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Journal of Agriculture & Forestry Research (JAFR)

Editorial message

Dear Readers, Authors, and Colleagues,

As we gather here for another edition of the Journal of Agriculture and Forestry Research, it is with immense pleasure and enthusiasm that we welcome you to the pages of this esteemed publication.

Our journal has long been committed to advancing the knowledge and understanding of agriculture and forestry practices, environmental sustainability, and the challenges facing our planet. We believe that by exploring innovative research, sharing valuable insights, and fostering collaboration among researchers, we can make a significant impact on the future of agriculture and forestry.

We are particularly excited about the growing interest in interdisciplinary research. Agriculture and forestry are intricately linked to environmental science, economics, technology, and more. We encourage authors to explore the intersections of these fields and submit articles that offer holistic perspectives on the complex issues we face.

As we look to the future, we remain committed to the principles of quality, relevance, and accessibility. We aim to maintain the high standards of peer review and editorial oversight, ensuring that the articles published in our journal are rigorously vetted and provide valuable insights to both researchers and practitioners. Furthermore, we will continue our open-access approach to make our content accessible to a global audience, fostering a culture of knowledge exchange and collaboration.

We would like to extend our heartfelt appreciation to our dedicated team of editors, reviewers, and staff who work diligently to make this journal a success. We also express our gratitude to our authors for their insightful contributions and to our readers for their continued support.

If you are an aspiring author, researcher, or practitioner in the field of agriculture and forestry, we invite you to consider the Journal of Agriculture and Forestry Research as a platform for sharing your work. Your contributions are instrumental in advancing our collective understanding and addressing the challenges that lie ahead.

Thank you for your continued support and trust in our mission. We look forward to the fruitful exchange of ideas and knowledge that will shape the future of agriculture and forestry research.

Sincerely,

Editor-in-Chief
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Journal of Agriculture & Forestry Research (JAFR)

Contents

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Research Article

Open access

Assessment of Changes in Land Use and Land Cover in Hadejia Nguru Wetland of Yobe State, Nigeria

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ABSTRACT

The study assessed the change in land use and land cover changes in HNWs. Parameters evaluated include; changes in land use land cover over a 40-year window (1979-2019). Data collection for. Changes in land cover/ land use were obtained for a period of 40 years from Satellite imageries of the study area were officially downloaded from the United States Geological Survey website. Changes in land cover/ land use. Results showed that thick vegetation reduced from 14,284.25ha in 1979 to 9,560.36ha in 2019, Grassland increased from 6,339.38ha to 7,306.72ha, water bodies decreased from 4,131.70ha to 1,095.62ha and bare surface increased 1556.35ha to 8,348.98ha during the same period. Results also showed an association between respondent's occupational change and changes in wetland resources availability. There was also a significant Chi-square value (33.481a) between respondents' effect of change in land use and increase in farm sizes (318.431a) majority of the respondents (78.25%) were males, 68% were married, Agric/farming (15.25%) topped the occupation list, age brackets of 45-50 was highest (23.75%) and 34.5% (highest) of the respondents had primary school education. The highest household size of the respondents (51.75%) was between 7 and 9 children, the highest previous yearly income (36.5%) was between N 50,000. 00 and N 100, 000. 00 per annum while the highest (36.5%) present yearly income was from N 1,000.000, 00 to N 1,500,000.00. majority of the respondents (80.25%) had awareness of change in land cover/land use, 89.25% were aware of the impacts of Changes in land cover/ land use on livelihood and 30.5% indicated sparse distribution as a major effect of Changes in land cover/ land use.. Result of LULC. There is the need to put in place right policies to protect and preserve wetlands. . It is therefore concluded that the changes in LULC which has led to changes in livelihood patterns of the wetland communities highly significant.

INTRODUCTION

Land use and land cover (LULC) describe the economic use of land and surface features, respectively. The Land cover reflects the biophysical state of the earth's surface including the soil material, vegetation, forest estate natural and man-made features, cultivated and

human settlements, and water. Land use, on the other hand, refers to the use of land by humans. It is the alterations done to Land cover as a result of human activities such as farming, road construction, human settlements/urbanization and industrialization (Panel, et al., 2023).

Land use and land cover are dynamic in nature and they provide a comprehensive understanding of the interaction and relationship of anthropogenic activities with the environment. Land use/cover changes also involve the modification, either direct or indirect, of natural habitats and their impact on the ecology of the area. Land use/cover change has become a central component in current strategies for managing natural resources and monitoring environmental changes (Zaitunnah et-al, 2018) Humans play a major role as forces of change in the environment, inflicting environmental change at all levels, ranging from the local to the global scale. The various uses of land for economic purposes have greatly transformed land cover at a global scale over the last 10,000 years, almost half of the ice-free earth surface has changed and most of the result was due to the use of land by humans (Joseph, et al., 2020). Land use and land cover changes are environmental issues mostly linked to climate change in a complex manner, and changes in both can have profound effects on an ecosystem‘s ability to provide goods and services to human society Land use and land cover changes play a key role in climate changes through the exchange of greenhouse gases, sensible heat, and local evapotranspiration Approximately 35% of the CO2 emissions to the atmosphere were from land use. In addition to climate change, the growth of human population and land cover changes have an effect on the biogeochemical cycles, habitat availability, biodiversity, soil erosion, water quality, water flow, and sediment flows (Ajibola, et al. 2016).

Intensification of land conversion for agriculture is accelerating land use land cover (LULC) change with its consequential impact on the natural landscape. For practical purposes, intensification occurs when there is an increase in the total volume of agricultural production that results from a higher productivity of inputs (FAO, 2005). Agricultural intensification in response to the government’s quest for economic diversification is aggravating LULC change across Nigeria particularly at the heart of wetland ecosystems. Despite the inherent dynamic system of wetlands, the ecosystem is suffering from great transformations worldwide (Arooba and Sheikh, 2017). These changes are fundamental obstacles in the country’s effort towards the attainment of food security, economic diversification, growth and sustainability of the physical environment. Similarly, Sebastiá et al. (2012) affirmed that a wide range of pressures affect these ecosystems and alter the quality and quantity of water. The increasing pressure on the ecosystem and the consequential land degradation are intensifying runoff, siltation of river channels, and flood events.

The wetland ecosystems in the country serve as a direct and indirect pool of resources for the population that derives maximum benefits from the exploitation of these essential resources for socio-economic and sustainable livelihood. Ehsan and Farhad (2014) described wetlands as the kidneys of the landscape because of their functions in chemical and hydrological cycles. The vast riverine wetland ecosystem is used most importantly for agriculture (farming, grazing, and fishing) and the inhabitants primarily depend on it for livelihood. The environmental destabilization of the wetlands and of the “dynamically” developing areas as far as the geomorphological processes are concerned is mainly due to certain anthropogenic interventions that alter “critical” parameters of the environment (Grundling et al., 2013). These alterations incorporate the greatest environmental concerns of human populations in recent time’s vis-a-viz loss of biodiversity, land, vegetal and water degradation, soil erosion, climate change, and its impact. Globally, the landscape and hydrological cycle have been modified by anthropogenic activity thereby, reflecting the socio-economic conditions and pattern of land resource utilization (Li et al. 2013). Monitoring and mitigating the negative consequences of LULC dynamics as well as sustaining the production of this vital riverine ecosystem should be the primary focus of most developing nations.

In spite of this there has not been any comprehensive documented information on the changes in land cover and land use viz-a-viz the interphase between the livelihood sustenance practices in the study area. Similarly, the range of change in land and land cover in the study area has not been documented. Furthermore, information on the changes in land use and land cover changes remains scanty.

The aim of this study is to assess the changes in land use/land cover in HNWs. The specific objectives are to; evaluate the changes in land use land cover over a

40-year window (1979-2019) in the study area, and assess the interphase of livelihood sustenance practices in relation to changes in land cover/ land use.

The intense infringements of land use systems into traditional forests and wetlands and also changes in land cover/ land use are contributing to the degradation of ecosystems leading to unsustainable development. Whereas such land developments could be contributing to the short-term socio-economic welfare of the people, they in the long run cause degradation and thus threaten the very livelihoods of the local people they were meant to sustain. History has it that, these lowlands were once occupied by a massive water body that has since receded, leaving behind patches. This shrinkage has been blamed on varied causes including changes in land use and anthropogenic factors. If this trend continues, the remaining wetland ecosystems may eventually be transformed into terrestrial landforms, losing a lot of their ecological and economic importance (Grundling et al., 2013).

Fishing, Nomadic pastoralism, hunting, collecting and gathering of vegetation resources constituted the main source of livelihood. Today, however, due to increased population and penetration of forces and influences of development have enormous competitive alternative and uses which includes; permanent human settlements, agriculture and forest resources commerce. Therefore, household welfare that was previously assured by a relatively smaller stable competing factors is no longer ascertain. This study will thus provide baseline information on livelihood sustenance practices, changes in climatic variables and changes in climatic variables scenario viz-viz the environmental, social, and economic responses of the communities who depend on the resources for their livelihood sustenance. The result can provide an avenue for strategic management and conservation options for the government and other stakeholders.

The study is limited to the assessment of livelihood sustenance practice in HNWs inhabitants in relation to changes in climatic factors and land use/land cover. Data collection was limited to parameters related to the stated objectives.

MATERIALS AND METHODS

Study Area

Location of the Study area

The HNWs is located at a point where Rivers Hadejia and Jama'are flow through a fossil dune field before converging and draining into Lake Chad (Barbier and Thompson, 1998) and lie between longitude 10°15'E and 11°30'E, and latitude 12°13'N and 12°55'N. The wetlands extend for approximately 120 km from West to East within Jigawa State and a further 60–70 km downstream in adjacent Yobe State (Barbier and Thompson, 1998). In width, the wetlands range from l0km to more than 50 km from North to South, with approximately 8000 km2 of floodplain covering three Nigerian States (namely Bauchi, Jigawa, and Yobe). The extent of the floodplain varies considerably from year to year depending on the volume of rainfall and complex interactions of river flow, dam releases, flood regimes, and topography. In Nigeria, wetlands cover about 28,000 km² (about 3%) of the 923,768 km2 of the country's land area (Abubukar et al., 2016). One of these is the HNWs named after two major towns (Hadeja and Nguru) in the area and are surrounded by many villages.

The Hadeja-Nguru Wetlands (HNWs) is an extensive floodplain created by the Hadeja and Jama'are Rivers to form the Komadugu- Yobe River which drains into Lake Chad. The wetlands cover an area of about 350, 000 ha and have an altitude of (asl) 152 - 305m (Bird Life International, 2015). The Nguru Lake and Marma Channel Complex Wetlands (located within the HNWs) were designated as the first Nigerian wetlands of international importance under the Ramsar Convention. According to Ramsar, (1994), the wetlands are notably known for the rrecharge and replenishment of underground water in the Komadugu-Yobe Basin, economically rich habitats for the biodiversity of various fauna and flora. The area is a major tourism site for the Palearctic and Afrotropical migrant water birds (Eaton and Sarch, 1997).

Vegetation of the study area

The general vegetation is characteristic of the Sudan savanna, – Sparse shrubs and isolated tall trees mostly Acacia Species. Three broad types of vegetation occur in HNWs. There is a scrub savanna, which consists of upland farmland areas and Acacia Woodlands. The second includes the "tudu" (raised areas) which are never inundated with tree species of Acacia, Ziziphus species, Balanites aegyptiaca, Tamarindus indica and

Adansonia digitata, while common grasses include Cenchrusbiflorus, Andropogon species. and Vetiveria nigritana.

In addition, pockets of riparian forests and woodlands, known as "kurmi" comprise species of Khaya senegalensis, Mitragyna inermis, and Diospyros mespiliformis. In some parts, the kurmi has been replaced with orchards of mango Mangifera indica, and guava Psidium guajava, (Ezealor, 2001). The third vegetation type consists of the seasonally flooded marshes in which the tree Acacia nilotica, is common while Dum palms (Hyphaene thebaica) grow on small, raised islands (Ezealor, 2001). Aquatic grasses include Echinochloa and Oryza species. While in drier parts Dactylocteniu maegyptium, Setaria species and Cyperus species, occur and extensive vegetation of Typhadomin gensis along the shore of the wetlands. The favorable moisture regime due to the high ground water table supported Mitragyna ground water woodland and seasonally flooded grassland. The woodland is becoming degraded due to falling water table as reported by Hadeja-Nguru Wetlands Conservation Projects (HNWCP, 1997).

The ecosystem comprises permanent lakes and seasonally flooded pools connected by a network of channels. The ecosystem is an important site for biodiversity, especially migratory water birds from Palearctic regions (Abubakar et al. 2016). For example, at one time, the floodplain supports over 423,000 birds of 68 species, including significant numbers of Ferruginous Duck (Aythyanyroca), Spur-winged Goose (*Plectropterus gambiensis*), Black-tailed Godwit (Limosalimosa), and Ruff (*Philomachu spugnax*) (Birdlife International, 2010). Other wildlife species found include species of gazelle (Gazella spp.), duiker (*Cephalophus* spp.), jackal (Canissp) and hyena (*Crocuta crocuta*) (Ogunkoya and Dami, 2007). In total, there are about 378 bird species listed for the wetland, 103 fish species, 250 species of flowering plants and more than 136 species of aquatic flora and fauna (Oduntan et al. 2010).

Population

The HNWs is the first Nigeria wetland to be named a RAMSAR site (RAMSAR, 1994). The people in the area depend on this wetland for water supply and other daily activities. Hausa, Kanuri, Fulani and Bade are the most dominant tribes in the wetlands where Hadeja has a population of 139,400 among which 54.6% are male and 46.4% are female (National Bureau of Statistics, 2016). The population including farmers, herders and fishermen who entirely depend on the ecosystem for their livelihoods (Kaugama and Ahmed, 2014; Birdlife international, 2015). The wetlands provide essential income and nutrition benefits in the form of agriculture, grazing resources, Non-Timber Forest Products, fuel wood and fishing (RAMSAR, 2007)..

Geology, Topography and Soil

Permeable sedimentary rocks of the Chad formation underlie this natural wetland, but a film of impervious layers has been formed at the bottom of the water body through successive years of clay deposition. This has significantly impeded percolation (Emmanuel, 2019). A monotonous low-lying plain that gently slopes northeastwards towards Lake Chad characterizes the relief around the site. River flow is highly seasonal and varies considerably depending on rainfall and run-off. Peak flow occurs between August and September when banks overflow and the area is inundated. The river regime in the area has however been affected by river regulation that peak discharge in the wetland is now in September-October (Emmanuel, 2019).

Drainage

Hydrology of the Hadeja-Nguru Wetlands The hydrological genealogy of the Hadeja-Nguru Wetlands sustains water from rainfall and runoff supplements from the wet season and is later depleted by other hydrological output like infiltration to underground, soil moisture recharge and evaporation (RAMSAR, 2007).

Climate

The Hadeja-Nguru wetland is located as part of the Komadugu-Yobe River basin, it has a semi-arid climate influenced by the strong convection storm of the Inter-Tropical Convergence Zone (ITCZ). The climate of the wetland is characterized by two distinct seasons; wet season (May- September) and dry season (October-April), The rainfall period is from June to October and has an annual mean of over 1,000mm in the upstream Basement complex area and approximately 500mm in the Hadeja-Nguru Wetlands (Sanyu, 1994). The dry season normally sets in October and remains until late May. The temperature recorded in the dry season ranges between 35°C and 40°C. Significant water flows to the wetlands begin in

late June or early July with peak discharges in August. Occasionally there may be a mean minimum temperature of 12°C from the month of December to January, (Ogunkoya and Dami, 2007).

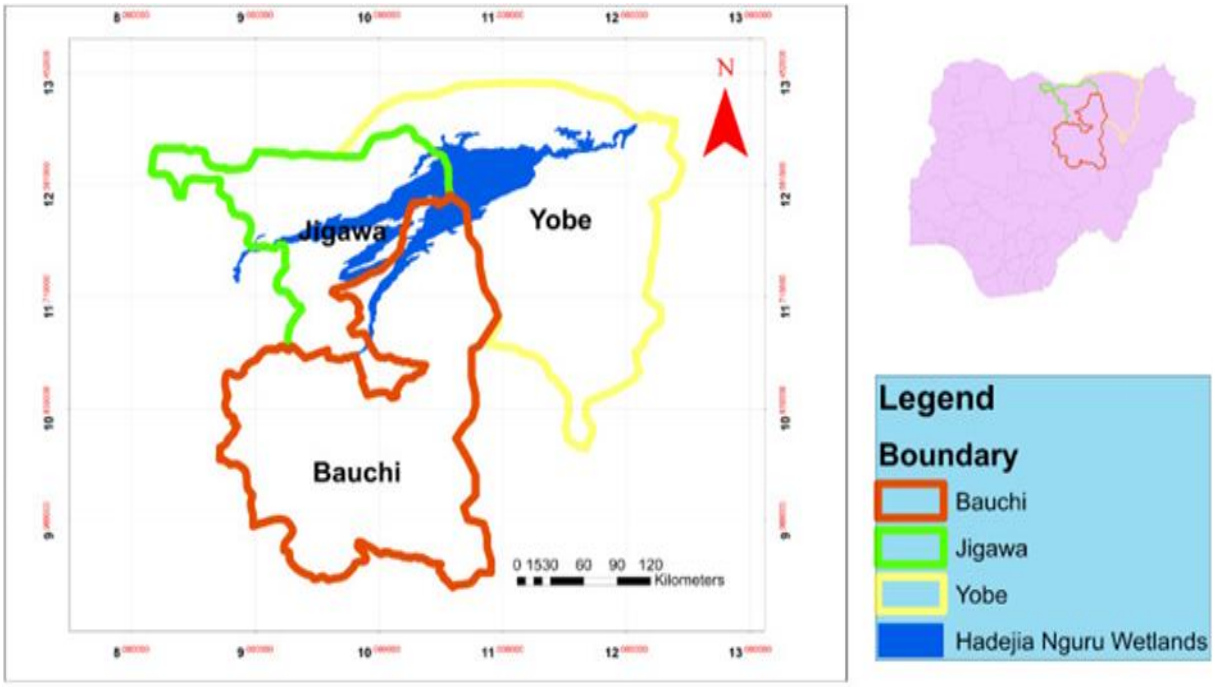

Figure: 1 Map showing boundary demarcations of HNWs between States Sources: GIS University of Maiduguri (2019)

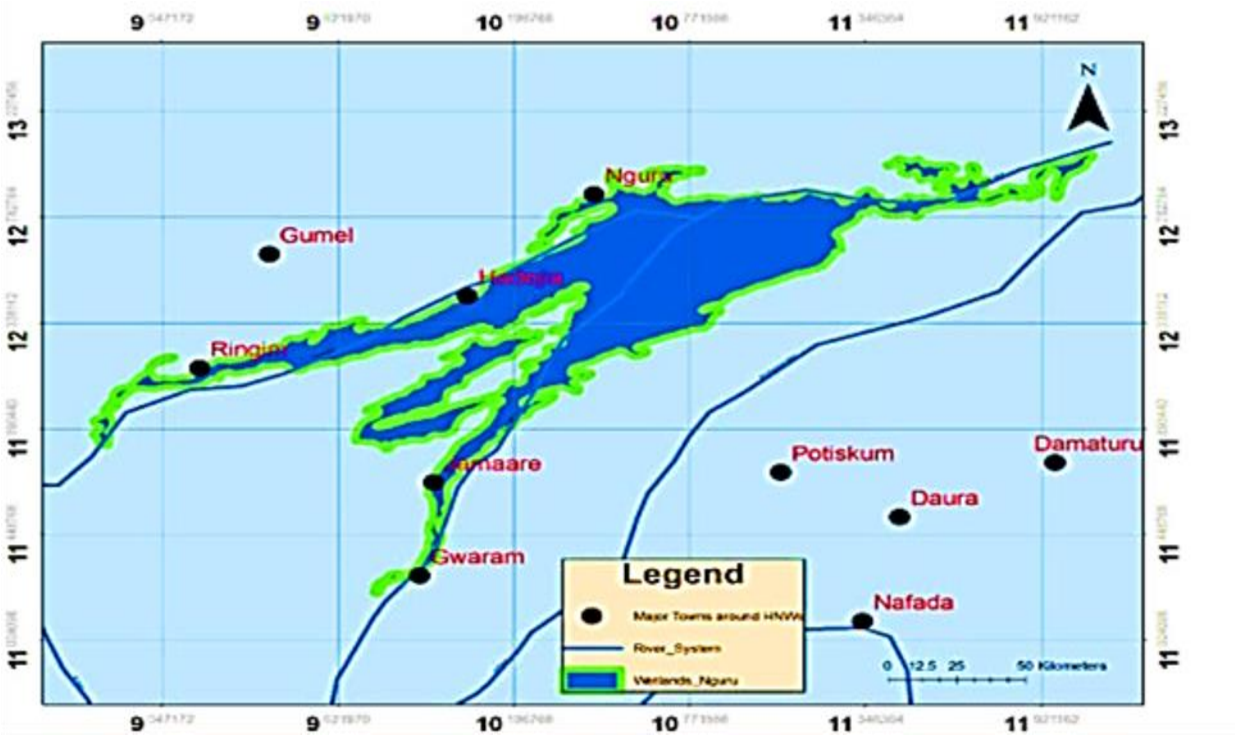

Figure: 2 legend map of HNWs

Source: GIS University of Maiduguri. (2022)

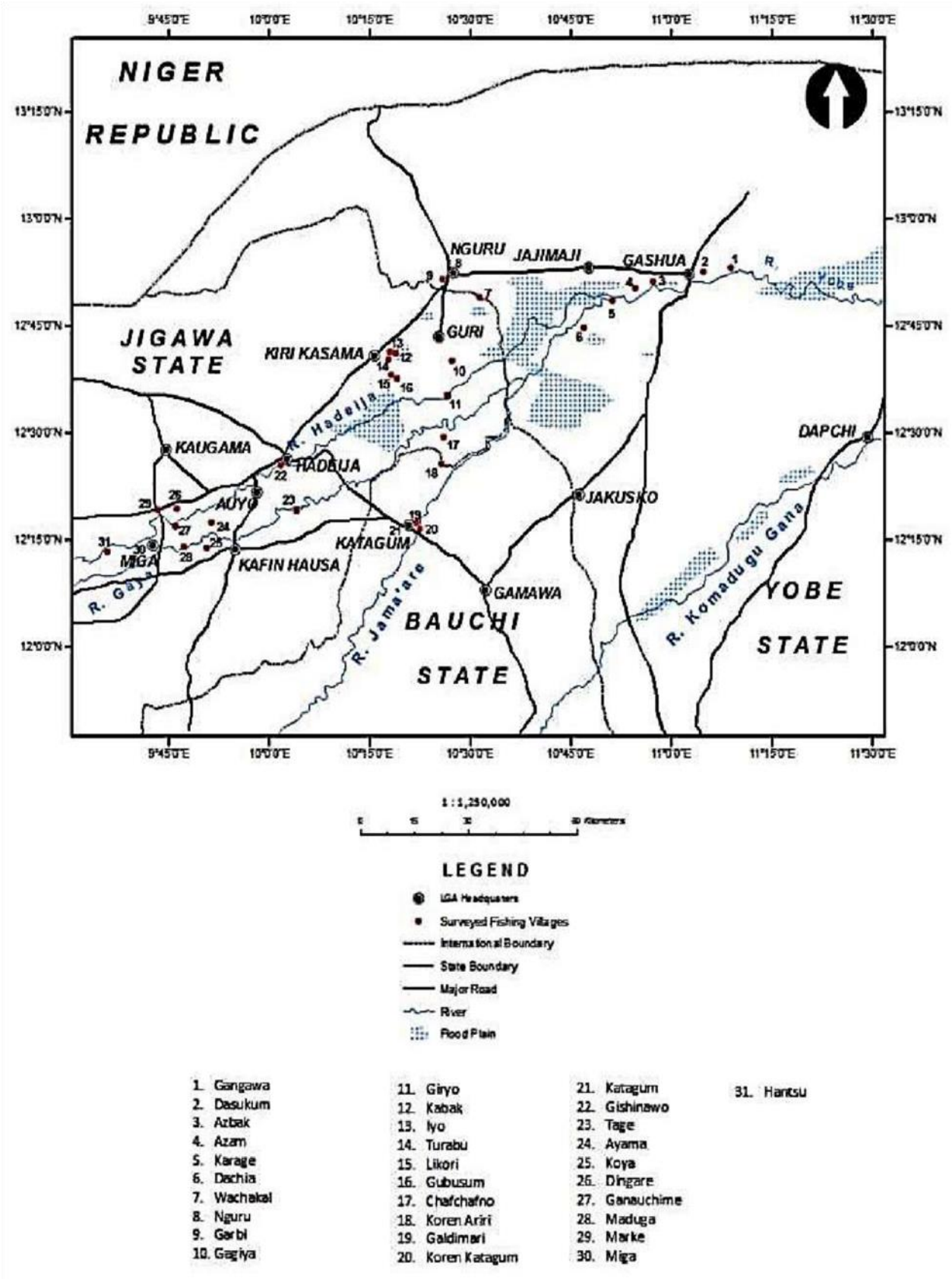

Figure 3: Map showing the extracted coordinate location of the study sites

Sources: GIS University of Maiduguri (2019)

Assessment of land use changes in HNW

The imageries of HNWs were officially sourced and downloaded from the official website of the United State Geological Survey (https://earthexplorer.usgs.gov/). The study area was classified into four classes or categories based on field study and personal experience of the study sites. Four land use/land cover (LULC) themes were decided for this research. These land use/cover categories or classes are: Water body, Thick vegetation, Grasses and bare land. The description and composition of these classes are presented in Table 2. This presents the remote sensing aspect of the study as it provides the land use/land cover change information for the selected study region. The detailed characteristics of the imageries used to produce the LULC maps are provided in (Table 2).

As can be observed in Table 3, the multispectral Landsat imageries covering the period from 1979 to 2019 were specifically selected from those available based on image quality. In all, three different epochs (1979, 1999, and 2019) were selected.

Data Analysis

Analysis of land use changes in HNW

To get the extent of the observed changes, post classification change detection approach was used to assess the five classified land cover maps using simple descriptive statistics. The Areal Statistics for the five land cover types were generated using the calculate area tool of ERDAS Imagine version 15 software, and this was generated in Hectares (H). Overall, from the statistics of the land cover maps, the change magnitude, change trend and Annual rate of change of the observed changes were then computed using the following formula (Abbas, 2012).

Magnitude =

$$Magnitude\ of\ the\ new\ year\ -\ Magnitude\ of\ the\ previous\ year$$

Percentage change (trend) for each LULC type was computed by dividing magnitude change by sum of observed changes between the years concerned and multiplied by 100 as shown in the equation:

$$Trend = \frac{Magnitude\ of\ change}{Sum\ of\ change} \times 100$$

To generate the annual rate of change for each LC type, the trend (percentage change) was divided by 100 and multiplied by the number of study years in between the two periods, for example 1972 – 1986, 1999-2009, 2009-2020 as shown below in the equation:

$$Annual\ rate\ of\ change = \frac{Trend \times Number\ of\ study\ years\ in\ between}{100}$$

(Abbas, 2012)

Assess the interphase of livelihood sustenance practices, and changes in land cover land use

Paired sample T-test of differences was used to test the differences in livelihood before and present while chi-square test of association was used to test the influence of the changes in LULC and climate on the livelihood as observed by the respondents in the area.

i. Students t-test

$$t = \frac{|X - Y|}{\sqrt{\left(\left(EX2/nx\right) - X2\right) + \left(\left(EY2/ny\right) - Y2\right)}} \quad \text{---------- Equation 1}$$

$$\frac{}{nx - 1} \qquad \frac{}{ny - 1}$$

Where t = t-test

x = Livelihood before

y = Livelihood After

n = number of observations

ii. Chi – square

$$X^2 = \sum \frac{(O-E)^2}{E} \quad - \quad - \quad - \quad - \quad - \quad - \quad \text{Equation 2}$$

Where X2 = chi – square

O = Observed frequency

E = Expected frequen

Assessment of land use changes in HNW

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As can be observed in Table 3, the multispectral Landsat imageries covering the period from 1979 to 2019 were specifically selected from those available based on image quality. In all, three different epochs (1979, 1999, and 2019) were selected.

Image Classification

Landsat 5 ETM, 7 ETM+ and 8 (OLI) images of 30m resolution with worldwide reference system (WRS) address of; path 187 row 051/052 (20/11/1979-20/11/1980), (08/12/1999-08/12/2000) and (05/01/2019-05/01/2019) for 1979, 1999 and 2019 respectively were downloaded from glovis.usgs.gov. The Landsat images were processed and mosaic to give a comprehensive coverage of the wetland area with help of image analysis and spatial analysis tool in Arc GIS 10.3. Unsupervised classification was adopted for the study.

Areas of the classes were calculated in km2 one after the other by highlighting the layer (class) in the attribute table, reclassified the map to produce the area of the selected class. The area of the reclassified layer (class) was then converted into a polygon and was letter calculated using calculates area tool in spatial statistics toolbox (Figures 4 and 5). All these procedures were applied in producing the LULC of each year and calculation of the areas.

