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Editorial message

Welcome to the first issue of the Journal of Agriculture & Forestry Research (JAFR) published in 2021 by the South Asian Research and Publishing Organization (SARPO). JAFR is an open-access multidisciplinary journal that publishes fundamental and applied research works in different areas of Agriculture and Forestry. JAFR is trying to become an advanced research journal dedicated to agricultural and forestry research. It functions as a strategic and applicable research publication on all aspects of agriculture and forestry, as well as publishes basic scientific articles reviewing the scientific aspects of current agriculture and forestry issue.

On behalf of our editorial team, I would like to thank our users, contributors, authors, and reviewers, all of whom have volunteered to contribute to the success of the journal and also for its development mission in the field of Agriculture & Forestry Research. We are publishing our articles with a particular emphasis on quality, safety, and better outcomes of research. We know that only advances in Agricultural and Forestry technology can help us in confronting the challenges of the 21st century, and to capitalize on the promises ahead.

Finally, we encourage the contribution of the scientific community in improving the agriculture and forestry sector to ensure the continuity of a successful journal. Writers, reviewers, and editors are always welcome. We longed for comments and suggestions that can improve the quality of the journal. We believe that JAFR will function as an insightful and inspiring platform for both the researcher and the user which will pave the way for an unprecedented future.

Editor-in-chief

Journal of Agriculture & Forestry Research

Journal of Agriculture & Forestry Research (JAFR)

Contents

J. Agric. For. Res. Vol. 1, No. 1, pp. 1-9, Year 2021

Journal of Agriculture & Forestry Research
Volume no.1, Issue no. 1, Year 2021
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Research Article

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Crown-Diameter Model for Madrid Tree (*Pterocapus erinacieus* Poir) in Okpokwu Area of Benue State, Nigeria

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ABSTRACT

Among the current most exploited and threatened species in Western Africa, *P. erinaceus* is an endemic and multipurpose species. Owing to its economic, social, and high cultural potential, the species is subjected to high pressures causing an important regression of its stands. There is a lack of emphasis on the effective management of open-growth tree species in most African nations; these have slow sustainability of economic tree species in most areas, especially in Nigeria. Also, this had led to a decline in the natural forest areas while this economic tree population is gradually becoming extinct. Crown-bole diameter relationship helps to predict growth space requirements for optimum planting and estimation of stand density or stocking for establishing plantations and sustainable management of economic tree species. This study aims to determine the natural spacing among *P. erinaceus* in Okpoga, area of Benue State, Nigeria. The data collected on every standing tree includes crown diameter (using a 30-meter measuring tape) and diameter at breast height (Dbh) using diameter tape. The data collected from the field were fitted to different modified models and the best-fitted model was used to predict crown diameter from stem diameter at breast height. The results of this study show that *P. erinaceaus* would require low densities for optimum planting, fast growth, and high yield for the purpose of timber and high densities for non-timber forest products in the study area; because low densities are required to produce maximum diameter growth throughout the life cycle of trees stand which is applicable to this tree species. For the maximum volume of timber, thinning should be administered at canopy closure; this is to create more spacing for continued growth until the trees only react minimally to thinning. The recommended planting spacing would enhance optimum planting, fast growth, high yield/production, and control competition within each tree species. This study showed that upper diameter class distributions were being over-exploited, without replanting or regeneration in the area. Therefore, model 4 is recommended for crown diameter prediction of *P. erinaceaus* in the study area; the model should be used outside the extent of the original data (dbh 10.00cm - 49.20cm) with caution; as extrapolating to values outside this range may yield misleading results.

INTRODUCTION

Pterocarpus erinaceus Poir. (Fabaceae), also called V`ene or West African rosewood, is a multipurpose endemic forest species of Sahelo-Sudanian and Sudano-Guinean savannas and forests of West Africa. In English, the plant is called Gambia gum, African kino, Senegal rosewood, African rosewood, African teak, molompi wood tree, kino tree and African gum. The trade names of the plant include lancewood, African teak, African rosewood, Senegal rosewood and cornwood.

Among the current most exploited and threatened species in Western Africa, P. erinaceus is an endemic and multipurpose species of Guineo-Sudanian and Sudano-Sahelian zones. In Togo, stands of P. erinaceus are present in the five ecological zones (Segla et al., 2015). Exploitation is mainly focused on its wood with technological qualities that are much appreciated. It is one of the best wood species of the sub region (Adjonou, et al., 2010), chiefly used for commercialization purposes and construction, cabinet making, and heating and for arts. Its different parts are used in the treatment of some diseases, as animals' fodder and for dyeing (Seglaet al., 2015). Owing to its economic, social, and high cultural potential, the species is subjected to high pressures causing an important regression of its stands (Segla et al., 2015).

During the last decades, deforestation constitutes the most disturbing second environmental problem after climate change for developing countries (Damett and Delacote 2011).There is generally lack of emphasis on effective management of natural forest in Nigeria. These have led to decline in the natural forest areas while some economic trees are gradually becoming extinct. Human activities such as deforestation, agriculture, overgrazing and bush fires, coupled with the adverse effects of climate change, are also contributing to the loss of many important native plant species (Assogbadjo et al., 2010) among which the most heavily traded tropical hardwood currently in the world, Pterocarpus erinaceus is now considered endangered (Dumenu, 2019; IUCN, 2018). Also Studies on growth space requirements for Pterocarpus erinaceus are generally lacking and inventory information on the plant is scarce especially in North Central Nigeria. Natural stands of the plant are under constant pressure and heavily exploited for timber, animal feeding and others uses.

Diameter at breast height and crown width are important tree characteristics where many of the forestry activities and process are related with it, therefore any attempt that can improve the accuracy of measuring, predicting and analyzing these variables should be given utmost importance (Elmugheiran and Elmamoum, 2014). The crowns of trees have been subjected to much less study than their stems, primarily due to their lower marketable value. However, crown size being closely related to the photosynthetic capacity of a tree, is an important variable in studies of the growth of individual trees (Helms, 1998). It is also very relevant in studies of the growth of stands due to the close correlation between crown size and stem diameter, and the 'packing' or density of trees in a stand (Amonum and Japheth, 2019).

The strong correlation between crown and bole diameter is particularly useful for predicting and estimating growth space, stand density and limiting stocking relationship (Goelz, 1996; Kigomo, 1998; Hemeryet al, 2005). It is also very relevant in studies of the growth of stands and the packing or density of tree stand (Hemery and Pryor, 2005). The growth of a tree mostly is determined by the tree crown characteristics, tree crown size can determine tree growth and survival; tree height and crown dimension determine length of its clear bole which is important in merchandizing of the tree into various wood products (Dau and Chenge, 2016).

This study aims to determine the natural spacing among Pterocarpus erinaceus in Okpoga, Okpokwu Local Government Area of Benue State. This study highlight the importance of promoting the development of innovative silvicultural strategies for the extension and modeling of natural stands of P. erinaceus in order to meet sustainably the timber needs of the West African region. Also, it helps to strengthening the roles of natural forests in providing ecosystem services and mitigating climate change effects. The study provided the needed data that are largely lacking at the moment for the documentation and improvement of the natural spacing among the plant species especially in Okpoga area of Benue State.

METHODOLOGY

Study area

Okpokwu is a Local Government Area in Benue State, Nigeria. Its headquarters is in the town of Okpoga located in the south of the area. Okpokwu has an area of 731 km2 and a population of 176,647 at the 2006 census and the postal code of the area is 973 (Wikipedia 2021). In Okpoga, the wet season is warm, oppressive, and overcast and the dry season is hot, muggy, and partly cloudy. Over the course of the year, the temperature typically varies from 17.2 °C to 32.2 °C and is rarely below 13.3°C or above 34.4 °C.The rainy period of the year lasts for 9.2 months, from February 20 to November 25, with a sliding 31-day rainfall of at least 0.5 inches. The most rain falls during the 31 days centered on September 5, with an average total accumulation of 8.1 inches.

The vegetation of the local government is that of a transition between the deciduous rain forest of Eastern Nigeria on the Southern part of the local government, and the grassland Savannah towards the North. Some common grass species found in the area include: Bahama grass (*Cynodam dactylon*), Wild sunflower (*Aspilia africana*), Elephant grass (*Pennisetum purpureum*), Carpet grass (*Axonopus compressus*), African Club Moss, African feather grass, African Olive etc. Some common tree species in the area include *Isoberlinia doka*, *I. tomentosa*, locust bean tree (*Parkia biglobosa*), *P erinaceus* etc. The area within 2 miles of Okpoga is covered by shrubs (59%) and cropland (36%), within 10 miles by shrubs (57%) and cropland (29%), and within 50 miles by shrubs (48%) and cropland (31%). The soil texture in the study area is loamy black and clay in some areas.

Data Collection and Analysis

Pterocarpus erinaceus was selected based on its economic value to the Okpoga community. Data from P. erinaceus were collected in Okpoga, Nigeria. A preliminary survey was carried out during which areas populated with the species were identified.

Enumerations of tree species in the study area was carried out and the measurements of the parameters of interest were taken trees with diameter less than 10cm were discarded because they were saplings and will not be good for the modeling exercise while trees with a diameter of 10cm and above were measured and the data which were useful to this study were recorded. The data collected on every tree species includes crown diameter by using a 30-meter measuring tape; DBH, diameter at the base, diameter at the middle, and diameter at the top.

Measurement of tree Parameters

Diameter of the sampled trees was determined with the use of diameter tape on winding the tape around the tree at 1.3 meters above the ground while total height was measured by the use of Haga altimeter.

Crown-diameter measurement was based on the assumption that the vertical projection of a tree crown is circular; four radii were measured (using 30-metre measuring tape) and in the direction forming equal angles (Foli et al., 2003; Zuhaidi, 2009). Along each radius of the tree crown, the diameter tape was held horizontally and extended until each person was vertically under the tip of the longest branch on both sides; a 3.00 - meters ranging pole was used to align vertically to the edge of the crown (Kigomo, 1991, 1998; Amonum and Japheth, 2019). The diameter tape was turned by 90 degrees and measurements were carried out repeatedly along the thinnest part of the tree crown and recorded (Foli et al., 2003).

Average crown diameter (Cd) was calculated by summing up the four radii and divided by 2, thus;

$Cd= \Sigma\, ri / 2$ --- (1)

Where Cd = average crown diameter; ri = projected crown radii measured on four axes.

DBH over bark was measured at 1.3 meters above the ground for all individual tree ≥ 10 cm by means of diameter tape. The points of the measurement were taken from the uphill sides of the trees and on the inside of the lean for leaning trees (Zuhaidi, 2009). For trees with deformations at 1.3m, the measurement was made at the sound point on the stem above the abnormality. During the measurement, loose bark, climbers and epiphytes were lifted above the measuring tape; this was to avoid measurement errors and reading.

Data Analyses

Model fitting

The data collected from the field were fitted to the following model forms for predicting crown diameter suggested by various authors with the aim of choosing the model form with the best fit characters.

Simple linear regression model

$Cd = b_0 + b_1\, dbh$ - - - [2] Lockhart et al. (2005)
Quadratic/power model
$Cd = b_0 + b_1\, dbh^2$ - - - [3] Foliet al. (2003)

Logarithm model

$Cd = b_0+b_1dbh+b_2\ln dbh$ - - - [4] Zuhaidi (2009)
$LnCd = b_0+ b_1 \ln dbh^2$ - - - [5] Dau and Chenge, 2016

Binomial model

$LnCd = b_0+b_2dbh^2 + b_1\ln dbh$ [6] Zuhaidi (2009)
Where: Cd = crown diameter; dbh = diameter at breast height; b0= intercept; b1 and b2= regression coefficient

Stand density/ basal area per hectare

S.D= D^2/40,000 - - -[7] Zuhaidi (2009).
Where: S. D = Stand density; D= diameter (dbh); = 3.142.

Model selection

The fitted models were assessed and selected with the view of determining their fitness for further use; the performance of each model in the below listed criteria were ranked and the best performed model in the overall rank was selected as the best. The assessment was based on all the following criteria.

(i) Squared multiple correlation coefficient (R^2)

This measures the degree of agreement between the regression model and the observed data. It measures the goodness of fit of the regression equation. The higher the R^2, the better the fit.
R^2=RSS÷YSS X 100; - - - (8)

RSS= regression sum of squares; YSS= total sum of squares

(ii) Root Mean Square Error (RMSE)

This is used to compare equations with the same dependent variable which do not differ. It is the overall error of the estimate. The smaller the RMSE, the more appropriate the model.
RMSS =√SSE/dfe - - -(9)
Where: SSE= error sum of squares; dfe = error degrees of freedom.

Model Ranking

Ranks were assigned to each model based on each criterion; the smaller the rank the better the performance of a model. Ranks were summed up to obtain the overall rank for each model. This highlighted the performance of each model with respect to all criteria considered.

RESULTS

Growth Trend of Madrid Tree (P. erinaceaus) Stand

The results of descriptive statistics of growth variables of Madrid tree species (*P. erinaceaus*) is shown in Table 1. The table showed that diameter at breast height had a mean value of 28.65 meters and standard deviation value of 8.10, Total height had a mean value of 13.87 meters and standard deviation value of 0.83, crown diameter had a mean value of 6.00 meters and standard deviation value of 1.40 while basal area had a mean value of 0.0696 meters and a standard deviation value of 0.0397.

The results of class distribution of Madrid tree (*P. erinaceaus*) stands from 15 -25cm had the highest frequency distribution of 65 tree stands; this was followed by the class diameter of 25.10 – 35.00cm which had a frequency value of 55 tree stands. Class range of 35.10 – 45.00cm had a frequency value of 30tree stands while diameter class greater than 45.00cm had a frequency value of 10 tree stands as shown on Figure 2.

Table 1: Descriptive Statistics of Growth Variables of Madrid Tree Species (*P. erinaceaus*) in the Study Area

Variables	Mean	SE	S. Dev.	C. V %	Min.	Max.
DBH (cm)	28.65	0.67	8.10	28.3	15.80	49.20
Total Height (m)	13.87	0.07	0.83	6.0	12.00	15.40
CD (m)	6.00	0.12	1.40	23.3	3.05	10.00
B.A (m^2)	0.0696	0.0033	0.0397	57.0	0.0196	0.1901

DBH = Diameter at breast height (cm), CD = Crown diameter (m), BA = Basal area (m^2), VOL. =Volume (m^3). Mean interval = ± standard error.

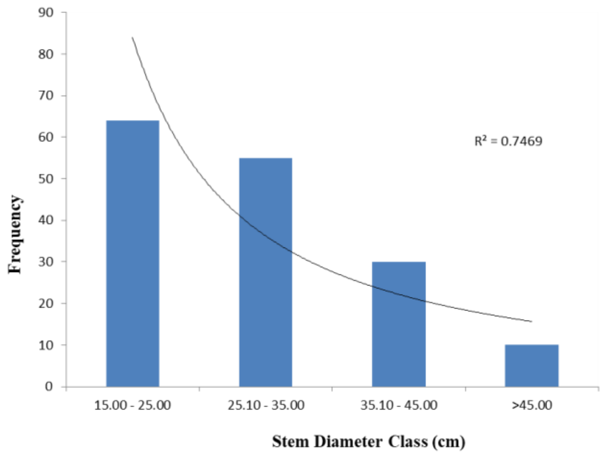

Figure 2: Dbh Class Distributions of Madrid Tree (*P. erinaceaus*) Stands in the Study Area

Crown diameter class distribution of Madrid tree species in the study area

The results of the crown diameter class distribution of Madrid tree species (*P. erinaceaus*) are shown in Figure 3. The Figure showed that the crown class distribution range of 4.2 - 6.00 m had the highest frequency value of 85; this was followed by the class range of 6.10 – 8.00 m with a frequency value of 30. A class range of greater than 8.00m had a frequency value of 25 while a class range of 3.00 – 4.1(m had a frequency value of 20 (Figure 3).

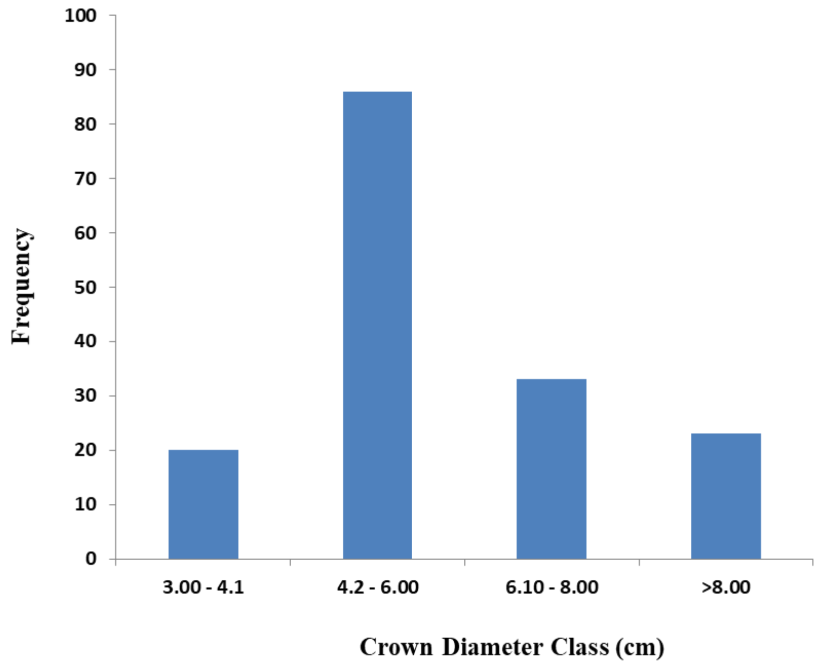

Figure 3: Crown Diameter Class Distribution of Madrid Tree Species (*P. erinaceaus*) in the Study Area

Summary of Regression Parameters for the Madrid Tree (P. erinaceaus) Stands

The results for the summary of regression parameters for the Madrid tree species are shown in Table 2. The Table showed a summary of regression analysis for the various model types and model coefficients. Models 1, 3, and 4 have the highest R2 value of 81 respectively while model 7 has the lowest R2 value of 75. Model 5 has the highest RMSE value of 0.723 while model 6 has the lowest RMSE value of 0.101 hence model 4 has the best fit (Table 2). The result for the Correlation between the Observed and Predicted Crown Diameter of Madrid Tree Species (*P. erinaceaus*) is shown in Figure 4, and the results for the predicted crown diameter and Residual Plot of Madrid Tree Species (*P. erinaceaus*) is shown in Figure 5.

