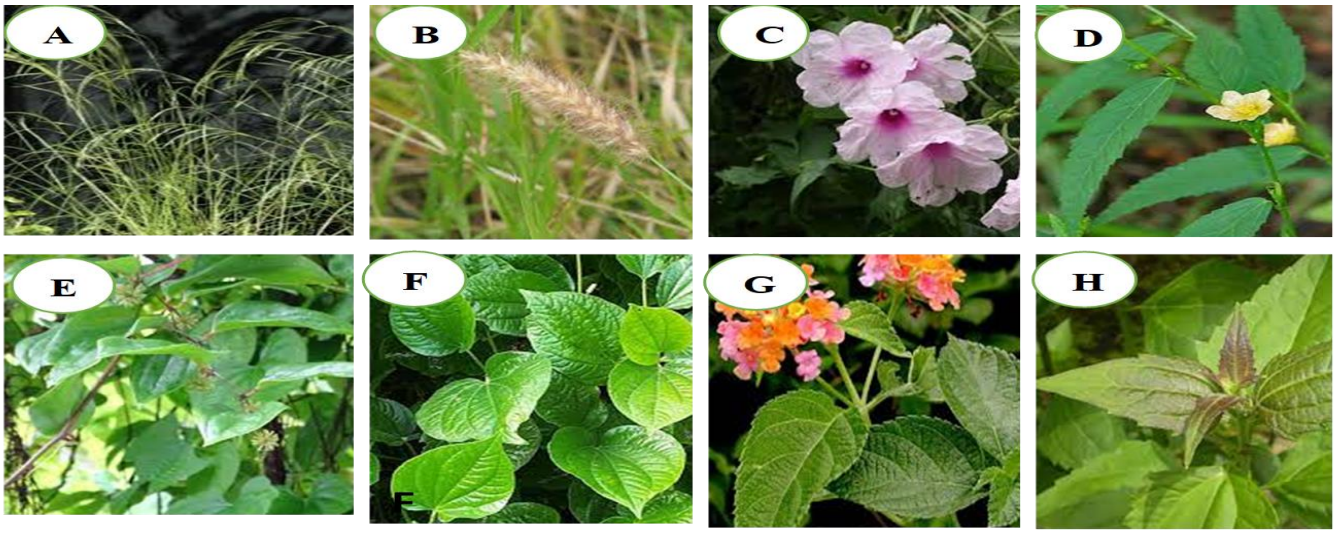


Figure 8: Pielou evenness index (A), Whitford index (B) and Margalef species richness index (C) in Bhomoraguri RF, Balipara RF, and Sengerimari RF.

Results on basal area (m2 ha-1) on the other hand, varied significantly. These results were within the data reported by other scholars from different forest types of India. For example, Kushwaha and Nandy (2012) recorded a basal are of 56.52 m2 ha-1 for tree; Nath et al. (2005) recorded a range of 0.63 to 3.77 m2 ha-1 for shrubs, and 7.81 to 98.58 m2ha-1 for tree species in Tropical wet evergreen forests of Arunachal Pradesh (India); Gogoi and Sahoo, (2018) recorded a range of 0.6 to 2.4 m2 ha-1 for shrubs, and 19.55-108.02 m2 ha-1 for trees, in Jeypore RF. The findings from the present study, reflects the general characteristics of most tropical dry forests of India. However, the differences in terms of individuals ha-1 and basal area (m2 ha-1) of the present results, when compared from other reported data could be due to

different in forest types, and maturation as well as the levels of anthropogenic activities. The high level of human intrusion observed requires a quick reaction; otherwise, they may convert these RFs into other land use system. Special attention should be given to Sengelimari RF, which is spontaneously become a treeless forest and soon will be a history.



***Aristida* spp. (A), *Cenchrus ciliaris* L. (B*), Ipomoea cheirophylla* O'Donell (C), *Sida acuta* Burm.f. (D), *Smilax ovalifolia* Roxb. ex D. Don (E), *Piper betle* L. (F), *Lantana camara* L. (G), and *Chromolaena odorata* (L.) R.M.King & H.Rob.(H).**

Figure 9: Species that indicate disturbed ecosystems (A, B, C, and D), and those that exhibits weed characteristics (E, F, G, and H) recorded in RFs.

Forest fire observed in Balipara RF (A), Trespass observed in Bhomoraguri RF (B), Bundles of firewood seen in the borders of Bhomoraguri RF (C), Tree stump observed in Sengelimari RF (D), Small scale rice field (farms) observed in Sengelimari RF (E), and harvesting of cultivated crops observed in Balipara RF (F).