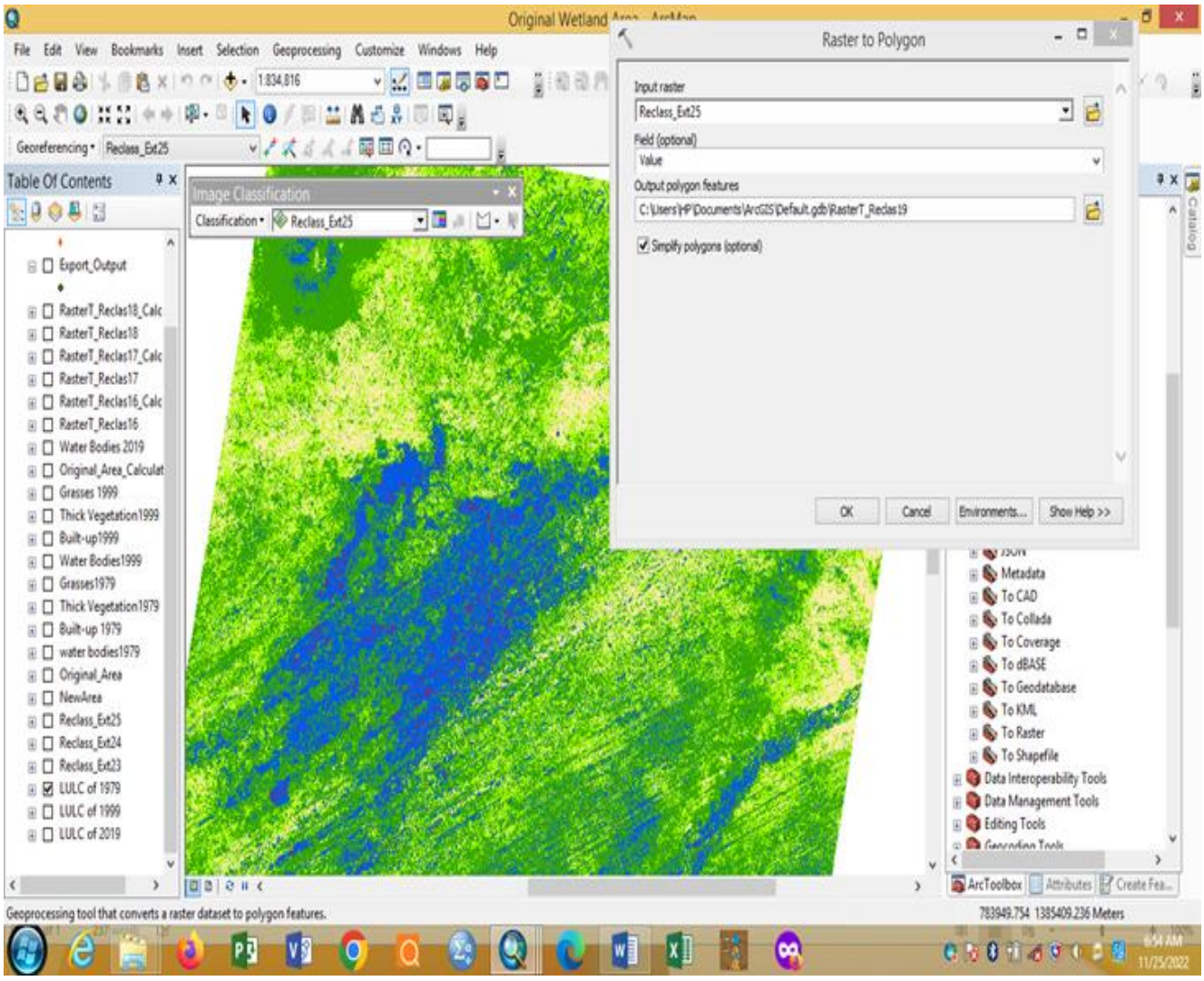

Figure: 4 Conversion of Images from Raster to Polygon

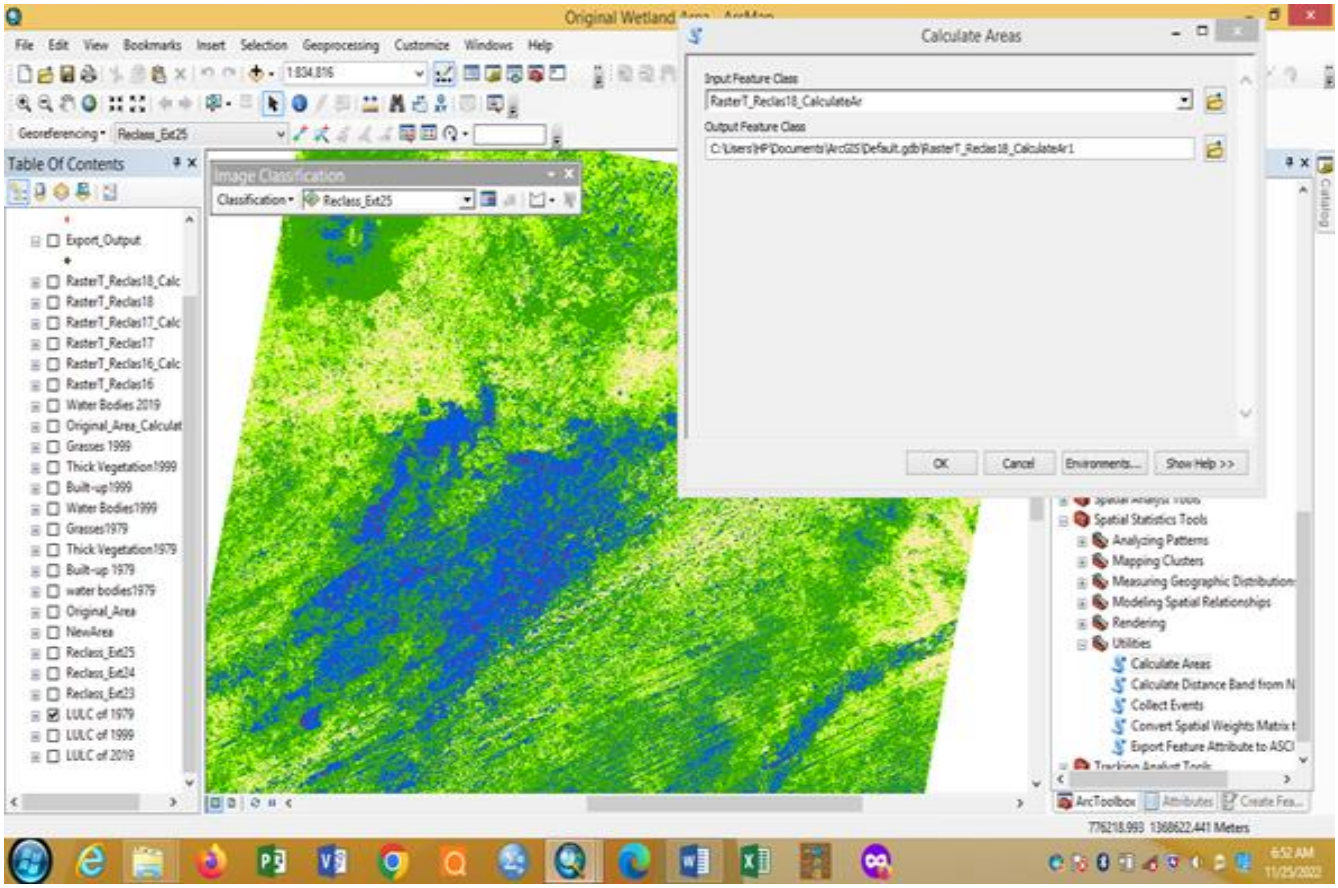

Figure: 5 calculation of total area of each class.

Table: 1 The description and characteristics of the LULC types used in the study

LULC Types	Description/composition
Water bodies -	This land cover type represents all areas of open water bodies, generally with less than 10% vegetal cover. This also represents all open water bodies irrespective of depth (both shallow and depth waters).
Thick Vegetation -	These are plants of an area that grow in disturbed or undisturbed conditions in wooded plant communities in any combination of trees, saplings, shrubs, vines, and herbaceous plants, including mature and successional forests and cutover stands.
Bare-land -	These are areas characterized by open land with little or no NTFPs. It also includes fallow agricultural fields and lands that are being subjected to continuous cultivation. Areas where soil exposure is apparent.
Grassland	These represent degraded and dried-off areas of the wetland or areas that before were occupied by NTFPs and water. It also represents inundated and dried floodplain areas. These areas largely lack vegetal cover or NTFPs and it appears dark and whitish in the raw satellite imagery.

Source: Field Work, (2021).

Analysis of land use changes in HNW

To get the extent of the observed changes, post classification change detection approach was used to assess the five classified land cover maps using simple descriptive statistics. The Areal Statistics for the five land cover types were generated using the calculate area tool of ERDAS Imagine version 15 software, and this was generated in Hectares (H). Overall, from the statistics of the land cover maps, the change magnitude, change trend and Annual rate of change of the observed changes were then computed using the following formula (Abbas, 2012).

$$Magnitude = Magnitude\ of\ the\ new\ year - Magnitude\ of\ the\ previous\ year$$

Percentage change (trend) for each LULC type was computed by dividing magnitude change by sum of observed changes between the years concerned and multiplied by 100 as shown in the equation:

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To generate the annual rate of change for each LC type, the trend (percentage change) was divided by 100 and multiplied by the number of study years in between the two periods, for example 1972 – 1986, 1999-2009, 2009-2020 as shown below in the equation:

$$Annual\ rate\ of\ change = \frac{Trend \times Number\ of\ study\ years\ in\ between}{100}$$ (Abbas, 2012)

RESULTS

The Trajectories of the LULC Change from 1979-2019

Table 1 presents the change trajectories of the four LULC classes identified in the area under study. The classified maps are also presented in Plates I and III which portrays the change trajectories of the entire landscape of HNW between 1979 and 2019. The result indicated that water body in HNW in 1979 was 4131.70 Km2 and in 1999 it occupied an area of about 1,255.99 Km2 which decreased by -69.60%. Bare surface which occupied a total area of 1,556.35 Km2 in 1979 increased to 9,466.04 Km2 in 1999 indicating an increase of 508.22%. Grassland had an area of 6,339.38 Km2 in 1979 and decreased to 2,709.38 Km2 in 1999 indicating a decrease by -57.26%, while thick vegetation which occupied a total area of 14,284.25 Km2 in 1979 decreased to 12,880.27 Km2 in 1999 indicating a decrease by -9.83%.

The percentage change in land use land cover classes between 1999 and 2019 as presented in Table 2 and 3 and Plates I and III. The result indicated that water body which occupied a total area of 1,255.99 Km2 in 1999 decreased to 1,095.62 Km2 in 2019 indicating a decrease by -12.77%. Bare surface which occupied a total area of 9,466.04 Km2 in 1999 decreased to 8,348.98 Km2 in 2019 indicating a decrease by -11.80%. Grassland had an area of 2,709.38 Km2 in 1999 and increased to 7,306.72 Km2 in 2019 indicating an increase of 169.68 %, while thick vegetation which occupied a total area of 12,880.27 in 1999 had reduced to 9,560.36 Km2 in 2019 indicating a decrease by -25.78%.

The percentage change in land cover classes between 1979 and 2019 is presented in Table 8 and 9, Plates I and III. The result indicated that water body which occupied an area of 4,131.70 Km2 in 1979 decreased to 1,095.62 Km2 in 2019 indicating a decrease by -277.11%. Bare surface which occupied a total area of 1,556.35 Km2 in 1979 increased to 8,348.98 Km2 in 2019 indicating an increase of 81.36%. Grassland had an area of 6,339.38 Km2 in 1979 but increased to 7,306.72 Km2 in 2019 indicating an increase by 13.24 %, while thick vegetation which was 14,284.25 Km2 in 1979 decreased to 9,560.36 Km2 in 2019, indicating a decrease by -49.41%. All land cover classes indicated losses of varying degrees and rates between 1979 and 2019. The rates at which selected surfaces changed were; Thick vegetation (118.10); water bodies (75.90), Bare surfaces (169.82) and grassland (24.18) respectively. The projected years of exhaustion of thick vegetation showed that in approximately 80.95 years there will not be vegetation while water body indicated that in approximately 14 years the HNWs will disappear.

Table: 2 Area and Percentages of Land cover classes in Hectare (H) during the Study Period 1979-2019

LULC Themes	1979	(%)	1999	(%)	2019	(%)
Thick Vegetation	14,284.25	54.29	12,880.27	48.95	9,560.36	36.34
Grass land	6,339.38	24.09	2,709.38	10.30	7,306.72	27.77
Water Bodies	4,131.70	15.70	1,255.99	4.77	1,095.62	4.16
Bare Surfaces	1,556.35	5.92	9,466.04	35.98	8,348.98	31.73
Total	26,311.67	100.00	26,311.67	100.00	26,311.67	100.00

Source: GIS Analysis, (2019)

Table: 3 Magnitudes of Change in the four identified LULC themes from 1979-2019 in HNWs

LULC Themes	1979-1989	%Δ	1999-2009	%Δ	1979-2019	%Δ	Rate	Projection
Thick Vegetation	-1,403.99	-9.83	-3,319.91	-25.78	-4,723.90	-49.41	-118.10	-80.95
Grass land	-3,630.00	-57.26	4,597.34	169.68	967.34	13.24	24.18	302.14
Water Bodies	-2,875.70	-69.60	-160.38	-12.77	-3,036.08	-277.11	-75.90	-14.43
Bare Surfaces	7,909.69	508.22	-1,117.05	-11.80	6,792.64	81.36	169.82	49.16

Source: GIS Analysis, (2019)

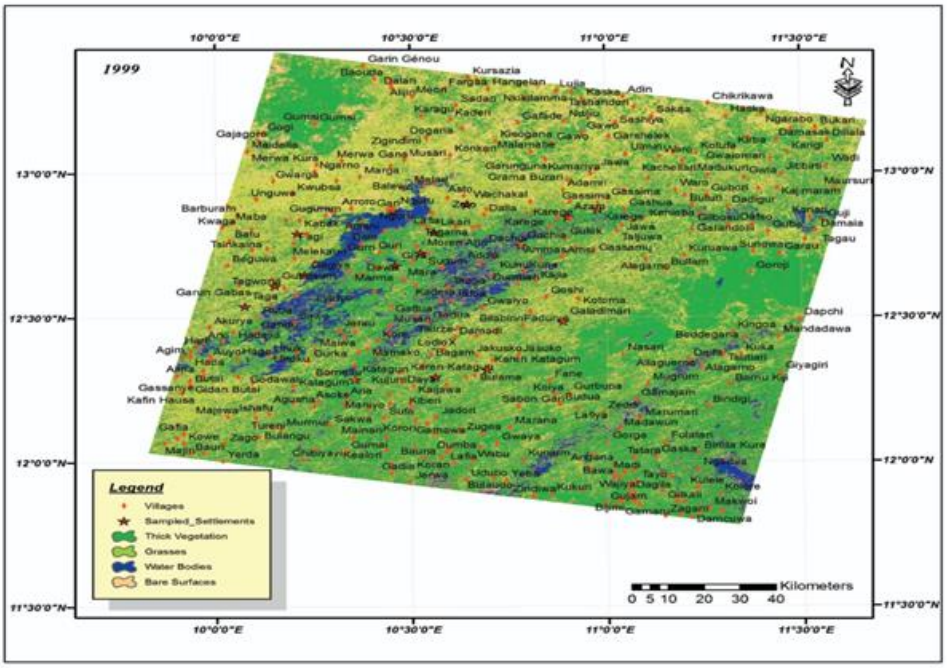

Plate: I The classified Land cover map of HNWs as at 1999
Source: GIS Analysis, (2019)

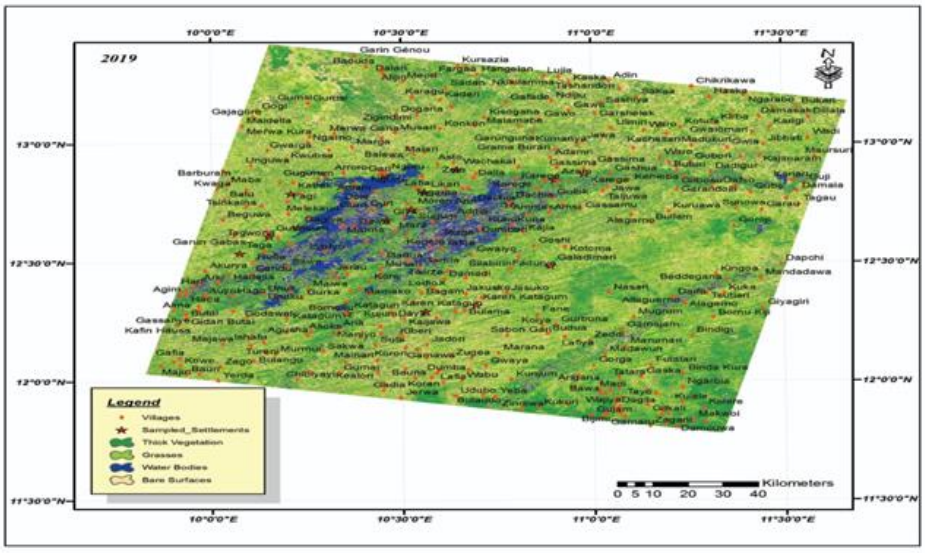

Plate: II The classified Land cover map of HNWs as at 2019
Source: GIS Analysis, (2019)

DISCUSSION

Findings from the classification of the imageries indicated continuous decrease in the water bodies from 1979 to 2019, which could be due to agricultural land use conversion. This could impact negatively on hydrological processes and ecosystem health. These observations agree with the findings of a similar study by Chen et al. (2009) on Impacts of land use change scenarios on storm-runoff generation in Xitiaoxi basin, China; Tadesse et al. (2015) who also reported on Assessing the impact of land-use land-cover change on stream water and sediment yields at an assessment of watershed level using SWAT; Woldesenbet et al. (2017) on Hydrological responses to land use/cover changes in the source region of the Upper Blue Nile basin, Ethiopia; Uluocha and Okeke (2004) on impacts of climate variability and land use change on streamflow in the Hailiutu river basin and Adepoju et al. (2019) on Vegetation Response to Recent Trends in Climate and Land use Dynamics in a Typical Humid and Dry Tropical Region under Global Change.

The increase in bare surfaces in the wetlands between 1979 and 2019 could be attributed to increased farming and grazing (including lopping of trees for livestock as well as for tradomedicinal uses) as observed during ground truthing. These findings are in consonance with those of Ikusemoran and Ezekiel (2011) in their study of Remotely Sensed Data and Geographic Information System Techniques for Monitoring the Shrinking HNWs, Nigeria where they affirmed human interventions especially agricultural practices to be the major cause of the changes in the wetlands, also grazing by herdsmen in the wetlands area for several generations. Geist and Lambin (2002) and FAO (2005) reported grouping of the land cover classes gave rise to three groups, which included the water body, the vegetation covers and the bare surfaces/farmland. This is in agreement with the fact that most areas in Africa, including Nigeria and HNWs in particular experience land tenure insecurity, particularly due to increasing land transactions for expansion of agri-business in conformity to the studies of Woldesenbet et al. (2017) and Pare, (2008).

Since, several factors contribute to a more complex land use dynamics pattern, the vegetation experienced losses as it decreased over the period in the study location. The overall change of thick vegetation in HNWs study sites between 1979 and 2019 was on the negative side (-49.41%) indicating a decrease. The marginal depletion of vegetation of the site may not be unconnected with the interruption of the natural flood regime via diverting flood water in the wet season and releasing damaging flood surges during the dry season and also as a result of several dams (including two large ones at Tiga and Challawa) and other hydro agricultural projects with intensive water demand have been commissioned at locations upstream as noted by Ikusemoran and Ezekiel (2011). The combined effects of these factors must have caused the dynamics of vegetation of the study site.

CONCLUSION AND RECOMMENDATION

Conclusion

Both natural and human activities are known to modify the natural environment, and HNWs is not an exception. The communities in the wetlands depend largely on the natural resources for their livelihood and survival. These natural resources have been significantly altered and continue to deplete due to unsustainable practices and over population. The natural resource scarcity that resulted from environmental changes have had severe impacts on wetland through loss of biodiversity, soil productivity and accelerated environmental degradation thereby increasing vulnerability and reduction in biodiversity. This hardship imposed led to a number of adjustments by individuals and communities to continue making out a living within the same environment. However, the current community level of adaptation measures may not be sufficient to meet the challenges of the current environmental change particularly in the face of change in LULC. It is therefore very important to improve the understanding of local populations and communities on the prevailing changes in their immediate environment because their behavior of removing vegetation cover , over the study period and the test of the relationship on vegetation cover as represented by the land use changes showed that there is an interwoven relationship among all the factors. The massive increased in removal of vegetation cover had the strongest impact amongst other factors that the research examined on the deterioration of the vegetation cover, wind speed increased steadily as observed during study periods. Based on the findings of this study, it is clear that the HNWs area should be protected because of the richness in biodiversity.

Recommendation

Based on the findings of the study the following are some of the recommendations

i. There is need to put in place right policies to protect and preserve wetland to enhance its sustainability and resilience to climatic changes and variability.

ii. Policy making as well as academic research on ecosystem changes should integrate people's testimonies and their stories as evidence of those changes. Such integration of local knowledge will help in foregrounding place-based sustainability models.

ii. Finally, there is the need for the government to have a plan action of mitigation and adaption measures in place and to provide a legal frame work for their adoption.

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Measuring the Degree of Adaptive Capacity of Farmers to Climate Change along River Niger in Kogi State, Nigeria

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ABSTRACT

Climate change threatens people with increased flooding, extreme heat, increased food and water scarcity, more disease, and economic loss. Human migration and conflict can also be a result. The World Health Organization calls climate change the greatest threat to global health in the 21st century. In Nigeria, the effects of climate change are expected not to stop at just affecting the agricultural production, it will surely affect the lives, health, and overall development of the country. In order to formulate appropriate programs and policies addressing this vulnerability, it is essential to understand their degree of adaptive capacity. This study measures the degree of adaptive capacity of farmers to climate change along River Niger in Kogi State. Primary data were collected from respondents, multistage sampling techniques were used to select respondents in Kogi State. Descriptive statistics, using the threshold concept for discrete variables results show that irrigation farming is the most used adaptation strategy to climate change in the study areas. It was concluded that the degree of adaptive capacities to climate changes in mulching material and planting of cover crops. The study recommends that to reduce the effect of climate change in these areas, there is a need for policymakers to engage communities when taking decisions relating to their livelihood.

INTRODUCTION

Climate change describes global warming, the ongoing increase in global average temperature, and its effects on Earth's climate system. Climate change BBC News. Nigeria floods: Overwhelming Disaster left more than 600 people dead in 2022. People with increased flooding, extreme heat, increased food and water scarcity, more disease, and economic loss. Human migration and conflict can also be a result. The World Health Organization (WHO) calls climate change the greatest threat to global health in the 21st century International Panel on Climate Change (IPCC), 2022. Climate change will affect people in Africa more than anywhere else in the world due to the nature of changes being witnessed, deteriorating terms of trade, inappropriate policies, high rates of population growth, the inequitable distribution of land, over-dependence on natural-resource-based livelihoods and over-reliance on rain-fed agriculture (Intergovernmental Panel on Climate Change (IPCC, 2022). Africa's climate is already changing Bennett, et al.,2018. In general, the continent is becoming warmer and drier. Rainfall is becoming less predictable. Meanwhile, storms, droughts and floods are becoming more common and intense. Africa's

average temperature rose at a rate of 0.05°C per decade from 1900 to 2000 for a total increase of 0.7°C (IPCC, 2022). Temperatures are due to rise by a further 0.2 to 0.5°C per decade, with the greatest warming occurring over the interior or semi-arid margins of the Sahara and central southern Africa (IPCC, 2022).

In an ever-progressing world, with an increasing demand for energy and animal agriculture, it is difficult to avoid climate change and its impacts on societies, both locally and globally Oppenlander et al. 2020. Climate change affects social development factors, such as poverty, infrastructure, technology, security, and economics across the globe. Although, climate change affects everything we see around us, the interrelation between climate change and social vulnerability and inequality is particularly evident in impoverished communities. In particular, impoverished communities experience reductions in safe drinking water, as well as food security as a result of climate change (IPCC, 2022). These typically rural, isolated communities do not exhibit sufficient financial and technical capacities to manage the risks associated with climate change Ekemhonye, et al. 2020. Recent events of the flood that affected 34 States in Nigeria displaced over 1.4 million people, killed over 603 people, and injured more than 2,400 persons. About 82,035 houses had been damaged, and 332,327 hectares of land had also been affected. The Nigerian government has blamed the floods of 2022 on unusually heavy rains and climate change BBC News, 2020. Gaps in knowledge of the degree of adaptive capacities to climate change research are still in a rather primitive stage and climate change in the region has not been fully identified or understood. Hence, although a lot is known about the science of climate change, there remain many uncertainties about its potential impact on the degree of adaptation BBC News, 2020. Yet, this message has failed to penetrate public discussions on climate change and adaptation policies. At the moment, few studies that have considered measuring degrees of adaptive capacities to climate change were from a global perspective or regional aggregates. This research has narrowed it down to a State along River Niger in Nigeria for easy use by policymakers. Thus, this study is expected to add to the scanty knowledge in this area of research.

METHODOLOGY

Data for this study were collected from primary sources. The data were obtained through the administration of a questionnaire to elicit information from the respondents, on the socio-economic characteristics of the farmers such as age, marital status, gender, education, household size, farming experience, farmland size, the extent of awareness of climate change, annual income, and various adaptation measures to climate change. The researcher was assisted by trained enumerators from the State's Agricultural Development Programme to carry out data collection.

METHODS OF DATA ANALYSIS

Objectives were achieved using descriptive statistics tools such as mean, frequency, and percentages.

Empirical measurement of adaptive capacity

Following the procedures adaptive capacities of farmers were determined using the threshold concept for discrete variables Okezie, et al. 2016 Nakuja, et al. 2012. Five attributes comprising knowledge of adaptation strategy, use of adaptation strategy, availability of adaptation strategy, accessibility to adaptation strategy, and farmers' consultation make on specific adaptation strategy. The adaptation strategies that were considered in this research are practices irrigation, use of improved seeds, livestock production, change in land preparation pattern, change of weeding pattern, application of fertilizer, change planting dates, fish farming, bush allowing, mono /sole cropping, diseases resistant variety, Okada service, early maturing variety, plant drought resistant variety, change from crop to livestock, engage in small-scale business, mixed cropping, crop rotation practice, practice water-harvesting scheme, planting of cover crops and change mulching material.

Climate change adaptation strategies

In measuring the adaptive capacities quantitatively, respondents were asked to indicate their degree of attainment of each attribute. The highest degree of attainment of each of the attributes or factors affecting adaptive capacities was scored 4.0 followed by a higher degree of 3.0, a high degree of 2.0 and the lowest degree was 1.0. Therefore, the degree of each respondent's knowledge of each adaptation strategy was sorted out. In terms of knowledge, the higher the degree, the better knowledge the respondents have

on a particular adaptation strategy. Table 1 summarizes how each attribute was measured.

The Adaptive Capacity (AC) is obtained by dividing the total score of the attributes for the respondent by the sum of the most desirable score of all attributes, thereby reducing the adaptive capacity to a scale of between 1 and 4.

Table 1: Rating level of respondents' achievements of adaptive capacity attributes

Degree	Scores	Knowledge	Use	Availability	Accessibility	Consultation
Highest	4.00	Very well	Several	Very regular	Easily accessible	Several
Higher	3.00	Well	Twice	Regular	Accessible	Twice
High	2.00	Fairly well	Once	Occasionally available	Not easily	Once
Low	1.00	Not well	Never	Never	Not accessible	Never

Source: Modified from Nakuja et al. (2012).

$$AdapCap_{ij} = \sum (K_{ij}, U_{ij}, V_{ij}, A_{ij}, C_{ij},)/T \quad (1)$$

Where:

AdapCapij = represents the ith farmer's to jth Adaptive capacity to climate change;

K = Knowledge;

U = Usage;

V = Availability;

A= Accessibility;

C = Level of consultation; and

T = the sum of the most desirable scores for all attributes.

The average adaptive capacity of respondents to the jth adaptation strategy was calculated using the equation (1).

$$Ave\ Adap\ Cap_j = \frac{\sum \text{Adap Cap}_i}{N} \quad (2)$$

Where N = the number of observations

Ave Adap Capj = Average adaptive capacity of respondents to jth adaption strategy

The cut-off point for each level was based on the dispersion of data by setting three intervals based on the median (1.33). These were namely: low, moderate, and high adaptive capacity levels.

Table 2: Respondent's degree adaptive capacities to climate change

Degree	Range	Ranges of indices
Low adaptive capacity	0 <*AdapCapij* < 1.33	0 <*AveAdapCap* < 1.33
Moderate adaptive capacity	1.34≤ *AdapCapij* <2.66	1.34≤*AveAdapCap*< 2.66
High adaptive capacity	2.67≤*AdapCapij* ≤ 4.00	2.67≤*AveAdapCapij* ≤ 4.00

Source: Modified from Nakuja et al. (2012)

RESULTS AND DISCUSSION

Respondents' adaptive capacities to climate change

Respondents' levels of adaptive capacities measures to climate change results are presented in Table 3. The results reveal that the practice of irrigation farming was ranked 1st out of the twenty-one adaptation questions raised on the level of respondent adaptive capacities to climate change with an adaptive capacities value of 3.91. This was followed by the use of improved seeds, livestock production, and change land preparation patterns which were ranked 2nd, 3rd, and 4th with adaptive capacities scores of 3.89, 3.70, and 3.66 respectively. The result implies that respondents residing in the study area practice irrigation farming as a major adaptive capacity to climate change since this is possible because of their proximity to the river which will enable them to practice both rain-fed and dry-season farming. The findings are in line with Oppenlander et al. 2020 who observed that the overall unreliability and inconsistency in the temporal and spatial distribution coupled with the inadequacy of the rainfall, recurrent droughts, and rapid population growth have all combined to make irrigation an essential factor in the food security strategies in Nigeria.

Use of improved seeds was the second most adaptive capacity to climate change used by respondents. This finding is in line with the Food and Agricultural Organization, 2022. The definition of improved seeds

is seeds that aim at increasing the quality and production of crops by having characteristics such as drought tolerance, high yielding, and early maturity. This implies that, because of its increases in quality of yield, farmers may tend to adopt it as an adaptive capacity to climate change compared to others in the study areas.