Table 2: Summary of Regression Parameters for the Madrid Tree Species (*P. erinaceaus*) in Okpoga, Nigeria

Model No.	Model type and model coefficients	R (%)	R^2 (%)	Adj. R^2 (%)	RMSE	F-ratio (p<0.05)	Rank Total
[1]	Cd = 1.558+ 0.155 dbh	80	81(1)	81	0.616(4)	599.438*	5
[2]	Cd = 3.809+ 0.002dbh ^{2}	89	80 (4)	80	0.632(6)	560.873*	10
[3]	Cd = 1.973+0.126 dbh + 0.001 dbh^2	81	81(1)	81	0.617(5)	298.792*	6
[4]	Cd = 4.683+0.201dbh - 1.340lndbh	90	81(1)	81	0.615(3)	301.042*	4
[5]	Cd = -8.502 + 4.373 lndbh	88	78(6)	76	0.723(7)	495.946*	13
[6]	LnCd = -0.615+ 0.718lndbh	89	80(4)	80	0.101(1)	571.215*	5
[7]	LnCd = 0.340+ -0.007 Lndbh +0.143Lndbh ^{2}	87	76(7)	75	0.116(2)	404.58 *	9

dbh =Diameter at breast height(cm); =Coefficient of Determination; RMSE= residual mean square error; * = significant at 0.05.

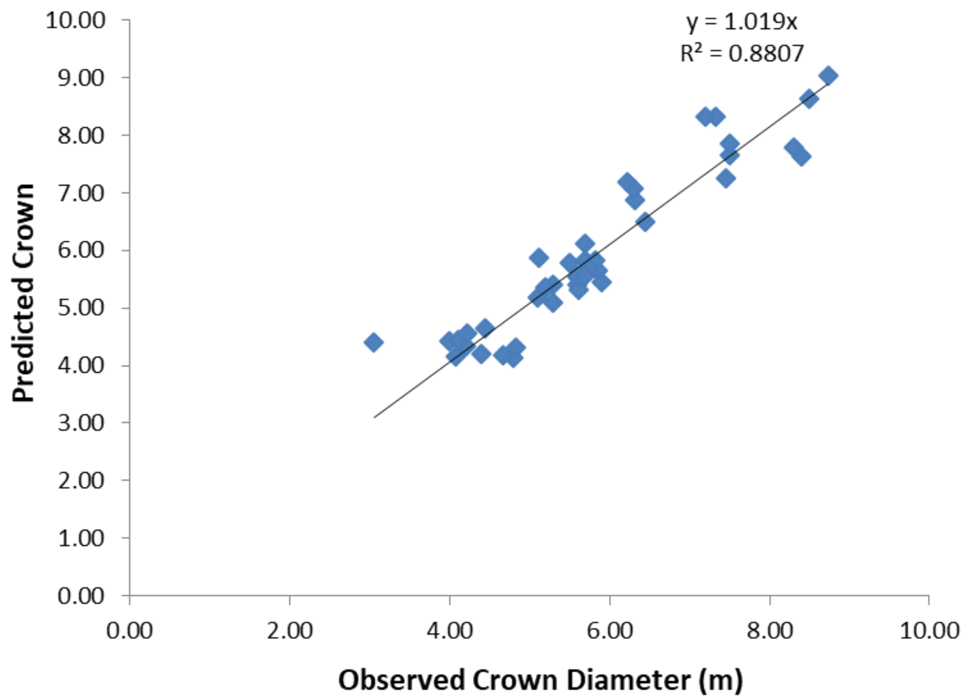

Figure 4: Correlation between Observed and Predicted Crown Diameter of Madrid Tree Species (P. erinaceaus) in Okpoga, Nigeria

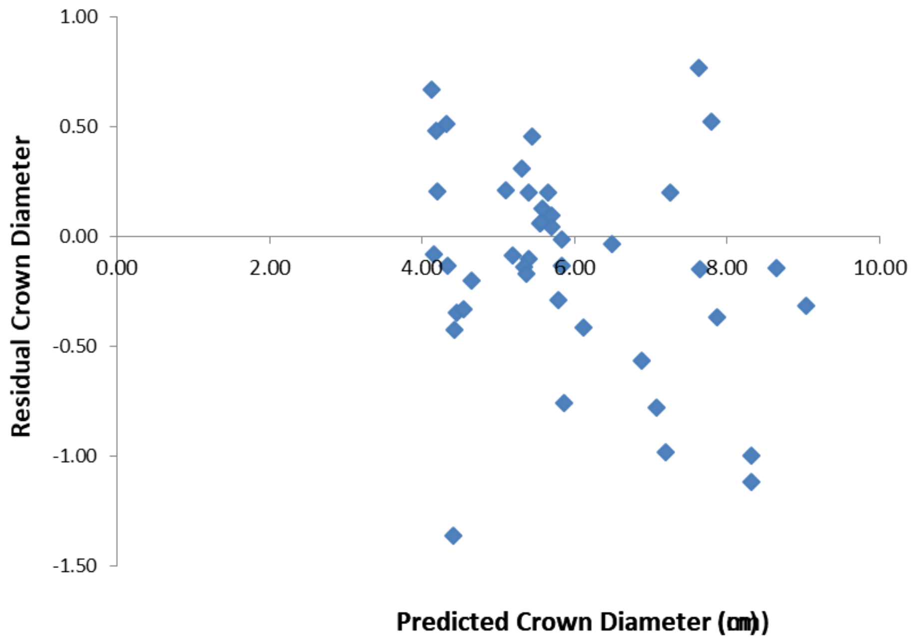

Figure 5: Residual Plot of Madrid Tree Species (*P. erinaceaus*) in Okpoga, Nigeria Models validation and assessment

The results for models validation and assessment is shown in table 4.3 the table showed a mean value of 5.78 for observed, 5.90 for predicted and -0.12 for residual. A minimum confidence interval value of 5.38 was recorded for observed, 5.49 were recorded for predicted and -0.26 was recorded for residual. A maximum confidence interval value of 6.17 was recorded for observed, 6.31 was recorded for predicted and 0.02 was recorded for residual (Table 3).

Predicted Crown Diameter (Cd), Growth Space (S), Stocking (N) and Stand Density (D) for Madrid Tree Species (P. erinaceaus) in the Study Area

The result on predicted crown diameter, growth space, stocking and stand density for Madrid tree species in the study area is shown in table 4. The value for Dbh (m) ranged between 0.158-0.492 while that of Cd (m) ranged between 4.20 - 8.80 Cd/dbh values ranged between 27.5823- 17.8862, Natural space (ha)value ranged between 0.0004 to 0.0019, Stock/ha-1 (1/S)values ranged between 2267.5737 - 516.5289 and SD (m2ha-1) values ranged between 0.0196 to 0.1901. Crown diameter = 1.558+ 0.155dbh; Natural spacing was derived using Cd2 /40, 000, stocking was obtained using 1/ natural space; while Stand Density (SD) = π D2/ 40,000.

Table 3: Models Validation and Assessment (Simple Linear model Cd = 1.558+ 0.155dbh)

Parameters	Observed(m)	Predicted(m)	Residual	% bias
Mean	5.78	5.90	-0.12	-2.59
Minimum Confidence Interval	5.38	5.49	-0.26	-5.49
Maximum Confidence Interval	6.17	6.31	0.02	0.32

Table 4: Predicted Crown Diameter (Cd), Growth Space (S), Stocking (N) and Stand Density (D) for Madrid Tree Species (*Pterocarpus erinaceaus*) in the Study Area

Dbh (m)	Cd (m)	Cd/dbh	Natural space (ha)	Stock/ha^{-1} (1/S)	SD (m^{2}ha^{-1})
0.158	4.20	26.5823	0.0004	2267.5737	0.0196
0.236	5.15	21.822	0.0007	1508.1535	0.0437
0.248	5.25	21.1694	0.0007	1451.2472	0.0483
0.251	5.30	21.1155	0.0007	1423.9943	0.0495
0.257	5.60	21.7899	0.0008	1275.5102	0.0519
0.286	6.10	21.3287	0.0009	1074.9798	0.0642
0.299	6.20	20.7358	0.0010	1040.5827	0.0702
0.314	6.52	20.7643	0.0011	940.9462	0.0774
0.343	6.71	19.5627	0.0011	888.4131	0.0924
0.368	6.85	18.6141	0.0012	852.4695	0.1064
0.396	6.98	17.6263	0.0012	821.0113	0.1232
0.392	6.92	17.6531	0.0012	835.3102	0.1207
0.402	7.20	17.9104	0.0013	771.6049	0.1269
0.404	7.21	17.8465	0.0013	769.4660	0.1282
0.412	7.30	17.7184	0.0013	750.6099	0.1333
0.413	7.35	17.7966	0.0014	740.4322	0.1340
0.457	8.45	18.4902	0.0018	560.2045	0.1640
0.483	8.59	17.7847	0.0018	542.0928	0.1832
0.492	8.80	17.8862	0.0019	516.5289	0.1901

The above table was derived using simple linear model Madrid Tree Species (*P. erinaceaus*): Cd = 1.558+ 0.155dbh; Natural space = Cd^2 π /40 000 and stocking = 1/ natural space; Stand Density (SD) = π D^2/ 40,000.

DISCUSSION

The results of this study highlight the importance of developing a model for the natural spacing of *P. erinaceaus* although the study did not consider the population of the plant species in the study area as distinct groups based on morphological characteristics despite the fact that Koura et al. (2013) showed that generally, morphological data analysis of plant species leads to the identification and determination of diverse groups in order to precise their constitution.

The results for growth variables for the plant species indicated to a large extent that P. erinaceaus has an optimal growth rate in the study area. The distribution of the plant species to a large extent was not sparse and by all indication, the plant is thriving in the study area although studies have shown that trees in protected area show better performance than those of the exploited areas of which the study is one. Furthermore, in the non-protected zones, in addition to exploitation, agriculture is one of the main activities leading to the regular clearing of lands for crop production, reducing available resources and consequently, the landscape become composed of forest fragments (Segla et al. 2016).

In all the data sets from the trees (Figures 4.1 and 4.2), there was more concentration of stem diameter at the upper diameter class (41.00 – 50.1cm) than in the lower diameter class distribution (15.00 – 40.00 cm); this may be as a result of the exploitation of the trees for socio-economic purposes by the people of Okpoga. It could also be due to poor regeneration on the part of the species. In this case, germination trials should be done in the nursery to improve the tree population. Constant farming activities in the area could also affect the regeneration of saplings; mostly saplings are always cleared alongside bushes for farming purposes. This results in the decimation of the population of younger ones.

The result of the crown diameter class can be used as an important visual indicator of tree and forest trends (healthy or unhealthy) in the study area. The tree's crown is a major part of the tree that can trap light for food production; trees with full and healthy crowns are generally associated with higher growth rates as a result of an increased rate of photosynthesis. When crowns become unhealthy, the rate of photosynthesis is reduced. These results described the current status and condition of the trees in the area, i.e. the crown conditions in the study area were healthy and free from high competition. This may be as a result of the low population (declining in population) of the tree species (open-grown trees) or the soil condition of the area. The result is in agreement with that of Dau and Chenge, (2016) who stated that crown degradation is typically the result of past and present stressors such as insects, diseases, weather events, drought, senescence, and competition or other stand conditions and when severe enough, may result in tree mortality.

Growth space requirements was determined based on the findings by Foli et al, (2003) and Dau and Chenge (2016), who stated that growth space was associated with crown size. Therefore, using the calculated crown diameter (Cd), the crown area (A) for each tree was estimated and expressed in hectare basis; to improve production, fast growth and quality of tree species in the study area, individual tree in a stand must have unrestricted continuous free-growing space; this requires knowledge of maximum occupancy [stock] of the sites with time. Thus, to control competition and ensure fast growth and high production/yields, this economic tree would require planting spacing of 4 x 4 meters in the study area; the results also provided a means of estimating the stocking per hectare (N ha-1) require for producing a complete canopy.

Seven models were tested and four criteria [Coefficient of determination (R2), Root mean square error (RMSE)/furnival index, Significance of regression (F-ratio) and Residual analysis] were used in this study. The essence is to select the best model for predicting DBH from CD. Table 4.2 showed that model 1, 3 and 4 had R2 values of 81 respectively indicating that they had the best fit as proposed by Dau et al.(2016) who stated that the higher the R2 values, the better the fit. All the values obtained in this study for F-ratio were significant at $P<0.05$. In interpreting the results, it must be pointed out that the model 4 has the best fit as the fewer the parameters, the better the fit. Model 4 (linear model) emerged the overall best and was used to predict crown diameter and stocking of P. erinaceaus in Okpoga.

CONCLUSION

Crown-bole diameter relationship helps to predict growth space requirements for optimum planting and estimation of stand density/stocking for establishing plantations and sustainable management of economic tree species. This study has shown that P. erinaceaus would require low densities for optimum planting, fast growth and high yield for the purpose of timber and high densities for non-timber forest products in the study area; because low densities are required to produce maximum diameter growth throughout the life cycle of trees stand which is applicable to the studied tree species as showed by the results obtained from the area. To maximize the use of land for timber and non-timber forest products purposes, the tree species would require growth planting spacing of 4 x 4 m (for fencing pole and electric-pole). For maximum volume of timber, thinning should be administer at canopy closure; this is to create more spacing for continue growing until the trees only react minimal to thinning. The recommended planting spacing would enhance optimum planting, fast growth, high yield/production and control competition within each tree species.

Restoration programs should be carried out within and outside the study area to ensure that the tree species does not reduce in the study area and eventually go into extinction as this study shown that, upper diameter class distribution were being over exploited, without replanting or regeneration in the area. Therefore, the study recommended model 4 for crown diameter prediction among *P. erinaceus* in the study area, the models should be used outside the extent of the original data (dbh 10.00cm - 49.20cm) with caution; as extrapolating to values outside this range may yield misleading results.

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Review Article

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Potentiality of RNA Interference Technology in Enhancing the Nutritional Status and Food Value of Plant Species

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ABSTRACT

Biofortification or enhancement of nutrient levels in plants via genetic engineering aims at alleviating hidden hunger and eradicating micronutrient deficiency-related malnutrition. A number of approaches have been attempted by plant biotechnologists for the same, of which manipulation of alien genes from diverse sources and their consequent integration within the genome of crop plants for expression of desired traits is quite a popular one. RNA interference (RNAi)-mediated gene silencing is an approach that has gained attention and special importance in the last two decades, particularly because of its ability to suppress genes, whose products lead to the formation of various anti-nutrients, allergens and toxins, with appreciable specificity. RNA-induced silencing or RNAi suppresses specific mRNAs in the host plants which leads to inhibition of specific enzymes that regulate many biosynthetic pathways. RNAi uses non-coding siRNAs (small interfering RNA) or miRNAs (micro RNA) to silence the target genes. These non-coding small RNAs have a sequence complementarity to the mRNA to be silenced. The targeted genes generally correspond to the key rate-limiting enzymes in multiple steps of the numerous interconnected pathways of the plant metabolome and hence are carefully chosen for avoiding off-target effects. The major implication of this technology to date has been on staple food crops like rice, wheat and maize, for the enrichment of micronutrients like iron and essential amino acids; however, recent experiments have also targeted plants like coffee, tea and oilseed crops, which equally form inseparable parts of daily food items, worldwide. This review elaborates several such existing examples, highlighting the genes, targeted by RNAi mechanisms, for nutrient and antioxidant enrichment, explores their practical use in daily life and briefly ponders upon the facets of possible initiatives to bring such genetically improved plants to the doorsteps of the masses.

INTRODUCTION

RNA interference (RNAi) is the method of blocking the function of any gene by the insertion of short ribonucleic acid (RNA) sequences that match part of the sequence of the target gene, preventing the production of the protein, encoded by that gene. The technique was discovered by Andrew Fire and Craig Mello in 1998, when they injected double-stranded RNA (dsRNA) into the worm Caenorhabditis elegans, triggering the silencing of genes that had identical sequences as that of the dsRNA. They were subsequently awarded the Nobel Prize for Physiology and Medicine in 2006 for this discovery (Wilson and Doudna, 2013). Cells recognize such dsRNAs as undesirable since they are not formed by the normal genetic mechanisms, and hence such RNAs are cleaved by the cytosolic ribonuclease, Dicer into 21-23 nucleotide-long microRNAs (miRNAs) and small interfering RNAs (siRNAs) with overhanging ends. The siRNA or the miRNA then associates with a pre-RNA-induced silencing complex (pre-RISC), containing an Argonaute protein that distinguishes between the two miRNA or siRNA strands as either sense or antisense, so that the sense strand with the identical sequence as the target gene (passenger strand) can get cleaved, while the antisense strand (guide RNA) gets incorporated to the RISC (Ender and Meister, 2010). The guide RNA-RISC complex then binds to the target complementary RNA sequence, cleaves it at a specific site, using the Argonaute protein, and subsequently degrades it, preventing it from getting translated into a protein. The miRNAs, however, differ from siRNAs in the initial step of their formation, since miRNAs are derived from single-stranded precursor sequences that possess complementary sequences, allowing them to fold back on themselves to form a stem-loop structure (pri-miRNA) at one end, which is cleaved by a nuclear endonuclease complex, Drosha to generate a pre-miRNA with a 3' overhang, which, in turn, is exported to the cytoplasm for Dicer to act on (Wilson and Doudna, 2013). In plants, the success of RNAi-mediated silencing depends on a number of factors, primarily on the architecture of the RNAi constructs. They usually have a spacer or an intron sequence between an inverted repeat, resulting in stem-loop structures like the miRNAs, which are here called hairpin RNAs (hpRNAs); these RNAi constructs can, in turn, be driven by either a strong constitutive promoter, such as the CaMV35S (dicots) or the maize ubiquitin1 (monocots) or tissue-specific promoters. The method of transformation is another crucial factor determining the efficiency of the technique. Although Agrobacterium-mediated transformation is the usual method of choice, direct introduction of RNAi vectors via particle bombardment and electroporation has also been employed (Saurabh et al., 2014).