Figure 10: Observed anthropogenic activities in studied RFs.

4.3 Analysis of diversity indices

Although results on species diversity varied significantly between plant life form and forests. However, the analysis of diversity indices illustrated a promising floristic diversity in the studied reserve forests. Shannon index (H') and Simpson diversity index (CD) were used to determine diversity and richness of a species present in the studied forests.

Higher values of H' indicate diverse and equally distributed community, and lower values represent less diverse community. Simpson diversity index (CD) on the other hand, the lower CD values represent higher diversity (Giri et al. 2019). Whitford index (WI) which is the ratio of abundance and frequency ratio (A/F) was used to assess the distribution pattern of species, while Pielou evenness index assessed species evenness. The high Shannon index (H') values for forbs in Bhomoraguri RF (3.34, dominated by Tectona grandis) and in Balipara RF (3.13, dominated by Shorea robusta) indicates that, the dominant tree species have less suppressing power on understory species. They provide an excellent opportunity for other plant species to coexist and populate the habitat. The present study's findings revealed a reasonably high flora species diversity that differed considerably ($p < 0.05$). Forb species showed a significantly higher diversity in all the studied RFs. Such results imply a relatively promising vegetation coverage in the studied RFs, which was within the range of 1.85 to 5.68 reported from other India's forests (Dibaba et al. 2019; Raha et al. 2020). For example, Nohro and Jayakumar (2020) reported Shannon index values of 3.73; Gogoi and Sahoo (2018) reported a range of 2.44 to 3.37; Dutta and Devi (2013) in Doboka Reserve Forest, recorded a range of 2.02 to 2.43; Karki et al. (2016) recorded a range of 2.65 to 3.47 in Central Himalaya Forest of Assam (India); Kushwaha and Nandy (2012) recorded (H' = 3.08-3.10); and Deori and Talukdar (2015) (H' = 3.61-4.01). Anthropogenic activities were the mostly reported factors which affected plant diversity (Begum et al. 2011; Konwar et al. 2009; Sumita et al. 2015). The indiscriminate felling of the species coupled with poor regeneration is likely to cause species to be vulnerable to extinction.

Species distribution patterns varied between forests, in Bhomoraguri RF (0.84-9.76), Balipara (0.89-14.62) and Sengelimari RF (0.84-6.18). The calculated values fall within the contagious categories of species distribution pattern, suggesting that the studied forests exhibit contagious patterns to all plant life forms. Pielous' evenness index (J), on the other hand, varied between forests in Bhomoraguri RF (0.72-0.93), Balipara (0.72-0.94) and Sengelimari RF (0.83-0.94). The Margalef index which justifies effective number of species in a particular habitat, differed between forest and plant life forms. In Bhomoraguri RF (1.25-4.7), Balipara (1.3-7.2) and Sengelimari RF (1.6-4.44). The Whitford index revealed that most of the species were contagiously distributed, suggesting that species in the studied forests performed contagious pattern of distribution. The domination of contagious distribution may be due to the fact that the majority of species reproduce vegetatively in addition to their sexuality (Shameem et al. 2010). An abundance of species on the other hand, increased proportionately with the value of Margalef index. The results suggested for the number of effective species in the studied forests. For instance, despite large number of tree species (n = 42) in Balipara RF, only 6 species namely Shorea robusta Roth, Syzygium cumini, Ficus hirta, Lagerstroemia speciosa, Altingia excelsa and Kayea floribunda were dominating more effectively in the entire ecosystem. These findings are comparable with the data reported by Gogoi and Sahoo (2018) (1.63-2.92 for herbs, 2.2-3.27 for shrubs, and 4.67-10.91 for tree) in Jeypore reserve forest of Assam, India.

4 CONCLUSION

Understanding species distribution, structure, and diversity patterns in reserve forests is essential for their effective planning, management, and sustainable use. The present study highlighted that reserve forests are potential biodiversity centers and play a significant role in floristic composition. However, the presence of species from the genus Aristida and Cenchrus, which are typically good indicators of disturbed ecosystems, suggests specific types of land degradation, mainly due to anthropogenic activities. The distribution of these species may have been accelerated by human encroachment. Therefore, immediate action is required; otherwise, these forests may be converted to other land uses. If the appropriate authorities do not take action, even the notable recorded forest biodiversity may become stressed by human activities.

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Declarations

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Authors' contributions

All authors contributed equally to this work: Gisandu K. Malunguja drafted the manuscript, conducted fieldwork, collected data, analyzed and interpreted the results, and compiled the findings. Ashalata Devi supervised the study, analyzed data, corrected errors, typeset the text, and assisted with fieldwork-related materials.

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