Table 3: Respondents adaptive capacities to climate change in Kogi State

Adaptations' strategies	Knowledge of attribute score	Accessibility of attribute score	Availability of attribute score	Consultation of attribute score	Uses of attribute Score	Adaptive capacity	Rank
Practices irrigation	3.83	3.93	3.84	3.98	3.97	3.91	1
Use of improved seeds	3.83	3.91	3.83	3.98	3.91	3.89	2
Livestock production	3.30	3.68	3.76	3.93	3.83	3.70	3
Change the land preparation pattern	3.26	3.66	3.63	3.92	3.83	3.66	4
Change of weeding pattern	3.26	3.66	3.63	3.60	3.66	3.56	5
Application of fertilizer	3.20	3.47	3.41	3.49	3.65	3.44	6
Change planting dates	3.11	3.38	3.40	3.37	3.58	3.37	7
Fish farming	3.06	3.33	3.28	3.31	3.48	3.29	8
Bush fallowing	2.92	3.3	3.20	3.29	3.46	3.23	9
Mono /sole cropping	2.84	3.16	3.19	3.27	3.41	3.17	10
Diseases resistant variety	2.65	2.92	3.17	3.19	3.30	3.05	11
Okada service	2.54	2.9	3.15	3.08	3.26	2.99	12
Early maturing variety	2.53	2.93	3.03	2.97	3.25	2.94	13
Plant drought-resistant variety	2.45	2.83	2.83	2.87	3.16	2.83	14
Change from crop to livestock	2.39	2.72	2.80	2.68	3.15	2.75	15
Engage in small-scale business	2.20	2.62	2.60	2.63	3.12	2.63	16
Mixed cropping	2.10	2.49	2.53	2.51	2.72	2.47	17
Crop rotation practice	2.05	2.16	2.35	2.51	2.68	2.35	18
Practice water-harvesting scheme	1.93	1.95	2.17	2.36	2.65	2.21	19
Planting of cover crops	1.2	1.17	1.27	1.19	1.21	1.21	20
Change mulching material	1.1	1.2	1.25	1.07	1.14	1.15	21

Source: Computation from field survey, 2021

Degree of adaptive capacities of respondents to climate change

The adaptive capacities of respondent to climate change is presented in Table 4. The result in Table 4 shows that most of the respondents interviewed have high adaptive capacities to practice irrigation farming, use of improved seeds, livestock production, change land preparation pattern, change of weeding pattern, application of fertilizer, change planting dates, fish farming, bush allowing, mono /sole cropping, diseases resistant variety, okada service, early maturing variety, plant drought-resistant variety and change from crop to livestock. This is because their adaptive capacities are within the range of 2.67 ≤Adap Capij ≤ 4.00. Among these adaptation strategies with high adaptive capacities, practices of irrigation farming and change mulching material recorded the highest and lowest with 3.91 and 1.15 degrees respectively.

The adaptation strategies with moderate adaptive capacities are small-scale business, mixed cropping,

practice crop rotation, and water-harvesting schemes. Out of 21 adaptation strategies used, farmers are moderately adaptive to only 4 of them. Among adaptation strategies in which farmers are moderately adaptive, small-scale businesses had the highest adaptive capacity value of 2.63 while crop rotation practice recorded the lowest with 2.21 degrees. This implies that farmers in the study area need additional knowledge, skills, and resources to improve their business activities. These will further assist respondents in increasing their degree of adaptive capacities to climate change.

Table 4: Degree of adaptive capacities of respondents to climate change

Adaptation's strategies	Adaptive capacities	Rank	Degree of adaptive capacities
Irrigation practices	3.91	1	High
Use of improved seeds	3.89	2	High
Livestock production	3.70	3	High
Change land preparation pattern	3.66	4	High
Change of weeding pattern	3.56	5	High
Application of fertilizer	3.44	6	High
Change planting dates	3.37	7	High
Fish farming	3.29	8	High
Bush allowing	3.23	9	High
Mono /sole cropping	3.17	10	High
Diseases resistant variety	3.05	11	High
Okada service	2.99	12	High
Early maturing variety	2.94	13	High
Plant drought resistant variety	2.83	14	High
Change from crop to livestock	2.75	15	High
Engage in small-scale business	2.63	16	Moderate
Mixed cropping	2.47	17	Moderate
Crop rotation practice	2.35	18	Moderate
Practice water-harvesting scheme	2.21	19	Moderate
Planting of cover crops	1.21	20	Low
Change mulching material	1.15	21	Low
Average	2.94	-	High

Source: Computation from field survey, 2021

CONCLUSION

It was concluded that the degree of adaptive capacities to climate change was high among the adaptation strategies sample across the State except for changes in mulching materials and planting of cover crops. The study recommends that to reduce the effect of climate change in these areas, there is a need for policymakers to engage community stakeholders when making decisions relating to their livelihood.

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Positive Role of Applied Chitosan as a Supplement Fertilizer on Okra Plants

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ABSTRACT

This experiment was conducted under modified greenhouse (net house) conditions at the Central Laboratory for Agricultural Climate (CLAC), Agricultural Research Center (ARC), to investigate the effect of using chitosan as a supplement fertilizer. Seeds of okra (*Abelmoschus esculentus* cv Balady) were sown on 15th February from each season in 2020 and 2021. Two factors were tested (i) applied method of chitosan (spry and adding to soil), and (ii) concentration of chitosan such as (100, 150, 200, 250 and 300 ppm) with fourth replicates designed in a randomized complete block. Results reflected the positive role of using a high concentration of chitosan on the growth, yield and quality of okra plants. The greatest values of all tested parameters i.e., vegetative growth (plant height, number of leaves and fresh and dry weights of leaves), chemical contents of leaves (N, P and K plus chlorophyll reading) and yield and its components (number of fruits/plant, average fruit weight, early and total yield, total protein, phosphorus and potassium) were obtained with 80% recommended doses from "N" chemical fertilizer + chitosan adding to soil 250 ppm "T10" and 80% recommended doses from "N" chemical fertilizer + chitosan spray 300 ppm "T6" treatments rather than grower treatment and reduced content of dietary fiber in okra fruit. While stem diameter was not affected by applied two tested factors.

INTRODUCTION

Okra (*Abelmoschus esculentus*) is an important vegetable crop with high demand and high economic value. Okra is an important and high-yielding vegetable of the mallow family grown in many countries. Okra, also known as 'Lady's cinquefoil' or 'Bendi' in Malaysia, is popular for its health benefits such as high fiber content, antioxidants, vitamin C, minerals, potassium and calcium. It is also important as a medicinal plant for plasma replacement in tropical and subtropical countries (Kumar et al., 2013; Sorapong, 2012). Popular for its short production time and ease of cultivation, okra is a versatile crop as fresh leaves, flowers, buds, pods, stems and seeds provide multiple uses. Additionally, the medicinal properties of okra have been recognized (Gemede et al., 2015). In addition, okra mucus can be used as a blood volume-increasing agent or plasma substitute for medical purposes. Okra mucilage binds cholesterol, toxins in bile acids are excreted by the liver, and most parts of okra are edible and used as food (Gemede et al. 2015; Maramag, 2013).

Chitosan is a biopolymer, a chitin derivative, and a compound that is completely harmless to the environment. That is mean it's very safe and environmentally friend. Moreover, this compound is characterized by unique properties such as bioactivity and biocompatibility (Dias et al., 2013). Its derivative, chitosan, is therefore described as a linear, semi-crystalline polysaccharide composed of glucosamine

(C6H13NO5) and N-acetylglucosamine linked by glycosidic β-linkages (1-4), with free amino groups. It differs from chitin polymers by the presence of in the polymer; we distinguish the second carbon atom of the D-glucose unit, not the acetamide group (Agbodjato et al. 2021). Also, literature results show that the use of chitosan in plants increases yield (Mondal et al., 2012), decreases transpiration (Dzung et al. 2011), and induces many metabolic changes to reduce plant viral load. It has been shown to increase tolerance to bacterial and fungal infections (Al-Hetar et al. 2011). In addition, chitosan-treated plants may be less susceptible to stress caused by adverse conditions such as drought, salinity, cold or hot temperatures (Lizarraga-Pauli et al. 2011; Jabeen and Ahmad, 2013; Pongprayoon et al. 2013). Chitosan stimulates important plant processes at all levels of biological organization, from single cells and tissues, through physiological and biochemical processes, to molecular changes associated with gene expression (Limpanavech et al. 2008; Hadwiger, 2013; Nguyen Van et al. 2013). Chitosan refers to a group of commercially available copolymers rather than a unique compound. In addition, treatment with chitosan makes plants more resistant to various soil and foliar pathogens and induces root-knot formation (Hamel and Beaudoin, 2010), making chitosan a useful tool for agricultural sustainability (Iriti and Varoni, 2015; Pichyangkuraa and Chadchawanb, 2015). Moreover, Kah et al. (2013) found that chitosan increases the absorption of bioactive compounds, allowing plants to absorb nutrients more effectively.

On other hand, the main goal of modern agriculture is to produce sufficient quantities of good quality food to meet the growing world population with low environmental impact (FAO, 2013). Agricultural production is severely affected by many pests and diseases, which can lead to huge losses. Chemical fertilizers and pesticides have been used over the past 100 years to combat these problems and increase yields. Large-scale development of these products has greatly increased productivity, but has also led to biodiversity loss and degradation of natural and agricultural systems. Furthermore, residue accumulation has caused environmental pollution and public health problems with the emergence of resistant pests (Sun et al. 2012). Therefore, alternative methods are needed to address these issues and reduce the environmental impact of activities without compromising agricultural productivity and achieving economic returns. Recently, chitosan-based materials have been used to create nanoparticles that can efficiently supply chemicals and nutrients to plants (Kah et al., 2013). Indeed, chitosan is readily absorbed by the epidermis of leaves and stems, prolonging the contact time and facilitating the absorption of bioactive molecules.

Furthermore, fertilizer requirements are important in early growth to improve okra productivity and quality. Currently, chemical fertilizers such as NPK (nitrogen, phosphorus, and potassium) are widely used in agricultural fields, including vegetable cultivation, because they can achieve high productivity in a short period of time. However, it is very expensive and causes nutrient imbalance and soil acidification (Akande et al. 2010). Furthermore, overuse of chemicals used to fertilize plants can lead to the accumulation of minerals and nutrients that are not readily available for plant consumption, ultimately leading to soil contamination and toxicity (Savci, 2012). Therefore, increasing crop production depends on improving soil fertility to ensure food for all in the current global food security scenario (Godfray et al. 2010). Therefore, it is imperative to develop various eco-friendly methods to improve soil fertility and increase agricultural production.

This investigation aims to evaluate the effect of using two ways for adding chitosan as a supplement fertilizer on the growth, yield, and quality of okra plants.

MATERIAL AND METHODS

This investigation was conducted at Central Laboratory for Agricultural Climate (CLAC), Agricultural Research Center (ARC), Giza, Egypt, under a modified greenhouse (net house). Through two summer seasons 2020 and 2021 to evaluate using chitosan as a supplement fertilizer on growth, yield and quality of okra plants.

Experimental layout

A modified greenhouse coved by a white net was 40m long, 8m wide and 5.25m height. This house was divided into 5 beds (1m wide and 40m long). Seeds of okra (*Abelmoschus esculentus* cv Balady) were sown on 15th February from each season by the spacing of 0.5m between plants inside the raw and separated 0.50m between beds. A drip irrigation system was placed in this experiment. Recommended doses from chemical fertilizer which should be added "200 Kg

ammonium nitrate (33.5% NH4NO3), 100 Kg potassium sulphate (48% K2O) and 150 Kg calcium super phosphate (15.5 % P2O5)/fad." was applied (as 100%) only with grower treatment (control), while, application chitosan treatments as supplement fertilizer were applied by 80% from nitrogen chemical fertilizer recommended doses. Fertilizer treatments were divided into three equal doses; the first was added after 30 days from the sowing date, the second at the flowering stage and the third after 30 days from the second does.

Treatments

Two factors were tested as follows compared to the grower treatment (as control):
(A) The applied method of chitosan (spry and adding to soil), and
(B)The concentration of chitosan such as (100, 150, 200, 250 and 300 ppm).
Treatments were arranged as follows:
1.100% recommended doses from chemical fertilizer (control treatment) "T1",
2. 80% recommended doses from "N" chemical fertilizer + chitosan spray 100 ppm "T2",
3.80% recommended doses from "N" chemical fertilizer + chitosan spray 150 ppm "T3",
4. 80% recommended doses from "N" chemical fertilizer + chitosan spray 200 ppm "T4",
5. 80% recommended doses from "N" chemical fertilizer + chitosan spray 250 ppm "T5",
6. 80% recommended doses from "N" chemical fertilizer + chitosan spray 300 ppm "T6",
7.80% recommended doses from "N" chemical fertilizer + chitosan adding to soil 100 ppm "T7",
8.80% recommended doses from "N" chemical fertilizer + chitosan adding to soil 150 ppm "T8",
9.80% recommended doses from "N" chemical fertilizer + chitosan adding to soil 200 ppm "T9",
10. 80% recommended doses from "N" chemical fertilizer + chitosan adding to soil 250 ppm "T10", and
11. 80% recommended doses from "N" chemical fertilizer + chitosan adding to soil 300 ppm "T11".

Data recorded

(1) ***Vegetative growth parameters***

Plant height, number of leaves, fresh and dry weights of leaves and stem diameter were measured at med of the season from fifth okra plants as random samples.

(2) ***Chemical contents of leaves***

Content percentages from N, P and K in leaves were recorded plus chlorophyll reading at med of season. Nitrogen was determined in leaves by the distillation in a Macro-Kjeldahle according to (FAO, 2008). Phosphorus was colorimetrically determined in leaves in the acid digest using ascorbic acid and ammonium molybdate as described by FAO (2008). Potassium was estimated in leaves photometrically as described by FAO, 2008. When, chlorophyll reading was measured in leaves by using a digital chlorophyll meter (model Minolta chlorophyll meter SPAD-501).

(3) Yield and its components

A number of fruits/plant, average fruit weight (from 10 fruits), and early and total yield (per plant) were measured at the harvest stage. Additionally, total protein and dietary fiber were determined according to A.O.A.C. (2005). Phosphorus and potassium concentration was determined according to FAO (2008).

Experimental design and data analysis

This experiment was designed in randomized complete block with fourth replications and obtained data were statistically analyzed using the analysis of variance method. Duncan's multiple range tests at a 5% level of probability were used to compare the means of the treatments (SAS, 2005).

RESULTS

Vegetative growth parameters

Data in Table (1) reflected the effect of applied chitosan as a supplement fertilizer on the vegetative growth of okra plants at tested two grown seasons 2020 and 2021.

Generally, indicated that, the application of okra plants with a high concentration of chitosan (spray or adding to soil) enhanced all tested vegetative growth parameters such as (plant height, number of leaves, fresh and dry weights of leaves) through two grown seasons compared to grower treatment. Contrary, the stem diameter parameter was not affected by the applied two tested factors at all two growing seasons. The greatest values of those parameters were obtained with applied treatments T10 (80% recommended doses from "N" chemical fertilizer +

chitosan adding to soil 250 ppm) and T6 (80% recommended doses from "N" chemical fertilizer + chitosan spray 300 ppm) more than other treatments without any significant difference. When application of okra plants by 80% of recommended doses from "N" chemical fertilizer + chitosan spray 100 ppm "T2" reduced all those parameters followed by 80% of recommended doses from "N" chemical fertilizer + chitosan spray 150 ppm "T3", respectively.

Table (1): Effect of using chitosan as supplement fertilizer by spraying or adding to soil on vegetative growth parameters i.e., plant height (cm), number of leaves, fresh and dry weights of leaves (g) and stem diameter (cm) of okra plants through 2020 and 2021 seasons.

Treatments	plant height	number of leaves	Fresh weight of leaves	Dry weights of leave	stem diameter
	Frist Season				
T_1	60.24D	16.25E	78.68D	16.20G	1.40A
T_2	52.63G	12.92J	64.94H	13.88K	1.34A
T_3	56.76EF	13.77I	68.65G	14.35J	1.36A
T_4	61.54D	15.55F	79.35D	16.66F	1.41A
T_5	64.33C	17.66D	80.55C	18.83E	1.44A
T_6	72.80A	24.96A	93.85A	23.54B	1.51A
T_7	54.35FG	14.25H	72.77F	14.99I	1.37A
T_8	58.85DE	14.86G	75.83E	15.53H	1.39A
T_9	65.42C	19.68C	82.66C	19.68D	1.46A
T_{10}	73.95A	25.22A	95.45A	24.15A	1.54A
T_{11}	68.53B	22.12B	88.67B	21.75C	1.49A
	Second Season				
T_1	58.43D	15.93E	75.53D	15.55G	1.39A
T_2	51.05G	12.66J	62.34H	13.32K	1.33A
T_3	55.06EF	13.49I	65.90G	13.78J	1.35A
T_4	59.69D	15.24F	76.18D	15.99F	1.40A
T_5	62.44C	17.31D	78.29C	18.08E	1.43A
T_6	70.62A	24.46A	90.10A	22.60B	1.49A
T_7	52.72FG	13.97H	69.86F	14.39I	1.36A
T_8	57.08DE	14.56G	72.80E	14.91H	1.38A
T_9	63.46C	19.29C	79.35C	18.89D	1.45A
T_{10}	71.73A	24.72A	91.61A	23.18A	1.52A
T_{11}	66.47B	21.68B	85.12B	20.88C	1.48A

Chemical Contents of Leaves

Illustrated data in Table (2) showed the effect of applied chitosan (spray or adding to soil) on the chemical contents of leaves (N, P, K and chlorophyll reading). The greatest contents in leaves from those parameters were observed with application treatments 80% recommended doses from "N" chemical fertilizer + chitosan adding to soil 250 ppm "T10" and 80% recommended doses from "N" chemical fertilizer + chitosan spray 300 ppm "T6", respectively, without any significant differences compared to other treatments. Although, the application of 80% recommended doses from "N" chemical fertilizer + chitosan spray 100 ppm "T2" led to reduced it.

Yield and its components

Presented data in Tables (3 and 4) indicated the effect of using chitosan as supplement fertilizer yield and its components such as (number of fruits/plant, average fruit weight fruits), early yield, total yield and fruit quality i.e., (total protein, dietary fiber, phosphorus and potassium).

Both tables observed that applying a high concentration of chitosan increased yield and

enhanced fruit quality more than the grower treatment (control). The highest yield and quality were obtained with okra plants applied by 80% recommended doses from "N" chemical fertilizer + chitosan adding to soil 250 ppm "T10" and 80% recommended doses from "N" chemical fertilizer + chitosan spray 300 ppm "T6" treatments, without any significant differences. When, applied 80% recommended doses from "N" chemical fertilizer + chitosan spray 100 ppm "T2" treatment reduced values of all tested yield and its components parameters. Furthermore, control treatment (100% recommended doses from chemical fertilizer (control treatment) "T1") replaced sixth rank after 80% recommended doses from "N" chemical fertilizer + chitosan spray 200 ppm "T4", without any significant differences.

Table (2): Effect of using chitosan as supplement fertilizer by spraying or adding to soil on chemical contents of leaves chlorophyll reading (SPAD), N, P and K (%) of okra plants through 2020 and 2021 seasons.

Treatments	Chlorophyll reading	N	P	K
	Frist Season			
T_1	40.75E	1.65D	0.39D	1.68D
T_2	31.20I	1.43G	0.31G	1.52G
T_3	33.34H	1.49F	0.33F	1.57F
T_4	41.95E	1.67D	0.40D	1.70D
T_5	45.87D	1.73C	0.42C	1.74C
T_6	56.53A	1.88A	0.47A	1.85A
T_7	35.42G	1.53F	0.34F	1.59F
T_8	39.65F	1.59E	0.37E	1.64E
T_9	49.84C	1.79B	0.44B	1.78B
T_{10}	57.76A	1.92A	0.48A	1.87A
T_{11}	53.27B	1.82B	0.45B	1.81B
	Second Season			
T_1	39.12E	1.58D	0.37D	1.61D
T_2	29.95I	1.37G	0.30G	1.46G
T_3	32.01H	1.43F	0.32F	1.51F
T_4	40.27E	1.60D	0.38D	1.63D
T_5	44.04D	1.66C	0.40C	1.67C
T_6	54.27A	1.80A	0.45A	1.78A
T_7	34.00G	1.47F	0.33F	1.53F
T_8	38.06F	1.53E	0.36E	1.57E
T_9	47.85C	1.72B	0.42B	1.71B
T_{10}	55.45A	1.84A	0.46A	1.80A
T_{11}	51.14B	1.75B	0.43B	1.74B

DISCUSSION

In this study obtained that using chitosan as a supplement fertilizer in high concentration had the best impact on improving and increasing all evaluated parameters of okra crop i.e., (plant height, number of leaves, fresh and dry weights of leaves, chlorophyll reading in leaves, content percentage from N, P and K in leaves, number of fruits/plant, average fruit weight fruits, early yield/plant, total yield/plant, total protein, dietary fiber, phosphorus and potassium).

This increase may be due to the use of chitosan which increases the activity of key enzymes of nitrogen metabolism (nitrate synthesis, glutamine synthetase, proteases), improves nitrogen transport in functional leaves, and promotes plant growth (Chibu and Shibayama, 2003; Shehata et al., 2012). Also, Hafez et al., 2019 found that chitosan application played a positive role in promoting functional leaf nitrogen transport, which promoted plant growth. Furthermore, Mondal and Malek et al., 2012 stated that vegetative growth parameters of okra increased with increasing chitosan application concentration. On the other hand, chitosan facilitated plant growth

by treating the plants with necessary mineral elements, which the plants could not supply in sufficient supply, probably due to soil problems or the supply of certain necessary amino compounds to the plants (Chibu and Shibayama, 2003). In addition, plants grew better due to improved root growth and greater root spread in the soil (Zubaidi and Zainab, 2016).

Table (3): Effect of using chitosan as supplement fertilizer by spraying or adding to soil on number of fruits/plant, average fruit weight (g), early yield/plant (g) and total yield/plant (Kg) of okra plants through 2020 and 2021 seasons.

Treatments	Number of fruits	Average fruit weight	Early yield/plant	Total yield/plant
	First season			
T_1	60.78E	3.05E	188.65E	2.850E
T_2	50.00I	2.32H	110.38I	2.150I
T_3	52.64H	2.54G	122.24H	2.335H
T_4	61.28E	3.08E	192.50E	2.995E
T_5	64.58D	3.32D	215.12D	3.162D
T_6	74.92A	3.86A	298.85A	3.724A
T_7	55.15G	2.58G	131.28G	2.510G
T_8	57.23F	2.83F	162.47F	2.680F
T_9	68.42C	3.55C	235.34C	3.388C
T_{10}	75.76A	3.98A	300.79A	3.853A
T_{11}	72.55B	3.62B	275.29B	3.558B
	Second season			
T_1	57.74E	2.93E	182.991E	2.793E
T_2	47.50I	2.23H	107.069I	2.107I
T_3	50.01H	2.44G	118.573H	2.288H
T_4	58.22E	2.96E	186.725E	2.935E
T_5	61.35D	3.19D	208.666D	3.099D
T_6	71.17A	3.71A	289.885A	3.650A
T_7	52.39G	2.48G	127.342G	2.460G
T_8	54.37F	2.72F	157.596F	2.626F
T_9	65.00C	3.41C	228.280C	3.320C
T_{10}	71.97A	3.82A	291.766A	3.776A
T_{11}	68.92B	3.48B	267.031B	3.487B

Chitosan has various functional groups such as hydroxyl groups and amine groups, and because it binds to metal ions by chemisorption or physical adsorption, it has a high adsorption capacity for various metal ions. Chitosan works with metallic elements because it fits the basic natural properties of multiple cations. Chitosan stimulates the activity of key enzymes in nitrogen metabolism and improves nitrogen transfer to leaves. This stimulates leaf function in growth and development. In addition, chitosan is a polysaccharide that is very important for plant defense and yield increase in plant nutrition, especially in horticulture. It causes a doubling of photosynthesis. The action of chitosan molecules varies from cell to cell and depends on their physiochemistry. It leads to increased root mass, flowering and final production (Abdel-Mawgoud et al., 2010; Monirul et al., 2018; Al-Hassani and Majid, 2019). Furthermore, chitosan had a positive effect on root effectiveness and nutrient uptake, leading to increased photosynthetic efficiency and carbohydrate and sugar production, resulting in increased internode growth and length and food accumulation led to an increase in stem diameter (Monirul et al., 2018).

Also, this result is harmony with Mondal et al., 2013, who, indicated that photosynthesis and chlorophyll in cowpea plants are increased by chitosan treatment,

which also influences the increase in biomass to water content by reducing transpiration (Bittelli et al., 2001). In addition, chitosan played an important role in plant nutrition and also had positive effects on growth rate, plant properties, and increasing metallic elements (Mondal and Malek, 2012; Mondal et al., 2013). Similarly, Guan et al., 2009 have shown that, this increases the availability and uptake of water and essential nutrients by modulating cellular osmotic pressure, and the release of harmful free radicals by increasing antioxidants and enzymatic activity. We believe this is due to a decrease in accumulation. Malerba and Cerana, 2016 reported that chitosan application enhances leaf chemistry, promotes water and nutrient uptake by vigorous roots, combats oxidative damage by reactive oxygen species (ROS), and enhances antioxidant enzymes. Furthermore, found that it can activate both defense systems and photosynthetic enzymes. Photosynthesis and biosynthesis of essential organic molecules are improved, increasing the accumulation of assimilates.

Table (4): Effect of using chitosan as supplement fertilizer by spraying or adding to soil on total protein (%), dietary fiber (%), phosphorus (%) and potassium (%) of okra fruits through 2020 and 2021 seasons.

Treatments	Total protein	Dietary fiber	Phosphorus	Potassium
	First season			
T_1	3.58E	2.18A	0.68E	2.42E
T_2	3.24I	1.55B	0.52I	2.04I
T_3	3.33H	1.52C	0.57H	2.13H
T_4	3.62E	1.37F	0.70E	2.44E
T_5	3.83D	1.34G	0.74D	2.53D
T_6	4.54A	1.24I	0.84A	2.82A
T_7	3.44G	1.46D	0.60G	2.22G
T_8	3.52F	1.41E	0.64F	2.33F
T_9	4.22C	1.30H	0.78C	2.64C
T_{10}	4.57A	1.25I	0.86A	2.88A
T_{11}	4.35B	1.29H	0.82B	2.73B
	Second season			
T_1	3.47E	2.09A	0.65E	2.40E
T_2	3.14I	1.49B	0.50I	2.02I
T_3	3.23H	1.46C	0.55H	2.11H
T_4	3.51E	1.32F	0.67E	2.42E
T_5	3.72D	1.29G	0.71D	2.50D
T_6	4.40A	1.19I	0.81A	2.79A
T_7	3.34G	1.40D	0.58G	2.20G
T_8	3.41F	1.35E	0.61F	2.31F
T_9	4.09C	1.25H	0.75C	2.61C
T_{10}	4.43A	1.20I	0.83A	2.85A
T_{11}	4.22B	1.24H	0.79B	2.70B

Furthermore, the enhancement at yield and its components parameters attribute to chitosan-containing plants were the best in most traits of vegetative growth and had a high proportion of mineral matter, which allowed the plants to build carbohydrate matter and high carbonation, reflected in an increase in average fruit weight (Al-Hassani and Majid, 2019). Application of chitosan increased all vegetative growth traits and yields and their constituents (Hafez et al., 2019). The important effects of chitosan on yield and its composition are likely due to the fact that chitosan has mimetic effects on physiological processes, improving nitrogen transport in functioning leaves and improving vegetative growth and development (Gornik et al., 2008).

The vast impact of chitosan is probably because of that chitosan is a brand new plant boom promoter which include GA3 that can be have impact at the plant boom and yield (El-Bassiony et al., 2014). On the other hand, for the impact of chitosan on macro and

micro elements (N, P, K, Fe, Zn, Cu and B), chitosan may be used as a remedy for mineral factors infected soil (Sheikha and Al-Malki, 2011). Also, the main position of chitosan in ameliorating plant roots potential to uptake water and vital elements and use them correctly inside plant in promoting of antioxidant enzyme sports, prevention of reactive oxygen species (ROS), activation of photosynthesis and biosynthesis of carbohydrates, proteins and different natural compounds that wanted for distinct plant metabolic sports and manufacturing of greater assimilates which translocated to end result in result of K and P mode of action, and there for the marketable end result yield and first-rate increased (El-saady, 2016). Moreover, the effective effect of chitosan on chemical additives leaves in phrases of photosynthetic pigments and NPK elements can be ascribed to the chitosan-mediated enhance root device performance to soak up greater to be had water and nutrients that wanted for critical physiological sports which include photosynthesis and biosynthesis of critical assimilates which ameliorate leaves chemical first-rate (Malekpoor et al., 2016).

In addition, chitosan increases the uptake and availability of water and essential nutrients by regulating intracellular osmotic pressure, promoting plant growth. Over the past decade, the signaling mechanisms of chitosan and its derivatives that regulate plant growth and developmental processes have been studied. Initial results show that chitosan helps activate the hydrolytic enzymes required to degrade and mobilize stored food materials such as starch and protein. Chitosan promotes root cell division by activating plant hormones such as auxin and cytokinin, further increasing nutrient intake. Furthermore, the increased yield can be attributed to chitosan's plant growth-promoting activity may be directly related to its effects on plant physiological mechanisms such as nutrient uptake, cell division, cell elongation, enzyme activation, and protein synthesis (Amin et al., 2007). In addition, Chissan improved the photosynthetic index by improving stomatal function and chlorophyll content, and also significantly increased crop yield. Polycationic chitosan increases the osmotic pressure of stomatal cells, resulting in increased stomatal opening and CO2 uptake (Chakraborty et al., 2020).