Hidden hunger, referring to overall poor quality of nutrition, not only causes a lack of nourishment or malnutrition, but also has collateral effects in impairment of the mental and physical development of children and adolescents, resulting in lower IQ, stunting, blindness, gradual deterioration of immune function and curbing of productivity with a consequent reduction in work capacity. The ill-effects of hidden hunger especially affect the socio-economically challenged population of the world with limited awareness and education of the various aspects of a truly nutritious diet, relying mostly on the meager staple diet for sustenance and satisfaction of hunger (Roychoudhury, 2020). As such, many techniques, ranging from pharmaceutical supplementation and industrial fortification to dietary supplementation have been attempted to alleviate human micronutrient deficiency. However, the sheer expense and inaccessibility of such sophisticated supplements serve little to the impoverished and underprivileged. Therefore, the unanimous solution to the problem has been the enhancement of the bioavailable micronutrients in food crops through the strategy called ‘biofortification’, precisely defined as “fortification in the field rather than in the factory”. Biofortification can be carried out by several techniques, such as agronomic biofortification, improvement of plant varieties via conventional breeding, and genetic engineering (Sharma et al., 2017).

Among the methods of genetic engineering, overexpression of a particular micronutrient by manipulating genes in the metabolic pathways, involved in its biosynthesis and catabolism, is a primary and major approach. A complementary approach involves the reduction or silencing of the genes involved in the synthesis of ‘anti-nutrients, allergens, etc. which inhibit the absorption of nutrients or render toxicity to food crops. This second approach is exemplified in plants, mostly by the use of genetic engineering techniques such as RNAi and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9-mediated gene silencing (Ali et al., 2010). This review focuses on some of the success stories in implementing RNAi technology in improving the levels of different nutrients and ensuring food safety in different plant species.

Iron biofortification in rice

The average daily allowance of iron for healthy males is 8 mg between 19 and 50 years of age, and 18 mg of iron for healthy females belonging to the same age group. The actual average daily intake of iron from food sources, however, is not adequate and often leads to varying stages of iron deficiency, especially in pregnant and pre-menopausal women. Characterized by World Health Organization (WHO) as one of the leading risk factors for worldwide disease, iron deficiency not only leads to anemia (Iron Deficiency Anaemia or IDA), but also disturbances of the gastrointestinal system, fatigue, weakness and impairment of normal immune function, and in its most severe stage, even to compromised neurocognition. The need for iron biofortification is thus obvious and evident, and rice being one of the major staple cereals in South East Asia, Latin America and some parts of Africa, is a suitable target crop for the same. The importance of biofortification specific to milled rice is further highlighted by the fact that post-harvest processing in brown rice has been found to reduce the iron content by around 4.8 times, which further decreases another 2.0-fold in the polished consumable grains. There are several approaches of iron biofortification in rice, such as endosperm-specific expression of the iron storage protein ferritin, overexpression of the nicotianamine synthase gene, NAS1, overexpression of iron transporters like IRT1, which target iron storage, chelation and transport at various levels in rice (Majumder et al., 2019).

RNAi technology has been used in the iron biofortification of rice by lowering the phytic acid content. Phytic acid is an antinutrient, since it prevents iron utilization and solubilization in plants due to its strong cation-chelating ability. Abbreviated as IP6 due to its chemical name myo-inositol-1, 2, 3, 4, 5, 6-hexakisphosphate, phytic acid accumulates in the aleurone layer of cereals, except for maize, as mixed salts called phytate. Phytate is a strong chelator of various cations like Fe^{2+}, Ca^{2+}, Mg^{2+} and Zn^{2+} due to the six negatively charged phosphate groups. This reduces the bioavailability of these micronutrients in the plant. Various enzymatic steps have been targeted in the phytic acid biosynthetic pathway to reduce its concentration in rice. The first enzyme in the biosynthetic pathway of phytic acid is myo-inositol-3-phosphate synthase (MIPS), the corresponding gene for which has been targeted for silencing. Though silencing of MIPS gene decreased the phytic acid levels indirectly by reducing the synthesis of myo-inositol, it exerted adverse effects on plants by making them susceptible to abiotic stress, since myo-inositol is an important osmolyte. Therefore, the RNAi approach was used to silence a different gene encoding the last enzyme, inositol-1,3,4,5,6-pentakisphosphate 2-kinase 1 (IPK1) in the phytic acid biosynthetic pathway. IPK1 gene was silenced using a seed-specific oleosin18 (Ole18) promoter, and the transgenic rice showed a 3.9-fold down-regulation in IPK1 transcripts in transgenic seeds, correlating to a 1.8-fold greater iron accumulation in the endosperm, without affecting the normal growth and development of the plant (Ali et al., 2013).

Manipulating the level of glutelin in rice and gliadin in wheat

Glutelin is a major seed-storage protein in rice, making up 60% of the total endosperm protein content. It is encoded by a multigene family, containing two highly similar subfamilies, GluA and GluB. It is synthesized as a 57 kDa precursor which is cleaved to 22-23 kDa basic subunit and 37-39 kDa acidic subunit. A dominant mutation, low glutelin content 1 (Lgc1) reduces glutelin content in rice grains. In Lgc1 homozygous lines, a tail-to-tail inverted repeat is formed by a 3.5 Kbp deletion between the two highly similar glutelin genes. This results in a dsRNA which can potentially induce gene silencing. Transgenic analysis of the lgc1 candidate region using reporter gene assay detected small interfering RNAs, supporting the hypothesis that lgc1 can suppress glutelin expression via RNAi. In the lgc 1 mutant line, a reduction of glutelin content was seen along with an increase in the levels of other seed storage proteins like prolamin. Such induction of prolamin synthesis was not found to be specific for LGC 1, but was simply a compensation for glutelin reduction. Prolamins accumulated in the protein body I, whereas glutelin accumulated in the protein body II. Since protein body I is barely digested in humans, the presence of LGC 1 can help in producing low-protein rice cultivar (Kusaba et al., 2003). This is highly beneficial for kidney disease patients, who are prescribed a restricted protein intake and cultivars harboring LGC 1 are now being used in low-protein diet therapy.

In bread wheat cultivar ‘Bobwhite’ lines, RNAi approach was used to down-regulate gamma-gliadin which caused an increase in glutenin content, along with a slight increase in the total protein content (Gil-Humanes et al., 2012). Reduced gliadin wheat is an alternative for consumers suffering from celiac disease. Moreover, reduced gliadin flour has high nutritional property, since it has increased lysine content.

Lysine biofortification in rice and maize

Lysine is an essential amino acid (EAA) that serves as an important source of energy and nutrition for humans and livestock. However, cereals such as rice and maize are deficient in lysine, which, being the limiting EAA, also restricts the absorption and utilization of other amino acids, leading to diseases like kwashiorkor or marasmus in cases of severe lysine deficiency and resultant protein deficiency. Thus, the biofortification of rice with lysine is a major interest of plant breeders. Of the several enzymes that have been targeted in the biosynthetic pathway of lysine, aspartate kinase (AK) and dihydrodipicolinate synthase (DHDPS) are particularly noteworthy, since mutant forms of these enzymes which are insensitive to lysine feedback inhibition have shown to increase lysine levels considerably. In addition, another key enzyme playing a major regulatory role in lysine catabolism is lysine ketoglutaratereductase/saccharopine dehydrogenase (LKR/SDH), which, if down-regulated or inhibited, has been shown to stimulate accumulation of lysine, preventing its breakdown. Further, overexpression of maize feedback-insensitive DHDPS coupled with an LKR/SDH RNAi construct in rice showed a 60-fold increase in free lysine level in matured seeds of transgenic rice, as compared to the wild-type seeds (Lee et al., 2001; Frizzi et al., 2008). In this regard, three constructs, 35S, Ri and 35R were respectively designed to (i) allow constitutive expression of the bacterial lysine feedback-insensitive AK and DHDPS genes, both driven by the CaMV35S promoter, (ii) inhibit expression of the LKR/SDH gene by LKR-RNAi construct under the endosperm-specific rice glutelin (Gt1) promoter, and (iii) express the combined transgenes of constructs for functions (i) and (ii) (Yang et al., 2021).

In maize, on the other hand, opaque 2 (o2) is a mutant with an opaque and floury kernel where the basic leucine zipper transcription factor O2, which regulates endosperm-specific genes like α-zein (22 kDa) and LKR/SDH, is mutated. The resultant reduced levels of α-zein were however compensated by other non-zein lysine-rich proteins, thus increasing the net lysine content. RNAi-mediated silencing of lysine-poor α-zeins has been shown to mimic the opaque kernel phenotype of the o2 mutant, along with high lysine content, pointing to the fact that decrease in α-zein is directly correlated with higher lysine content, thereby enhancing its nutritional quality (Houmard et al., 2007).

Increasing amylose content in wheat and sweet potato

Wheat is a major staple crop that forms an important source of carbohydrates, proteins, fats, vitamins and minerals, contributing to a healthy human diet, with starch being the major component of the wheat kernel. Starch is composed of amylopectin (70-80% of dry weight), a highly branched polysaccharide, together with amylose (20-30% of dry weight), a linear chain of D-glucose molecules with few branches, for 70-80% and 20-30% of the starch dry weight, respectively. While amylopectin is easily digested by humans and other mammals, amylose tends to form complexes that are resistant to digestion, mimicking the dietary fiber. When cooked food cools down, amylose molecules re-associate rapidly, resisting digestion, whereas amylopectin molecules re-associate slowly and get more readily digested. Therefore, starch with higher amylose content is called resistant starch (RS), and increased consumption of RS is shown to be associated with several health benefits, such as the lower risk of type II diabetes, obesity, cardiac diseases, and colorectal cancers. A reduction in starch digestion in the small intestine also leads to a decrease in the rate of entry of glucose into the bloodstream, which in turn lowers the insulin demand and the glycemic index of the food items consumed. Besides, the undigested starch moves to the large bowel from the small intestine, underlining their fecal bulking and laxative action, and is finally converted to short-chain fatty acids by fermentation, which is shown to be associated with increased satiety (Williams, 1995). Therefore, engineering wheat to have a higher percentage of inefficiently-digested amylose is of special interest, given its enhanced nutritional and health-promoting roles. RNAi-mediated gene silencing has been used to suppress the expression of two different isoforms of starch-branching enzyme of class II, viz., SBEIIa and SBEIIb in the durum wheat endosperm, to increase the amylose content by decreasing amylopectin biosynthesis. Hairpin-RNA (hpRNA) molecules, corresponding to the sequences of SBEIIa and SBEIIb were expressed in inverted repeats in wheat, under the endosperm-specific glutenin (high-molecular-weight Dx5 subunit gene) promoter, which resulted in more than 70% increase in amylose content. Rats fed with this high amylose wheat grain as a whole meal showed improved bowel function in comparison to standard wholemeal wheat, further emphasizing its beneficial role in human health due to its enhanced resistant starch content (Regina et al., 2006; Sestili et al., 2010). Similar RNAi techniques were applied in the case of sweet potato (Ipomoea batatas) which yielded higher amylose content. The storage roots of sweet potato (Ipomoea batatas) contain starch which is made up of unbranched linear amylose (10-20%) and branched amylopectin, constituting the rest. The starch branching enzymes which are responsible for producing branched amylopectin are of two classes – class A (potato SBEII) and class B (potato SBEI). A dsRNA interference vector was constructed based on the cDNA of sweet potato SBEII (IbSBEII) and introduced in the sweet potato genome via Agrobacterium-mediated plant transformation (Shimada et al., 2006).

Lowering the level of sucrose phosphate in potato

The two enzymatic steps of sucrose biosynthesis involve the catalysis of the synthesis of sucrose-6-phosphate (Suc6P) by sucrose-phosphate synthase (SPS), followed by hydrolysis of Suc6P by sucrose phosphatase (SPP) to yield sucrose and inorganic phosphate (Pi). Accumulation of glucose and fructose due to sucrose hydrolysis occurs by a process called hexogenesis when potato tubers are stored at 4°C. This process is called 'cold sweetening', which causes the browning of the potato, imparts a bitter taste to it, thereby deteriorating the nutritional and commercial value. During potato chip and French fry productions, the reducing sugars in potato react with free amino acids in a non-enzymatic Maillard reaction to produce brown- to the black-pigmented product along with carcinogenic acrylamide that is not acceptable to the consumers. Reducing sugars and asparagine are the two major substrates for acrylamide formation in processed potato products. CaMV35S promoter-driven hairpin RNAi constructs, containing part of the coding region of the tobacco NtSPP2 gene were used by Chen et al. (2008) to reduce SPP expression in transgenic potato tubers. Suc6P was reported to be accumulated in RNAi mediated-SPP-silenced potato tubers when stored at 4°C, along with decreased sucrose accumulation. It was revealed that cold-induced expression of vacuolar invertase (VINV) was blocked in SPP-silenced tubers. This further explained the presence of a reduced sucrose-to-hexose conversion. Further silencing of the VINV gene through RNAi showed dramatically reduced acid invertase activity and no reducing sugar was accumulated during cold storage of potato.

Improving starch accumulation in Arabidopsis and maize

Starch phosphorylation and dephosphorylation are the important events regulating starch accumulation. Glucan water dikinase (GWD) adds phosphate to starch, while phosphoglucan phosphatase (SEX4) removes these phosphates. RNAi constructs were introduced in Arabidopsis thaliana to reduce the expression of AtGWD and AtSEX4. The starch build-up was manipulated with ethanol-inducible and senescence-induced gene promoters. Ethanol induction of RNAi lines lessened transcript accumulation for AtGWD and AtSEX4 by 50%. At the end of the dark period, the transgenic lines exhibited seven times more starch, but they exhibited similar growth rates and total biomass, as the wild-type plants. Likewise, RNAi construct against AtGWD-homologous gene from maize under the constitutive ubiquitin promoter elevated starch content in maize with no impact on total plant biomass (Weise et al., 2012).

Increasing grain yield

RNAi-mediated suppression of GA20 oxidase (controlling gibberellin biosynthesis) gene resulted in semi-dwarf plants from taller rice variety, QX1. The transgenics showed an increase in panicle length, increased number of seeds per panicle and higher grain weight (Qin et al., 2013). OsSPL14 (Squamosa promoter binding protein like14) was reported to be regulated by microRNA (miRNA), OsmiR156 in rice (Jiao et al., 2010). A point mutation in OsSPL14 perturbs OsmiR156-directed regulation of OsSPL14, generating an 'ideal' rice plant with reduced tiller number, increased lodging resistance and enhanced grain yield.

Increasing shelf life of tomato

1-Aminocyclopropane-1-carboxylate (ACC) oxidase catalyzes the oxidation of ACC to ethylene, a phytohormone that plays an important role in the ripening process of tomato fruit. A double-stranded (ds) RNA expression unit of tomato ACC oxidase was introduced into tomato cultivar Hezuo 906. The sense and antisense configurations of DNA fragments were linked with 1,002 bp or 7 nt artificially-synthesized fragments, respectively, and placed under the control of a modified CaMV35S promoter. With respect to the construct with the 1,002 bp linker, the severity of the phenotypes indicated that 72.3% of the transformed plants had non-RNA interference, about 18.1% had semi-RNA interference, and only 9.6% had full-RNA interference. However, when the construct with the 7 nt linker was used for transformation, the results were 13.0%, 18.0%, and 69.0%, respectively, indicating that the short linker was more efficient in RNAi of transgenic tomato plants. Transgenic tomato plants were produced where ACC oxidase gene was shut down, producing fruits that released traces of ethylene and had a prolonged shelf life of more than 120 days (Xiong et al., 2004).

Enhancing flavonoid and carotenoid levels

The downregulation of carotenoid cleavage dioxygenases (CCD) can increase the overall carotenoid content in rice seeds and leaves by preventing the cleavage or degradation of carotenoids, the precursor for Vitamin A. It was found that CCD4a-suppressed variants of rice showed a greater accumulation of carotenoids in seed endosperm and leaves. Among the three CCD genes in rice, the CCD1 expression is generally higher in leaves, CCD4a is expressed in young seedlings and to some extent in seeds (lesser expression in leaves, as compared to CCD1), and CCD4b expression is generally up-regulated in seeds during later stages. Three different RNAi vector constructs were made, using cDNA sequences for each of the CCD genes, including untranslated regions. OsCCD4a suppression lines showed the maximum overall increase in carotenoid levels (Ko et al., 2018).