On other hand, soil-applied chitosan also significantly enhanced seedling growth and induced early flowering in many ornamental plants (Pichyangkura and Chadchawan, 2015). Similarly, Xu and Mou (2018) found that applying chitosan to soil increased lettuce leaf number, area, fresh weight, dry weight, and chlorophyll index. Suppression of plant diseases, insects and nematodes, increased biomass and beneficial microbial activity, high nitrogen and calcium content, improved soil physical structure and nutrient availability, direct stimulation of plant growth the synergistic effect of many factors, may play a role derived from chitosan as a soil conditioner.

Furthermore, addition of chitosan alters rhizosphere conditions, shifting the microbial balance in favor of beneficial organisms and against plant pathogens (Sharp, 2013). Chitosan provides a carbon source for soil microorganisms, promotes the conversion of organic matter to inorganic matter, and helps roots to absorb more nutrients from the soil (Xu and Mou, 2018).

Chitosan and all other chitin derivatives have a high nitrogen content of 6% to 9%. Plants can access nitrogen in chitin through microbial degradation and release of inorganic nitrogen, or by direct uptake of monomers as organic nitrogen (Roberts and Jones, 2012). Chitosan can be used to add organic matter to soil without increasing the carbon-to-nitrogen ratio. In addition to nitrogen, chitosan also contains large amounts of calcium minerals that provide structural strength to crustacean exoskeletons (Boßelmann et al., 2007). Although chitosan contains nitrogen and calcium, its beneficial effects on plant growth and yield are not due to that nutrient alone, and some studies have shown that the control plots treated with mineral fertilizers have been shown to contain chitosan nutrients was balanced.

Chitosan significantly enhanced the seedling growth of several plants compared to conventional mineral fertilizers. Due to its cationic properties, chitosan is also suitable as a vehicle to provide additional essential nutrients (Sharp, 2013). Hydroxyl and amino functional groups on deacetylated chitosan allow the formation of coordination compounds with ions such as copper, zinc, iron, but not alkali metals (e.g. potassium) or alkaline earth metals (e.g. calcium or magnesium) is not possible (Ramírez et al., 2010). For this reason, chitosan is a sustainable alternative to synthetic chelators such as ethylenediaminetetra acetic acid, which are routinely used to supply iron and other nutrients to overcome their low solubility in calcareous/neutral soils. It has become a viable alternative (Strawn et al., 2019). Due to its high molecular weight and porous structure, chitosan

forms a gel that absorbs large amounts of water and enhances the water-holding capacity of soil (Jamnongkan and Kaewpirom, 2010).

Application of chitosan to soil increased levels of nitrogen, phosphorus, potassium, total sugars, soluble protein and total amino acids (Farouk et al., 2011). Chitosan application to soil has been reported to increase chlorophyll content in leaves of many crops (Farouk et al., 2011; Sheikha and Al-Malki, 2011). As a bio-stimulant, chitosan may also enhance the fluorescence of chlorophyll and enhance the photosynthetic rate.

Furthermore, the use of chitosan as a bio-stimulator in plant development can increase leaf and shoot size. Chitosan has been found to exert molecular effects on flowers, directly affecting growth and physiological parameters (Salachna and Zawadzińska, 2014).

CONCLUSION

From this investigation indicated that application chitosan by high concentration started by 200 ppm have a positive role for increasing and enhancing all tested parameters of okra plants such as (vegetative growth, chemical contents in leaves, yield and fruits quality. On other hand, the suitable concentration depending on method applies of chitosan. The greatest values of almost i.e., vegetative growth, chemical contents in leaves, yield and best fruits quality parameters were obtained with applied 80% recommended doses from chemical fertilizer + chitosan adding to soil 250 ppm "T10" and 80% recommended doses from chemical fertilizer + chitosan spray 300 ppm "T6" treatments, without any significant differences followed by 80% recommended doses from chemical fertilizer + chitosan adding to soil 300 ppm "T11", 80% recommended doses from chemical fertilizer + chitosan adding to soil 200 ppm "T9", 80% recommended doses from chemical fertilizer + chitosan spray 250 ppm "T5" and 80% recommended doses from chemical fertilizer + chitosan spray 200 ppm "T4" treatments, respectively. More that, control treatment (100% Recommended doses from chemical fertilizer (control treatment) "T1") placed the six place.

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Population Effect on Individual Crop Growth, Development, and Yield in Rainfed Maize in Southern Guinea Savanna Ecological Zone of Nigeria

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ABSTRACT

Maize yield is closely related to plant population; more plants mean higher yield. However, there are limitations to increasing plant population in tropical conditions. A 3 x 4 Factorial experiment with four replications was conducted to evaluate four plant populations (53, 333, 106, 666, 159,999, 213,332 plants/ha) on crop growth, development, yield and yield components of three maize varieties (Local variety, Oba Super and Samaz 52). The crops were spaced 25 cm x 75 cm and seeds were sown in line with the expected plant population per hectare: 53, 333, 106, 666, 159,999, 213,332 plants/ha. Six plant stands were randomly selected and tagged from the net plot for height measurement, average numbers of leaves per plant, leaf areas, and stem girth at 3, 5, 7 and 9WAS, as well as for yield and yield components. Significant ($p \leq 0.05$) influence of population was observed on days to 50% tassel, ear weight, ear length, kernel/ear, 100-kernel weight and grain yield per plant. Generally, increasing plant population led to the processive delay in days to 50% heading, reduction in ear weight, ear length, kernel/ear, 100-kernel weight as well as grain yield. There were significant interactions between variety and population on days to 50% tassel, ear weight, ear length,kernel/earr, 100-kernel weight and grain yield per plant. The better response of hybrids to population stress was evident in this trial compared with the local variety where the highest grain yield per plant was recorded in Oba Super II (779.13g and 890.01g, respectively in 2021 and 2022 cropping seasons), while the least grain yield per plant was in the local variety (530.80g and 475.00g, respectively in 2021 and 2022 cropping seasons). Sowing seeds at one seed/hole gave the highest grain yield/plant, 790.27g and 970.00g, respectively in the 2021 and 2022 cropping seasons. The least grain yield/plant, 513.27g and 322.50g, respectively in 2021 and 2022 cropping seasons were observed when four seeds were sown/hole. The highest amount of grain yield/ha was obtained in Oba super II (103,883.74kg and 118,667.70kg), respectively in the 2021 and 2022 cropping seasons, with the local variety giving the lowest grain yield in both seasons. While the lowest grain yield/plant was recorded in P4 (213,332 pop/ha), in the first year, the plot

compensated for the yield reduction/plant with an increase in plant population cumulating in significantly greater harvest/ha (109,496.94 kg/ha), with P1(53,333 plant/ha) recording significantly the lowest grain yield/ha in both seasons. However, P4(213,332 pop/ha) did not repeat the same feat attained in the first trial as it trailed behind P3 (160,000 pop/ha) and P2 (106,666 pop/ha) in yield/ha; an indication that the population may not be able to maintain stable yield. The most consist population relative to yield/ha was P3 (160,000 pop/ha).

INTRODUCTION

It has been reported that maize producers in many parts of the world, particularly in developing countries where good-quality data from local trials are not available, rely on published information to make agronomic decisions. Observing that many papers have been published on the effects of plant population on yield, but the results are associated with prevailing local environmental conditions and agronomic practices of each study. Stating that this could lead to confusion among maize producers regarding the most appropriate agronomic management decision for their specific conditions and farming systems. Thus, there is a need to indigenize such studies to identify how plant population affects maize grain yield under local conditions.

It has generally been observed that maize yield in Nigeria is low compared to some other countries in Africa (Oloyede-Kamiyo and Olaniyan, 2020). While the authors observed that the reason adduced to this yield disparity has been low soil nitrogen, the problem of pests and diseases as well as poor access to quality seeds, they stressed that apart from these factors, one other major cause of poor yield of maize in Nigeria is suboptimal plant population. When adequate plant population is not maintained, low yield results, are observed Oloyede-Kamiyo and Olaniyan (2020). They reported that the recommended plant population for maize is 53,333 plants/haa at a spacing of 25cm within a row and 75cm between rows at one plant per hill, while a population density of 80,000 plants/ha was found to be optimal for hybrids (Olaniyan, 2014; Oloyede-Kamiyo and Olaniyan, 2020).

Erenstein et al. (2022) observed that, since its domestication some 9,000 years ago, maize (*Zea mays* L.) has played an increasing and diverse role in global agri-food systems. Stressing that global maize production has surged in the past few decades, propelled by rising demand and a combination of technological advances, yield increases and area expansion (Erenstein et al. 2022). The authors added that maize is already the leading cereal in terms of production volume and is set to become the most widely grown and traded crop in the coming decade. In 2021, world maize production was 1,210 million thousand tonnes; an increase from 308 million thousand tonnes in 1972 with an average annual rate of 3.18% (Erenstein et al. 2022). Maize (Zea mays L.) plays a critical role in meeting the high food demand and is globally one of the most widely cultivated crops (FAO, 2017). Both the land area used for maize grain production and the amount of maize produced per unit area been increasing in recent years (FAO, 2017). It is one of the highest-yielding and most versatile cereals, adding that the global demand for maize has shown an increasing trend in the past decade (FAO 2009); with maize productivity increasing globally as a result of improved genetics and agronomic practices.

Oyewole et al. (2010) opined that the establishment of adequate plant stands is a prerequisite for successful crop production. In maize production, plant population and row spacing are two key agronomic factors known to have a strong influence on maize grain yield (IPNI Canada (2018), stating that maize yield is closely related to plant population, with more plants meaning higher yield. However, there are limitations to increasing plant population. The most favorable planting densities for high yield in the tropics are probably in the range of 65,000 to 75,000 plants/ha. Stressing that a population of less than 65,000 plants/ha is not advisable because a 10 percent loss of plants is not uncommon under rainfed field conditions (IPNI Canada, 2018). The report further added that having more than 75,000 plants/ha will not increase yield unless growing conditions are very favorable with a yield potential of >13 t/ha. Adding that for drought-prone environments, it is not advisable to have more than 75,000 plants/ha (IPNI Canada, 2018).

The optimum plant population depends on several crucial factors, including soil fertility, soil water-holding capacity, and hybrid maturity group, observed

Sangoi et al. (2002). Modern hybrids possess the ability to withstand greater stress attributable to high population densities than older hybrids, which in turn enables producers to establish higher plant populations, leading to higher yields per unit area (Russell, 1984; Duvick, 1997).

The number of plants per unit area is influenced by the distance between rows, the distance between plants in a row, and the number of plants in a hill. Farmers have been advised to, select an optimal plant spacing that allows for ease of field operations, such as fertilizer application or weeding, minimizes competition among plants for light, water, and nutrients, and creates a favorable micro-climate in the canopy to reduce the risk for pests and diseases (IPNI Canada, 2018). Narrow row widths of about 50 to 70 cm are recommended to ensure that sunlight falls on the plants and not on bare soil.

The agronomic practices implemented in a production system should allow the selected germplasm to react positively to the increased plant populations when favorable environmental conditions occur (Haegele et al. 2014) while also being tolerant to increased plant-to-plant competition under suboptimal growing conditions (Tokatlidis and Koutroubas, 2004). Changes in agronomic practices such as fertilization, effective weed control, and tillage practices can further alter the relationship between population density and maize grain yield. Thus, it is important to adjust the plant population accordingly to achieve optimal grain yields. Interactions between plant genotype and plant population can also affect maize grain yield, with a recent study conducted by DeBruin et al. (2017) finding a positive relationship between maize grain yields and plant population in modern hybrids, but a contrasting response in older hybrids. Previous reports observed that during the past six decades, much work has been done to evaluate the effects of plant population on maize grain yield in a wide variety of environments and regions (Duncan, 1958; Pretorius and Human, 1987; Ciampitti and Vyn, 2012; Hörbe et al. 2013; Assefa et al. 2016; Qin et al. 2016); with the observation that rainfall is a major determinant of differences in agronomic practices used between regions.

The authors reported that in arid and semiarid regions, rainfall is scarce and variable, and soil water is often the most limiting factor for grain production. Climatic conditions affect soil water content throughout the growing season, influencing the number of plants per unit area the soil can maintain throughout this period and, therefore, the optimal plant population. Both plant population and row spacing affect leaf canopy architecture (Sharratt and McWilliams, 2005) and, in turn, affect crop uptake of water and nutrients, as well as light interception. They pointed out that to justify the establishment of low plant populations, rapid canopy closure is needed for efficient resource use. Hammer et al. (2009) found that at high plant populations, root architecture was more important than canopy architecture and light interception for increasing grain yield.

As the human population is increasing with the total land area remaining fixed, the problem of scarcity of land for agricultural purposes is becoming pronounced. To feed this increasing population, the productivity of available land must be increased. As agricultural land becomes limiting, with the increasing human population, planting more seeds/hole maybe a justifiable means of addressing food scarcity. The study was to determine the effect of increasing the number of seeds/holes on plant growth, development, yield components and yield of maize under rainfed condition.

MATERIALS AND METHODS

A 3 x 4 Factorial experiment with three replications was conducted to evaluate four plant population (53, 333, 106, 666, 159,999, 213,332 plants/ha) on growth, development, yield and yield components of three maize varieties (Local variety, Oba Super and Samaz 52). Variety was a main treatment factor with population as sub treatment factor. The experiment was conducted in the rainy seasons of 2021 and 2022 in Kogi State University Anyigba Students' Research and Demonstration Farm (Latitude 70 301 and Longitude 70 091 E). The land was ploughed, harrowed and ridged. The crops were spaced 25 cm x 75 cm in subplots measuring 4m by 5m and seeds sown in line with the expected plant population per hectare. To achieve the experimental population, seeds were sown at either 1, 2, 3 or 4 seeds / hole, to give, respectively 53, 333, 106, 666, 159,999, 213,332 plants/ha. Nitrogen fertilizer (NPK 20:10:10) was applied in 2 split doses, starter doze at 2 weeks (60kg N/ha, 30kg P2O5/ha and 30 kg K2O/ha) after planting while crops were top dressed with Urea (46 % N) just before tasseling. Weed management was manually done with hoe at 2 and 6 weeks after sowing. However, after tasseling, emerging weeds were hand-

pulled. For the control of insect pests such as grasshoppers, stem borers and Fall Army Worms (FAW), Emamectin Benzoate was sprayed at the rate of 30ml/16litres of water using a knapsack sprayer.

Data Collection

Six stands of crops were tagged in each net plot (3.5m by 4.5m) for data collection throughout the period of the experiment. Growth and development data were collected at 2, 4, 6, 8 and 10WAS, while data on yield components and yield were determined at the termination of the trial. Data were collected on plant height, being a measure of plant height from ground level to the pick of the longest leaf (before tassel) or the tassel (after tassel); Number of leaves per plant, numerical counting of all fully unfolded leaves. Other parameters collected were leaf area, and stem girth in accordance with Oyewole et al. (2015 a & b). While data on yield and yield components, such as cob yield, cob weight and grain yield were also obtained over a Metler weighing scale to two decimal places.

Growth Parameters

• Plant height (cm): The heights of each of the tagged plants were measured using a meter rule; from the soil surface to the apex and recorded an average of the total plants measured.
• Number of leaves per plant: This parameter was obtained by a simple count of the total functional leaves produced by tagged plants and recorded as average numbers of leaves/plant.
• Leaf area per plant (cm2): This was determined by measuring the lamina length and maximum width, multiple by a constant of 0.75 as described by Oyewole (2011).
• Stem girth (cm): This was determined with the aid of veneer calipers and recorded as an average of six tagged stands. Measurements were taken just above the ground level.

Yield Parameters

• Number of ears/plant: The number of ears from tagged plants in each net plot was averaged over the number of tagged plants to obtain a mean number of ears for the plant.
• Ear length (cm): Lengths of harvested ears were measured with the aid of measuring tape and averaged over the number of harvests.
• Ear weight (g): Harvested ears were weighed and averaged over total harvests per net plot.
• Threshing percentage: The harvested cobs were weighed, threshed and the grains were weighed. The result was expressed as ratios of grain weight over total cob or ear weight expressed in percentage.
• Number of kernels/ear: Kernels on the harvested ears were manually striped counted and averaged over the total number of sampled cobs/plot.
• 100-grain weight: Samples of three batches of hundred kernels per plot were drawn and weighed and recorded as mean of three batches.
• Grain yield: Cobs in the net plots were separately harvested, threshed, winnowed and weighed to give grain yield per plot (tons/ha).

Analysis of Data

Data collected were subjected to Analysis of Variance (ANOVA) as described for Factorial Experiment (Statistical Analysis System (SAS), 1998) and means found to be statistically significant at 5% probability were separated using LSD.

RESULTS AND DISCUSSION

Effect of increasing maize population per stand on height (cm) of three varieties of maize

Plant height at maturity (cm) is an important component which helps in the determination of the growth attained during the growing period (Abuzar et al. 2011), however height is dependent on many variables, among which are variety used, nutrient available, as well as plant population among other factors. Generally, it has been observed that agronomic practices implemented in a production system should allow the selected varieties to react positively to increased plant populations when favorable environmental conditions occur (Haegele et al. 2014) while also being tolerant to increased plant-to-plant competition under suboptimal growing conditions (Tokatlidis and Koutroubas, 2004). While stressing that changes in agronomic practices such as fertilization, effective weed control, and tillage practices can further alter the relationship between population density and maize grain yield. Thus, it is important to adjust the plant population accordingly to achieve optimal grain yields. Analysis of data in this trial showed that variety investigated significantly ($p \leq 0.05$) influenced crop heights at 4, 8 and 10WAS in the 2021 cropping season (Table 1) and at 4, 6, 8 and 10WAS in the 2022 cropping season. At the end of the trial, Sammaz 52 recorded the tallest crops in both

seasons (385.71 cm and 340.83 cm, respectively in the 2021 and 2022 seasons), while the local variety recorded the tallest crops (363.11 cm) in the 2021 trial, while coming behind the other two varieties in 2022 trials. It should be expected that taller plants will lodge easily and are likely to break as a result of the wind effect (Oyewole et al. 2015a & 2015b). This will be more pronounced where an increase in plant height is not complemented by thicker plant stems / girths and where cobs are also borne high up the stems, which put more weight towards the top of the crop; such weight may make the plant tilt over under the influence of wind.

Table 1: Effect of increasing maize population per stand on height (cm) of three varieties of maize (*Zea mays*) in 2021 and 2022 cropping seasons

Treatment	2021 Cropping Season				2022 Cropping Season			
	Height (cm)							
	4WAS	6WAS	8WAS	10WAS	4WAS	6WAS	8WAS	10WAS
Variety								
V1: Sammaz 52	78.95^{a}	139.99	304.91^{b}	385.71^{a}	77.79^{a}	140.83^{a}	303.00^{a}	340.83^{a}
V2: Oba super-II	63.56^{b}	138.78	290.02^{c}	356.25^{c}	65.95^{b}	143.08^{a}	301.06^{a}	338.83^{a}
V3: Local Variety	64.53^{b}	140.49	318.04^{a}	363.11^{b}	63.61^{b}	121.42^{b}	275.39^{b}	297.92^{b}
LSD (0.05)	3.671*	NS	12.754*	7.891*	6.885*	13.895*	17.987*	12.761*
Population								
P1: 53,333 pop/ha	62.05^{d}	125.54^{d}	281.70^{b}	337.48^{c}	60.56^{b}	112.67^{d}	228.39^{d}	253.67^{c}
P2: 106,666 pop/ha	64.95^{cd}	137.36^{c}	293.41^{b}	356.29^{b}	65.67^{b}	134.78^{c}	303.21^{c}	336.44^{b}
P3: 160,000 pop/ha	67.33^{bc}	141.94^{b}	293.73^{b}	367.50^{b}	73.59^{a}	140.22^{b}	309.70^{b}	344.44^{b}
P4: 213,332 pop/ha	72.74^{a}	155.18^{a}	348.44^{a}	412.15^{a}	77.74^{a}	152.77^{a}	331.27^{a}	368.89^{a}
LSD (0.05)	2.781*	4.721*	15.932*	13.732*	6.223*	4.881	3.612	9.657*
Interaction								
V1P1	67.59^{bc}	125.22^{f}	271.02^{f}	351.99	72.40	118.00	288.40	318.00
V1P2	69.27^{b}	141.21^{cd}	297. 10d^{e}	377.21	72.80	144.33	304.23	344.33
V1P3	70.17^{b}	141.55^{cd}	300. 45^{cd}	388.41	81.30	144.66	313.23	344.66
V1P4	81.76^{a}	151.99^{b}	351. 05^{b}	425.22	84.66	156.33	306.13	356.33
V2P1	58.85^{e}	128.17^{f}	282. 31^{e}	338.77	59.66	130.00	289.44	318.00
V2P2	61.58^{cde}	134.64^{e}	287. 01^{e}	344.66	62.26	135.00	300.21	330.00
V2P3	65.95^{bc}	142.87^{c}	282. 54^{e}	342.77	70.23	147.00	304.23	347.00
V2P4	67.93^{bc}	149.44^{b}	308. 23^{c}	398.78	71.63	160.33	310.34	360.33
V3P1	59.70^{de}	123.22^{f}	291. 77d^{e}	321.67	49.63	90.00	107.34	125.00
V3P2	64.00^{bcde}	136.23^{d}	296. 12^{d}	346.99	62.66	125.00	305.20	335.00
V3P3	65.87^{bcd}	141.41^{cd}	298. 22^{d}	371.33	69.23	129.00	311.67	341.66
V3P4	68.53^{b}	161.11a	386. 04^{a}	412.45	72.93	141.66	377.34	390.00
LSD (0.05)	6.781*	5.329*	8.712*	4.672*	3.526*	8.782*	3.884*	9.563*
CV%	14.45	8.83	13.35	21.72	16.56	11.56	18.67	17.77

Means with the same letter(s) are not significantly different at 5% level of probability

Table 2: Effect of increasing maize population per stand on number of leaves of three varieties of maize (Zea mays) in 2021 and 2022 cropping seasons

Treatment	2021 Cropping Season				2022 Cropping Season			
	Leaf Number							
	4WAS	6WAS	8WAS	10WAS	4WAS	6WAS	8WAS	10WAS
Variety								
V1: Sammaz 52	8	10	12	12	8	10	12	12
V2: Oba super-II	7	10	11	12	7	10	11	13
V3: Local Variety	7	10	11	13	7	10	11	13
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS
Population								
P1: 53,333 pop/ha	8	10	11	13	8	10	12	13
P2: 106,666 pop/ha	8	10	11	13	8	10	11	13
P3: 160,000 pop/ha	7	9	11	12	7	9	11	12
P4: 213,332 pop/ha	7	10	11	12	7	10	11	12
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS
Interactions								
V1P1	8	10	12	12	7	10	12	12
V1P2	8	11	11	13	8	11	13	13
V1P3	8	9	12	12	7	10	12	13
V1P4	8	9	11	12	8	9	11	12
V2P1	7	9	12	12	8	10	12	11
V2P2	7	10	11	12	6	10	10	12
V2P3	7	10	10	13	6	10	11	13
V2P4	7	10	11	13	8	9	9	12
V3P1	8	9	11	13	8	9	11	12
V3P2	7	10	11	13	5	9	11	12
V3P3	7	9	11	13	7	9	11	12
V3P4	7	10	11	13	8	9	10	12
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS
CV%	11.37	7.99	8.04	5.17	17.24	4.73	7.29	15.02

Means with the same letter(s) are not significantly different at 5% level of probability

There was a significant ($p \leq 0.05$) influence of population investigated on crop heights at 4, 6, 8, and 10WAS in both seasons, with early signs of leaf etiolation observed due to competition for solar radiation (Hay and Walker, 1989) as seeding rates increase (Oyewole, 2011). Seeding at four seeds per hole consistently gave the tallest crops, followed by seeding three seeds per hole, with one seed per hole giving the shortest crops at 4, 6, 8 and 10WAS in both seasons; definitely because this experienced the least competition for solar radiation, compared with other treatment. Understandably, as crops are clustered together, they compete for solar radiation (Hay and Walker, 1989), and this competition leads to leaf elongation (Oyewole, 2011; Hay and Walker, 1989). It is expected that the higher the population per unit area, the stiffer the competition for solar radiation (Oyewole, 2011; Hay and Walker, 1989), thus producing taller crops as observed in this trial. The observation is in line with the outcomes of trials conducted by Oyewole et al. (2015a and 2015b). In a similar experiment conducted by Abuzar et al. (2011) data showed that plant height was significantly affected by plant population densities, which they observed was due to crowding effect of the plant and higher intra-specific competition for resources. Sangakkara et al. (2004) explained that as the number

of plants increased in a given area the competition among the plants for nutrients uptake and sunlight interception also increased, with competition for sunlight leading to increase in plant heights. It is, however not uncommon to find stunted plants with increasing plant population, particularly in an environment where major crop nutrients are critically limiting. Planting at one seed / hole gave the shortest average crop height at the end of the trial (337.48 cm in 2021 and 253.67 cm in 2022 cropping seasons), while the tallest average plant height at the end of the trial were observed when four seeds were sown per hole (412.15 cm 368.89 cm, respectively in 2021 and 2022 cropping seasons).

Table 3: Effect of increasing maize population per stand on leaf area (cm^2) of three varieties of maize (*Zea mays*) in 2021 and 2022 cropping seasons

Treatment	2021 Cropping Season				2022 Cropping Season			
	Leaf Area (cm^2)							
	4WAS	6WAS	8WAS	10WAS	4WAS	6WAS	8WAS	10WAS
Variety								
V1: Sammaz 52	149.94	405.07	414.79	340.34	149.94	405.17	415.59	341.34
V2: Oba super-II	126.50	388.09	414.17	355.67	128.25	388.09	414.43	355.67
V3: Local Variety	123.72	353.22	407.07	349.60	124.22	350.04	406.51	349.60
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS
Population								
P1: 53,333 pop/ha	150.77	420.18	464.67^{a}	416.85^{a}	150.77	420.32	465.74^{a}	417.33^{a}
P2: 106,666 pop/ha	138.06	397.31	426.74^{ab}	359.06^{ab}	138.06	398.02	427.09^{ab}	359.39^{b}
P3: 160,000 pop/ha	126.01	357.11	381.28^{b}	317.92^{b}	128.13	356.45	381.59^{b}	318.10^{b}
P4: 213,332 pop/ha	117.44	353.91	375.35^{b}	300.31^{b}	119.59	349.62	374.30^{b}	300.65^{c}
LSD (0.05)	NS	NS	57.960*	66.580*	NS	NS	58.160*	55.370*
Interactions								
V1P1	146.79	413.19	435.51	383.23	171.65	517.34	497.96	436.45
V1P2	165.53	473.79	475.24	347.41	182.62	497.26	447.96	378.98
V1P3	151.39	377.64	379.16	333.08	180.92	438.80	413.33	376.57
V1P4	136.02	355.66	369.23	297.61	150.08	359.99	379.34	288.42
V2P1	123.70	399.41	375.17	350.34	150.09	435.27	363.66	396.40
V2P2	123.70	438.22	469.71	379.63	84.47	403.81	437.70	297.56
V2P3	109.96	361.34	399.89	326.71	154.33	443.69	459.23	361.20
V2P4	141.50	353.36	411.92	399.33	106.30	302.91	358.68	367.50
V3P1	145.20	380.65	449.05	467.99	88.12	302.68	324.95	425.95
V3P2	106.33	347.19	432.79	350.12	80.60	364.64	444.87	331.60
V3P3	107.54	332.35	364.78	303.67	120.48	398.63	420.09	293.70
V3P4	135.74	352.69	381.65	276.59	123.62	338.86	307.10	135.77
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS
CV%	26.63	18.10	16.73	21.57	12.64	11.56	19.91	22.19

Means with the same letter(s) are not significantly different at 5% level of probability

Table 4: Effect of increasing maize population per stand on stem girth (cm) of three varieties of maize (Zea mays) in 2021 and 2022 cropping seasons

Treatment	2021 Cropping Season				2022 Cropping Season			
	Stem girth (cm)							
	4WAS	6WAS	8WAS	10WAS	4WAS	6WAS	8WAS	10WAS
Variety								
V1: Sammaz 52	3.52	4.84	5.51	5.69	3.49	5.11	5.75	6.00
V2: Oba super-II	3.23	5.37	5.49	5.80	3.23	5.55	5.69	5.77
V3: Local Variety	3.24	5.17	5.54	5.70	3.24	4.81	5.42	5.81
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS
Population								
P1: 53,333 pop/ha	3.44	5.61^{a}	5.87^{a}	6.22^{a}	3.58	5.84^{a}	6.29^{a}	6.43^{a}
P2: 106,666 pop/ha	3.53	5.52^{a}	5.67^{a}	5.91^{ab}	3.41	5.79^{ab}	5.64^{a}	5.93^{ab}
P3: 160,000 pop/ha	3.09	4.84^{b}	5.45^{ab}	5.55^{bc}	3.58	5.29^{b}	5.69^{a}	6.03^{bc}
P4: 213,332 pop/ha	3.24	4.52^{b}	4.93^{b}	5.23^{c}	3.11	4.55^{c}	4.85^{b}	5.05^{c}
LSD (0.05)	NS	0.496*	0.521*	0.603*	NS	0.543*	0.642*	0.592*
Interactions								
V1P1	3.53	5.02	5.35	5.76	4.26	5.83	6.70	6.70
V1P2	4.14	5. 21	5.92	6.07	4.20	5.23	5.96	6.43
V1P3	3.27	4.66	5.72	5.72	3.53	4.40	5.13	5.60
V1P4	3.14	4.45	4.97	5.19	3.40	4.96	5.20	5.26
V2P1	3.31	5.83	6.13	6.26	3.67	6.63	6.50	6.60
V2P2	3.35	5.76	5.76	6.30	2.80	5.20	5.53	5.60
V2P3	3.04	5.10	5.46	5.50	3.86	5.56	6.16	6.23
V2P4	3.20	4.77	4.82	5.13	2.86	4.80	4.56	4.63
V3P1	3.49	5.97	6.13	6.65	2.83	5.06	5.66	6.00
V3P2	3.11	5.58	5.32	5.35	3.23	5.43	5.43	5.76
V3P3	2.97	4.76	5.18	5.43	3.36	4.83	5.78	6.23
V3P4	3.39	4.35	4.99	5.37	3.06	3.90	4.80	5.26
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS
CV%	16.59	10.19	9.95	10.76	6.61	12.87	8.54	12.48

Means with the same letter(s) are not significantly different at 5% level of probability

There were statistically significant interactions between variety and plant population on plant height at 4, 6, 8 and 10WAS in both seasons; an indication that the variety investigated were significantly influenced by variation in population per unit area. The observed interaction is not unexpected, as it has been observed that agronomic practices implemented in a production system should allow selected varieties to react to plant population manipulations when favorable environmental conditions occur (Haegele et al. 2014).

Effect of increasing maize population per stand on number of leaves, leaf area and stem girth of three varieties of maize

Plant leaves play crucial role in crop photosynthesis, any effect of imposed treatment on either leaf number or leaf area which may impact on photosynthesis should probably be expected to affect

crop yield. Worthy of note, however is the fact that the process of yield formation involves complex interplays of various yield determining factors (Jamileh and Moghadam, 2015), besides leaf number and leaf area (Hay and Walker, 1989) with usually unpredictable outcomes. Such varying factors which may affact sink-source relation may moderate expectations away from basic principles. However, in this trial analysis of data indicated that variety as well as population investigated did not significantly ($p \geq 0.05$) influence number of leaves at 4, 6, 8 and 10WAS in 2021 and 2022 cropping seasons (Table 2). There were also no observed interaction effects between variety and population on leaf number in both seasons (Table 2). Oyewole et al. (2015b) observed that where leaf formation is gene dependent, leaf number may not respond to agronomic practices; such as population manipulation; this may be particularly so in determinate crops such as maize. While it could be deduced that the non-significant effect of the treatment imposed on leaf numbers, could be an indication of a possible non-significant effect on yield outcomes, seeing that leaves are vital in crop photosynthesis; with Valadabadi and Farahani (2010) reporting that photosynthesis increases by development of leaf area. However, reports have shown that soil water is often the most limiting factor for grain production in arid and semiarid regions (Sharratt and McWilliams, 2005), while Hammer et al. (2009) found that at high plant populations, root architecture was more important than canopy architecture and light interception for increasing grain yield.

Leaf area is an important parameter of maize. Data analysis indicated that variety did not significantly ($p \geq 0.05$) influence leaf area at 4, 6, 8 and 10WAS in 2021 and 2022 cropping seasons (Table 3). Generally, leaf area increased among the variety though not significantly at 4WAS to 8WAS then dropped at 10 WAS. Plant population significantly ($p \leq 0.05$) influenced leaf area at 8 and 10WAP in both seasons, but not at 4 and 6WAS in 2021 and 2022 cropping seasons. Leaf area was observed to diminish as plant population per stand was increased from one to four plants / stand. Thus, the highest leaf areas were observed in one plant / stand. Just as observed in outcome of variety above, leaf area increased among studied population through 4WAS to 8WAS then dropped at 10WAS. The drop in leaf areas could be attributed to leaf senescence. There were no observed interaction effects between variety and population on leaf area in both seasons throughout the period of data collection (Table 3). The significant effect of population on leaf area conformed to the report of Valadabadi and Farahani (2010) who reported that leaf area, among other things, is influenced by plant population. Observing that the highest physiological growth indices are achieved under high plant density, because photosynthesis increases by development of leaf area. Previous research findings also indicated that in high maize density, leaf area index and crop growth rate increased than low maize density throughout crop growth season (Saberali, 2007).

Data analysis showed that variety investigated did not significantly ($p \geq 0.05$) influence stem girth at 4, 6, 8 and 10WAS in 2021 and 2022 cropping seasons (Table 4), while population had significant effect on stem girth at 6, 8 and 10WAS. At the termination of the trial, the widest stem girth was recorded in the single maize stand in 2021 and 2022 cropping seasons (6.22 and 6.43 cm, respectively), while the least stem girth was among four plants / stand (5.23 and 5.05 cm). Stem girth was found to reduce with increasing plant population per stand; a phenomenon that may encourage easy lodging or stem breakage.

Effect of increasing maize population per stand on yield component and yield of three varieties of maize

Analysis of data showed that variety investigated significantly ($p \leq 0.05$) influenced days to first tassel, days to 50% tassel, as well as grain yield per plant (Table 5), but no significant effect ($p \geq 0.05$) of variety was not observed on ear weight, ear length, kernels per ear as well as 100-kernel weight. Significant ($p \leq 0.05$) influence of population was observed on days to 50% tassel, ear weight, ear length, kernel/ear, 100-kernal weight and grain yield per plant (Table 5). Generally increasing plant population led to processive delay in days to 50% heading, reduction in ear weight, ear length, kernel/ear, 100-kernal weight as well as grain yield per plant. There were significant interactions between variety and population on days to 50% tassel, ear weight, ear length, kernel / ear, 100-kernal weight and grain yield per plant (Table 5).

Previous researchers have observed that interactions between plant genotype and plant population can affect maize parameters, especially grain yield, with DeBruin et al. (2017) finding a positive relationship between maize grain yields and plant population in modern hybrids.

Though explaining that modern hybrids possess the ability to withstand greater stress attributable to high population densities than older hybrids, which in turn enables producers to establish higher plant populations, leading to higher yields per unit area (Russell, 1984; Duvick, 1997).

The observation in this trial quite agreed with those previous reports of positive response of yield components and yield to varietal and population influence (Russell, 1984; Duvick, 1997; DeBruin et al. 2017). Similarly, in line with the experimental outcome, Abuzar et al (2011) reported that biomass yield was significantly affected by different plant population densities in a maize plot. They reported that treatments having a population of 60000 and 80000 plants/ ha produced the maximum biomass yield of 16890 kg/ha each, while the lowest biomass yield (13330 kg/ha) was recorded with a population of 140,000 plants/ha. Several studies show that biomass yield decreases progressively as the number of plants increases in a given area because the production of the individual plant is reduced (Hamidia et al. 2010).

Similarly, they also observed that grain yield was significantly affected by plant population densities. Emam (2001) verified that kernels/ear and kernels/ear row are the most important yield adjustment components in response to plant population density in maize; an observation which was quite in line with this experimental outcome. The better response of hybrids to population stress was evident in this trial where the highest grain yield per plant was recorded in Oba Super II (779.13g and 890.01g, respectively in 2021 and 2022 cropping seasons) while the least grain yield per plant was in the local variety (530.80g and 475.00g, respectively in 2021 and 2022 cropping seasons). Sowing seeds at one seed/hole gave the highest grain yield/plant, 790.27g and 970.00g, respectively in 2021 and 2022 cropping seasons. The least grain yield/plant, 513.27g and 322.50g, respectively in 2021 and 2022 cropping seasons were observed when four seeds were sown/hole.

The highest amount of grain yield/ha was obtained in Oba super II (103,883.74kg and 118,667.70kg), respectively in 2021 and 2022 cropping seasons, with the local variety giving the lowest grain yield in both seasons. While the lowest grain yield/plant was recorded in P4 (213,332 pop/ha), in the first year, the plot compensated for the yield reduction/plant with increase in plant population cumulating in significantly greater harvest/ha (109,496.94 kg/ha), with P1(53,333 plant/ha) recording significantly the lowest grain yield/ha in both seasons. However, P4(213,332 pop/ha) did not repeat the same feat attained in the first trial as it trailed behind P3 (160,000 pop/ha) and P2 (106,666 pop/ha) in yield/ha (Table 6); an indication that the population may not be able to maintain stable yield. The most consist population relative to yield/ha was P3 (160,000 pop/ha), thus recommended for the experimental area, thus maintaining a mean population of 133, 333 plants/ha is predicted to give better performance for the varieties. While Oba super II is recommended for the experimental area.

CONCLUSION

It has been observed that stand density affects plant architecture, alters growth and developmental patterns and influences carbohydrate production. High population increases interplant competition for light, water and nutrients, which may be detrimental to final yield because it stimulates apical dominance, induces barrenness, and ultimately decreases the number of ears produced per plant and kernels set per ear, observed Sangoi (2000). Keeping this in view, the present study was formulated to optimize the planting density of maize under the Southern Guinea savannah agro-ecological zone in Nigeria.

The better response of hybrids to population stress was evident in this trial where the highest grain yield per plant was recorded in Oba Super II (779.13g and 890.01g, respectively in 2021 and 2022 cropping seasons) while the least grain yield per plant was in the local variety (530.80g and 475.00g, respectively in 2021 and 2022 cropping seasons). Sowing seeds at one seed/hole gave the highest grain yield/plant, 790.27g and 970.00g, respectively in the 2021 and 2022 cropping seasons. The least grain yield/plant, 513.27g and 322.50g, respectively in 2021 and 2022 cropping seasons were observed when four seeds were sown/hole. The highest amount of grain yield/ha was obtained in Oba super II (103,883.74kg and 118,667.70kg), respectively in the 2021 and 2022 cropping seasons, with the local variety giving the lowest grain yield in both seasons. While the lowest grain yield/plant was recorded in P4 (213,332 pop/ha), in the first year, the plot compensated for the yield reduction/plant with an increase in plant population cumulating in significantly greater harvest/ha (109,496.94 kg/ha), with P1 (53,333 plant/ha) recording significantly the lowest grain yield/ha in both seasons.

Table 5: Effect of increasing maize population per stand on yield component and yield of three varieties of maize (Zea mays) in 2021 and 2022 cropping seasons

Treatment	Days to first tassel		Days to 50% tassel		Ear weight (g)		Ear length (cm)		Kernel / ear		100-kernel weight (g)		GY / Plant (g)	
	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
Variety														
V1: Sammaz 52	56^{b}	55^{c}	62^{b}	61^{b}	99.23	124.00	12.52	13.52	249.93	301.58	26.17	27.98	621.03^{ab}	782.50^{b}
V2: Oba super-II	57^{a}	58^{b}	64^{a}	64^{a}	124.45	137.50	14.12	14.43	310.43	345.38	25.83	26.38	779.13^{a}	890.01^{a}
V3: Local Variety	57^{a}	60^{a}	65^{a}	65^{a}	103.34	83.03	13.50	12.93	287.78	244.40	23.67	22.97	530.80^{b}	475.00^{c}
LSD (0.05)	0.3	0.2	1.1	1.4	NS	NS	NS	NS	NS	NS	NS	NS	197.65	85.98
Population														
P1: 53,333 pop/ha	57	57	64^{a}	63^{b}	145.30^{a}	146.22^{a}	14.87^{a}	15.14^{a}	337.66^{a}	336.13^{a}	28.67^{a}	30.59^{a}	790.27^{a}	970.00^{a}
P2: 106,666 pop/ha	56	58	62^{b}	63^{b}	120.20^{a}	155.33^{a}	14.19^{a}	14.72^{a}	313.92^{a}	342.47^{a}	25.56^{ab}	24.63^{b}	753.30^{b}	840.00^{b}
P3: 160,000 pop/ha	57	57	65^{a}	63^{b}	87.83^{b}	106.33^{b}	12.57^{b}	13.09^{b}	253.26^{b}	298.21^{b}	24.22^{b}	25.88^{b}	517.77^{c}	623.34^{c}
P4: 213,332 pop/ha	56	58	65^{a}	65^{a}	82.73^{b}	72.33^{c}	11.88^{b}	11.44^{c}	226.01^{b}	211.67^{c}	22.44^{b}	22.00^{c}	513.27^{c}	322.50^{d}
LSD (0.05)	NS	NS	1.4	1.7	30.310*	29.810*	1.480*	0.861*	41.810*	38.270*	3.300*	2.170*	27.250*	56.860*
Interactions														
V1P1	57	54	62	60	123.30	185.00	13.61	15.84	295.50	373.60	28.00	34.00	777.60	1190.00
V1P2	55	55	59	60	122.30	130.00	14.04	14.39	283.20	337.80	27.30	24.00	766.60	850.00
V1P3	54	54	62	59	86.30	100.00	11.83	12.56	242.80	273.60	26.00	30.00	533.30	620.00
V1P4	56	56	63	63	65.00	81.00	10.59	11.31	178.30	221.30	23.30	23.90	406.60	470.01
V2P1	57	57	65	63	166.30	201.00	15.90	17.26	356.30	394.60	28.60	28.00	1046.60	1260.00
V2P2	54	60	60	63	137.30	144.00	14.83	14.77	331.30	353.90	26.60	27.90	863.30	930.00
V2P3	60	57	66	63	97.60	124.00	12.58	13.22	269.50	343.40	24.60	27.65	600.00	790.02
V2P4	56	59	64	66	96.60	91.00	13.14	12.46	284.50	289.60	23.30	22.00	606.60	580.00
V3P1	57	60	64	66	146.30	80.00	15.10	12.31	361.06	240.20	29.30	29.78	546.60	460.01
V3P2	59	60	66	66	101.00	112.10	13.68	15.36	327.40	335.70	22.60	22.00	630.00	740.00
V3P3	58	59	66	66	79.60	95.00	12.47	13.48	232.41	277.63	20.60	20.00	420.00	460.00
V3P4	55	59	62	66	86.60	45.00	12.73	10.56	230.16	124.10	22.00	20.10	526.60	240.00
LSD (0.05)	NS	NS	NS	NS	29.581*	19.610*	3.348*	2.091*	24.891*	33.457*	2.127*	4.671*	245.341*	123.119*
CV%	2.87	2.97	2.33	2.97	5.68	22.31	11.39	11.27	21.66	21.53	13.97	14.20	12.47	12.22

Means with the same letter(s) are not significantly different at 5% level of probability

Table 6: Effect of increasing maize population per stand on yield of three varieties of maize

Treatment	2021 cropping season		2022 cropping season	
	Grain yield/ Plant (g)	Grain yield /ha Kg/ha	Grain yield/ Plant (g)	Grain yield /ha Kg/ha
Variety				
V1: Sammaz 52	621.03	82,803.64	782.50	104,333.07
V2: Oba super-II	779.13	103,883.74	890.01	118,667.70
V3: Local Variety	530.80	70,773.16	475.00	63,333.18
Population				
P1: 53,333 pop/ha	790.27	42,147.47	970.00	51,733.01
P2: 106,666 pop/ha	753.30	80,351.50	840.00	89,599.44
P3: 160,000 pop/ha	517.77	82,843.20	623.34	99,734.40
P4: 213,332 pop/ha	513.27	109,496.92	322.50	68,799.57

However, P4 (213,332 pop/ha) did not repeat the same feat attained in the first trial as it trailed behind P3 (160,000 pop/ha) and P2 (106,666 pop/ha) in yield/ha; an indication that the population may not be able to maintain stable yield. The most consist population relative to yield/ha was P3 (160,000 pop/ha), thus recommended for the experimental area. Maintaining mean population of 133, 333 plants/ha is predicted to give better performance for the varieties. While Oba super II is recommended for the experimental area; as it performed better than the other varieties investigated.

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DNA Fingerprinting of Date Palm Varieties (*Phoenix dactylifera* L.) Grown in Sudan Using ISSR Markers and SDS-PAGE

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ABSTRACT

Date palm (*Phoenix dactylifera* L.) family (Arecaceae) is the most important and ancient cultivated species in Sudan. Protein-based (SDS-PAGE) and Inter- Simple Sequence Repeat (ISSR) PCR) were used to identify genetic distance and design the phylogenetic tree for the five different date palm varieties (Mishriq, Barhi, Khadrawi, Sagay, and Khenaizy). EightI SSR- PCR primers which were used to amplify DNA segments from five date palm varieties were annealed with 60 loci across all variety genomes with an average of 7 loci per primer with a range of 200 to 950bp. Among those loci scored, 51 loci were polymorphic with (85%) polymorphism for at least one of the varieties with an average of 6 polymorphic bands per primer. A total of 159 bands from all analyses with an average of 19.8 fragments per primer, were enough for the identification and evaluation of these five date palm varieties. According to ISSR analysis, UPGMA (Unweight Pair Group of Arithmetic Averages) classified the fifty-one polymorphic loci into two main clusters, the first one contained two varieties: Mishriq and Barhi. While Khenaizi, Sagay, and Khadhrawi grouped in the second one which consisted of two sub-clusters, the first one consisted of Khenaizi and Khadhrawi, and the second sub-cluster consisted of Sagay variety. The combined tree of ISSR and SDS-PAGE analysis classified the date palm varieties under study into two main clusters. The first one consisted of two varieties: Mishriq and Barhi, which were closely related varieties. While the second one consisted of two sub-clusters, the first one consisted of two varieties, Khenaizi and Sugay and the other sub-cluster contained of Khadhrawi variety.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is an angiosperm that belongs to monocots and is considered the most important and ancient cultivated species in the Arab world (Elshibli, 2009). Date Palm has a major socio-economic importance because of its high nutritional value, great yields and its long life span. Date palms in Sudan have been traditionally grown using old, local varieties, mainly of the dry type, for 3000 years. (Elshibli and Korpelainen, 2009).

Numerous males were used as the source of pollen for hand pollination of the female trees. (Osman and Boulos, 1978). Female trees are cultivated mainly for their nutritive fruits. Although the average economic life of a date palm tree is estimated to be up to 50 years, the tree may stay productive for up to 150 years (Chao and Krueger, 2007).

Date contains many nutrients such as carbohydrates, proteins, fat, minerals and vitamins (Al-Qarawi et al. 2004). It is a good source of high nutritional value

food. Indeed it is rich in carbohydrates, dietary fibers, proteins, minerals, and vitamin B complex, such as thiamine (B1), riboflavin (B2), niacin (B3), pantothenic (B5), pyridoxine (B6), and folate (B9) (Eoin, 2016).

Carbohydrates form 70% of date fruit and are mostly fructose and glucose in equal ratio while date proteins are rich in amino acids that contain acidic side chains but poor in methionine and cysteine, which their side chain are composed of sulfur. Minerals in date fruits are calcium, iron, magnesium, selenium, copper, phosphorus, potassium, zinc, sulfur, cobalt, fluorine, manganese, and boron (Chao and Krueger, 2007; Al-Harrasi et al. 2014). Date fruits are highly nourishing and may have numerous potential health benefits. The protective effects of fruits against chronic diseases are ascribed to bioactive non-nutrients called phytochemicals. Phytochemicals have gained increased interest among several investigators, including clinicians due to their antioxidant activity, cholesterol-lowering properties, and other potential health benefits such as chemoprevention of cancer, prevention of diabetes and cardiovascular diseases (Chao and Krueger, 2007; Al-Harrasi et al. 2014).

DNA markers have proved valuable in crop breeding, especially in studies of genetic diversity and gene mapping, phylogenetic studies, gene tagging, genome mapping, and evolutionary biology in a wide range of crop species (Gupta and Varshney, 2000).

ISSRs markers are powerful tools to study the inter- and intra-specific genetic variations in date palms and an easy approach with highly reproducible and multiple genomic loci target ability. To assess the genetic diversity on the basis of geography, the genetic relationship among genotypes was inferred using UPGMA cluster analysis representing the closeness and divergence among date palm cultivars. The present study will be helpful for germplasm management in order to improve the conservation and production of elite cultivars.

MATERIALS AND METHODS

Plant materials

The green and yellow leaflets female samples were collected from EL Slate farm in north Khartoum (Barhi, Khenaizi, Khadhrawi, Sugay andMishriq Wad Laggai). Sagay has been recently introduced from the Kingdom of Saudi Arabia, (Khonaizi) from the United Arab Emirates, (Khadhrawi and Barhi) from Iraq and Mishriq Wad Laggai which was domesticated in Sudan, the plant materials were collected and transferred in Liquid nitrogen (-190°C).

DNA extraction: (CTAB Method)

Cetyl trimethyl ammonium bromide (CTAB) method (Allen et al. 2006).) 50-mg samples of young leaflets tissues were ground to a fine powder in liquid nitrogen, the powder was then placed in .5- mL microtubes containing 700 µL 3% CTAB extraction buffer. The solution was incubated at 65ºC for 60 min, gently mixing by inversion every 15 min; an equal volume of chloroform-isoamylalcohol (24:1) was added to the tubes and gently mixed for 1 min. Samples were centrifuged for 10 min. at 10,000 rpm; 600 µL of the supernatant was then transferred to a fresh tube following the addition of 500 µL chloroform-isoamylalcohol (24:1); this procedure was repeated twice; 500 µL of the supernatant was then transferred to a fresh tube with 700 µL of cold isopropanol (-20ºC) and 1/10th volume of 3 M sodium acetate samples were gently mixed by inversion and centrifuged at 12,000 rpm for 10 min, and so it was possible to visualize the DNA adhered to the bottom of the tube. The liquid solution was then released and the DNA pellet was washed with 700 µL of 70% ethanol and set to dry for approximately 12 h, or until the next day, with the tubes inverted over a filter paper, at room temperature; the pellet was then re-suspended in 100 µL TE buffer plus 5 mL ribonuclease (RNase 10 mg mL–1) in each tube; this solution was incubated at 37ºC for 1h, and after stored at -20ºC. They were used as template DNA for ISSR primer analysis.

Qualitative and Quantitative Analyses of Extracted DNA

DNA yield was measured using a UV-visible spectrophotometer (PerkinElmer, Waltham, MA, USA) at 260nm. DNA purity was determined by calculating the absorbance ratio at A260/280nm. (Wilson and Walker, 2005). For quality and yield assessments, electrophoresis was performed for all DNA samples on 0.8% garose gels that were stained with ethidium bromide; the bands were observed and compared with a known standard lambda DNA marker sample.

ISSR-PCR

Primers Selection: three types of primers, produced by (Sigma Aldrich, Banglore),six anchored dinucleotides repeat primer (AG)10C, (AG)10T, (CT)10A, (CT)10T, (CT)10G, and (CA)8GT, one nonanchored tetra-nucleotides repeat primer (GACA)4, and one nonanchored tri- nucleotides (CAG)5. Which were preselected for their performance with date palm DNA were tested to arrive at the primitive primer which gives descriptive segments (polymorphism, Table 1).

Table 1: The ISSR primers used in the study

No	Primer	Sequence 5′--- 3′	Annealing Temp. C^0/Sec
1	DPISR1	AGCAGCAGCAGCAG	50.9 / 57.8
2	DPISR2	CACACACACACACACAGT	50.9 / 57.8
3	DPISR3	GACAGACAGACAGACA	48 / 47.5
4	DPISR4	AGAGAGAGAGAGAGAGAGAGC	50.9 / 57.8
5	DPISR5	AGAGAGAGAGAGAGAGAGAGT	50.9 / 57.8
6	DPISR6	CTCTCTCTCTCTCTCTCTCTA	50.9 / 57.8
7	DPISR7	CTCTCTCTCTCTCTCTCTCTT	50.9 / 57.8
8	DPISR8	CTCTCTCTCTCTCTCTCTCTG	50.9 / 57.8

The polymerase chain reaction (PCR) Optimization

According to Williams 1990, the polymerase chain reaction (PCR) mixture (25 µl)consisted of 2 µl of total genomic DNA, 12.5 µl of Ampli Taq Gold 360 Mastermix (Applied Biosystems), 2 µl (5 pmol/ µl) of each primer and 8.5 µl of nuclease-free water. Amplification took place in DNA amplification The rmocycler (Biorad, icycler), is programmed as a denaturation step of 4 min at 94 ºC followed by 35 cycles compose of 30 seconds at 94 ºC, for 30 seconds at annealing temperature and 3 minutes at 72ºC. a final extension of 72 ºC for 5 minutes, and hold at 4ºC.

Agarose Gel Electrophoresis

Amplification products were electrophoresed on 1.8% agarose gel (Sigma) in 100 mil 0.5XTBE buffer. The gel was run at 120V constant voltages for 45 minutes. The 100 bp standard DNA size marker (ladder) (Sigma Aldrich, Banglore) was run along with the samples to compare the molecular weight of amplified products. Gels were stained with 0.5 µg/mL ethidium bromide for 15 min (Caetano-Anolles, 1997).

Visualization and analysis of PCR products

Visualization of amplification products and data analysis of reproducible bands visualized on agarose gels 1.8% were scored using a binary code in a data matrix 1 and 0 for their present and absent respectively for the eight primers. Fragments with the same mobility were considered identical, irrespective of the intensity of the fragment.

Statistical Analysis

ISSR, SDS-PAGE, and combined analysis data were converted into binary data in an Excel worksheet and were analyzed using the SPSS-16 program to find the genetic distance between and within the five different date palm varieties. Unweight Pair Group Method with Arithmetic Average (UPMGA) analysis was used for cluster analysis using ISSR, data based on the Jaccard similarity matrix which were computed with the SPSS-10 program to produce a genetic distance matrix using Dice similarity coefficients19. A dendrogram was generated by cluster analysis using the unweighted pair group method of the arithmetic averages (UPGMA).

RESULTS AND DISCUSSION

ISSR polymorphism

The results obtained through eight ISSR primers (as listed in Table 2 and Fig 1) showed the eight ISSR-PCR primers were used to amplify DNA segments from five date palm varieties (Khenaizi, Sugay, Khadhrawi, Mishriq and Barhi), were annealed with 60l ocia cross all variety genomes with an average of 7l ociperprimer with a range of 200 to 950 bp. Among those lociscored, 51l oci were polymorphic with (85%) polymorphism for atleast one of the varieties with an average of 6 polymorphic bands per primer.

A total of 159 bands from all analysis with an average of 19.8 fragments perprimer, which were enough for the identification and evaluation of genetic diversity and designing the phylo genetic tree for these five different date palm varieties. These results are in agreement with those of Zehdi et al. (2004), using ISSR on Tunisi a date palm that generated 100 bands were identified at 14 microsatellite loci with average of 7.14 all elesperlocus. Adawy et al. (2002) using seven ISSR primers generated 53 fragments ranging from 298 to 1200 bp in size.

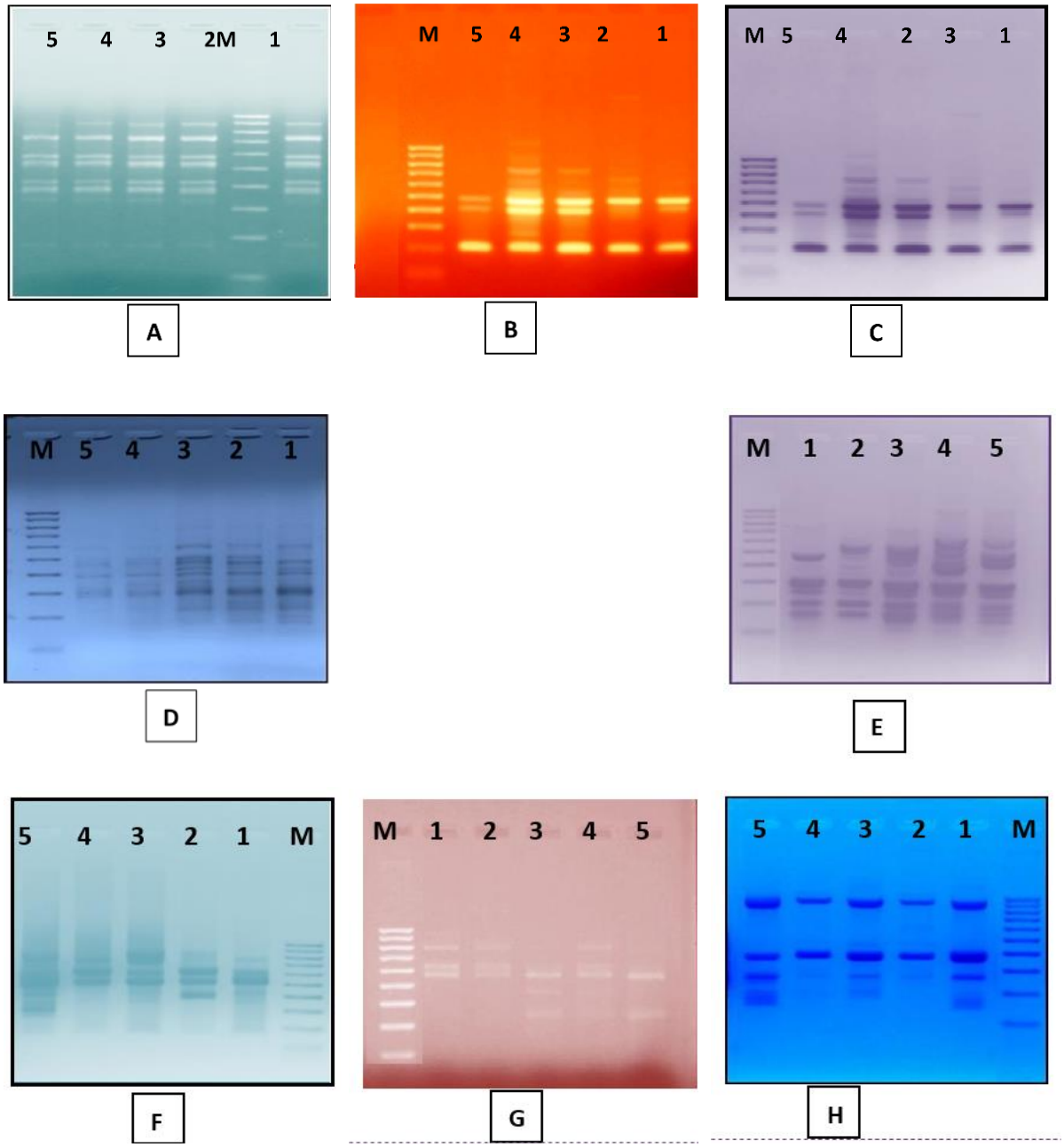

Fig.1: ISSR profile of five date palm varieties amplified with eight different ISSR primers. (A)Primer DPISR-1, (B) Primer DPISR-2, (C)DPISR-3 (D)DPISR-4 ,(E)DPISR-5(F)DPISR-6,(G)DPISR-7and,(H) DPISR-8.M: 100bp ladder marker. Lanes 1 through 5 refer to date palm varieties Khenaizi, Sugay, Khadhrawi, Mishriq and Barhi, respectively.