RNAi approach was used to enhance the carotenoid and the flavonoid content of tomato (Lycopersicon esculentum), both of which are highly beneficial as antioxidants for human health. RNAi was used to suppress Tomato DE-ETIOLATED 1 (TDET1, an endogenous photomorphogenetic regulatory gene) with the help of DET-1 derived inverted repeat constructs which were driven by three different fruit-specific promoters. The transgenic tomatoes showed specific degradation of TDET1 transcripts, along with an increase in carotenoid and flavonoid content (Davuluri et al., 2005). Similarly, RNAi has been used to down-regulate the expression of the lycopene epsilon cyclase (ε-CYC) gene to enhance the carotenoid content of rapeseed (Brassica napus). The transgenic Brassica seeds showed increased levels of β-carotene, zeaxanthin, violaxanthin and lutein (Yu et al., 2007).The β-carotene hydroxylase (BCH) enzyme converts β-carotene to zeaxanthin in potato (Solanum tuberosum). Silencing of BCH gene through RNAi using GBSS (granule bound starch synthase) promoter led to increase in two health-promoting carotenoids, β-carotene and lutein, in potato (Eck et al., 2007). The SlNCED 1 gene encodes for 9-cis-epoxycarotenoid dioxygenase (NCED) which is the key enzyme in abscisic acid (ABA) biosynthesis in tomato fruit. This gene was silenced via RNAi under fruit-specific E8 promoter that led to lowering in ABA level due to a significant reduction in the NCED activity. Thus, carbon that is normally channelized to free ABA metabolic pathway during ripening is partially blocked or 'backlogged', and on the contrary, is diverted to the carotenoid pathway in the RNAi lines, causing an increase in the assimilation and accumulation of lycopene and β-carotene (Sun et al., 2012).

Increasing the levels of ascorbic acid in tomato

Ascorbic acid/Vitamin C helps in the enhancement of iron absorption, boosting immunity, and decreasing blood cholesterol among the various functions. The deficiency of vitamin C is known to cause scurvy. The primary enzymes for the oxidation of ascorbate in plants are ascorbate peroxidase (APX) and ascorbate oxidase (AO), which oxidize ascorbate to monodehydroascorbate. The levels of ascorbate can be increased in fruits by suppressing their oxidation. RNA-interference vector was constructed using a 597 bp fragment from the 5' end of mitochondrial SlAPX gene of tomato, cloned into the vector, which was then transformed into Agrobacterium tumefaciens through electroporation. The transgenic plants thus produced showed a 1.4-2.2-fold increase in the ascorbic acid levels in tomato, as compared to the wild-type control (Zhang et al., 2011).

Manipulating the levels of fatty acids in cotton seed, soybean and flax

Cotton (Gossypium hirsutum) is one of the most important economic crops for the production of fiber, but it is also the sixth-largest source of vegetable oil in the world. The composition of cotton seed oil is such that it has a relatively high level of palmitic acid (C16:0), which is a saturated fatty acid, stabilizing the oil and making it suitable for frying at high temperature. Moreover, hydrogenation of cotton seed oil to produce solid margarine hard stock also leads to the formation of trans-fatty acids. Both saturated fatty acids and trans-fatty acids have been shown to possess equivalent properties of raising the undesirable low-density lipoprotein (LDL) cholesterol and lowering the desirable high-density lipoprotein (HDL) cholesterol.

Due to growing awareness about the ill effects of LDL and its correlation with coronary heart diseases, there is an increasing trend in the use of favoring oils, rich in unsaturated fatty acids such as oleic acid (C18:1), and low in palmitic acid, but rich in stearic acid (C18:0). Such oils are both nutritionally beneficial and provide the required function without the need for hydrogenation. The major enzymes controlling the synthesis and catabolism of such desired fatty acids are desaturases, especially stearoyl-acyl-carrier protein (ACP) Δ9-desaturase (encoded by ghSAD-1 in cotton seed), which converts stearic acid to oleic acid, and microsomal ω6-desaturase or Δ12-desaturase (encoded by ghFAD2-1 in cotton seed), which converts oleic acid to linoleic acid.

RNAi-mediated seed-specific silencing of ghSAD-1 led to the accumulation of stearic acid, resulting in high-stearic (HS) cotton seed oil, while silencing of ghFAD2-1 resulted in the accumulation of oleic acid, leading to high-oleic (HO) cotton seed oil. Cotton cv Coker 315 was transformed with constructs, containing seed-specific soybean lectin promoter, and ghSAD-1 or ghFAD2-1 cDNA clone in the inverted repeat to generate hpRNA-mediated gene silencing. It was observed that stearic acid increased substantially to 40%, compared to the normal levels of 2-3%, and oleic acid content soared to as high as 77% in contrast to 15% in non-transgenic seeds. These results were also concomitant with a significant reduction in palmitic acid levels to only 12% of the total fatty acids in double homozygous F2 plants that carried both the gene silencing constructs, corresponding to ghSAD-1 and ghFAD2-1, obtained on intercrossing of stable lines expressing the HS and HO traits (Liu et al. 2002; Segal et al. 2003).

Consumption of α-linolenic acid (18:3) was found to be unhealthy for human beings. Improvement of soybean oil flavor and stability required a reduction in its α-linolenic acid levels. The omega-3 fatty acid desaturase (FAD3) gene family comprising GmFAD3A, GmFAD3B and GmFAD3C produces the FAD3 enzyme which converts linoleic acid (18:2) to α-linolenic acid in the polyunsaturated fatty acid pathway. A 318-nucleotide long conserved sequence, common to all the three gene family members, was used as an inverted repeat in the RNAi expression cassette placed under a seed-specific glycinin promoter. The transgenic line was found to contain 1-3% of α-linolenic acid, as compared to 7-10% in non-transgenic soybean seed. This siRNA-mediated silencing of FAD3 was also seen to be stably inherited in transgenic soybean lines. Marked reduction in α-linolenic acid by this approach enhanced the agronomic value of the seed (Flores et al. 2008). Flax possesses two isoforms of FAD2 enzymes that convert monounsaturated oleic acid to polyunsaturated linoleic acid. RNAi approach was used to silence both the FAD2 genes simultaneously in flax that led to high levels of oleic acid, instead of linoleic acid and this trait remained stable across several generations (Dar et al., 2017).

Enhancing secondary metabolitesfornutraceutical and pharmaceutical applications

Squalene epoxidase enzymes (encoded by the genes pgSQE1 and pgSQE2) catalyze the rate-limiting step in the biosynthesis of phytosterol and triterpenoid saponin. RNA interference of PgSQE1 in transgenic Panax ginseng completely suppressed PgSQE1 transcription. Concomitantly, the interference of PgSQE1 resulted in the reduction of ginsenoside production. Interestingly, silencing of PgSQE1 in the roots of RNAi-engineered plants strongly up-regulated PgSQE2 and PNX (cycloartenol synthase) and resulted in enhanced phytosterol accumulation. These results indicated that the expression of PgSQE1 and PgSQE2 were regulated differently and that PgSQE1 regulates ginsenoside biosynthesis, but not that of phytosterols in P. ginseng (Han et al., 2010). Artemisinin is an anti-malarial drug obtained from Artemisia annua, but the content of artemisinin is low. Suppressing the expression of SQS (squalene synthase), the key enzyme of sterol pathway (a pathway competitive with that of artemisinin biosynthesis) using a hairpin-RNA-mediated RNAi technique was attempted, which showed desirable results in increasing artemisinin content in the transgenics (Zhang et al., 2009). In another work, DNA-encoded hairpin RNA-mediated suppression of the gene encoding morphinan pathway enzyme, salutaridinol 7-O-acetyltransferase (SalAT) in opium poppy resulted in the novel accumulation of the pharmacologically important alkaloid, salutaridine at up to 23% of total alkaloid; this alkaloid was not detectable in the parental genotype (Allen et al. 2008). Earlier, Allen et al. (2004) also reported high-yield accumulation of the non-narcotic alkaloid, reticuline, at the expense of morphine, codeine, oripavine and thebaine, by silencing of codeinone reductase (COR) in opium poppy, Papaver somniferum, using a chimeric hairpin RNA construct, designed to silence all members of the multigene COR family through RNAi. Since reticuline is quite an early precursor of codeinone, opium poppy was metabolically engineered by this approach to prevent entry into the morphine synthesis branch.

Lowering caffeine content in coffee and tea

Caffeine (1, 3, 7-trimethylxanthine) is a purine alkaloid found in tea and coffee plants. It is most commonly used as a stimulator of the central nervous system; however, studies indicated several adverse effects of caffeine as well, such as palpitations, gastrointestinal disturbances, anxiety, high blood pressure and insomnia. This led to more interest in recent times in the consumption of decaffeinated or 'decaf' beverages, particularly coffee (Ashihara and Crozier 2001). Transgenic coffee plants have been engineered, targeting several methyltransferases in the caffeine biosynthetic pathway. First, xanthosine is methylated by xanthosine methyltransferase (XMT), producing 7-methylxanthosine. Next, its ribose residue is removed and the resulting 7-methylxanthine is methylated by 7-N-methylxanthine methyltransferase (MXMT or theobromine synthase), producing 3,7-dimethylxanthine (theobromine) as the product. Theobromine in turn is methylated by 3, 7-dimethylxanthine methyl ransferase (DXMT or caffeine synthase) to caffeine (1, 3, 7-trimethylxanthine). Therefore, genes encoding MXMT and DXMT enzymes were used as targets for RNAi-mediated gene silencing. The cDNAs encoding the three enzymes were cloned and designated as CaXMT1, CaMXMT1 and CaDXMT1 and these were used to construct dsRNA which could lead to RNAi-mediated gene silencing in Coffea arabica and C. canephora under a constitutive CaMV35S promoter. The transformed tissues showed reduced transcript levels corresponding to all the three enzymes, concomitant with a reduction in the levels of theobromine and consequently, caffeine, with 50-85% lowering of theobromine for the non-transgenics, 100% decaffeination in embryonic tissues and 70% decaffeination in plantlets (Ogita et al., 2004).

Alleviation of the adverse effects of caffeine on human physiology is a promising impetus for not only generating transgenic coffee plants, but also transgenic tea, especially green tea, for its added health benefits. Suppression of caffeine by RNAi-mediated gene silencing by targeting the same three enzymes as mentioned above was undergone in tea, resulting in 44-61% reduction in caffeine and 46-67% decrease in theobromine contents, as compared to the controls (Mohanpuria et al. 2011).

Lowering the level of sinapate esters

Removal of sinapate ester can improve the canola oil seed value by decreasing the overall polyphenol content and increasing the nutritional value. Hüsken et al. (2005) used the RNAi method to down-regulate the sinapate ester synthase genes. The main biosynthetic gene in the sinapyl ester pathway is UDP-glucose: sinapate glucosyltransferase (BnSGT1). The dsRNA construct was made with BnSGT1 under the influence of seed-specific napin promoters. An overall 76% decrease in sinapate ester levels was noted in transgenic canola seeds. Removal of sinapate esters enhanced the flavor of canola seeds, along with an increase in the content of resveratrol that is known to reduce heart disease, arteriosclerosis and cancer. However, no other properties like fatty acid content and oil content were influenced by this approach. Moreover, the heritability of this trait ensured constant maintenance of nutritional and agronomic status.

Removal of toxic compounds in edible plants

Cassava is a major staple food crop in many tropical countries. However, it contains toxic cyanogenic glycosides within the tuber. Antisense down-regulation of genes encoding cytochrome P450 enzymes, CYP79D1 and CYP79D2, which catalyze the first step of the synthesis of linamarin and lotaustralin, generated transgenics with more than 90% reduction of cyanogenic glycosides in the tubers (Siritunga and Sayre, 2003). In another work, RNAi technology was successfully used to down-regulate the expression of Mald1, which leads to the production of a prominent allergen in apples. Transgenic apple harboring intron-spliced hpRNA construct showed an approximately10-fold reduction in Mald1 expression in the leaf. This interference event also suppressed the synthesis of sorbitol that affects the quality of fruit via starch accumulation and acid-sugar content (Teo et al., 2006). RNAi approach was also used to simultaneously silence two asparagine synthase genes (StAs1 and StAs2) to generate potatoes with reduced asparagine, one of the main precursors for the neural toxin, acrylamide (Zhu et al., 2016). Subsequent genetic manipulation led to the creation of transgenic lines with reduced levels of toxic steroidal glycoalkaloids by silencing the gene encoding the enzyme, sterol side chain reductase 2. People consuming Lathyrus sativus (a leguminous crop), suffer from a paralytic disease called lathyrism, due to the presence of a neurotoxin called β-oxalylaminoalanine-L-alanine (BOAA). The gene responsible for the production of BOAA was therefore suppressed using RNAi method (Zhang et al. 2016). Sunilkumar et al. (2006) observed that transgenic cotton plants expressing RNAi construct of the δ-cadinene synthase gene (encoding the enzyme responsible for the synthesis of the toxic compound, gossypol), when fused to a seed-specific promoter, caused reduction in the gossypol content. Lyc e3, an allergen present in tomato has been lowered through RNAi. Lyc e 3 encodes a hydrophilic non-specific lipid transfer protein that directs specific intermembrane lipid transfer. Human IgE antibody shows a strong reactivity to Lyc e3 which leads to IgE sensitization and causes allergenic reactions. Two cDNAs, LTPG1 and LTPG2, with high sequence homology to the N-terminal sequence of Lyce 3 were used for dsRNAi construct. To assess the allergenic potential of Lyc e 3-deficient tomato fruits, the histamine released from sensitized human basophils stimulated with transgenic and parental lines were measured, when a strong reduction in the release of histamine was observed from the basophils, challenged with transgenic tomatoes, as compared with the control plants (Le et al., 2006).

CONCLUSION

Food security in the wake of an ever-increasing world population is a challenge that has no single solution to date. RNAi-mediated gene silencing has led us to understand and discover valuable targets to enhance micronutrient availability in a variety of plants, some of which have been exemplified in this review. The issue of "off-target" silencing by RNAi has also been addressed in plants by the use of tissue-specific and inducible promoters, allowing targeted silencing only in specific tissues. It is a favored technology since it is cost-effective, producing RNAi inducers throughout the life of the plant. RNAi-mediated gene silencing suffers from certain limitations as well. It may cause chromatin modification which leads to the formation of heterochromatic regions. Such alterations can be heritable and may have ill effects, thus compromising biosafety. Hence, plants produced via RNAi must be assessed for potential risks involved, concerning food safety and its effect on the environment. Complete knockouts or gene silencing may not always be achieved by employing RNA silencing techniques. Furthermore, many endogenous plant promoters are resistant to siRNA-directed transcriptional silencing. Such siRNA or long dsRNA produced by the current RNAi vectors trigger RNA-dependent protein kinase (PKR) pathways in plants which could activate plant stress response and cause certain side effects. A solution to this problem could result from the development of the next-generation RNAi vectors with characteristics of miRNA vectors. The miRNA structures have been selectively evolved to evade the PKR pathway and show no adverse effects other than its programmed role. In conclusion, while on the one hand, further research efforts for plant nutrient enhancement through RNAi are necessary, it is also essential to generate awareness among the masses and make the transgenic products reach them after rigorous assessment of biosafety, allergenicity and toxicity, so that they can be practically put to use, to assure nutritional security for all, rather than remaining a distant elusive goal.

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Fuelwood Utilization and Health Effects among Farming Households in Ekiti State, Nigeria

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ABSTRACT

Fuelwood has long served as a major energy source known and used for cooking in developing countries. The combustion of fuelwood emits smoke with particles that have adverse effects on the health of users. This study investigated the health effects of fuelwood utilization among farming households in Ekiti State, Nigeria. Primary data was obtained from 120 farming households with the aid of a pre-tested questionnaire and focus group discussion. A multistage sampling technique was employed in selecting respondents for the study. Data were analysed using descriptive statistics and a probit regression model. Results revealed that the majority (75.8%) of the respondents were female with a mean age of 42 years and mean household size of 9 persons, 42.5% had primary education and earned a mean monthly income of ₦26,380. The major health problems associated with fuelwood utilization in the study area were eye irritation (56.6%), sneezing (50.8%) and breathing difficulty (43.3%). Sex ($p<0.05$), marital status ($p<0.05$), household size ($p<0.01$), primary occupation ($p<0.01$) and income ($p<0.05$) were the factors determining the health effects of fuelwood utilization among the farming households in the study area. The study recommended that renewable energy sources such as kerosene and cooking gas should be made readily available to farm families at subsidized and affordable prices.

INTRODUCTION

Wood is considered mankind's very first source of energy. Today, it is still the most important single source of renewable energy providing about 6% of the global total primary energy supply (FAO, 2019). Wood fuel or fuelwood may be available as firewood, charcoal, chips, sheets, pellets and sawdust (Ojo et al. 2012). Fuelwood is a source of energy derived by burning wood materials like logs and twigs and is common among rural dwellers. It is a traditional source of energy, which has remained the major source of fuel among farming households (Kadafa et al. 2017). Globally, about 3 billion people depend on fuelwood to meet their domestic energy needs. Private households' cooking and heating with fuelwoods represent one-third of the global renewable energy consumption, making wood the most decentralised energy in the world (Jagger and Shively, 2014; FAO, 2019). Nearly 90% of the households in developing countries depend on fuelwood for cooking and/or heating (Scheid et al. 2019). Poor access to modern energy rates in less developed countries (LDCs) and Sub-Saharan (SSA) countries remain high at 91% and 83%, respectively.

In Nigeria, it is estimated that about 91% of the household energy needs are met by biomass (Babalola, 2010). The over-dependence on fuelwood for energy is chiefly because of its affordability and easy accessibility relative to alternatives, such as bottled gas, kerosene or electricity. Clean energy such as kerosene and gasoline are scarce in the rural areas and often sold at a price far above the official pump prices, the supply of these alternatives is highly constrained and unreliable. This is especially true of electricity which is subject to frequent outages (Nnaji et al. 2012; Sa'ad and Bugaje, 2016).