The average number of fragments per primer was 7.6 fragments with 64.1%polymorphism. While, Adawy et al. (2004) generated 159 fragments when using 19 ISSR primers to analyze bulked DNA samples representing five date palm varieties and the average number of fragments/primer was 8.4.

In Table:4.6 the number of amplified fragments per primer varied from 13bands for the primer DPISR7, which showed the lowest primers efficiency(8.2 %,) to 29 bands for the primer DPISR-1 which represented the highest efficiency (18.2 %,) Table 3.19 The most informative primers, considering the percentage of polymorphism (% P = 100) were DPISR-5, DPISR-6 and DPISR-8 with 12, 8 and 7 polymorphic bands respectively indicating their abundance over other in date palm genome while the non –anchored (CAG)tri-

(DPISR1) nucleotides exhibited the lowest polymorphism (16.6 %).

On the other hand polymorphic band with discrimination power (2%) indicated the rareness of such repeat among the five varieties analyzed. This is due to the absence of sites that complement the sequences of these primers in the palm genome and the extent of polymorphism varies with the nature of the primer used and the sequence of repeats (motif) in the primer employed. This result together with those obtained by Ruas (2003) and Perezdela Torre et al. (2010) indicates that the level of polymorphism detected by ISSR primers depends on the species or genus and the repetitive ISSR used in the primer utilized to generate the amplification profiles, additionally, this result is in agreement with what was reported by Zhao et al. 2012 who stated that the AG–ISSR repeat is the most abundant and polymorphic among di-nucleotide and comprises 85.7 % of date palm genome.

Table 2: List of the used primers and the complementary information of the ISSR assay

Primer	No. ofloci	NMB*	NPB*	TBN*	P%*	%E*	%D*	ABL(bp)*
DPISR1	6	5	1	29	16.6	18.2	2.0	380-900
DPISR2	6	1	5	16	83.3	10	9.8	200-700
DPISR3	6	1	5	19	83.3	12	9.8	200-800
DPISR4	10	1	9	24	90	15.1	17.6	200-600
DPISR5	12	0	12	26	100	16.3	23.5	200-580
DPISR6	8	0	8	16	100	10.1	15.7	280-850
DPISR7	5	1	4	13	80	8.2	7.8	350-800
DPISR8	7	0	7	16	100	10.1	13.7	200-950
Total	60	9	51	159	85	100	100	
Average	7	1.2	6	19.8	81.7	12.5	12.5	

*TBN: Total band number, NPB: Number of polymorphism bands, NMB: Number of monomorphism bands, P%: Polymorphism percentage, ABL: The amplified band length, D%: Discrimination power, E%: Primer Efficiency.

It is clear that the ISSR markers differed among them in the number of bands according to the marker. This was shown by several studies, Karim el al. (2010), studied the genetic convergence between the ten date palm varieties in Tunisia to find the genetic convergence between the ten date palm varieties they used the same primers, and only seven of them gave a resultwith11bandsperprimer (AG10C, AG10T, CT10A, CT10T, CT10A, CT10G and GACA4).

Genetic distance and phylogenetic tree analysis

In the present study ISSR, SDS-PAGE and combined analysis data were converted into binary data and were analyzed using the SPSS-16 program to construct the genetic distance between the five different date palm genotypes (Table 3, 4 and 5). Three phylogenetic trees which were generated had shown six similar clusters (Fig. 2, 3 and 4).

The genetic distances matrix was calculated for the 51 polymorphic bands of ISSR, 8 protein patterns and combined analysis of the five varieties on the basis of present and absent of the polymorphic bands. The genetic distance and separation of each variety varied according to the type of analysis used. The range is between (0.922-0.645 Table 3), (0.816- 0.289 Table 4) and (0.887-0.627 Table 5) with a mean of (0.783-0.552-0.757) for the three analyses respectively. Thus the genotypes that tested in this study are highly divergent at the DNA level.

The smallest distance value observed between Khenaizi and Sagay varieties for the SDS-PAGE and combined-based analysis was 0.289, and 0.627 respectively, which appear to be the most similar varieties and can be closely related. While the Sugay

variety was highly divergent from Barhi variety with a distance of 0.922 - 0.887 for the ISSR and combined-based analysis respectively.

It is noteworthy that Mishriq presented a very limited average distance (from 0.780 to 0.849) with Khenaizi, Sugay and Khadhrawi, thus Mishriq could be characterized by a high divergence at the DNA level and could be unlikely regrouped with them. All the varieties displayed different intermediate levels of dissimilarity (0.627 to 0.658) and are grouped with the other ones.

Table 3: Genetic distance values of Nei's coefficient revealed by ISSR markers analysis

	Matrix File Input				
	Khenaizi	Sagay	Khadhrawi	Mishriq	Barhi
Khenaizi	.000				
Sagay	.664	.000			
Khadhrawi	.645	.683	.000		
Mishriq	.852	.774	.751	.000	
Barhi	.797	.922	.659	.761	.000

Table 4: Genetic distance values of Nei's coefficient revealed by SDS- PAGE analysis

	Matrix File Input				
	Khenaizi	Sagay	Khadhrawi	Mishriq	Barhi
Khenaizi	.000				
Sagay	.289	.000			
Khadhrawi	.516	.408	.000		
Mishriq	.548	.577	.816	.000	
Barhi	.447	.500	.707	.408	.000

Table 5: Genetic distance values of Nei's coefficient using combined data of ISSR and SDS-PAGE analysis

	Matrix File Input				
	Khenaizi	Sagay	Khadhrawi	Mishriq	Barhi
Khenaizi	.000				
Sagay	.627	.000			
Khadhrawi	.637	.658	.000		
Mishriq	.849	.780	.793	.000	
Barhi	.780	.887	.701	.749	.000

The trees constructed were shown in Fig 2, 3 and 4 explained the molecular phylogenetic relationships between the five varieties. Unweight Pair Group Method with Arithmetic Average (UPMGA) analysis classified date palm varieties into two main clusters in ISSR, SDS-PAGE, and ISSR, SDS-PAGE combined analyses.

Fifty-one polymorphic loci, UPGMA classified the date palm varieties into two main clusters according to combined analyses that were revealed by ISSR and SDS-PAGE combined analyses. The first one consisted of two varieties: Mishriq and Barhi it is similar to those based on agronomic traits (Fig 4). And the second one consisted of two sub-clusters, the first one consisted

of two varieties, Khenaizi and Sagay and the second sub-cluster consisted of Khadhrawi variety (Fig. 4).

UPGMA ordered the 51 polymorphic loci of ISSR analysis of the date palm varieties into two main clusters the first one contained two varieties: Mishriq and Barhi. While Khenaizi, Sugay and Khadhrawi grouped in the second one which consisted of two sub-clusters, the first one consisted of Khenaizi and Khadhrawi, and the second sub-cluster consisted of Sagay variety (Fig 2).

The combined tree of ISSR and SDS-PAGE analysis in (Fig.4) classified the date palm varieties under study into two main clusters according to UPGMA analysis similar to the SDS-PAGE analysis tree (Fig 3). The first one consisted of two varieties: Mishriq and Barhi, which were closely related varieties. While the second one consisted of two sub-clusters, the first one consisted of two varieties, Khenaizi and Sagay and the second sub-cluster consisted of Khadhrawi variety.

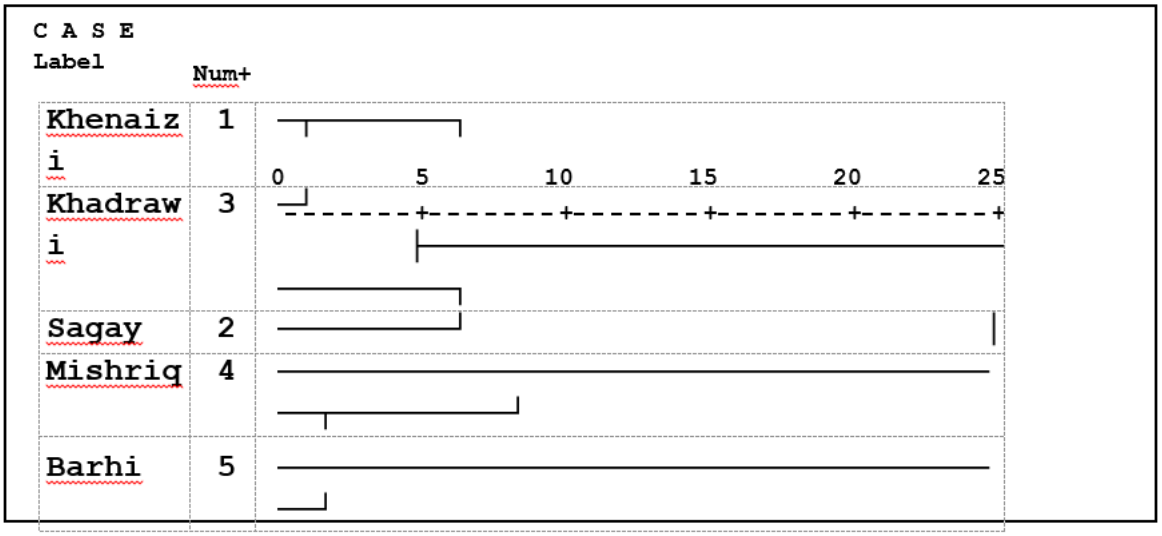

Fig 2: Cluster analysis with UPGMA method of five date palm varieties using ISSR, data based on Jaccard similarity matrix.

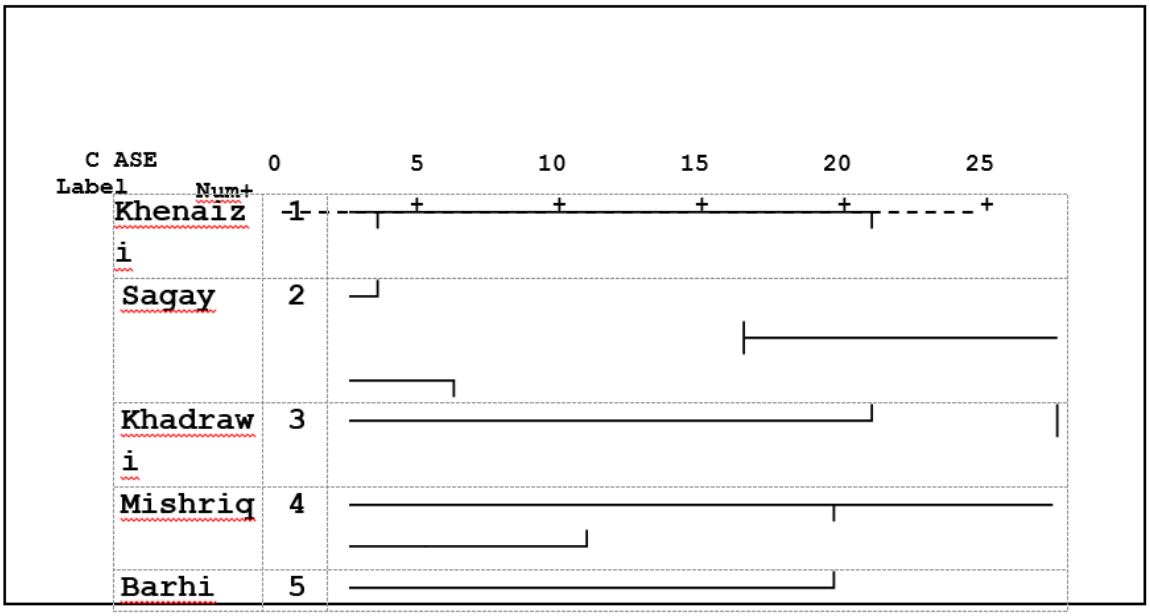

Fig 3: Cluster analysis with UPGMA method of five date palm varieties using SDS-PAGE data based on Jaccard similarity matrix.

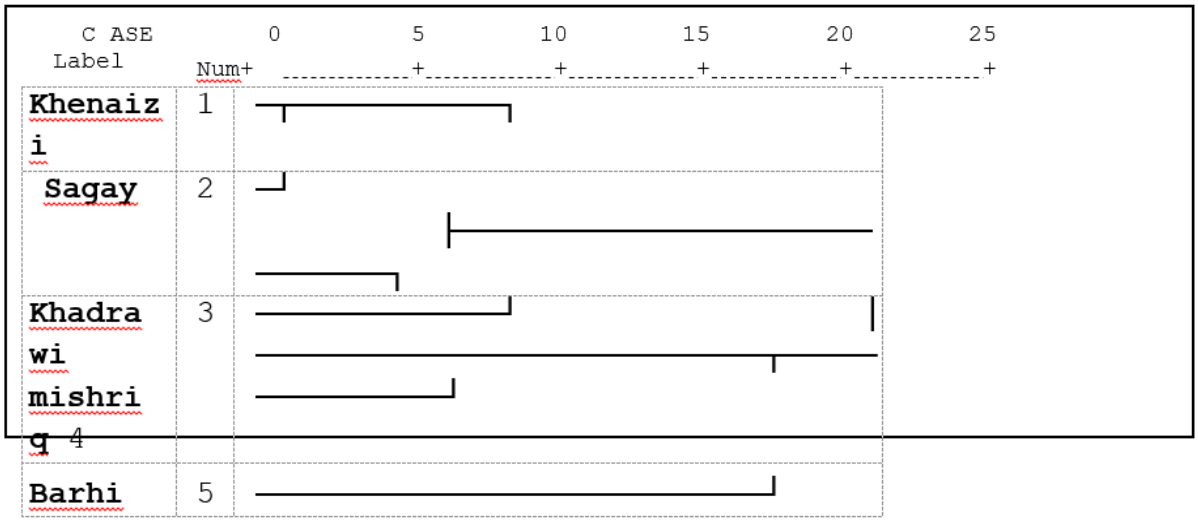

Fig 4: Cluster analysis with UPGMA method of five date palm varieties using combined data of ISSRand SDS-PAGE based on Jaccard similarity matrix.

Generally, the genotypes tested revealed that the geographic origin was not affected the cluster divisions. Accordingly, the sister varieties `Khenaizi` and 'Sagay with (0.289, 0.627 distances Table 2 and 3) which have different geographical origins (The United Arab Emirates and the Kingdom of Saudi Arabia respectively) fell in one sub-cluster (Fig 4 and 3). Also, the two varieties Mishriq and „Barhi" (Sudan and Iraq, respectively) fell in the same cluster, This result agrees with other reports for Moroccan, Algerian and Tunisian date palm varieties based on analyses using microsatellite markers (Zehdi et al. 2004) and isozyme markers (Ould et al. 2001).

A high degree of independence between the geographical origin and molecular data was indicated. The RAMPO and AFLP data applied on Tunisian date palms (Rhouma et al. 2007; Rhouma-Chatti et al. 2011) showed that the studied varieties clustered independently of their geographic origin. The two varieties Mishriq and Barhi (Sudan and Iraq, respectively) which grouped into the same cluster, based on agronomic traits particularly the fruits which were characterized by dates of medium size and brown color. This could be explained by the presence of a common genetic origin among the tested varieties in spite of their origin and this agrees with Hammadi et al. (2012) who found that fruit consistency which is an important characteristic of date fruit has an association with genetic markers because clustering based on fruit consistency is in accordance with clustering by microsatellite markers.

The varieties Barhi and 'Khadhrawi originated in Iraq and were grouped in a different cluster, this observation suggested that genetic variation range with each of the Iraqi varieties exits grouped, this result confirmed what was obtained by Al-Najm et al. (2017) when they used inter-primer binding site (iPBS) markers to assess the molecular variation and genetic diversity of 54 and 12 date palm genotype collected from Australia and Iraq, they found that Barhi and 'Khadhrawi originated in Iraq in a different group to those collected in Iraq , their observations suggests that a range of genetic variation within each of the Iraqi varieties exists. So this could be helpful in Sudan where date palm breeding is highly dependent on seed propagation with subsequent selection based on specific characteristics such as fruit quality and plant vigor as determined by local farmer preferences (Khierallah et al. 2011). Results also showed that the imported date palm varieties recently introduced to Sudan groves are closely grouped in one cluster this could be explained by the presence of a common genetic origin among them, Al-Khalifah et al., (2013), added that over the years many date palm varieties have been transplanted to areas other than the area of their origin, and there may have been adapted with different names. On the whole, our data augment those describing the application of molecular tools in date palm variability analysis and previously reported (Trifi et al. 2000). Dendogram showed that accession grouping in relation to their geographical origin is not well defined.

ISSR data allowed the discrimination of five varieties. However, the use of SDS- PAGE, in spite of the low numbers of bands could distinguish Khadhrawi and

Mishriq varieties by negative unique bands, while ISSR assay could be distinguished the five varieties through 16 unique bands.

CONCLUSION

The combined cluster analysis of ISSR and SDS-PAGE data clearly showed high degree of independence between the geographical origin and molecular data which were indicated. The imported date palm varieties recently introduced to Sudan are closely grouped in one cluster this could be explained by the presence of a common genetic origin among them. Generally, the genotypes tested revealed that the geographic origin was not affected the cluster divisions. Moreover, ISSR profiles can be used in developing molecular identities for date palm varieties in order for their proper identification, registration and conservation.

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Screening of Mineral Solubilizing Microbes and Rhizobium for Growth Promotion and Development of *Acacia leucocephala* Grown under Nursery Conditions

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ABSTRACT

The application aspect of microbial strains especially mineral solubilization in individual and /or combination under glasshouse and field conditions with respect to enhancement in plant of *Acacia leucocephala* was the main objective of the present for which 11 fungi, 5 bacteria, and 11 isolates of Rhizobia had been used. Screening of ten fungal species and 5 bacterial isolates for their effects on the growth of *Acacia leucocephala*, done under pot culture in conditions, elucidated the best performance of the combination of PF3 (*Penicillum crysogenum* Thom. 1) and IF5 (*Cunninghmella elegans* Lendn.) and *Rhizobium* isolates R10 and /or R11 in *A. leucocephala*. The combination of selected microbial inoculants for the individual test tree species was evaluated for their individual performance and /or in combination with the other selected inoculants in a specific ratio of their inoculums. Under the dual inoculation experiment, selected mineral solubilizers (fungi/bacteria) were evaluated along with the combination of different isolates of Rhizobium and resulted in the final formulation of *Aspergillus* sp. + *Cunnighmella elegans* with *Rhizobium* isolate no. 11 tree legumes tested. The present study done under controlled greenhouse conditions has provided an experimental approach to adopt pre-inoculation of the symbiotic microbes to seedlings in the nursery for better plantation results.

INTRODUCTION

The application of chemical fertilizer provides nutrition in high concentration in the soil and plants. However, the entire contents would not be absorbed by the plants and the remaining parts would bind to the soil. Microorganisms are useful for biomineralization of bound soil and make nutrients available to their host and / or its surroundings. Microorganisms facilitate plant mineral nutrition by changing the amounts, concentrations and properties of minerals available to plants. These changes lead to change in growth, development and chemical composition of plant that are common and substantial enough to encourage the exploitation of plant microbe interaction for improvement of crop productivity. There are various groups of organisms that can solubilize and / or leaching of phosphate, iron and other mineral metals. (Kumar et al.2004; Afzal et al.2005; Mehrvarz et al. 2008; Patil et al. 2002; Mehrvarz and Chaichi, 2008; Reis et al. 2008). All

tropical legumes fix the atmospheric nitrogen by Rhizobium which requires optimum level of phosphorus in plant tissue. Their seedlings establish better in presence of mineral solubilizers because more of the tropical soils are phosphate fixing and make it unavailable to the plants (Dabas and Kaushik, 1998; Sahgalet al. 2004; Tilaket al. 2005; Hameeda et al. 2008; Gupta et al. 2007;Huda et al. 2007).

It is known that a large number of seedlings of forest species useful for afforestaion in dry deciduous conditions are required for raising plantations. Microbial application at the nursery stage was also found to be useful in enhancing productivity in some forest trees like Albizzia, Acacia and Dalbergia etc. (Rahangdale and Gupta, 1998; Sahgalet al. 2004; Thatoi et al. 1993). *Acacia auriculoformis*, *Acacia nilotica*, and *Acacia leucocephala* are three legumes suitable for agroforestry because their litter contains more than 2% nitrogen (Puri, 1960; Mishra and Sharma, 2004). It is however equally important to screen some suitable bioinoculants for these species.

Acacia leucocephala or *Leucaena leucocephala* is the most productive and versatile multi-purpose legume tree in tropical regionsits plantation in degraded land helps in the recovery of soil microbiological properties (Valpassos et al. 2007; Ruiz et al. 2006; Forrester et al. 2006; Line et al.2006).*Acacia leucocephala* has been the focus of a great deal of research in the past few decades for its nitrogen fixing potential (Hogberg and Kvarnstrom, 1982;Sanginga et al. 1985 a and b). The high nitrogen fixing potential of this tree is related to its abundant nodulation under specific soil conditions (Hogberg and Kvarnstrom, 1982; Lulandala and Hall, 1986; Halliday and Somasegaran, 1982). In view of the above-cited aspects of plant-microbe interaction, their potential towards biomineralization of unavailable sources of minerals and elements and application in transplantation tree legumes useful for the revegetation and reclamation of disturbed land, encourage us to evaluate the microflora for their effect on growth and development of some tree legumes at nursery conditions.

MATERIALS AND METHODS

1. Microbial strains used

Six phosphate solubilizing fungi, PF1 (*Penicillium grisefulvum* Diercks.), PF2 (*Penicillium restrictum* Gilman &Abott.), PF3 (*Penicillium chrysogenum* Thom. 1), PF4 (*Aspergillus species* 1), PF5 (*Aspergillus ornatus* Raper, Fernele, Tresner.) and PF6 (*Aspergillus wentii*), four iron leaching fungi IF1 (*Penicillum expansum*), IF4 (*Paecilomyces variotii*), IF5 (*Cunnighamella elegans*) and IF6 (*Penicillium chrysogenum* Thom 2), five phosphate solublizing bacteria and 11 *Rhizobium* were used for the inoculation studies (The *Rhizobium* strains 1a, 18, 20, 28a, 6, 23, 5a, 16a, 29, 13 and 7a were selected and re-coded as R1, R2, R3, R4, R5, R6, R7, R8, R9, R10 and R11 respectively).

2. Experimental

1) The experiment was set at the glass house of the Regional Plant Resource Centre at a temperature of 35±2ºC & and 80±5% relative humidity in Pot size: 8x11" polybags containing 2.5 kg soil. The soil contains 83.8% of sand, 8.8% of slit, and 7.4% of clay. The textural class of the soil was loamy sand the soil pH was 6.27. The salt content of the soil was 0.504. The nitrogen (N), phosphate (P2O5), and potassium (K2O) of the soil were 168.7Kg/Ha, 237.2kg/Ha, and 645.12kg/Ha respectively.

2) Screening experiment

One factor at a time i. e. individual microbial culture was used to inoculate the poly pot 25ml of 7-day-old culture prepared in Czapekdox medium (4.5 pH) was added to each pot prior to seed sowing. 25 ml of 5-day-old culture prepared in potato dextrose broth (7.0 pH) was added to each pot prior to seed sowing. The experiment was set in 20 replications. Pretreated, and healthy seeds (3 no.) per pot. Daily watering was done through a sprinkler mist system. Finally, observations were recorded of 120 days of plants for Shoot height, Number of leaves, leaflets, Branches developed, and fresh and dry biomass of leaves.

3) Dual inoculation experiment

In the second phase of the experiment, the two best microbial strains were selected for each tree legume on the basis of their performances in the previous experiment. The experiment was set up according to the first experiment done on screening except the different microbial inoculums used for specific tree legumes. The experimental sets were taken into consideration. [1] Control: without any inoculation, [2] PF4: 50ml in each pot., [3] IF5: 50ml in each pot, [4] PF4+IF5: 50ml of PF4 + 50ml of IF5 in each pot, [5] PF4 (more)+IF5: 100ml of PF4 + 50ml of IF5 in each pot, [6]

PF4+IF5 (more): 50ml of PF4 + 100ml of IF5 in each pot.

In second phase of experiment, the two best microbial strains were selected on the basis of their performances in the previous experiment. The experiment was set up according to the first experiment done on screening except for the different combinationsof microbial inoculum used for specific tree legumes. [1] controluninoculated, [2] inoculated with PF4, [3] Inoculated with IF5 [4] Individual inoculation of Rhizobium (11 no.), [5] Inoculation with PF4 and Individual Rhizobium, [6] Inoculation with IF 5 with individual Rhizobium.

5. Growth analysis

Growth parameters were recorded for the experimental plants such as shoot height (in cm), root length (in cm), number of leaves, leaflets and branches, total seedling height (in cm), collar diameter (in mm), fresh and dry biomass of leaf, stem, root, shoot and seedling (in gram) (Al-Garni, 2006, Sahet al. 1998 and Tewari et al. 2006). Statistical analysis for one-way ANOVA was done by following Sockal et al.1981).

6. Soil analysis

Basic Soil analysis was done by Solution Analyser (Sandeep Instrument). After the experiment was completed the potting soil was subjected to analysis. For each treatment, the soil was pooled for each replication. 1 gram soil was added to 10 ml of distilled water and stirred. The soil dilution was subjected to analysis for pH, (Mishra et al. 2002; Sangha and Jalota, 2005;Chanderet al. 1998). Soil N, P, K was analyzed by wet oxidation method through commercial laboratories (Greenwood et al. 2001).

RESULTS

Effect of microbial inoculation on Acacia leucocephala

The plants of *A. leucocephala* grown under different treatments along with control untreated plants exhibited good growth in terms of plant height, biomass, and other plant parts. Besides this, significant variations could be observed among all the treatments in affecting plant growth performances as compared to uninoculated control. However, fungal strains PF 4 (Aspergillus species 1), IF 1 (PaecilomycesvariotiiBainier.), and IF 5 (*Cunnighamella elegancs*) showed better effects in enhancing plant height, number and biomass of leaves as compared to other microbial inoculants. Bacterial strains also performed well in improving plant health. Finally, these two fungal strains PF4 and IF5 were selected for further experimentations on dual inoculations (Table1). In *Acacia leucocephala* different growth parameters were observed. In the number of leaves, the highest value exhibited by PF4 i.e. 69.60 ± 15.66 and then in IF5 i.e. 47.60 ± 14.51. In the case of a number of leaflets 324.60 ± 80.39 was observed in the case of PF4 which is highest than239.40 ± 68.82 in the case of IF5. The best result in the case of root length was observed in the case of PF3 i.e. 24.68 ± 2.67 and in PF2 i.e. 23.06 ± 2.55. In the case of shoot height highest result was observed in the case of PF4 i.e. 62.72 ± 12.42 then in IF5 i.e. 53.62 ± 21.77. In the case of fresh biomass of leaves best result exhibited by PF4 i.e. 5.52 ± 1.37 and PF6 i.e. 4.15 ± 0.90. In the case of dry biomass of leaves PF6 and PF1 exhibited better results than others i.e. 3.34 ± 1.75 and 2.28 ± 0.90. In the case of collar diameter IF4, IF5, and IF6 were found to be the best among others. By analyzing the growth parameters we have selected PF4 and IF5 from the primary screening to be used for the secondary screening. The result was found in PF3 i.e. 21.40 ± 1.67mm than in IF5 and PF2 i.e. 21.00 ± 0.70mm and 21.00 ± 2.54mm, respectively. From the primary screening fungi PF3 and bacteria PB6 found to be the best among treatments were selected for the secondary screening.

4) Dual Inoculation with Rhizobium:

Effect of dual inoculation on Acacia leucocephala

The plants of *A. leucocephala* grown under different treatments along with control untreated plants exhibited good growth in terms of plant height, biomass, and plant parts. Besides this, significant variations could be observed among all the treatments in affecting plant growth performances as compared to uninoculated control. However, fungal strains PF 4, and IF 5 in equal quantity showed better

effects in enhancing plant height, number, and biomass of leaves as compared to other treatments. (Table 2). In the case of secondary screening in case of the number of leaves and leaflets best result was by the single inoculation of IF5 i.e. 28.29 ± 6.15 cm root length and 67.86 ± 16.20cm shoot height, but the combination of PF4 and IF5 in 1: 1 ratio attained a shoot height of 65.42 ± 19.96cm. In the case of fresh biomass of leaves, the best result was obtained by PF4 (M) + IF5 i.e. 2.42 ± 1.75. In the case of the exhibited by the combination of PF4 and IF5 in the combination of 1: 1 i.e. 63.00 ± 32.54 no. of leaves and 269.60 ± 142.89 no. of leaflets. In the case of root length and shoot height, the best result was obtained collar diameter of the plant best result was obtained by a single inoculation of IF5 (28.20 ± 10.28mm). By observing all the parameters we found that the combination of PF4 and IF5 in the ratio of 1: 1 found to be the best.

Table -1: Growth performance of *Acacia leucocephala* under screening experiment

Treatments	No. of Leaves	Shoot height (cm)	Fresh biomass leaves (gm)	Dry biomass leaves (gm)
Control	29.80 ±10.77	29.96 ±11.47	1.08 ±0.87	1.63 ±0.57
PF1	45.40 ±15.04	39.90 ±5.10	2.28 ±0.90	3.19 ±1.00
PF2	24.60 ±4.82	20.22 ±9.55	0.99 ±0.69	0.73 ±0.21
PF3	17.00 ±4.35	21.10 ±14.01	3.06 ±0.91	1.45 ±2.29
PF4	69.60 ±15.66	62.72 ±12.42	5.52 ±1.37	1.41 ±0.57
PF5	38.40 ±9.71	38.14 ±9.47	1.90 ±1.14	0.92 ±0.79
PF6	43.20 ±19.48	34.50 ±11.20	4.15 ±0.90	3.34 ±1.75
IF1	47.40 ±8.50	44.56 ±4.50	4.00 ±1.26	2.62 ±1.00
IF4	41.00 ±9.19	48.24±9.00	2.68 ±1.76	0.57 ±0.61
IF5	47.60 ±14.51	53.62±21.77	3.18 ±3.24	3.16 ±1.61
IF6	38.00 ±15.52	42.12±8.32	3.63 ±1.00	2.65 ±1.21
PB2	46.00 ±17.14	42.96±7.46	2.94 ±1.30	1.52 ±0.43
PB3	40.40 ±14.65	47.38±8.54	2.12 ±1.61	0.99 ±1.26
PB4	32.60 ±17.68	40.20±13.93	3.07 ±0.95	2.12 ±1.61
PB5	28.60 ± 4.72	45.38±7.40	2.36 ±0.84	0.73 ±0.62
PB6	39.40 ±10.73	51.88±8.74	3.03 ±0.53	2.62 ±0.60

PF1 (*Penicillium grisefulvum* Diercks.), PF2 (*Penicillium restrictum* Gilman &Abott.), PF3 (*Penicillium rysogenum* Thom. 1), PF4 (*Aspergillus* species 1), PF5 (*Aspergillus ornatusRaper*, Fernele, Tresner.) and PF6 (*Aspergillus wentii*), four iron leaching fungi IF1 (*Penicillum expansum*), IF4 (*Paecilomyces variotii*), IF5 (*Cunnigham ellaelegans*) and IF6 (Penicillium crysogenumThom 2), PB2: *Streptomyces* sp.1, PB3: *Micrococcus luteus*, PB 4 : *Micrococcus luteus*, PB5: *Micrococcus varians*, PB6 : *Streptomyces* sp. 2

Table -2 Effect of dual inoculation of selected microbes on the growth of *Acacia leucocephala*

parameters	1	2	3	4	5	6
Leaf no.	38.60 ± 23.86	41.40 ± 16.16	42.60 ± 10.74	63.00 ± 32.94	50.80 ± 9.52	50.60 ± 16.83
Leaf lets no.	165.40 ± 90.15	167.20 ± 76.32	248.20 ± 76.32	269.60 ± 142.89	308.00 ± 46.92	265.00 ± 97.49
Root length (cm)	21.64 ± 8.45	26.14 ± 6.15	28.20 ± 6.15	22.68 ± 5.14	24.50 ± 5.66	23.26 ± 1.93
Shoot length (cm)	55.52 ± 18.71	49.60 ± 8.83	67.86 ± 16.20	65.42 ± 19.96	73.18 ± 21.99	73.36 ± 8.46
Biomass leaf (g)	1.64 ± 0.67	1.52 ± 0.63	2.65 ± 0.58	2.61 ± 1.68	6.09 ± 2.10 **	4.34 ± 2.52*

Dry biomass leaf(g)	0.85 ± 0.43	1.07 ± 0.59	1.80 ± 0.32	2.42 ± 1.75	2.38 ± 0.86	2.40 ± 1.26
± Standard deviation						
Abbreviations for treatments						
1- control, 2- PF4 (*Aspergillus* sp. 1) 3 - IF5 (*Cunnighmella elgans*) , 4 -both fungal strains equal amount , 5- both fungal isolates in 2:1 ratio, 6- both fungal isolates in 1:2 ratio						

Effect of microbial inoculants and Rhizobium on *Acacia leucocephala*

The plants of *A. leucocephala* grown under different treatments (47) along with control untreated plants exhibited good growth in terms of plant height, biomass and plant parts. Besides this, significant variations could be observed among all the treatments in affecting plant growth performances as compared to uninoculated control. However, the combination of PF4, IF5 and R11 showed better effects in enhancing the number and biomass of leaves as compared to other treatments (Table -3). In the dual inoculation or the third experiment, the parameter shoot height showed the best result in single inoculation of IF5 (60.08 ± 9.43cm), second best was single inoculation of PF4 (52.56 ± 6.55cm) and then PF4 + R9 (51.08 ± 7.87cm). In case of root length highest value obtained from PF4 + R9 i.e. 27.46 ± 12.32cm then PF4 + R6 i.e.22.46 ± 2.30cmthen PF4 + R5 i.e. 26.58 ± 2.79. In the case of total seedling height best result was obtained by a single inoculation of IF5 (80.38 ± 11.25cm) then single inoculation of PF4 (43.34 ± 6.56cm) then single inoculation of R2 (73.06 ± 7.74cm). In case of no. of leaves highest value obtained from PF4 + IF5 + R11 (39.80 ± 9.98) then PF4 + IF5 + R10 (39.20 ± 7.56) then PF4 + R6 (37.60 ± 8.32).in the case of no. of primary leaflets highest value obtained from PF4 + IF5 + R11 (210.20 ± 49.97) then IF5 + R5 (192.20 ± 45.36) then PF4 + IF5 + R10 (189.00 ±19.09). In case of collar diameter best result found in PF4 + IF5 + R10 (22.60 ± 1.52mm), then PF4 + IF5 + R11 (22.60 ± 1.10mm) then PF4 + R6 (21.80 ± 1.80mm). In the case of fresh biomass of leaves best result was found in the case of IF5 + R6 (7.47 ± 1.26g) then PF4 + IF5 + R11 (7.28 ± 0.87g) then PF4 + R11 (7.26 ± 1.24g). In the case of dry biomass of leaves best result was obtained from PF4 + IF5 + R11 (4.71 ± 1.19g) then PF4 + R11 (4.55 ± 1.39g) then PF4 + IF5 + R8 (4.41 ± 1.27g). In the case of fresh biomass of root best result obtained by PF4 + IF5 + R3 (2.85 ± 1.53g) then in PF4 + IF5 + R5 (3.81 ± 0.57g) then PF4 + IF5 + R11 (2.57 ± 0.49g). In the case of dry biomass of root highest value was obtained by PF4 + IF5 + R5 (1.63 ± 0.96g), then PF4 + IF5 + R3 (1.60 ± 1.11g) then PF4 + IF5 + R11 (1.43 ± 0.27g). By analyzing the result of the growth parameter of *Acacia leucocephala* we found out that the combination of PF4 (*Aspergillus* species 1), IF5 (*Cunnighamella elegans*) and R10 (*Rhizobium* species) and the combination of PF3, IF5 and R11 (*Rhizobium* species) found to be the best inoculums for the plant. Though the test plants were found to be infected with Rhizobium a very poor performance was observed as far as root nodule formation is concerned. Hence total number of nodules, size and structure could not be recorded.

DISCUSSION

The present study revealed the suitability of some inoculantsfor enhancing growth and biomass and indirect P uptake in test plants when compared with the uninoculated control. Among them, fungal inoculants exhibited better performance in providing benefits of mineral uptake to these plants in general. The findings on better growth performance of test plants showed the impact of inoculation and affirmed their potential in afforestation of problematic soil. The plants of *Acacia leucocephala* grown under different treatments along with control untreated plants exhibited good growth in terms of plant height, biomass, and plant parts with significant variations. Though all microbial strains perform better in laboratory conditions may vary in field conditions, and screening and selection of microbial inoculants for the development of biofertiliser are needed. Their affectivity depends upon the type of host they associate with. It is clearly evident that the uninoculated plants of *A. leucocephala* exhibited better growth as compared to the few fungal inoculants used in this study. Inoculations with bacterial strains did not show better performance in seedling growth compared to control.

Test plants of *A. leucocephala* were inoculated by the most effective mineral solubilizers. Such plants have

double symbiotic benefits in terms of nitrogen and phosphorus that allow plants to grow well under experimental conditions. Inoculation results have been quite promising in the tree species taken. Microbial inoculation of tree legume species with mineral solubilizes and nitrogen-fixing organisms not only enhanced the nutrient content in the above-ground plant material but also provided a well-balanced and regulated nutrient supply due to an enlarged and symbiotically associated root system developed in such plants. The amelioration of microflora to developing seedlings is useful for certain plantation programs.

Table 3- Effect of selected inoculants in combination with Rhizobium isolates

	SHOOT HEIGHT (IN CM)	ROOT LENGTH (IN CM)	TOTAL SEEDLING HEIGHT (IN CM)	NUMBER OF LEAVES	NUMBER OF PRIMARY LEAFLETS
CONTROL	43.66 ± 8.16	15.16 ± 4.43	58.82 ± 5.46	21.20 ± 4.02	136.80 ± 75.29
PF4	52.56 ± 6.55	20.78 ± 1.53	73.34 ± 6.56	28.20 ± 6.10	144.20 ± 28.71
IF5	60.08 ± 9.34**	20.30 ± 2.72	80.38 ± 11.25**	28.00 ± 4.74	146.00 ± 14.78
R1	47.06 ± 7.79	19.88 ± 2.13	66.94 ± 8.27	16.40 ± 8.38	140.80 ± 53.22
R2	51.38 ± 5.56	21.68 ± 4.70*	73.06 ± 7.74	24.00 ± 1.58	156.80 ± 35.52
R3	45.32 ± 9.51	19.68 ± 2.29	65.00 ± 9.35	23.80 ± 3.63	124.20 ± 19.20
R4	28.12 ± 2.93	17.92 ± 5.30	46.04 ± 6.57	26.60 ± 5.77	145.20 ± 24.63
R5	37.52 ± 6.95	18.14 ± 2.23	55.66 ± 6.86	32.40 ± 7.13*	161.80 ± 36.24
R6	38.38 ± 3.19	26.16 ± 2.26**	64.54 ± 4.53	34.80 ± 5.31**	178.40 ± 28.04
R7	35.12 ± 4.87	16.70 ± 3.47	51.82 ± 6.22	23.40 ± 5.46	125.60 ± 33.09
R8	34.70 ± 4.50	17.42 ± 4.39	52.12 ± 5.87	24.60 ± 1.67	125.20 ± 15.58
R9	49.08 ± 6.43	19.64 ± 3.10	68.72 ± 7.74	35.00 ± 11.22**	182.40 ± 54.59
R10	39.94 ± 2.44	20.76 ± 2.83	60.70 ± 1.29	29.00 ± 2.65	179.20 ± 41.97
R11	46.06 ± 5.02	18.04 ± 3.25	64.10 ± 6.05	24.40 ± 7.89	130.00 ± 38.63
PF4 + R1	48.16 ± 7.75	21.72 ± 2.26*	69.88 ± 5.66	30.00 ± 4.30	152.40 ± 16.41
PF4 + R2	43.08 ± 12.25	13.58 ± 2.31	56.66 ± 13.78	26.80 ± 3.70	146.60 ± 17.81
PF4 + R3	39.64 ± 9.32	19.64 ± 1.29	59.28 ± 9.04	26.40 ± 3.13	146.60 ± 26.49
PF4 + R4	40.06 ± 7.42	22.08 ± 2.10*	61.14 ± 7.70	31.80 ± 7.22*	163.40 ± 34.83
PF4 + R5	44.24 ± 6.99	26.58 ± 2.79**	70.82 ± 4.63	33.00 ± 9.62*	172.60 ± 52.69
PF4 + R6	40.08 ± 8.41	22.46 ± 2.30*	62.54 ± 7.17	27.40 ± 7.30	144.00 ± 38.65
PF4 + R7	41.50 ± 8.17	19.62 ± 6.35	61.12 ± 8.20	31.00 ± 5.15	169.20 ± 11.34
PF4 + R8	49.42 ± 5.97	20.38 ± 3.09	69.80 ± 7.00	27.40 ± 2.19	147.00 ± 12.53
PF4 + R9	51.08 ± 7.87	27.46 ± 12.32**	79.26 ± 15.13**	26.00 ± 4.74	132.00 ± 27.99
PF4 + R10	48.82 ± 9.65	19.12 ± 5.82	67.94 ± 14.20	19.80 ± 3.11	102.60 ± 16.40
PF4 + R11	47.80 ± 9.70	21.60 ± 1.10	69.40 ± 10.14	28.80 ± 8.53	143.40 ± 48.67

IF5 + R1	50.66 ± 4.91	20.04 ± 2.41	70.70 ± 7.23	30.60 ± 6.15	153.00 ± 33.48
IF5 + R2	42.96 ± 4.81	20.30 ± 2.11	63.28 ± 4.10	30.80 ± 5.12	162.60 ± 17.77
IF5 + R3	40.64 ± 5.72	19.76 ± 4.91	60.40 ± 9.28	28.00 ± 2.00	141.00 ± 11.96
IF5 + R4	44.20 ± 3.85	22.00 ± 2.90*	66.20 ± 5.75	26.00 ± 3.81	132.00 ± 23.10
IF5 + R5	44.30 ± 12.55	21.50 ± 3.74	65.80 ± 12.83	37.60 ± 8.32**	192.00 ± 45.76
IF5 + R6	48.58 ± 13.34	22.44 ± 3.77*	71.02 ± 14.72	34.80 ± 7.26**	180.20 ± 33.12
IF5 + R7	52.08 ± 6.83	20.24 ± 2.48	72.32 ± 5.22	27.00 ± 8.46	167.40 ± 53.95
IF5 + R8	40.40 ± 5.21	21.04 ± 3.37	61.44 ± 2.00	24.80 ± 3.42	154.80 ± 50.53
IF5 + R9	41.48 ± 5.47	18.68 ± 2.36	60.26 ± 6.63	23.00 ± 2.45	119.60 ± 11.35
IF5 + R10	50.56 ± 7.43	18.58 ± 2.79	69.10 ± 9.53	24.60 ± 5.03	137.40 ± 35.62
IF5 + R11	45.82 ± 6.26	18.38 ± 2.62	64.20 ± 5.47	26.00 ± 2.92	145.60 ± 16.01
PF4 + IF5 + R1	44.08 ± 7.89	18.98 ± 2.36	63.06 ± 9.54	26.40 ± 2.61	156.00 ± 20.05
PF4 + IF5 + R2	38.24 ± 6.51	13.82 ± 4.73	52.06 ± 4.46	21.40 ± 3.05	138.40 ± 62.14
PF4 + IF5 + R3	49.22 ± 4.59	15.38 ± 4.58	64.60 ± 8.31	28.80 ± 5.36	151.00 ± 29.78
PF4 + IF5 + R4	48.86 ± 6.97	19.90 ± 4.45	68.76 ± 6.41	32.80 ± 11.69*	173.60 ± 45.42
PF4 + IF5 + R5	44.54 ± 6.69	16.22 ± 3.08	60.76 ± 6.05	29.80 ± 4.44	155.20 ± 23.97
PF4 + IF5 + R6	43.58 ± 6.07	18.26 ± 2.24	61.84 ± 5.83	31.40 ± 6.91	163.20 ± 25.59
PF4 + IF5 + R7	40.46 ± 7.90	20.40 ± 0.84	60.86 ± 8.20	34.80 ± 2.17**	166.60 ± 16.56
PF4 + IF5 + R8	37.00 ± 8.33	20.26 ± 3.08	57.26 ± 10.11	33.40 ± 6.69*	173.20 ± 34.69
PF4 + IF5 + R9	39.30 ± 6.53	18.14 ± 1.31	57.44 ± 7.23	30.80 ± 3.56	158.60 ± 7.60
PF4 + IF5 + R10	46.68 ± 7.80	19.48 ± 3.62	66.16 ± 6.54	39.20 ± 7.56**	189.00 ± 19.05
PF4 + IF5 + R11	45.10 ± 12.59	22.34 ± 3.32*	67.44 ± 14.28	39.80 ± 9.98**	210.20 ± 49.97*

	COLLAR DIAMETER (IN MM)	**FRESH BIOMASS OF LEAF (IN GRAM)**	**DRY BIOMASS OF LEAF (IN GRAM)**	**FRESH BIOMASS OF ROOT (IN GRAM)**	**DRY BIOMASS OF ROOT (IN GRAM)**
CONTROL	19.80 ± 0.84	3.00 ± 0.81	1.58 ± 0.41	1.56 ± 0.17	0.70 ± 0.16
PF4	20.20 ± 3.63	4.36 ± 2.07	2.50 ± 1.19	2.05 ± 0.81	0.88 ± 0.24
IF5	19.00 ± 2.00	4.96 ± 0.99*	2.70 ± 0.51	1.93 ± 0.49	1.24 ± 0.61
R1	20.80 ± 0.84	4.97 ± 0.89*	2.99 ± 0.60*	1.40 ± 0.25	0.67 ± 0.20
R2	21.20 ± 5.81	5.03 ± 0.99*	3.05 ± 0.92*	2.32 ± 1.14	1.22 ± 0.88
R3	20.00 ± 1.41	5.13 ± 1.81*	3.14 ± 1.36**	2.23 ± 0.68	1.25 ± 0.45
R4	18.80 ± 2.17	4.62 ± 0.70	2.77 ± 0.50	1.73 ± 0.39	1.00 ± 0.14
R5	18.60 ± 2.68	4.92 ± 0.99	2.86 ± 1.08*	1.93 ± 0.62	1.02 ± 0.36
R6	20.00 ± 0.71	6.58 ± 0.75**	4.14 ± 0.56**	2.03 ± 0.82	1.04 ± 0.42

R7	21.00 ± 1.41	4.60 ± 2.03	2.81 ± 1.14	1.84 ± 0.92	0.94 ± 0.45
R8	20.00 ± 1.22	5.12 ± 1.60*	2.67 ± 0.75	1.98 ± 0.78	1.03 ± 0.51
R9	19.40 ± 2.41	3.94 ± 0.63	2.23 ± 0.46	1.95 ± 0.50	1.02 ± 0.45
R10	19.80 ± 1.30	6.04 ± 1.60**	3.68 ± 1.50**	1.42 ± 0.41	0.75 ± 0.33
R11	19.80 ± 2.39	5.43 ± 0.82**	3.25 ± 0.60**	1.46 ± 0.34	0.79 ± 0.28
PF4 + R1	20.00 ± 0.71	5.56 ± 0.66*	3.19 ± 0.47**	1.72 ± 0.94	0.97 ± 0.54
PF4 + R2	20.80 ± 0.84	5.32 ± 1.16*	2.81 ± 0.74	1.58 ± 0.44	1.05 ± 0.47
PF4 + R3	20.60 ± 1.14	5.03 ± 0.45*	2.49 ± 0.28	1.77 ± 0.62	0.89 ± 0.34
PF4 + R4	21.80 ± 1.30	5.29 ± 0.47*	2.79 ± 0.32	2.02 ± 0.44	1.03 ± 0.29
PF4 + R5	21.00 ± 1.00	4.67 ± 1.36	2.78 ± 0.75	1.85 ± 0.53	0.91 ± 0.34
PF4 + R6	21.80 ± 2.68	5.31 ± 1.81*	3.00 ± 1.18*	1.49 ± 0.66	0.85 ± 0.34
PF4 + R7	20.00 ± 1.22	5.36 ± 0.74**	3.00 ± 0.96*	1.76 ± 0.48	0.97 ± 0.32
PF4 + R8	20.00 ± 3.00	5.34 ± 0.81**	3.07 ± 0.45*	1.81 ± 0.65	0.91 ± 0.39
PF4 + R9	19.20 ± 2.68	4.64 ± 1.04	2.85 ± 1.14*	1.80 ± 0.35	0.97 ± 0.36
PF4 + R10	20.80 ± 3.11	4.58 ± 0.34	2.68 ± 0.24	1.65 ± 0.80	0.83 ± 0.42
PF4 + R11	20.60 ± 1.14	7.06 ± 1.24	4.55 ± 1.39**	1.28 ± 0.47	0.78 ± 0.33
IF5 + R1	18.40 ± 2.61	4.20 ± 0.72	2.51 ± 0.65**	1.50 ± 0.42	0.70 ± 0.38
IF5 + R2	20.60 ± 1.67	5.92 ± 0.84**	3.26 ± 0.65**	1.32 ± 0.63	0.64 ± 0.31
IF5 + R3	19.40 ± 3.85	6.66 ± 0.80**	3.78 ± 1.01**	1.63 ± 0.46	0.93 ± 0.23
IF5 + R4	19.60 ± 2.97	5.92 ± 0.71**	3.53 ± 0.84**	1.67 ± 0.53	1.00 ± 0.19
IF5 + R5	18.30 ± 2.28	5.92 ± 0.43**	3.45 ± 0.37**	1.92 ± 0.40	1.13 ± 0.23
IF5 + R6	20.00 ± 2.00	7.47 ± 1.25**	5.04 ± 1.02**	2.10 ± 0.07	0.99 ± 0.47
IF5 + R7	20.00 ± 1.22	5.04 ± 0.20*	3.34 ± 0.63**	1.79 ± 0.32	1.02 ± 0.33
IF5 + R8	20.80 ± 1.30	5.07 ± 2.48*	3.18 ± 1.54**	1.54 ± 0.18	1.00 ± 0.38
IF5 + R9	19.80 ± 2.39	5.15 ± 0.78*	3.10 ± 0.66**	1.53 ± 0.31	0.94 ± 0.36
IF5 + R10	19.80 ± 1.30	6.08 ± 1.07**	3.87 ± 0.53**	1.78 ± 0.56	0.99 ± 0.44
IF5 + R11	21.20 ± 1.10	5.00 ± 0.96*	3.17 ± 0.59**	1.96 ± 0.48	1.03 ± 0.25
PF4 + IF5 + R1	18.40 ± 1.82	4.99 ± 1.03*	3.20 ± 0.92**	2.09 ± 0.34	1.33 ± 0.30
PF4 + IF5 + R2	19.80 ± 1.48	6.58 ± 0.78**	4.36 ± 0.81**	2.15 ± 0.38	1.36 ± 0.38
PF4 + IF5 + R3	20.80 ± 1.64	5.78 ± 0.90**	3.47 ± 0.88**	2.85 ± 1.53*	1.60 ± 1.11*
PF4 + IF5 + R4	21.20 ± 1.48	4.72 ± 0.76	2.77 ± 0.27	1.84 ± 0.25	0.94 ± 0.32
PF4 + IF5 + R5	21.00 ± 1.00	6.41 ± 1.07**	4.01 ± 0.97**	2.82 ± 0.58*	1.65 ± 0.96**
PF4 + IF5 + R6	19.80 ± 2.28	4.61 ± 0.86	2.61 ± 0.63	1.83 ± 0.44	0.98 ± 0.43
PF4 + IF5 + R7	20.40 ± 2.19	4.55 ± 1.22	2.64 ± 0.80	1.88 ± 0.29	1.08 ± 0.33
PF4 + IF5 + R8	21.60 ± 0.89	6.37 ± 0.69**	4.41 ± 1.27**	1.98 ± 0.47	1.34 ± 0.68
PF4 + IF5 + R9	21.40 ± 0.89	5.85 ± 0.49**	3.68 ± 0.73**	2.03 ± 0.21	1.27 ± 0.15
PF4 + IF5 + R10	22.60 ± 1.52	6.07 ± 1.50**	3.58 ± 1.41**	2.15 ± 0.70	1.18 ± 0.33
PF4 + IF5 + R11	22.20 ± 1.10	7.28 ± 0.87**	4.71 ± 1.19**	2.57 ± 0.49	1.43 ± 0.27

Screening of ten fungal species and 5 bacterial isolates for their effects on the growth of Acacia leucocephala done under pot culture in greenhouse conditions, elucidated the best performance of the

combination of PF3 (Penicillumcrysogenum Thom. 1) and IF5 (CunninghmellaelegansLendn.) and Rhizobium isolates R10 and /or R11 in A. leucocephala.

Penicilliumchrysogenum Thom. (1) showed to be the suitablefor A. leucocephalain increasing the plant dry biomass of leaves and total shoot dry biomass, as the biomass increment ($P<0.001$). Variation among different inoculants used in this study is well reflected in the growth and development of host species (Rahangdale and Gupta, 1998).

Microbial inoculations may also increase the dualroot shoot ratio, leaf numbers and length of the branches of host plants (Al-Garni, 2006). The findings of increment in the number of branches, and leaf area of inoculated A. leucocephala plants (12 no. per plant) over control (3.0 per plant). Are corroborated with studies of Weih and Nordh (2005) on Salix sp.. Who stated that the total leaf area of the pot-grown plants is a better predictor of shoot biomass and branches in the field than the pot-grown plants. The result showed that the selected inoculants contributed to a higher rate of shoot growth amongst 15 no. of microbial strains tested, Pencilliumchrysogenum Thom. 1 was found to be the most effective in increasing plant height, biomass and morphological quality of seedlings among all the inoculants used.

Nodulated legumes generally have a high requirement for phosphorus to generate ATP which is required for nitrogenase function. It was observed that growth and nodulation increase by use of phosphate fertilizers (Huda et al.2007). Most tropical soils are phosphate fixing, use of mineral solubilizers from microbial origin may make them available to the host plants. Microbial inoculants are also found to be useful in enhancing the growth of forest tree seedlings grown under stress conditions (Dabas and Kaushik, 1998; Dash et al. 2013). The plants of Acacia leucocephala inoculated with different phosphate solubilizersand iron ore leaching fungi also exhibited good growth in terms of plant height, biomass and plant parts compared to the uninoculated control. Besides this, significant variations could be observed among all the treatments in affectingplant growth performances. Bacterial strains performed poorly in improving plant health grown under this experiment. Increase in plant height of tree seedlings over uninoculated control is indicative of the potential effect of these inoculants. The suitability of Penicilliumchrysogenum and Cunninghmellaelegans. (1) are observed to be an effective inoculants for this tree species influencingthe plant dry biomass of leaves and total shoot dry biomass ($P<0.05$ & $P<0.001$).

It is clearly evident that microbial inoculations respond differentially towards the growth and development of host species. It became evident that fungal inoculation is likely to increase the number of branches in seedlings. Enhancement in the number of branches may lead to the development of tree crowns. Jankiewicz and Stecki (1976) reported that branching patternsindicate the type and form of tree crown.

The leguminous species possess physiological specialization relative to rhizobial affinities and also exhibit symbiotic promiscuity. Allen and Allen (1958) have considered that plant is the dominant partner in symbiosis and that nodule formation merits recognition. In the present study, the nodulation pattern in A. leucocephala was very poor. Since the study was carried out for only four months; the role of inoculated Rhizobium and their effects on the performance of host plants is difficult to be interpreted. However, during screening experiments for evaluation of bioinoculants of mineral solubilizersespecially phosphate solubilizingmicrobes and Rhizobium isolates, the seedlings of Acacia leucocephala was found to be manifested with root nodule though it was very poor and could not be recorded for its number, size and structure. Hence, the role of Rhizobium inoculants in the performance of test plants can not be interpreted well.

Many of the tropical leguminous trees are reported to be fixing atmospheric nitrogen through nodule development, endowed with VA mycorrhizaland other microbial associations. Plantation of such species enriches poor soils, seedlings fortified with microbial inoculations may lead to the successful in establishment of plants in poor sites (Sahet al. 1998 and Sahgalet al. 2004). The effects of mineral solubilizersneed to be evaluated under field conditions, on a long-term basis, before the application of these inoculations to plantation seedlings is considered a viable proposition.

CONCLUSION

The present study done under controlled greenhouse conditions has provided an experimental approach to

adopt pre-inoculation of the symbiotic microbes to seedlings in the nursery for better plantation results. This aspect may become very practicable and cost-effective proposition in plantation programs in tropicalconditions. The present study was restricted with the time schedule and the working environment, further studies may be taken for the development of the package of practice for the tree legumes useful for of reclamation of wastelands and revegetation of barren and/or overburdened mine lands. However, the outcome of the present study is very important due to the requirement of microbial manifestation in the tree improvement programin a stressful environment.

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Seed Scarification and Plant Extracts Enhanced Germination, Seed Health and Seedlings Vigour of *Tetrapleura tetraptera*

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ABSTRACT

Seed dormancy and seed-borne fungi are main constraints to the domestication of edible non-timber forest products such as Tetrapleura tetraptera. This study aimed to evaluate the effect of thermal and mechanical scarification on seed dormancy and the efficacy of four plant aqueous extracts (*Cymbopogon citratus*, *Callistemom viminalis*, *Tephrosia vogelii*, *Eucalyptus saligna*) against seed-borne diseases and seedlings vigour of *T. tetraptera*. Treatments consisted of soaking the seeds in water at 70°C for 2 and 4 hours; and seed scarification with abrasive paper at 1 mm and 2 mm depths. The biological activity of plant extracts was evaluated both in vitro (by the dispersion method on agar medium) and in vivo at 0.25, 0.50 and 0.75 mg/ml. Momtaz (Imidacloprid 250 g/kg + Thiram 200 g/kg) was used as a positive control. Dormancy was lifted by scarification at 2 mm depth; this treatment had the highest germination percentage (92.33%) and the lowest infection rate (20.67%). The more frequent seed-borne fungi isolated belong to *Aspergillus* spp. (18.43-21.78%). The pathogenicity test was positive with Alternaria alternata, *A. fumigatus* and *Cercospora* sp. *T. vogelii* extract totally inhibited the growth of the pathogenic fungi at all the concentrations tested. Seeds infection with *C. viminalis* (5.33%) and *T. vogelii* (4.12%) extracts at 1 mg/mL were significantly similar to Momtaz (3.33%). The extract of C. viminalis had the highest vigour index (674.42) at 0.75 mg/ml. Mechanical scarification using abrasive paper and seeds treatment with plant extracts of *T. vogelii* could be used in the domestication process of the species.