The combustion of solid wood fuel indoors and outdoors emits smoke with particles that have adverse effects on the health of users. Several studies (Vivan et al. 2012; Odunuga and Akinbile, 2015; Kadafa et al. 2017; Piabuo and Puatwoe, 2020) have reported significant health effects of wood fuel combustion for cooking in rural areas. Fuelwood, especially, through open-fire three-stone stoves has implications for individuals' health, particularly, for women and children who spend hours beside the cooking fire and inhale smoke because they are primarily responsible for cooking (Sa'ad and Bugaje, 2016). About 1.3 million people, mostly women and children die prematurely every year because of exposure to indoor air pollution from biomass (Vivan et al. 2012). A child exposed to indoor air pollution is more likely to catch pneumonia, which is one of the world's leading killers of young children. In addition, indoor smoke has been linked to low birth weight, infant mortality, tuberculosis, cataracts and asthma (Sa'ad and Bugaje, 2016). Among women, there is a high association between fuelwood combustion and a high risk of chronic bronchitis and chronic obstructive pulmonary disease, especially asthma and cataract. Indoor combustion of fuelwood has been called the 'kitchen killer' because about 1.6 million deaths have been registered as a result, accounting for 2.7% of the global disease burden (WHO, 2007; Piabuo and Puatwoe, 2020). The most common forms of respiratory tract infections as a result of exposure to smoke due to cooking fuel are dry cough, breathing problems, neurologic problems, cardiopulmonary, cardiovascular diseases, asthma and lung cancer (Zidago et al. 2016; Mbanya and Sridhar, 2017; Mohapatra et al. 2018). Lower life expectancy has equally been reported by Badamassi et al. (2018). Other diseases such as asthma, stroke, and immune system impairment have equally been attributed to indoor and outdoor pollution as a result of fuelwood combustion (Tao et al. 2016). The poor in developing countries, therefore, pay much more in terms of health impacts, collection time, and stress compared to their counterparts in the developed world (Vivan et al. 2012). From the foregoing, this study investigates the health effects of fuelwood utilization among farming households in Lagos State with the following specific objectives: i) describe the socio-economic characteristics of farming households in the study area ii) identify the major health effects experienced as a result of fuelwood utilization iii) determine the health effects of fuelwood utilization among farming households in the study area.

METHODOLOGY

The study was conducted in Ekiti State, Nigeria. Ekiti State was created on the 1st of October, 1996. Its capital is Ado-Ekiti. It is located between latitude 7025' 8005'N and longitudes 4045' 5046E. The state is bounded in the north by Kwara State and Kogi State while Osun State occupies the west and Ondo State lies in the south and extends to the eastern part. The state is made up of 16 Local Government Areas (LGAs) with an estimated population of 3,270,800 (NPC-NBS, 2016) and a total landmass of 6,353 square kilometres. The two prominent climatic seasons in the area include the rainy season, lasting from April to October and the dry season within November and March. Temperature ranges between 21 °C and 28 °C with high humidity. The people of the state are predominantly farmers. Cocoa is the main cash crop while yam, cassava and grains like rice and maize are the major food crops produced in the state. Besides farming, the inhabitants of the state are artisans, traders, civil and public servants.

Sampling procedures

A multistage sampling technique was used to select respondents for the study. The first stage involved the purposive selection of two local government areas (Ekiti East and Ijero LGAs) due to the prevalence of farming activities in the LGAs. In the second stage, a simple random sampling technique was used to select five villages from each of the LGA making a total of 10 villages. The third stage involved the random selection of 12 farming households from each of the selected villages making a total of 120 farming households for the study.
The study was based on the primary data obtained from farming households in the study area using an interview schedule with the aid of a pre-tested questionnaire and supported by focus group discussion. Data were collected on socio-economic characteristics of the respondents, sources of fuelwood used for cooking and health issues experienced as a result of fuelwood utilization in the study area.

Analytical techniques

The following analytical tools were employed in this study.

Descriptive analysis

Descriptive statistics such as frequencies, percentages, means and standard deviation were adopted to describe information on the socio-economic characteristics of the farming households, sources of fuelwood utilized for cooking and health problems experienced as a result of cooking with fuelwood in the study area.

Probit regression analysis

The model was used to determine the health effects of fuelwood utilization among farming households in the study area. The model is explicitly specified as:

$$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_4 + \beta_5X_5 + \beta_6X_6 + \beta_7X_7 + e_i \quad (1)$$

Where: Y is the dependent variable that takes 1 if the respondents experience any health issue as a result of fuelwood utilization and 0 otherwise. The explanatory variables are: X_1 = Age (years), X_2 = Sex (dummy), X_3 = Education (level), X_4 = Marital status (1 if married, 0 if otherwise), X_5 = Household size (No of people), X_6 = Primary occupation (1 if farming, 0 otherwise) X_7 = Income (₦), β = parameter to be estimated, e_i = error term. SPSS version 20 and Stata version 12 were used for data analysis.

RESULTS AND DISCUSSION

Socio-economic characteristics of the respondents in the study area

Table 1 presents the results of the socio-economic characteristics of the respondents in the study area. The results reveal that the majority (75.8%) of the respondents were female while 24.2% were male. This is not unexpected as women are traditionally responsible for cooking in households and are therefore more likely to be exposed to health problems associated with fuelwood utilization in the study area. This result corroborates the findings of Kadafa et al. (2017) that most females use fuelwood primarily for cooking as the major source of domestic energy utilization, hence they are more adversely affected by the use of fuelwood compared to men. A larger proportion (38.3%) of the respondents fell within the 41 to 50 years age range, 25% were within the age range of 31 to 40 years and 16.7% were between 51 and 60 years while 15% were within the age range of 21 and 30 years. This implies that fuelwood utilization, especially for cooking is employed by all age groups in the study area. The mean age of 42 years implies that the respondents were still young and could cope with the stress of firewood gathering and utilization in the study area. This supports the findings of Odunuga and Akinbile (2015) that the majority of the respondents who exploited and utilized fuelwood in Ogun State were young and still possess enough strength, vigour and vitality to exploit fuelwood in a substantial amount. Distribution by educational qualification reveals that 42.5% of the respondents had primary education, 35% had secondary education and 9.2% had tertiary education while 13.3% had no formal education in the study area. This implies that the majority of the respondents, though had one form of education or the other, had a low educational level and this might have an implication on their standard of living as well as healthy living in the study area. This agrees with the findings of Ojo et al. (2012) that education is a major factor, especially in an economic unit where human hygiene matters. The majority (72.5%) of the respondents were married. This implies that the respondents were responsible for their family needs. The majority (57.5%) had between 6 and 10 people in their households. The mean household size of 9 persons implies that the respondents had a large household size and this might influence fuelwood utilization, especially for cooking in the study area. This is in line with the reports of Isma'il et al. (2014) that family size influences the demand for fuelwood in a household. The results further reveal that the majority (51.7%) of the respondents were farmers, 18.3% engaged in petty trading, 16.7% were in paid employment jobs and 13.3% were artisans. This implies that farming being the major occupation of the respondents can predispose them to utilize fuelwood in the study area.

Furthermore, 45.8% of the respondents earned between ₦20,100 and ₦40,000 monthly, 31.7% earned less than ₦20,000 while 22.5% earned more than ₦40,000 monthly in the study area. The mean monthly income of ₦26,380 implies that the economic status of the respondents is low and this could be a predisposing factor to fuelwood utilization which is cheaper and readily available when compared to other energy sources.

Sources of fuelwood in the study area

The result in Figure 1 shows that the majority (51%) of the respondents obtained their fuelwood from their farmland, 25% got it from nearby bushes and 18% got it from the forest while 6% purchased their fuelwood. This result implies that the dependence on fuelwood as an energy source may be connected to its availability, easy accessibility and affordability as 94% of the respondents got their fuelwood at no cost in the study area. This result is in tandem with the findings of Isma'il et al. (2014) that 53% of rural households in Ikara LGA of Kaduna State obtained their fuel-wood from their farmlands.

Health problems associated with fuelwood utilization in the study area

The results in Table 2 reveal that majority (85%) of the respondents had experienced at least one of the health problems listed in Table 2 as a result of fuelwood utilization in the study area. The results show that 56.6% of the respondents had experienced eye irritation, 50.8% sneezed while cooking with fuelwood and 43.3% had breathing difficulty. Other health problems experienced by the respondents were cough (30.8%), catarrh (12.5%), asthma (11.7%) and headache (10%). This result implies that, although fuelwood is readily available and a cheap source of domestic energy, it poses a great threat to the health of the farming households in the study area. This result corroborates the findings of Kadafa et al. (2017) that respondents at Yelwa Village, Nasarawa State suffered from a variety of health problems such as eye irritation, cough, persistent sneezing and shortness of breath as a result of fuelwood utilization.

Table 1. Socio-economic characteristics of respondents in the study area (n = 120).

Variables	Frequency	Percentage (%)
Sex		
Female	91	75.8
Male	29	24.2
Age		
21-30	18	15.0
31-40	30	25.0
41-50	46	38.3
51-60	20	16.7
Above 60	6	5.0
Mean	**42.13 (±12.041)**	
Educational Qualification		
No Formal Education	16	13.3
Primary Education	51	42.5
Secondary Education	42	35.0
Tertiary Education	11	9.2
Marital Status		
Single	12	10.0
Married	87	72.5
Divorced	7	5.8
Widowed	14	11.7
Household Size		
1-5	40	33.3
6-10	69	57.5
Above 10	11	9.2
Mean	**9(±1.063)**	
Occupation		
Farming	62	51.7
Self-employed	16	13.3
Trading	22	18.3
Paid employment	20	16.7
Monthly Income		
≤20,000	38	31.7
20,100-40,000	55	45.8
>40,000	27	22.5
Mean	**26,380 (±1212.275)**	

Source: Field survey, 2020

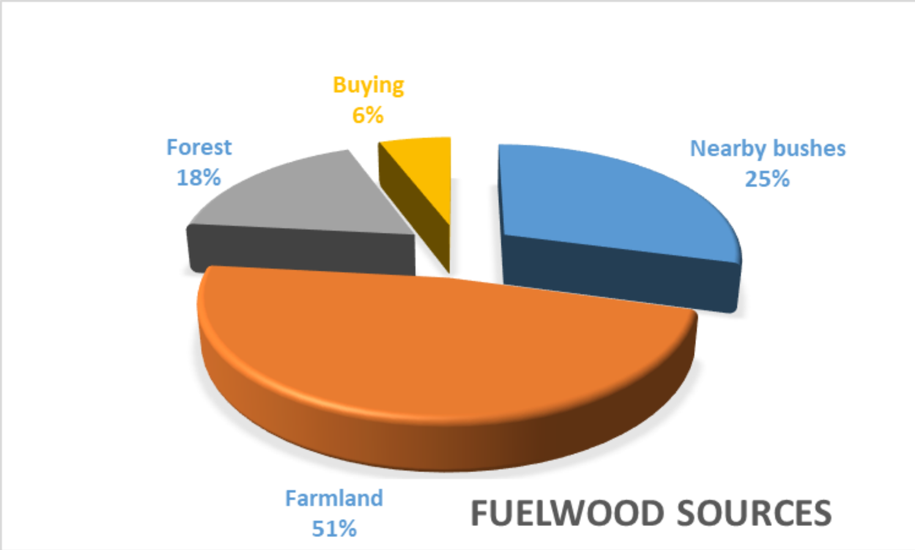

Figure 1. Sources of fuelwood used for cooking in thestudy area

Determinants of health effects of fuelwood utilization among farming households in the study area

The result of the probit regression analysis on the determinants of the health effects of fuelwood utilization among farming households in the study area is presented in Table 3. The Chi-square value of 230.961 ($p<0.01$) implies that the model fits the data well. The result reveals that sex ($p<0.05$), marital status ($p<0.05$), household size ($p<0.01$), primary occupation ($p<0.01$) and income ($p<0.05$) were the factors determining the health effects of fuelwood utilization among the farming households in the study area.

Table 2. Health problems associated with fuelwood utilization in the study area.

*Health problems	Frequency	Percentage %
Suffered health problems	102	85
Eye irritation	68	56.6
Sneezing	61	50.8
Breathing difficulty	52	43.3
Cough	37	30.8
Catarrh	15	12.5
Asthma	14	11.7
Headache	12	10.0

Source: Field Survey Data, 2020 *Multiple responses

The marital status of the respondents was found to have a positive significant relationship with the health effects of fuelwood utilization in the study area. This implies that married respondents were more likely to experience health problems occasioned by fuelwood utilization than unmarried respondents in the study area. This is because married respondents require more domestic energy to cater to their family needs. Odunuga and Akinbile (2015) and Kadafa et al. (2017) reported similar results. In the same vein, household size and primary occupation of the respondents were positive and significant at 1% alpha levels respectively. This implies that these variables increase the probability of the respondents experiencing health problems as a result of fuelwood utilization in the study area. The larger the household size, the more fuelwood required, the more exposure to smoke emitted from fuelwood combustion and the higher the adverse effects on the health of users. This result is in consonance with the findings of (Vivan et al. 2012; Piabuo and Puatwoe, 2020) that the combustion of solid wood fuel emits smoke with particles that have adverse effects on the health of users. Also, the primary occupation of the respondents being farming predisposed them to utilize fuelwood due to its affordability and accessibility, this increases their exposure to smoke from fuelwood combustion and consequently, health problems associated with fuelwood utilization in the study area. The income of the respondents was also found to have a positive significant relationship with the health effects of fuelwood utilization at 5% alpha levels in the study area. This implies that farming households with higher incomes have a higher likelihood of experiencing health problems as a result of fuelwood utilization in the study area. This result is surprising as higher income is expected to enhance the capacity of using renewable energy sources such as kerosene or LPG thereby reducing the health effects of fuelwood utilization but this result is a pointer to the fact that renewable/cleaner energy sources such as kerosene, electricity and LPG were not only very expensive but also very scarce in the study area. This result confirms the findings of Onyekuru and Eboh (2011) that those with better income buy more fuelwood which was cheaper in the rural areas thereby increasing their exposures to health consequences of using fuelwood. Conversely, the sex of the respondents was found to have an indirect significant relationship with the health effects of fuelwood utilization at a 5% level of probability in the study area. This implies that the likelihood that farming households would experience health problems as a result of fuelwood utilization is higher in women than men. This is not unexpected as women were chiefly responsible for cooking and therefore have higher exposures to fuelwood combustion than men in the study area. This result supports the reports of WHO, (2007); Piabuo and Puatwoe (2020) that, there is a high association between fuelwood combustion and a high risk of chronic bronchitis and chronic obstructive pulmonary disease, especially asthma and cataract among women. Badamassi et al. (2018) equally reported that combustion of particulate matter (PM2.5) has a greater adverse effect on women's life expectancy in the long run.

Table 3. Determinants of health effects of fuelwood utilization among farming households in the study area.

Variable	Coefficient	S.E	Z-value
Age	0.137	0.100	1.370
Sex	-0.143**	0.076	-2.168
Education	-0.015	0.041	-1.374
Marital status	0.133**	0.066	2.008
Household size	0.095***	0.038	2.525
Occupation	0.148***	0.057	2.593
Income	0.000**	0.000	2.212
Constant	-2.955	0.290	-10.177
chi^2	230.961***		9.675

*** Significant at 1%; ** Significant at 5%
Source: Computed from Field Survey Data, 2020

CONCLUSION

Based on the findings of this study, it can be concluded that fuelwood utilization had negative effects on the health of farming households in the study area. The major health problems experienced by the respondents were eye irritation, sneezing, and breathing difficulty. Consequently, the sex of the respondents, marital status, household size, primary occupation and income were the drivers of health effects of fuelwood utilization among the farming households in the study area. Therefore, an alternative source of fuel such as kerosene and cooking gas should be made readily available to farm families at subsidized and affordable prices. This will encourage a shift from the reliance on unhealthy and environmentally unfriendly fuelwood to more sustainable sources of energy. Also, Government, both at federal and state levels, may also promote a solar-powered cooking stove program in the rural area. Improved versions of biomass stoves developed locally by the Energy Commission of Nigeria through its energy research centres at the University of Nigeria, Nsukka and Usman Dan Fodio University in Sokoto should be promoted. An elaborate awareness programme by the government to disseminate and encourage the adoption of such technology by the households nationwide as the case of our neighbouring countries like Niger, Cameroon and Senegal should be put in place. Furthermore, Local government agencies and non-governmental organizations should embark on public enlightenment campaigns to inform the farm families on the consequences of fuelwood consumption on their health, climate, environment and biodiversity.

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Review Article

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Park, People, Policy: Synergy towards a Holistic Approach to Sustainable Management of Protected Areas

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ABSTRACT

Nature exists with people and for human development, hence, bodies like Community Based Development, Global Environment Facility and United Nations Development Program are interested in conservation-development synergy for guaranteed nature protection. Conservation has broadened its dimensions due to species and ecosystem extinction cum booming human population. Social emancipation, economic empowerment, cultural continuity and political stability within the frame of legislative allowance are as vital as ecological sustainability in protected areas management. To save existing Protected Areas from imminent extinction, this review addressed the rationale behind the participatory approach to conservation; the significance of policy in nature protection; and the necessity to balance conservation and rural development. The significance of rural communities within protected wildlands on the spate of biodiversity and ecosystem loss cannot be neglected because sustainable livelihood is crucial to the people as sustainable conservation is to resource managers. However, enforcement shapes both parties in achieving peaceful coexistence. Socioeconomic and cultural atmospheres mainly influence the success of conservation efforts and should be considered to guarantee biodiversity in perpetuity.

INTRODUCTION

Conservation in the 21st century needs to expand its dimension along spatial, temporal and social scales owing to species and ecosystems extinction. Ravaging habitat loss has been highlighted to be responsible for approximately 80% of globally threatened species (Ervin et al., 2010) and has triggered concerns locally as well as globally towards evolving approaches to conservation. Strategic and timely expansion in the landmass of protected areas is the most immediate and effective response to the imminent biodiversity crisis in the world today. Humans rely on vital ecosystem functions of protected areas. As an example, 33 out of 105 of the world's largest cities source clean water from protected areas (Ervin et al., 2010). Economies of many developing countries especially in Africa; Latin America and The Caribbean, depend solely on tourism revenue associated with protected areas. As the human population booms, there is a resultant escalation in demand for a resource with its associated loom in tension due to inappropriate resource allocation and ecological injustice. Prior to the 19th century, there has always been a 'Fortress Conservation' where people are completely excluded from nature. The birth of Yellow Stone National Park in the late 19th century, precisely 1872 put a turn in the paradigm. The experimental park was not completely participatory as it ejected locals from the resource though putting a dot on economic development. However, there has been a metamorphosis of approaches since then, in the management of natural lands under political protection. Political protection comes with a level of enforcement. Renewable natural resources protection is not an exception. Rigid; flexible; the result is always significant. In the bid to save existing protected areas and future ones from imminent extinction, this review serves as a reference for highlighting the critical factors that are fundamental to a successful conservation project. This would be through addressing the rationale behind the participatory approach to conservation; the significance of policy in nature protection; and the necessity to strike a balance between conservation and rural development all in an atmosphere that spells locals' participation and experiences in conservation projects under current policy environments. Rural communities of course benefit from biodiversity as explained by Ervin et al. (2010) that many great cities and municipals across the globe tap their clean water from protected areas. However, this does not negate some externalities borne by the same people as a result of its protection. Overlooking this will turn the park into an ordinary print.

Significance of Protected Areas in Sustainable Conservation

Parks across the globe render not only ecosystem functions but also social, political, cultural, economic and peace functions. They are pools of biological diversity as well as zones of trade and cultural continuity. Some of the rural development strategies are summarized in Figure 1 where environmental education seems to be the most applied strategy. Although, it may not be as acceptable to park inhabitants as much as local empowerment would (Sotolu et al. 2016).

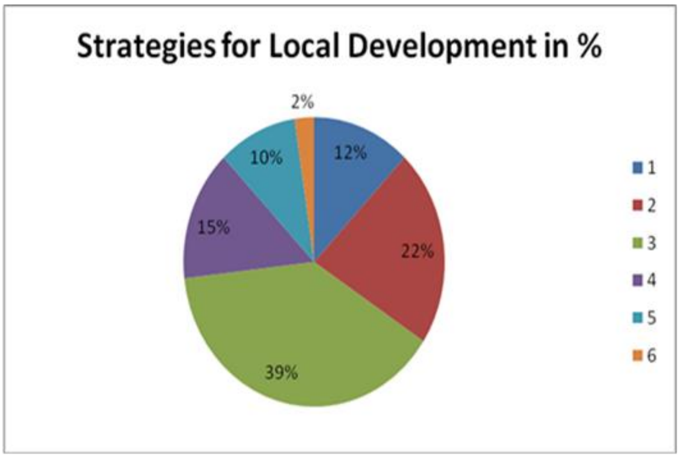

Figure 1. Strategies for Rural Development in Protected Areas in Brazil
Source: Adapted from Chiaravalloti et al. (2015)
Key: 1: conservation unit's job; 2: income generation from biodiversity exploitation; 3: environment education; 4: empowering local associations; 5: scientific research; 6: participatory monitoring.

International Efforts towards Conservation and Development

Parties to the Convention on Biological Diversity (CBD) in February 2004 committed to a strategic set of actions known as the Programme of Work on Protected Areas with the goal to establish comprehensive, ecologically representative and effectively managed networks of terrestrial protected areas by 2010 and marine protected areas by 2012. Measurable targets and specific timelines were set for the programs and proposed to be a guiding framework for protected areas in the next decade. Presently, a global network of protected areas is directly or indirectly responsible for employment generations that rival in number with those provided by some global economy icons (Ervin et al. 2010).

Global environmental facility (GEF), the operating entity of the financial mechanism of the CBD, is globally recognized as the world's leading facility for aiding nations in the implementation of their obligations under the CBD Programme of Work on Protected Areas. Enhancing the sustainability of protected area systems is pivotal to the GEF biodiversity strategy. This is achieved through improving: financial sustainability; protected area coverage, representativeness and connectivity; and protected area capacity and management effectiveness. Over 2,300 protected areas covering a land area of more than 634 million hectares have benefited from GEF funding. GEF has solely invested 1.89 billion in protected areas with an additional 4.5 billion in co-financing from project partners.

United Nations Development Programme (UNDP), one of the implementing agencies of GEF, is the world's most significant contributor of technical assistance to protected areas. Since the ratification of the CBD Programme of Work on Protected Areas in 2004, UNDP has supported over 700 protected areas in 55 countries, covering nearly every goal, target and action of the Programme of Work on Protected Areas. UNDP has helped to improve protected area management effectiveness across more than 85 million hectares and to establish new protected areas covering more than 15 million hectares. UNDP's rationale for making such a significant investment in protected areas is simple: protected areas and community conserved areas together represent as much as a quarter of the world's land surface, and this land and sea mass represent an enormous potential to contribute to human development by securing ecosystem services, maintaining the livelihoods of hundreds of millions of people, and buffering humanity from the impacts of climate change. Nations across the globe benefit from activities of these international bodies and more.

Strength of a Legal Resolve

When wild animals raid farmlands within natural areas, farms owned by the impoverished locals; compensation for loss is not paid (Sotolu et al. 2017); locals are excluded from park management; and poverty lingers on in the communities, there is bound to be conflict not only involving park managers and the rurals (Ayivor et al. 2013), but also between the different land users in those communities. Hunting; encroachment; killing wild animals for their trophies, all lead to arrests and forced evictions which have raised eyebrows of the local victims and are seen as an impediment to putting a stop to illegal activities (Ayiyor et al. 2013). This is because the people feel embittered, cheated and taken advantage of, on a land that was originally theirs. Human-Wildlife Conflict with its associated retaliatory killing of wild animals (Sotolu et al. 2017); Illegal Wildlife Trade; farmland encroachment into parks; and more will all need a resolve that involves legal facet as well as dialogue. As explained by Sotolu et al. (2016), park communities are unlikely to be satisfied with enforcement and park protection laws. Until and unless unlimited access is given for resource exploitation, there will always arise issues of concern between nature and people. Limitless access, however, is uncompromisingly unachievable, since no system involving people can be sustainably functional without spelling out offenses with associated penalties that should be effectively instituted. From poaching and disagreement over park boundaries in Zakouma National Park in Chad, to eviction from Digya National Park in Ghana (Ayivor et al., 2013), to retaliatory killing of wild animals in Cross River National Park in Nigeria (Sotolu et al. 2016), friction continues and would always require resolution.

Dot on Participatory Conservation

Protected areas are vital to reducing, if not putting a halt to, biodiversity loss. They are in situ repositories of genetic materials and ancient relics of landscapes that are pivotal to socio-cultural, aesthetic, spiritual and traditional relationships of human existence (Harmon and Putney, 2003). However, these roles are still blinking red hence, the terms ‘paper park’, ‘island parks’ describing failures (Laurance, 2008). Preservationists' approach of ‘fences and fines’, ‘fences and guns’, and ‘colonial approach’ viewed people as exploiters of biodiversity and excluded them from resource protection (Vig and Kraft, 2012). A serious issue in nature protection is a conflict between protected area managers and support zone communities. These include disagreement and disputes over access to resources and its control involving arrests, prosecutions, violent confrontations and even deaths sometimes (Ayivor et al., 2013). As there are benefits from a collective system for multiple objectives of resource management, so also are there challenges to be overcome as depicted in Table 1.

Table 1. Potential benefits and risks in managing protected areas for multiple objectives.

Synergy	Benefits	Trade-off
Conservation- Climate change	maintaining large tracts of intact ecosystems, such as grasslands and forests, which are ideal for climate change adaptation and mitigation because they are more likely to be resilient to climate impacts	mitigation can involve practices that reduce biodiversity, for example, managing forests for short rotations and favoring fast-growing, early successional species, at the expense of mature, climax species.
Conservation-Ecosystem services	through restoration efforts, such as removing invasive species from grasslands and restoring fire processes typically restores ecosystem services	Managing a riparian system to sustain the volume of water flows maybe inconsistent with the hydrological regimes needed to sustain key ecological processes (e.g., flooding)

Conservation-Sustainable livelihoods	Intact, functioning ecosystems are much more likely to provide reliable and secure livelihoods than more vulnerable systems, reducing the vulnerability of resource dependent communities	Managing wild biodiversity for sustainable livelihoods, such as non-timber forest products frequently lead to substitution, domestication or extinction, particularly if safeguards are not in place
Ecosystem services-Sustainable livelihoods	Managing biodiversity to maintain ecosystem services disproportionately benefits the poor, who depend on natural resources and ecosystem services the most	Managing grasslands to sustain grazing through annual fires may harm important medicinal plants and thatch resources

Source: Adapted from Ervin et al. (2010)

Case Scenarios

From Ayivor et al. (2013), it was learned that 'In 2006, a border dispute in Kyabobo National Park, Ghana resulted in the tragic death of two Wildlife Officials' (Ghanaweb, 2006). Another incident occurred in Bui National Park, also in Ghana in 2007, when a poacher lost his life for resisting arrest and attacking a Wildlife Official (Ayivor, 2007). Local communities attacked Wildlife Officials and burnt down one of their campsites. Both incidents were resolved through the intervention of local chiefs and Wildlife Officials from the national headquarters.

In addition, 'In 1989, 2002 and 2006, three major eviction exercises were carried out in Digya to move mainly migrant communities and their families (squatters) who were allowed entry into portions of the park by local chiefs. These chiefs claimed that cash compensation for expropriation of their lands had been paid to wrongful claimants and, therefore, considered themselves as rightful owners of these portions of the park. The exercises mostly targeted squatters who often resisted eviction, thus, compelling Wildlife Officials to seek the support of the military to evict them. During the 2006 eviction exercise, nine people lost their lives through a boat accident that occurred while they were being ferried across Volta Lake. The eviction exercise of 2006 was abandoned due to public outcry and a court injunction (Myjoyonline, 2006).'

Metamorphosis of Global Approaches to Conservation

In the bid for biodiversity protection, national parks were established across Sub-Saharan Africa primarily for hunting and tourism by the colonials. This did not go without the expulsion of the original custodians of the resource- the locals (Adams and Mulligan 2003; King 2007). The late 19th century witnessed the birth of a reconsideration of the 'fortress' approach by the establishment of the Yellowstone National Park in 1872 in the United States of America. It was an 'experiment park', based on attempting to merge conservation with economic development (King, 2009). Of course, the transformation was not sudden but gradual, as even at this time people and the park were still mutually exclusive. Colonialism and the increasing promotion of the need for sustainable development aided further designation of nature for conservation across the globe (King, 2009).

Concern over loss of biodiversity and the fear of consequent extinction has greased the push for the establishment of more protected areas worldwide. From the beginning to the mid-20th century which marked the end of the Classic Model of Protected Area management (Table 2), a meager 600 protected areas were in operation.

In about 1999, there were nearly 3000 protected areas (Ghimire, 1994). Approximately 5% of the earth's landmass was protected as a result of more than 25,000 protected areas towards the end of the 20th century characterized by the era of the Modern Model of protected area management as shown in Table 2. Currently, over 11.5% of the planet's landmass is protected due to the creation of more than 100,000 protected areas (IUCN and UNEP, 2003). The Emerging Models as against Traditional models (Figure 2) have not been receiving so much attention from parks all over the globe (Ervin et al. 2010). Regions of higher biological diversity have been established to also be areas of higher human population densities which manifest higher rates of social and economic poverty (King, 2009) but higher levels of cultural diversity. Thus, raising eyebrows over the alienation of the rural from the resource through which they derive sustenance. This resultantly makes the implementation of management strategies more difficult (Sotolu et al. 2016).

Table 2. The succession of models in protected areas management.

Issues	Classic Model (Mid 1800s-1970s)	Modern Model (1970s-Mid 2000s)	Emerging Model (Mid 2000s and Beyond)
The rationale for Establishing Protected Areas	Set aside for productive use	Concurrent social, ecological and economic objectives	Strategy to maintain critical life support systems
Purpose of Protected Areas	Established primarily for scenic values rather than functional values	Established for scientific, economic and cultural reasons	Established to support ecosystem services, promote climate change adaptation, resilience and mitigation
Management Purpose	Managed mostly for park visitors	Managed for the Locals	Managed for social, economic and ecological values emphasizing maintenance of ecosystem services

Source: Ervin et al. (2010)

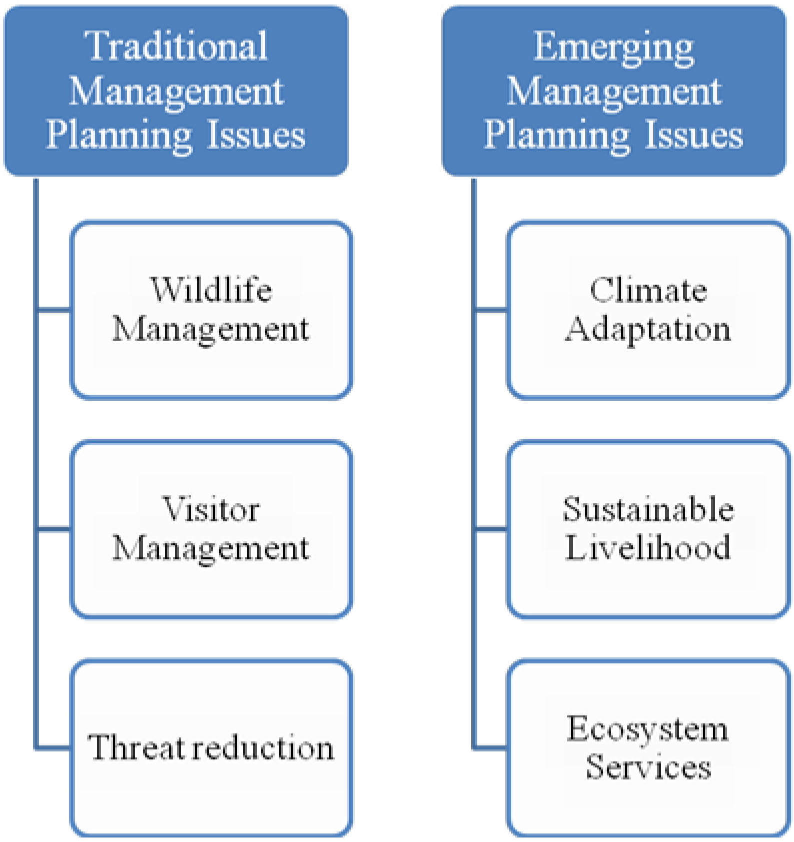

Figure 2. Traditional versus Emerging Management Planning Issues As Conservation Strategies

Source: Adapted from Ervin et al. (2010)

Realization of the significance of the locals' social and economic empowerment through conservation has led park management in the 21st century to pay more attention to that angle (Figure 3).

Impediments to a Collective System

Nature areas across the globe are characterized by human population pressure, putting a lot of stress on biodiversity through overexploitation. As noted by Ayivor et al. (2013), the high human population density of indigenous fishing and farming communities in addition to migrants who moved into the area with the creation of the Volta dam surround Digya National Park in Ghana, putting socio-economic pressure on the park. Some of these are poverty, illegal entry, poaching and livestock grazing among others. Adequate well-trained and equipped staff per unit patrol area is essential for effective enforcement. This capacity is lagging especially in West African parks. Ghanaian Digya National Park and Shai Hills Resource Reserve had 0.016 and 0.198 effective patrol staff per km^2 and operational budget of US$2.5/km2 and US$58/km2 respectively (Jachmann, 2008), while James et al. (2001) estimated ideal cost for effective protected area management to be US$250/$km^2$. Park officers often decry insufficient funds and equipment to access difficult terrains, sometimes requiring chopper or high-powered motorboats over lakes (Ayivor et al. 2013).

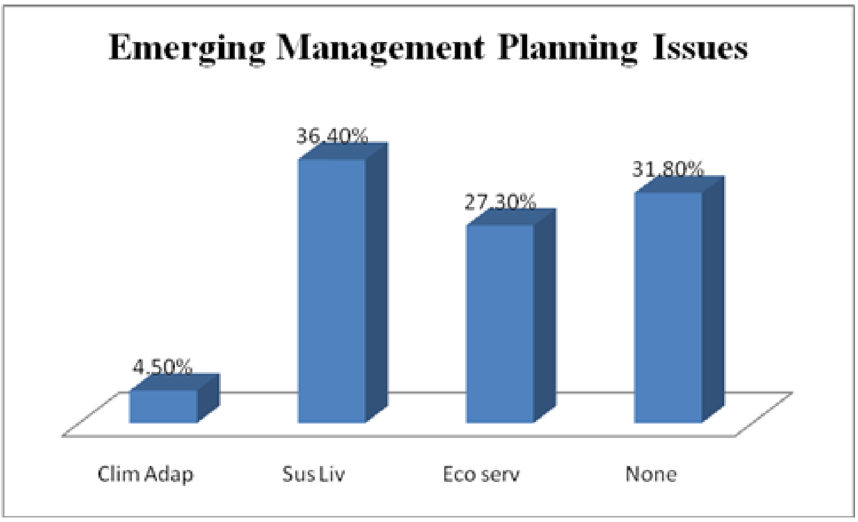

Figure 3. Evolution of park management planning issues across the globe.
Source: Adapted from Ervin et al. (2010)
KEY: Clim Adap- Climate Change Adaptation; Sus Liv- Sustainable Livelihood; Eco Serv- Ecosystem Services.