INTRODUCTION

Forests provide essential goods and services for food, health, and income for 1.2 billion people worldwide (Betti et al. 2016). Among these resources, edible non-timber forest products (ENTFP) occupy a prominent place correlated with their food, socio-economic, and cultural values, which are very decisive for raising the standard of living of the populations (Manfo, 2018). NTFPs constitute a reserve or safety net, a source of subsistence and income in the event of crop losses, shortages, unemployment, or other emergencies or difficulties resulting from disasters or calamities (Muyambo et al. 2017). However, production, conservation, and processing techniques are still rudimentary, and parasitic attacks are significant on ENTFP (Awono et al. 2016). These attacks cause many losses in the production and conservation of ENTFP and considerably reduce the contribution of the ENTFP sector in the fight against food insecurity. Among the ENTFP of tropical Africa, *Tetrapleura tetraptera* is a perennial Mimosaceae tree that reaches 35 to 50 cm in diameter at maturity and bears buttresses at the base (Eyog et al. 2006). Its fruits have four protruding dark green sides when they are formed and become dark brown and shiny when ripe. The ripe fruits of *T. tetraptera* are dried and used as spice condiments, as well as for their medicinal properties in the treatment of digestive diseases, cysts, myomas, and obesity (OAU/STRC, 1996). The result of a study conducted by the Tropenbos Cameroon program in 2017 in southern Cameroon reveals that a cut pile of *T. tetraptera* costs between FCFA 50 and 200 (USD 0.08 to 0.33) and that a pod is sold between FCFA 150 and 300 (USD 0.25 to 0.50) per unit (Walter, 2001). This spice is also exported and sold in the European markets (Sunderland, 2000). In 2000, the United Kingdom imported 20 tonnes from Nigeria, Ghana, and Cameroon (Tabuna, 2000). Despite this socio-economic importance, the production of *T. tetraptera* faces several difficulties including difficult seed germination and high seed infection of seed-associated fungi (Ndoye et al. 2000). In fact, because of the rigidity of the pod and the seed coat, and because of the un-permeability of the seed to water, it remains in a state of dormancy. This makes the natural germination of *T. tetraptera* almost impossible without the disseminator action of elephants or people (Alexandre, 1978). To overcome this constraint, various techniques have been applied to *T. tetraptera* seeds to lift the dormancy. Wakaya and Akinyele (2016) used 98% concentrated sulfuric acid, lime juice, overnight soaking, and hot water to stimulate *T. tetraptera* germination and found that dormancy can be broken by sulfuric acid. However, this technique has proven difficult to apply, and not accessible to low-income producers. The establishment of techniques within the reach of producers and easily applicable is, therefore, necessary to efficiently germinate the seed of T. tetraptera. To manage these fungal diseases of seeds (damping off, seed rot, *fusarium*, etc.) whose pathogens are mostly telluric (*Rhizoctonia*, *Cercospora*, *Aspergillus*, *Colletotrichum*, *Fusarium*, etc.), several synthetic fungicides are recommended. Unfortunately, their judicious use in accordance with the prescriptions of use is rather rare because the local nursery workers are inexperienced and untrained in the management of pesticides. The sometimes anarchic use of synthetic pesticides presents risks of environmental pollution, development of resistance to pathogens and intoxication for the user (Lulia et al. 2012; Kausik and Sayan, 2015). For these reasons, research in the field of plant protection is increasingly oriented towards the development of alternatives to chemical control by resorting to the use of non-toxic substances of natural origin (Murray, 2000; CABI, 2011). Hence, this study was carried out to evaluate the effect of thermal and mechanical scarification on seed dormancy and the effectiveness of four plant extracts against seed pathologies and seedling vigour of *T. tetraptera*.

MATERIAL AND METHODS

Collection and Conservation of Plant Material

The fruits of *T. tetraptera* were picked under five trees about 20 years old in the locality of Kekem, in the West region of Cameroon. These fruits were dried at room temperature in the laboratory for two months before the test. Using a hammer, the fruits were carefully broken to obtain the seeds which were stored in sterilized and hermetically sealed glass boxes. The leaves of the plants used for the preparation of the extracts, namely *Eucalyptus saligna*, *Tephrosia vogelii*, *Callistemon viminalis* and *Cymbopogom citratus*, were harvested from 6 - 8 a.m. at the Application and Research Farm of the Faculty of Agronomy and Agricultural Sciences (FASA) of the main campus of the University of Dschang in the West region of Cameroon.

Scarification of Tetrapleura tetraptera Seeds

The seeds were disinfected in a 2% hypochlorite solution for 3 min, then rinsed 3 times with distilled water for 5, 10 and 15 minutes respectively to remove traces of the disinfectant. The disinfected seeds were then scarified either by heat infusion of the seeds in water at 70°C for 2 hours or for 4 hours; either mechanically with abrasive paper by rubbing the proximal part of the seed (hilum) to a depth of 1 mm or 2 mm. The control batches consisted of seeds that had received no treatment. The scarified seeds were then placed in 15 cm-diameter glass Petri dishes previously lined with three layers of blotting paper soaked in water and then incubated at 22 ± 1°C under a 12 h of light and 12 h of darkness photoperiod, and watered every other day. There were 100 seeds per treatment and each treatment was repeated 3 times. The date of first germination, germination rate and seed infection rate were assessed daily. The Germination (G) was calculated as follows: G = g x 100 /N, where g = number of seeds germinated and N = total number of seeds sowing (Djeugap et al., 2014). The infection rate (IR) reflects the susceptibility of seeds to fungal infections during the germination period: IR = i x 100 /N with i = number of seeds infected and N = total number of seeds sowing. The germination rate (GR) was calculated through the relationship GR = ∑n / ∑ (n x DAS) with n = number of seeds germinated on day d and DAS = the number of days after sowing.

Isolation and Identification of Seed-borne Fungi of *Tetrapleura tetraptera*

The medium used for the culture of fungi was potato dextrose agar (PDA) supplemented with 1 g/L of chloramphenicol and sterilized at 121 °C for 15 min. *Tetrapleura tetraptera* seeds, whether symptomatic or not, were disinfected in a 3% hypochlorite solution for 2 min and then rinsed with sterile distilled water (Djeugap et al. 2015). Ten disinfected seeds were aseptically deposited in 90 mm Petri dishes containing 20 ml of PDA medium and placed in an incubator at 22°C. The fungal colonies visible around the inoculated seeds observed 5 days after inoculation, were then purified on the PDA medium (Djeugap et al. 2017). The isolation frequency (IF) of each fungus was calculated using the following formula: IF = (NF/NT) x 100 where NF represents the total number of samples from which a particular fungus was isolated and NT, the total number of samples from which the isolations were made (Iqbal and Saeed, 2012). The identification was made on the basis of the morphological characteristics of the purified fungi (mycelium and fructification) as observed under the microscope using the identification keys of Mycology (Champion, 1997).

Pathogenicity Test

For each 10-day-old isolated fungus, a spore suspension was prepared for inoculation by adding 10 ml of sterilized distilled water to the Petri dishes containing the PDA culture medium, and then gently rubbing with a thin brush. A drop of Tween 80 was added to homogenize the spores in the suspension, then the suspension was filtered using a filter cloth (mesh diameter < 1mm) to remove the mycelial fragments (Imathiu et al. 2014). Finally, 10µl of each suspension was deposited on a hemacymeter (Thoma cell) and mounted on a microscope to count the number of spores and subsequently determine the concentration of the suspension through the formula of Mathur and Kongsda (2003): (Number of spores x volume of spore suspension in mL)/Counting area in mm^2 x depth of counting area in mm)/1000. This number of spores was calibrated at 2.4 x 105 spores/ml for all the fungal species used for the pathogenicity test. The seeds disinfected as indicated above (Djeugap et al. 2015) were placed in Petri dishes containing 3 layers of sterilized blotting paper and moistened with sterile distilled water at the rate of 100 seeds per box. Then, a 10 ml spore suspension of each fungus was sprinkled on these seeds and the dishes were finally sealed with parafilm and incubated at room temperature (22±1°C). Each treatment was repeated 3 times and the seed infection rate was determined.

Preparation of Plant Extracts

Fresh leaves harvested from the plants were washed with tap water to remove microorganisms and dust, and then dried in the shade for two weeks. The dried leaves were ground to obtain a fine powder. To obtain aqueous extracts, 100 g of the plant powder was macerated with 500 ml of sterile distilled water for the aqueous extract, for 24h with two shaking, in the dark. The mixture was filtered using muslin, cotton, and Whatman No 4 paper and the filtrate constituted the crude plant extract (Falleh et al. 2008).

Evaluation of the Antifungal Potential of the Plant Extracts

The evaluation of the in vitro activity of the extracts was carried out following the dispersion method on

agar medium (PDA) at concentrations of 2.5, 5.0 and 7.5 mg/ml on the three fungi which were positive in the pathogenesis test namely: *Alternaria alternate*, *Aspergillus fumigatus* and *Cercospora* sp. Sterilized distilled water and the synthetic fungicide Momtaz (Imidacloprid 250 g/kg + Thiram 200 g/kg) served as a negative and positive control, respectively. The radial growth (RG) of the pathogen was evaluated by the formula: RG= (d1+d2- 2d0) / 2 by measuring the growth diameters every day from two orthogonal lines drawn on the back of the boxes and crossing at the level from the point of deposition of the explant. In this formula, d0 is the diameter of the explant, d1 and d2 are the two measured orthogonal diameters of the culture. The percentages of inhibition (%I) were determined by the relationship %I = 100 (Dc - Df) / Dc where Dc is the growth diameter of the control and Df is the growth diameter of the fungal colony on medium supplemented with extracts (Yaouba et al. 2017).

Evaluation of the Efficacy of Plant Extracts on Seed Germination and Infection, and Vigour Index of Seedlings of Tetrapleura tetraptera

Seeds scarified and disinfected (200 seeds each were considered per treatment) were soaked with 25 ml of aqueous extracts at concentrations of 0.25, 0.5, 0.75 and 1.0 mg/ml. A volume of 25 ml of sterilized distilled water and Momtaz fungicide mixture (Imidacloprid 250 g/kg + Thiram 200 g/kg) at 5 mg/kg respectively served as negative (T-) and positive (T+) controls. After soaking, the seeds were placed in an oven at 40°C for 15 min following the modified protocol of Djeugap (2013) then inoculated in Petri dishes layered with moist paper. The Petri dishes containing the seeds were stored at room temperature with a 12 h of light and 12 h of darkness photoperiod. Each treatment was repeated 3 times. The daily observations carried out focused on counting the number of germinated seeds and the number of healthy seeds in order to calculate the infection rate and the seed germination rate. The seedling vigour index was obtained as follows. Vigour Index = Germination Rate x (Root length + Stem length) (Abdul-Baki and Anderson 1973).

Statistical Analysis

The analyses were carried out using the statistical analysis software R version 3.5.1 at the 5% probability threshold. Since the data did not follow the normal distribution and the homogeneity of the variance was not respected, the Kruskal-Wallis test was used for the separation of the means.

RESULTS AND DISCUSSION

Effect of Scarification on Germination and Infection of T. tetraptera seeds

Thermal and mechanical scarification significantly increased the germination rates of *T. tetraptera* seeds compared to the control where the germination rate was zero (Table 1). The germination rate was maximum with the mechanical scarification at 2 mm depth (92.33%) followed by the mechanical scarification treatment at 1 mm (81.52%), while thermal scarification treatments for 2 hours and thermal scarification for 4 hours showed very low with germination rates of 1.27% and 0.67% respectively. There was no significant difference between the infection rates obtained from the different treatments, but the seed infection rate was higher in the heat scarification treatment boxes for 2 hours (42.00%). The control boxes had no germinated seeds, but a fairly high infection rate (28.67%). Figure 1 shows the germinated and infected seeds at 3 DAS where visible mycelium of the pathogens is observed on seeds. The different results between thermal and mechanical scarification could be explained by the fact that at 2 mm the embryo is already visible and develops without braking due to the integument. Nkongmeneck et al. (1996) showed that scarification using a nail clipper could increase germination capacity by 80 to 90% after three days. The dormancy breaking methods used have been evidenced by other studies, namely Pérez-Garcia & Gonzalez (2006) and Rao et al. (2006) for mechanical scarification which consists of using sandpaper; and Li et al. (1999) for thermal scarification with hot water (80°C) which made it possible to remove the waxy cuticle from the seeds. Some authors highlighted the interest in involving local populations in the domestication and in situ and ex-situ conservation strategies of plant species of interest (Harivel et al. 2006; Meunier et al. 2010).

Effect of Treatments on the Date of First Germination and the Germination Rate

The first germination was observed in the thermal scarification treatment boxes for 4 hours and in the mechanical scarification treatment boxes at 2 mm with a duration of 2.33 and 2.67 days respectively (Table 2). These dates differed significantly from those

obtained in the boxes with mechanical scarification treatments at 1 mm and thermal scarification for 2 hours (4.91 and 4.67 respectively). The germination speed was higher in the mechanical scarification treatment boxes (19.85 and 14.10 respectively at 2 mm and 1 mm) unlike the speeds obtained in the thermal scarification treatment boxes (0.10 and 0.27 respectively for 2h and 4h).

Table 1. Germination and infection rate of *Tetrapleura tetraptera* seeds submitted to different scarification techniques, 14 days after sowing.

Treatment	Germination (%)	Infection rate (%)
Control	0.0±0.0^{c}	28.67±1.98^{b}
Thermal scarification for 2h	0.67±0.58^{c}	42.00±4.88^{a}
Thermal scarification for 4h	1.23±0.63^{c}	27.67±2.81^{b}
Mechanical scarification at 1 mm	81.52±3.42^{b}	23.81±4.84^{c}
Mechanical scarification at 2 mm	92.33±4.73^{a}	20.67±5.86^{c}

a,b,c Data are means ± standard deviations. Means with different superscript letters in the same column are significantly different as per the Kruskal-Wallis test at p= 0.05.

Table 2. Effect of scarification on the germination date and germination rate of de *Tetrapleura tetraptera* seeds at 14 days after sowing.

Treatment	Day of the first germination	Germination rate (seeds/day)
Control	0.0±00^{c*}	0.0±00^{d}
Thermal scarification for 2h	4.67±1.16^{a}	0.10±0.10^{c}
Thermal scarification for 4h	2.33±0.04^{b}	0.27±0.14^{c}
Mechanical scarification at 1 mm	4.91±1.19^{a}	14.10±2.19^{b}
Mechanical scarification at 2 mm	2.67±0.58^{b}	19.85±1.59^{a}

*Data are means ± standard deviations. Means with different superscript letters in the same column are significantly different as per the Kruskal-Wallis test at p= 0.05.

Seed-borne Fungi of Tetrapleura tetraptera Seeds and their Pathogenicity

Seven species of seed-borne fungi were been identified: *Rhizoctonia* sp., *Aspergillus niger*, *Aspergillus flavus*, *Aspergilus fumigatus, Trichoderma* sp., *Alternaria alternata*, *Cercospora* sp. and *Pestalotiopsis* sp. The more frequent species isolated were: *A. flavus* (21.78%), *A. ni*ger (18.48%) and *A. fumigatus* (16.31%); *Pestalotiopsis* sp had the lowest isolation frequency (Figure 2). The pathogenicity test was positive for *A. alternata*, *A. fumigatus* and *Cercospora* sp. with infection rates of 45.75%, 32.25% and 22.12%, respectively, 5 days after inoculation (Table 3). The seed's response to artificial inoculation shows a rapid death of seed by these three seed-borne pathogens within 5 days (Figure 3). The isolated fungal species are well known as damaging species of ENTFP seeds. Most of these species have already been identified on *Ricinodendron heudelotii* and *Garcinia kola*, two ENTFP of high socio-economic value in Cameroon, as being responsible for the seed losses (Dongmo et al. 2017). Also, Djeugap et al. (2017) reported species like *A. flavus* and *Cercospora* sp. as fungi responsible for post-harvest losses of *Monodora myristica*. Most of these fungi are responsible for the high post-harvest losses of some fruits (Onuorah and Orji, 2015). *Cercospora* sp. was also isolated from seeds of *Persea americana* with high occurrence frequencies (Erute and Oyibo, 2008). The high frequencies of certain species such as *Aspergillus* in seeds of *T. tetraptera* could be explained by the fact that they are extremely polyphagous and likely to live on more diverse media than other species (Agrios, 2005). Poor storage conditions may also explain their presence in *T. tetraptera* grains. Some of the fungi inoculated on the grains of *T. tetraptera* developed and others did not; they latter can be considered as opportunists.

Bio-efficacy of Plant Extracts on the Inhibition Percentage of Pathogenic Fungi of Tetrapleura tetraptera seeds

The extract of *C. viminalis* at a concentration of 0.75 mg/mL significantly reduced the radial growth of *A. alternata* and *Cercospora* sp. by 80.89% and 69.08%,

respectively. The extract of *C. citratus* at the concentration of 0.75 mg/ml was significantly reduced by 60.25%, 41.77%, and 19.49% respectively, the radial growth of *A. alternata* and *Cercospora* sp., and *A. fumigatus*. The extract of *Eucalyptus saligna* totally inhibited the growth of *A. fumigatus* at the concentrations of 0.75 mg/mL. The radial growth inhibition was absent in the negative control dishes for all the fungi, while in the positive control dishes enriched with Momtaz, the radial growth of the fungi was completely inhibited (Table 3). *Tephrosia vogelii* extract totally inhibited the development of the three fungi at all the concentrations tested. The effectiveness of aqueous extracts of *T. vogelii* and *C. viminalis* on the development of these microorganisms varies according to concentrations and has been demonstrated by Salem et al., (2017) on *C. viminalis* and Masete (2021) on *T. vogelii*. Other studies showed that *C. citratus* and *T. vogelii* extracts exhibited antifungal activity against potato late blight pathogen *Phytophthora infestans* (Galani et al. 2013).

Figure 1. Scarified and germinated seeds of *Tetrapleura tetraptera* at 3 days (A) and 7 days after sowing (B) in the control (non-treated).

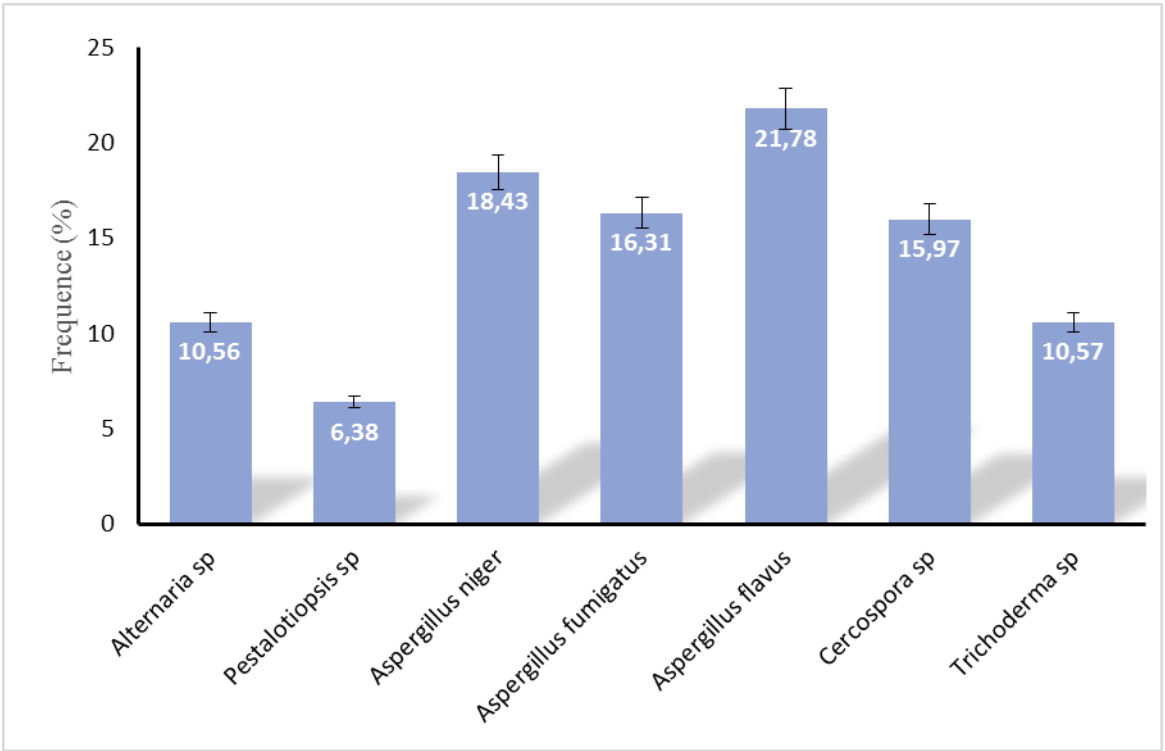

Figure 2. Occurrence/frequency of fungi isolated from *Tetrapleura tetraptera* seeds.

Figure 3. Pictures showing seed infection after inoculation with *Alternaria alternata* (left), *Aspergillus fumigatus* (center) *Cercopora* sp. (right), 5 days after inoculation.

Effect of Plant Extracts on Germination, Seed Infection, and Seedlings Vigour of Tetrapleura tetraptera

T. vogelli and *C. viminalis* extracts gave the highest germination percentage at 0.25 mg/mL (97.33%) and 0.5 mg/mL (96.67%), respectively. However, there was no significant difference between the different concentrations of the extracts and the two controls on the germination of *T. tetraptera* seeds (Table 5). Also, the lowest seed infection was obtained with the same extracts and the values obtained were significantly ($p<0.05$) comparable to the positive control (synthetic fungicide). In fact, seed infection with *C. viminalis* extract was 5.33% at 1 mg/mL and 4.12 % with T. vogelii at the same concentration while in the positive control, it was 3.33%. Significant differences were observed between the different concentrations of the aqueous extracts and the two controls on the percentage of infection of the seeds of *T. tetraptera* (Table 5). Table 6 shows that the maximum vigour index (674) was obtained with *C. viminalis* at 0.75 mg/mL while the positive control obtained the lowest vigour index (506). However, significant differences were observed between the different doses of aqueous extracts and the two controls on the vigour index of *T. tetraptera* seeds.

Table 3. Pathogenicity and infection rate of seed-borne fungi isolated from *Tetrapleura tetraptera*, 5 days after inoculation.

Fungi	Pathogenicity	Infection rate (%)
Alternaria alternata	+++	45.75±4.65^{a*}
Pestalotiopsis sp	-	0.0±0.0^{d}
Aspergillus niger	-	0.0±0.0^{d}
Aspergillus fumigatus	++	32.25±2.88^{b}
Aspergillus flavus	-	0.0±0.0^{d}
Cercospora sp	+	22.12±3.66^{c}
Trichoderma sp	-	0.0±0.0^{d}

***Data are means ± standard deviations. Means with different superscript letters in the same column are significantly different as per the Kruskal-Wallis test at p= 0.05. +++ = highly pathogenic; ++ = moderately pathogenic; + = pathogenic; - = not pathogenic.**

The *T. vogelii* extracts protect the seeds against fungal attack more than the others, followed by *C. viminalis*, *E. saligna* and *C. citratus* extracts. This activity of the aqueous extracts could be due to their chemical composition in antifungal substances. These results corroborate those of Yaouba et al. (2019) that showed the antifungal potential of *E. saligna* extracts against the fungi responsible for the deterioration of green

beans post-harvest; and those of Djeugap et al. (2011) who demonstrated the in vitro and in vivo efficacy of plant extracts including *C. viminalis* against black nightshade downy mildew; Helal et al. (2017) who showed that *C. citratus* extract caused inhibition of *Aspergillus* sp.; and Kpatinvoh et al. (2007) findings showed an inhibitory activity of *T. vogelii* on the radial growth of fungi in stored cowpea seeds.. This suggests that the extracts of these plants would be effective in the fight against fungi associated with ENTFP seeds. The antifungal activity of these plant extracts could be due to the action of oxygenated monoterpenes (Yoshimura et al., 2010) and phenolic compounds including sterols, flavonoids, condensed tannins, coumarins and alkaloids (Galani et al. 2013).

Table 4. Inhibition of the mycelial growth of seed-borne fungi of *Tetrapleura tetraptera* by plant aqueous extracts.

Plant extracts	Concentration (mg/mL)	Growth inhibition (%)		
		Alternaria alternata	***Cercospora* sp.**	***Aspergillus fumigatus***
Callistemom viminalis	0.25	27.67±3.76^{c*}	18.07±2.33^{c}	0.0±0.0^{d}
	0.50	73.33±6.55^{bc}	67.07±4.87^{bc}	0.0±0.0^{d}
	0.75	80.91±11.32^{bc}	69.08±15.26^{bc}	0.0±0.0^{d}
Cymbopogom citratus	0.25	0.0±0.0^{d}	0.0±0.0^{d}	62.65±18.78^{b}
	0.50	0.0±0.0^{d}	0.0±0.0^{d}	28.11±6.77^{bc}
	0.75	60.25±12.41^{b*}	41.77±13.27^{b*}	19.49±5.64^{c}
Eucalyptus saligna	0.25	85.16±9.95^{ab}	77.10±8.29^{ab}	54.21±16.23 ^{b}
	0.50	82.36±13.11^{ab}	78.31±11,34^{ab}	79.27±20,44^{ab}
	0.75	95.66±12.78^{ab}	84.34±10,94^{ab}	100±0.0^{a}
Tephrosia vogelii	0.25	100±0.0^{a}	100±0.0^{a}	100±0.0^{a}
	0.50	100±0.0^{a}	100 ±0.0^{a}	100±0.0^{a}
	0.75	100±0.0^{a}	100±0.0^{a}	100±0.0^{a}
Momtaz (synthetic fungicide)		100±0.0^{a}	100±0.0^{a}	100±0.0^{a}
Control (distilled water)		0.0±0.0^{d}	0.0±0.0^{d}	0.0±0.0^{d}

*Data are means ± standard deviations. Means with different superscript letters in the same column are significantly different as per the Kruskal-Wallis test at 5%.

Table 5. Influence of plant aqueous extracts on the germination (%) of *Tetrapleura tetraptera* seeds.

Concentration (mg/ml)	***Cymbopogom citratus***	***Callistemon viminalis***	***Eucalyptus saligna***	***Tephrosia vogelii***
0.25	86.67±5.77^{a*}	90.00±7.32^{a}	86.67±5.27^{a}	97.33±5.77^{a}
0.50	90.00±7.00^{a}	96.67±5.77^{a}	84.67±5.77^{a}	83.33±5.27^{a}
0.75	92.00±7.00^{a}	90.00±7.32^{a}	80.33±5.27^{ab}	80.33±6.54^{a}
1.00	86.67±5.27^{a}	80.00±4.10^{b}	76.67±5.27^{c}	76.67±5.27^{b}
Momtaz (fungicide)	94.67±5.77^{a}	94.67±5.77^{a}	94.67±5.77^{a}	94.67±5.77^{a}
Distilled water (control)	92.33±11.54^{a}	92.33±5.54^{a}	92.33±6.54^{a}	92.33±6.54^{a}

*Data are means ± standard deviations. Means with different superscript letters in the same column are significantly different as per the Kruskal-Wallis test at 5%.

Table 6. Effect of plant aqueous extracts on seeds infection (%) of *Tetrapleura tetraptera* during germination.

Concentration (mg/mL)	*Cymbopogom citratus*	*Callistemon viminalis*	*Eucalyptus saligna*	*Tephrosia vogelii*
0.25	19.33±5.77^{b}	20.03±2.07^{b}	25.63±17.66^{b}	23.33±15.27^{b}
0.50	13.91±6.27^{b}	12.04±7.55^{c}	19.33±5.77^{bc}	16.67±9.77^{b}
0.75	12.33±5.77^{b}	10.22±7.32^{c}	16.67±5.77^{b}	4.54±1.96^{c}
1.00	8.67±4.01^{b}	5.33±2.82^{c}	13.33±7.27^{bc}	4.12±1.77^{c}
Momtaz (fungicide)	3.33±1.27^{c*}	3.33±1.27^{c}	3.33±1.27^{c}	3.33±1.27^{c}
Distilled water (control)	86.67±15.27^{a}	86.67±15.27^{a}	86.67±15.27^{a}	86.67±15.27^{a}

(a-c) Data are means ± standard deviations. Means with different superscript letters in the same column are significantly different as per the Kruskal-Wallis test at p= 0.05.

Tableau 7. Effect of aqueous plant extracts on the vigour index of *Tetrapleura tetraptera* seedlings.

Concentrations (mg/mL)	*Cymbopogom citratus*	*Callistemon viminalis*	*Eucalyptus saligna*	*Tephrosia Vogelii*
0.25	541.94±10.86^{b*}	553.00±14.01^{b}	410.13±9.28^{d}	603.43±11.92^{a}
0.50	446.96±15.73^{d}	464.69±36.99^{c}	533.99±6.78^{c}	467.23±4.11^{dc}
0.75	634.27±7.84^{a}	674.42±11.25^{a}	562.44±6.05^{b}	530.33±7.52^{b}
1.00	448.60±10.66^{d}	388.74±6.30^{ab}	597.02±12.37^{a}	432.61±5.04^{d}
Momtaz (fungicide)	506.74±10.95^{c}	506.74±10.95^{bc}	506.74±10.95^{c}	506.74±10.95^{c}
Distilled water (control)	238.31±7.57^{e}	238.31±7.57^{d}	238.31±7.57^{e}	238.31±7.57^{e}

*Data are means ± standard deviations. Means with different superscript letters in the same column are significantly different as per the Kruskal-Wallis test at p= 0.05.

CONCLUSION

This work investigates effective and easily applicable solutions to the dormancy and therefore to the germination of *T. tetraptera* seeds and proposes a biological control measure for seed-borne fungi. Scarification through mechanical abrasive at 2 mm depth improves the germination rate by up to 92%. The most frequently identified fungi on *T. tetraptera* seeds were: *A. flavus*, *A. niger* and *A. fumigatus* which may be mycotoxigenic. The pathogenic fungi were: *A. alternata*, *A. fumigatus* and *Cercospora* sp. Aqueous extracts of *T. vogelii, E. saligna*, *C. viminalis,* and *C. citratum* exhibited higher antifungal properties and inhibited the growth of the three pathogenic fungi as well as the synthetic fungicide Momtaz. These plant extracts promoted germination, vigour and seed protection. Since key domestication constraints of *T. tetraptera* have been underpinned and seed-borne fungi have been identified and controlled by this work, studies on the cultivation of *T. tetraptera* in the nursery and in the field should now be emphasized.

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