Figure 4. Schematic representation of the people-nature relationship.

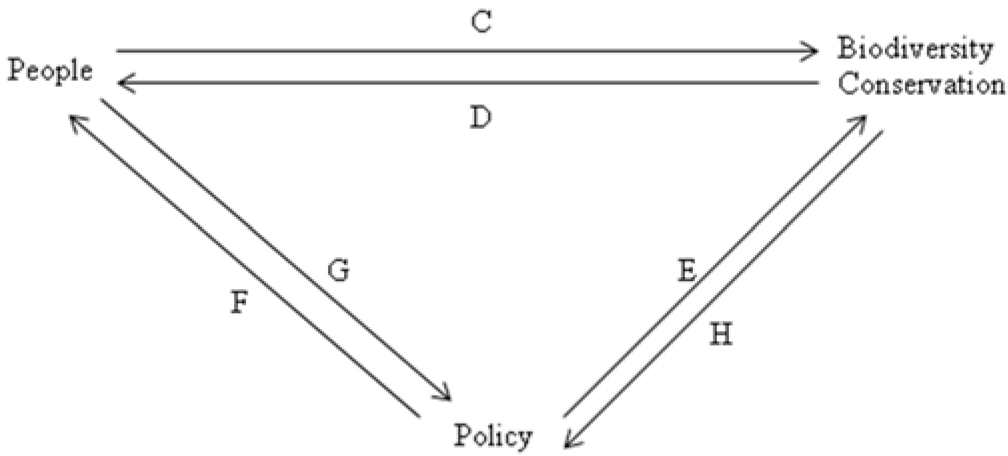

Figure 5. Schematic representation of a people-park policy scenario.

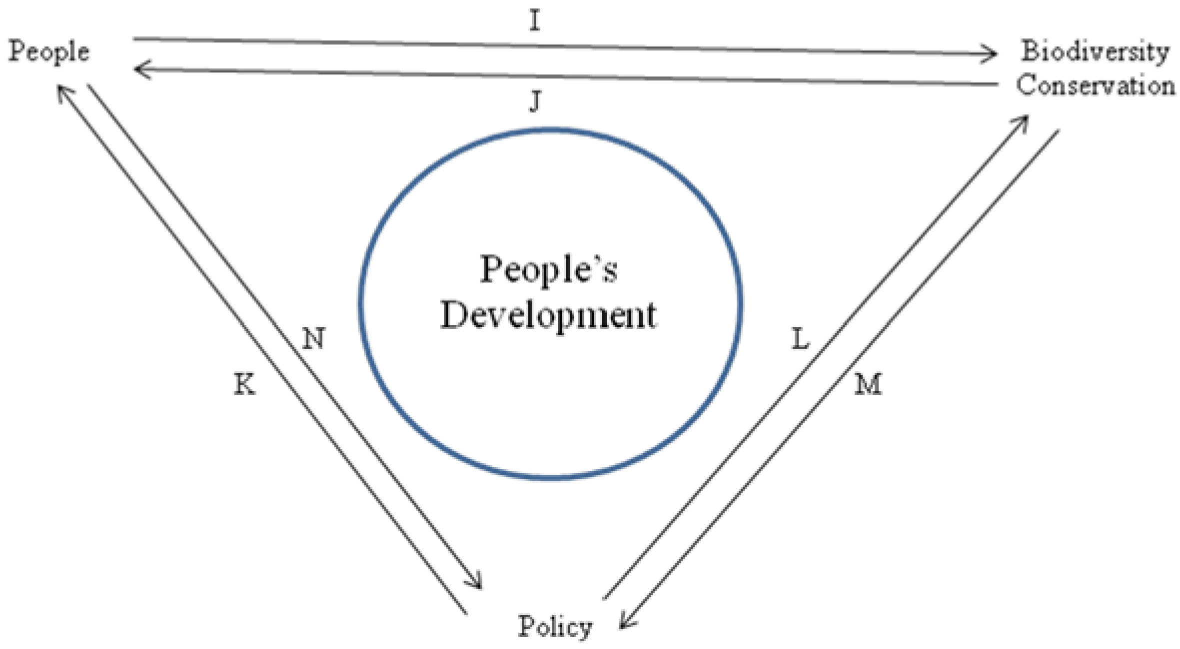

Figure 6. Schematic representation of a people-centered conservation initiative.

Ecological justice describes the fair treatment and meaningful involvement of all people regardless of race, colour, national origin, or income with respect to the development, implementation and enforcement of environmental laws, regulations and policies. The participatory approach that fringes on ecological injustice are bound to collapse and lead to mistrust. Benefits from protected area management should be equitably shared and its challenges collectively accounted for. As narrated by Ayivor et al. (2013), an elderly woman reported 'I derive no benefit from the park but instead crop losses. When I get to my farm and encounter an elephant feeding on my crops, I can only create noise to drive it away. If that fails, I just look on helplessly as my farm is destroyed. Often, I get so devastated and have no option but to weep all the way back home. In another narration, locals were reported to have complained that, 'We were served an eviction notice without us being told where to go. Two weeks after the notice, we were forcefully evicted and were not allowed even to salvage our belongings, including food crops and livestock. Wildlife officials were highhanded on us and there was no one to speak for us. We had to move at night to the opposite side of the Sene River with our children without any protection against the harsh environment. We had to pitch tents using improvised local materials as temporary houses…….' Situations like this do not exclude fatalities as was described during an eviction exercise in 2006 when settlers were overloaded in privately operated boats leading to deaths of tens of evacuees in a protected area in Ghana (Ayivor et al. 2013). The conservation that risks the lives of the communities will meet with public outcry and violence.

Synergy Itself

Most African nature lands were put under protection by the colonials who ruled their respective countries before the 1960s when those nations got their independence. Prior to park protection, people exploit biodiversity without fear of prosecution. Although resource use in most rural environments was guided by traditional and customary laws which were not based on empirical deductions, this kind of protection was not going to yield sustainable results as it did not involve arrest or prosecution. Moreover, traditional protection of resources only existed in few natural areas, not in all. At these periods, people relied solely on nature for sustenance. There were killing of wild animals (hunt and kill); grazing by livestock; logging; wood collection; non-timber forest products (NTFPs) harvesting; farming; fishing; and encroachment which were all unrestricted (A). Wildlife on the other hand was a part of the people's cultural and traditional setting (B). Then, issues were only between people and nature as depicted in Figure 4.

After protection, the policy comes in to act on both the people and the resource itself. People exert more pressure on nature alongside encroachment and urbanization, seeing the resource as a 'lost glory' (C). Wild animals raid locals' farmlands; carnivores kill and transmit zoonosis to livestock and then to people (D). The policy sought to protect nature (E) while restraining the locals (F). The resultant scenario is that where people are evicted from 'their lands' and alienated from resource management; conflicts arose between park managers and rural communities over illegal resource exploitation; arrests are made, and offenders are prosecuted (G) although resources are protected and 'fortress conservation is achieved (H) as seen in Figure 5.

There had to be adjustments on the part of the people and nature managers in order to bring a synergy tailored towards a combined and correlated action aimed at bringing harmony into the system with minimized friction. This requires inputs from other stakeholders as well – government; non-governmental organizations (NGOs), local and international conservation bodies; conservation researchers and research institutes; the private sectors; and the like, focusing people at the centre of conservation programs. This will give locals a sense of ownership and stewardship over the resource, hence regulating exploitation to ensure sustainability (I). The resource remains a part of the people's culture (J); policy protects lives, limbs (K) and nature (L); conservation goal is achieved (M), and people no more see park management as infringement but realize the need to support conservation efforts (N). Conservation-hinged benefits provided to the people on regular basis would ensure that resources are safe with minimal enforcement and patrol duties as seen in Figure 6.

CONCLUSION

Biodiversity is an endowment for human development-renewable, regenerative, but exhaustible and hence should be jealously protected. Parks are managed by people while policy guides and guards both towards a peaceful co-existence. Parks require adequate, well-armed paramilitary functions per square kilometers of the park territory for effective enforcement. People require alternative means of livelihood and involvement in park management for efficient participation. The policy requires to be flexible towards sustainable conservation. Dialogue is a vital tool in finding ways forward. Regular outreach programs between the park and the people would create a soft pedestrian for mutual trust and cooperation. Creating alternative livelihoods like beekeeping, handicrafts using local materials and small-scale livestock production, would go a long way in synergizing economic development and sustainable conservation within the limits of effective enforcement. Renewable natural resource under political and legal protection is more likely to be sustainable if guiding policy and principles are all-encompassing and effectively implemented. From the on-going, factors responsible for both the success and failure of conserving biodiversity in National Parks in various contexts were socioeconomic and cultural. These realizations pitch that future conservation approaches in parks should place more emphasis on the human dimension of biodiversity conservation rather than purely scientific studies of species and habitats.

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Research Article

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Effects of Long-Term Fertilization Methods on Rye Yield Components

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ABSTRACT

To reveal the effect of different fertilization methods on rye yield components, plant samplings were carried out in Westsik's crop rotation long-term experiment of the Research Institute of Nyíregyháza, IAREF, University of Debrecen, Hungary during the 2018 and 2019 seasons. Fertilization methods are based on different variations and combinations of organic manure and NPK fertilizers. One of the 15 crop rotations is maintained without fertilization, here we apply fallow in one phase (I). In four crop rotations straw manure (IV, V, VI, VII), in two crop rotations farmyard manure (X, XI), in one crop rotation (II) lupine green manure as the main crop, in two crop rotations (III, VIII) lupine for seed, in CR VIII lupine as main crop plus second crop, in one crop rotation (IX) lupine for green forage, in four crop rotations, lupine green manure as a second crop (XII, XIII, XIV, XV) are applied. Eleven crop rotations are treated with NPK fertilizer (II, III, IV, V, VI, VIII, IX, XI, XII, XIII, XIV). Results showed that all studied characters significantly differed due to fertilization methods in both years. Organic manure form plus chemical fertilizers resulted in the higher of analyzed rye plant parameters (plant weight per m2, seed weight per m2, spike number per m2, and 1000 seed weight). Comparing the effects of the three organic fertilization methods without chemical fertilizer, the farmyard manure was the most effective on the winter rye yield components. In addition, there were positive and close correlations between the rye seed weight, the rye plant weight, and 1000 seed weight, in both seasons. It could be concluded applied three organic manure (farmyard manure, straw manure, and lupine green manure) with chemical fertilizers were the most effective on rye yield component production.

INTRODUCTION

Rye (*Secale cereale* L.) is widely grown in Eastern, Central, and Northern Europe as a most important cereal crop for both human and animal feeding, especially in sandy soil (Bushuk, 2001). The total cultivated area of rye plants reached about 4.21 million ha and 25.940 thousand ha in the world and Hungary, respectively. A long-term field experiment is well known worldwide and has a positive effect on soil productivity, this effect is attributed mainly to the release of the constituent nutrients of the organic matter during decomposition and the improvement of the soil's physical condition, increases soil organic material and soil carbon content, which helps to maintain the soil fertility (Hemalatha and Chellamuthu, 2013; Balkcom et al. 2018) and promote to increase soil carbon sequestration, enhance crop growth characters and yield productivity of rye plants (Casarano et al. 2006). By organic manure using, we can increase the organic material content of the soil. The soil's organic material content is a very important factor in soil fertility (Rahman and Parkinson, 2007).

Using chemical fertilizers was increased worldwide for cereal production to face the big difference between production and consumption. With long-term and large-scale use of NPK mineral fertilizers, a lot of environmental issues will appear, such as changing soil pH, disturbances in beneficial microbial ecosystems, increase pests, and even contributing to the release of greenhouse gases, soil acidification, and crust. Possible methods to reduce chemical fertilizer use could be the adoption of leguminous crops in cereal-based cropping systems and the recycling of organic wastes (Patil et al. 2001). Involve legumes in cereal crop production reduced the dependence on chemical fertilizers and improved the soil condition, consequently the yield of rye plants (Rochester et al. 2001; Achu et al. 2013). The soil nutrient content is positively influenced by growing legumes and applying farmyard manure. Several investigations indicated positive effects of farmyard manure especially on soil organic carbon content and soil biological properties in many field experiments (Mäder et al. 2002; Marinari et al. 2006; Heinze et al. 2010) and in conventional farming systems too (Edmeades, 2003; Böhme et al. 2005; Elfstrand et al. 2007). In addition, the farmyard manure had a positive effect on the soil's organic carbon content and winter rye yield opposite the applied green manure fertilizer (Heinze et al. 2011). The interaction between organic and mineral fertilizers was studied by (Fageria et al. 2009; Stępień et al. 2016; Qiuchen, 2018) They found that rye yield and yield attributes significantly increased due to organic and mineral fertilizers compared to no fertilization. In the same way, the nutrient supply greatly influenced the growth characteristics and yield components of winter wheat (Bulman and Hunt, 1984). Rye yield was strongly determined by the genotype, the environment, the nutrient supply, and their interactions (Simmonds, 1981; Sattelmacher et al. 1994). Also, seed weight was considered one of the most important factors of winter rye yield and is genetically determined (Chmielewski and Köhn, 2000).

The objective of the experiment reported herein was to assess the effect of all fertilization methods of Westsik's crop rotation field experiment on some yield components of rye plants, as the main crop of this long-term experiment.

MATERIALS AND METHODS

Description of the experiment

Our plant samples were collected in the field of Westsik's crop rotation long-term experiment at the Research Institute of Nyíregyháza, IAREF, University of Debrecen, Hungary during the 2018 and 2019seasons. The soil of this experiment is classified as acidic sandy soil (pHKCL 4.47) with low humus content (0.64%) in the 0-20 cm soil layer. The experiment includes 14 three-year-long and 1 four-year-long crop rotations (C.R.) as presented in Table 1.

The fertilization methods are based on different variations and combinations of organic manure and NPK fertilizers. One of the 15 crop rotations is maintained without fertilization, here we apply fallow in one phase (I). In four crop rotations straw manure (IV, V, VI, VII), in two crop rotations farmyard manure (X, XI), in one crop rotation (II) lupine green manure as the main crop, in two crop rotations (III, VIII) lupine for seed, in CR VIII lupine as main crop plus second crop, in one crop rotation (IX) lupine for green forage, in four crop rotations, lupine green manure as a second crop (XII, XIII, XIV, XV) are applied. Eleven crop rotations are treated with NPK fertilizer (II, III, IV, V, VI, VIII, IX, XI, XII, XIII, XIV).

Sampling and measured parameters

Samples of rye plants were collected randomly from each plot after all plants reached the maturity stage on the 2nd and 11th of July during the 2018 and 2019 seasons, respectively using a square wooden frame 100 × 100 cm (1m2) in three repetitions/parcels, to determine the following characters: plant weight (g m^{-2}), spike number per m-2, seed weight m-2 and 1000-grain weight (g).

Statistical analysis

All collected data were analyzed according to the IBM SPSS Statistical Software Package 21.0 version. One-way ANOVA as described by (Snedecor and Cochran, 1980), then Tukey's test, P<0.05 as mentioned by (Tukey, 1977) was used to determine the treatment effect.

RESULTS

Plant weight

Data presented in Table 2 clearly show that the averages of rye plant weight/m^2 were between 325 and 1652 g m^{-2} and 539 and 1771 g m^{-2} in both the 2018 and 2019 seasons, respectively. The straw manure plus chemical fertilizer in C.Rs IV, V and VI recorded higher values of rye plant weight, which were1219, 1077, 1499 and 1545, 1251, 1202 g m^{-2} respectively as compared with using straw manure alone without chemical fertilizer in C.R. VII in both seasons. The farmyard manure plus chemical fertilizer produced higher rye plant weight in C.R. XI (1238 g m^{-2}) compared with using farmyard manure alone without chemical fertilizer in C.R. X (1166 g m^{-2}) in the 2018 year. The highest values of plant weight resulted in C.R.X (1771 g m^{-2}) and C.R. XI (1637 g m^{-2}) in 2019 and without significant differences between them. In addition, C.R. VIII resulted in higher values of rye plant weight (1652 and 1764g m^{-2}) in both seasons, respectively. Moreover, chemically fertilized lupine green manure in C.Rs VIII, XII, XIII, and XIV resulted in higher plant weight/m^2 compared with lupine green manure alone, without chemical fertilizer in C.R. XV in both years. This can be concluded that those C.Rs, where lupine was grown for seed (III and VIII), for forage (IX) and lupine green manure(II, XII, XIII and XIV) produced higher rye plant weight with chemical fertilizer than those without chemical fertilizer (XV). Applying fallow in C.R. I produced high plant weight/m^2 (1212 and 1356 g m-2) in the 2018 and 2019 seasons.

Table 1. Number of crop rotations, fertilization methods, and fertilization doses of the rye before its sowing in the Westsik's crop rotation experiment.

Number of crop rotation	N (kg ha^{-1})	P_2O_5 (kg ha^{-1})	K_2O (kg ha^{-1})	Farmyard manure (t ha^{-1})	Straw manure (t ha^{-1})	Lupine as the main crop	Lupine green manure as a second crop
I						-	-
II		31	28			green manure	-
III		31	28			for seed	-
IV	65	47	56		3.48	-	-
V	65	47	56		11.30	-	-
VI	65	47	56		26.10	-	-
VII					26.10	-	-
VIII	43	31	28			for seed	+
IX	43	31	28			for green forage	-
X				26.1		-	-
XI		31	28	26.1		-	-
XII		31	28			-	+
XIII	43	31	28			-	+
XIV	43	31	28			-	+
XV						-	+

Seed weight per m^{-2}

Data presented in Table 3 clearly showed that seed weight per m-2 was significantly affected by C.R. Straw manure with chemical fertilizer in C.Rs. IV, V and VI resulted in higher seed weight compared with straw manure alone, without chemical fertilizer in C.R. VII, in both years. Farmyard manure plus chemical fertilizer resulted in higher seed weight in C.R. XI (429.3 and 577.7 g m-2) compared with using farmyard manure alone, without chemical fertilizer in C.R. X (405.1 and 573.7 g m-2), in both years. The highest values of seed weight/m2 were recorded in C.R. VIII (606.6 g m-2) in 2018 and in C.R.XI (577.7 g m-2) in 2019. Application of lupine green manure as a second crop plus chemical fertilizer resulted in higher seed weight in C.Rs VIII, XII, XIII and XIV as compared with lupine green manure as a second crop alone, without chemical fertilizer in C.R. XV, in both years. Generally, the data also showed that not only organic fertilizer but chemical fertilizer is very important to increase the rye yield components.

Spike number per m^{-2}

The data presented in Table 4 revealed the significant differences in spike number measured in crop rotations with different fertilization methods in both years. The data of C.R. VII was significantly different from those of the C.Rs VIII and X but was not different from all other C.R. data in 2018. The data of C.R. VI was significantly different from C.Rs II, III and IV data, but was not different from the remained C.Rs data in 2019. The C.Rs II, III and IV data were significantly different from C.Rs VI, VII and VIII data, but were not different from all the remained ones in 2019. Straw manure plus chemical fertilizer in C.Rs IV, V, VI)(523, 483, and 641 spike m^{-2}) resulted in a higher number of spike/m^2 than using straw manure only, without chemical fertilizer as in C.R. VII (372 spike m-2) in 2018. The C.R. X resulted in the highest number of spikes (745 spike m^{-2}) in 2018, while in the 2019 season, the highest number of spikes (640 spikes m-2) resulted in the C.R. XI. In addition, the data in Table 4 clearly showed that among the lupine C.Rs (II, III, VIII, and IX) during 2018, the C.R. VIII produced the highest number of spikes (679 spike m-2). In 2019 CR II produced the highest result (717 pc m-2), but in 2018 this C.R. produced the lowest number of spikes per m-2 (515 pc m-2). Growing lupine green manure as a second crop with or without chemical fertilizer (C.Rs XII, XIII, XIV, and XV) did not produce a high number of spikes. Also, C.R. I (fallow) was nearly the same in both analyzed years (599 and 604 pc m^{-2}).

Table 2. Rye plant weight/m2as affected by fertilization methods and fertilization doses (mean ± Standard deviation, n=3).

Number of crop rotation	Rye plant weight g m^{-2}	
	2018	**2019**
I	1 212 ^{bcde} ±154.00	1 356 ^{cd} ±102.84
II	1 362 ^{def} ±42.75	1 489 ^{cd} ±134.42
III	1 341 ^{def} ±177.85	1 764 ^{d} ±44.54
IV	1 219 ^{bcde} ±216.07	1 545 ^{cd} ±114.41
V	1 077 ^{bcd} ±92.72	1 251 ^{bc} ±154.79
VI	1 499 ^{ef} ±231.39	1 202 ^{bc} ±196.00
VII	325 ^{a} ±69.89	539 ^{a} ±86.31
VIII	1 652 ^{f} ±29.59	1 345 ^{cd} ±91.09
IX	1 311 ^{def} ±85.44	1 637 ^{cd} ±72.23
X	1 166 ^{bcde} ±128.18	1 771 ^{d} ±294.48
XI	1 238 ^{cde} ±118.05	1 670 ^{cd} ±170.56
XII	1 025 ^{bcd} ±52.16	1 395 ^{cd} ±130.08
XIII	871 ^{bc} ±162.85	1 379 ^{cd} ±201.06
XIV	855 ^{b} ±149.48	1 640 ^{cd} ±348.26
XV	379 ^{a} ±41.48	871 ^{ab} ±108.45
Main averages	**1 102**	**1 390**

1000 seed weight

The results of statistical analysis show significant differences between the 1000 seed weights in both years (Table 5). The data of C.R. VII and XV were significantly different from all the other data. These crop rotations produced the least 1000 seed weight in 2018. The data of C.R. VIII and XI were significantly different from C.Rs. I, VII, X, XII, XIII, XIV and XV data but was not significantly different from C.Rs. II, III, IV, V, VI and IX data. The C.Rs. VIII and XI produced the highest values of 1000 seed weight. Also, the data of C.R. VII was not significantly different from C.Rs. II, IV and XV but were significantly different from all other crop rotations data in 2019. As presented in Table 5 C.Rs. IV, V and VI resulted in a higher 1000 seed weight than in C.R. VII in both years. The farmyard manure treatment in C.R. XI produced the highest 1000 seed weight (27.700 g) in 2018 while in CR X (29.667g) during the 2019 season. Also, the results showed that among the lupine-grown C.Rs II, III, VIII and IX, the highest values of 1000 seed weight resulted from C.R. VIII in 2018 (27.567) and CR III in 2019 (27.833). The lowest 1000 seed weight was measured in CR IX (25.900 g) in 2018, while in CR II (25.167 g) in 2019.

Table 3. Seed weight g m^{-2} as affected by fertilization methods and fertilization doses (mean ± Standard deviation, n = 3).

Number of crop rotation	Seed weight g m^{-2}	
	2018	**2019**
I	411.1 ^{bcde} ±62.93	387.9 ^{bcde} ±69.39
II	537.3 ^{ef} ±39.82	429.5 ^{cde} ±80.47
III	474.2 ^{def} ±22.54	543.9 ^{de} ±21.69
IV	452.5 ^{de} ±103.84	496.5 ^{cde} ±65.93
V	402.6 ^{bcde} ±37.25	362.1 ^{bcd} ±58.50
VI	542.7 ^{ef} ±83.77	362.1 ^{bcd} ±69.60
VII	86.9 ^{a} ±31.64	126.3 ^{a} ±22.62
VIII	606.6 ^{f} ±27.32	432.9 ^{cde} ±47.40
IX	488.7 ^{ef} ±23.67	528.7 ^{cde} ±22.90
X	405.1 ^{bcde} ±11.93	573.7 ^{e} ±106.65
XI	429.3 ^{cde} ±49.42	577.7 ^{e} ±54.64
XII	339.6 ^{bcd} ±17.22	412.7 ^{cde} ±37.02
XIII	287.9 ^{b} ±73.58	459.5 ^{cde} ±78.01
XIV	292.2 ^{bc} ±33.89	534.7 ^{cde} ±107.04
XV	113.9 ^{a} ±27.17	237.2 ^{ab} ±47.72
Main averages	**391.3**	**430.5**

Correlation

Correlation analysis results indicated a significant positive correlation between the seed weight per m-2 and other measured rye yield components as presented in Table 6 in both analyzed years. The correlation was significantly positive and very strong between the seed weight/m2 and plant weight (0.977), and between the seed weight and 1000 seed weight (0.776). In terms of yield, the seed weight, the plant weight and 1000 seed weight were the most important component in the 2018 year. There were a significant medium and positive correlation between the seed weight and spike number (0.592). It can be explained that the yield of rye was not determined mainly by the spike number. Furthermore, during 2019 the correlation was significantly positive and very strong between the seed weight and rye plant weight (0.975) and between the seed weight and 1000 seed weight (0.704). In terms of yield, the seed weight, the plant weight and 1000 seed weight were the most important component in the 2019 year. There was a significantly positive and medium correlation between the rye seed weight and spike number (0.550). These results agree with those obtained by (Leilah and Al-Khateeb 2005; Gulmezoglu et al. 2010; Bhushan et al. 2013; Nouraein 2019.

Table 4. Spike number (pc m^{-2}) as affected by fertilization methods and fertilization doses (mean ± Standard deviation, n=3).

Number of crop rotation	Spike number pc m^{-2}	
	2018	**2019**
I	599 ^{ab} ±170.47	604 ^{abc} ±52.11
II	542 ^{ab} ±105.14	717 ^{c} ±40.01
III	555 ^{ab} ±89.71	701 ^{c} ±52.54
IV	523 ^{ab} ±156.08	709 ^{c} ±14.74
V	483 ^{ab} ±31.06	605 ^{abc} ±44.73
VI	641 ^{ab} ±101.04	515 ^{a} ±79.10
VII	372 ^{a} ±76.86	539 ^{ab} ±50.01
VIII	679 ^{b} ±31.26	534 ^{ab} ±21.63
IX	581 ^{ab} ±80.90	573 ^{abc} ±61.97
X	745 ^{b} ±87.75	599 ^{abc} ±66.58
XI	574 ^{ab} ±27.78	640 ^{abc} ±33.28
XII	523 ^{ab} ±68.03	595 ^{abc} ±46.23
XIII	497 ^{ab} ±48.22	610 ^{abc} ±44.67
XIV	509 ^{ab} ±54.60	678 ^{bc} ±102.52
XV	503 ^{ab} ±92.91	567 ^{abc} ±40.61
Main averages	**554**	**612**

DISCUSSION

Rye yield components

Applying straw manure plus chemical fertilizer in C.Rs IV, V and VI recorded higher values of rye plant weight, seed weight m-2, number of spike m-2 and 1000-seed weight as compared with using straw manure alone without chemical fertilizer in C.R. VII in both seasons. Straw manure treatments could be able to improve the soil's physical and chemical properties, consequently, increase the rye yields parameters (Broumand et al., 2010). Also, organic fertilization promoted the rye plant development significantly as mentioned by (Gill and Meelu, 1982; Jate, 2012; Grantina-Ievina and Ievinsh, 2015). C.R. VIII resulted in higher values of rye plant weight in both seasons, respectively. This can be explained that the C.R. VIII consists of four parts, and this is a complex manuring system with green lupine manure as a main and second crop besides chemical fertilizer, which has a good effect on soil fertility and increases growth characters as well as the yield components of rye plants (Hardarson, 1993; Grantina-Ievina and Ievinsh, 2015; Stępień et al. 2016; Pietrzykowski 2017; Qiuchen 2018). Moreover, green manure has a beneficial effect on plant production, its application reduces the nitrogen losses from the soil, which applies higher nitrogen supply for the next plant, consequently, results in higher yield (Thorup-Kristensen and Bertelsen, 1996). Also, C.R. I (fallow) was nearly the same. These results are in harmony with those obtained by (Sadras and Slafer, 2012; Würschum, 2018; Dreccer et al., 2019). Also, the good effect of organic and chemical fertilization application together on rye weight m-2 was noticed by (Saleque et al. 2004; Bokhtiar and Sakurai, 2005; Mottaghian et al. 2008). Moreover, C.Rs VIII, XII, XIII and XIV resulted in higher plant weight/m2 compared with lupine green manure alone in C.R. XV in both years.

Table 5. 1000 seed weight (g) as affected by the Number of crop rotations, fertilization methods, and fertilization doses (mean ± Standard deviation, n=3).

Number of crop rotation	1000 seed weight (g)	
	2018	**2019**
I	24.967 ^{b}±0.86	27.000 ^{bc}±1.80
II	26.167 ^{bc}±0.40	25.167 ^{ab}±1.04
III	26.000 ^{bc}±1.83	27.833 ^{bc}±0.57
IV	26.233 ^{bc}±1.38	26.000 ^{abc}±0.50
V	26.567 ^{bc}±0.63	25.167 ^{ab}±2.36
VI	25.933 ^{bc}±0.73	26.500 ^{bc}±0.86
VII	21.500 ^{a}±0.26	22.500 ^{a}±0.50
VIII	27.567 ^{c}±0.46	26.167 ^{bc}±0.28
IX	25.900 ^{bc}±0.75	27.167 ^{bc}±1.60
X	24.233 ^{b}±0.28	29.667 ^{c}±1.60
XI	27.700 ^{c}±0.70	28.333 ^{bc}±2.56
XII	24.933 ^{b}±0.73	27.167 ^{bc}±0.28
XIII	24.933 ^{b}±0.45	29.833 ^{c}±0.76
XIV	24.733 ^{b}±0.80	29.000 ^{bc}±0.86
XV	21.667 ^{a}±0.23	25.167 ^{ab}±1.15
Main averages	**25.269**	**26.844**

This can be concluded that those C.Rs, where lupine was grown for seed (III and VIII), for forage (IX) and lupine green manure (II, XII, XIII and XIV) produced higher rye plant weight with chemical fertilizer than without chemical fertilizer (XV). These results are in agreement with those obtained by (Nedzinskiene, 2006; Cioremele and Contoman, 2015; Wojtkowiak et al. 2015; Stępień et al. 2016). 1000 seed weight is an important factor in terms of rye yield and is significantly affected by fertilization methods and the doses used (Sadras and Slafer, 2012; Dreccer et al. 2019).

Table 6. The correlation coefficients of the linear relationship (R-values) among rye seed weight and other rye yield parameters (n=3).

Person's correlation	Plant weight (g m^{-2})	Spike number (pc m^{-2})	1000 seed weight (g)
Seed weight (g m^{-2})in 2018	0.977**	0.592**	0.776**
Seed weight (g m^{-2})in 2019	0.975**	0.550**	0.704**

Person's correlation P<0.05** Correlation is significant at the 0.01 level. *Correlation is significant at the 0.05 level.

As presented in Table 5 C.Rs. IV, V and VI resulted in a higher 1000 seed weight than in C.R. VII in both years. Applying lupine green manure as a second crop with chemical fertilizer in C.Rs XII, XIII and XIV resulted in a higher 1000 seed weight than applying lupine green manure as a second crop alone, without chemical fertilizer in C.R. XV in both years. Using all of the lupine forms (grown lupine for seed or green manure) has high importance due to its nitrogen-fixing capability and role in sustainable crop production systems (Bhardwaj et al. 1998). The green manure application promotes not only crop growth but soil microbial activity, too (Tejada et al., 2008). Our results proved that farmyard manure with chemical fertilizer was more effective than the other applied organic manure form with chemical fertilizer on the 1000 seed weight. Applied fertilization (organic manure with or without chemical fertilizer) has a very important role in terms of the development of grain yield. According to (Erhart et al. 2005) composting with and without chemical fertilizer resulted in higher grain yield (10% on average) than the unfertilized control treatment. Higher rye yield parameters at organic manure plus chemical fertilization application can be explained by the manuring system effect. On the soil N content increase, the soil pH decreased, and P and K deficit will arise in the soil. Applying together organic and chemical fertilizers results in higher microbial and enzyme activity in the soil. Consequently, the long-term application of organic manure with chemical fertilizer has a positive effect on grain yield and soil quality (Liu et al. 2010).

CONCLUSION

Our research indicated that all studied characters were significantly affected by fertilization methods and doses in both years. Different fertilization methods resulted in a different effect on the yield component of the rye plant in both years. Straw manure without chemical fertilizer produced the lowest rye yield components, in both years. Also, growing lupine green manure as a second crop without chemical fertilizer produced the second-lowest data of rye plant weight and seed weight in both analysed years. It could be noticed that farmyard manure from the applied three organic manure (farmyard manure, straw manure, and lupine green manure) with chemical fertilizer was the most effective for the rye yield component production. Moreover, the results of the statistical analysis proved a positive, medium, and strong correlation between the rye yield components in both years.

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Survey and Identification of Major Insect Pests of Seed Spices in Ethiopia

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ABSTRACT

Insect pests inflict damage to humans, farm animals and crops. Subsistence cash crop production is essential for the growing population of Africa. Seed spices occupy an important place for their flavoring, culinary uses and essential oil derivatives. Survey was conducted in major growing areas of Ethiopia during 2016 and 2018 main cropping season with an aim of identifying problematic insect pests and providing baseline information on pests of black cumin, fenugreek, coriander and white cumin in different agro-ecologies of Ethiopia. Samples were randomly collected from insect infested plants from each study location for laboratory inspection. Specimens of unidentified insects were kept in vials containing ethanol for identification. All specimens were classified into their respective orders, families, genera and species. Generally, the result reveled that pod borer was a common insect pest on black cumin and fenugreek and aphid was a common insect pest recorded on fenugreek. Management study which solves the problem of insects on seed spice must be designed in the future on pod borer, aphids, cut worm and leaf minor.

INTRODUCTION

Insects are the most diverse species of animals living on earth. Insects are undoubtedly the most adaptable form of life as their total numbers far exceed that of any other animal category (Mohamed and Sallam, 2011). The majority of insects are directly important to humans and the environment. For example, several insect species are predators or parasitoids of other harmful pests; others are pollinators, decomposers of organic matter, or producers of valuable products such as honey or silk. Some can be used to produce pharmacologically active compounds such as venoms or antibodies. Less than 0.5 percent of the total number of known insect species are considered pests, and only a few of these can be a serious menace to people. Insect pests inflict damage on humans, farm animals, and crops. Subsistence cash crop production is essential for the growing population of Africa. In Ethiopia highland seed spices are produced in different areas. But till now detailed assessments of insect pests are not undertaken in different parts of the country. So, the objective of this study was to identify major insect pests of seed spices and know their distribution across the country.

MATERIAL AND METHODS

Study site and materials

The study was conducted at different locations namely; Shirka, Hela Zembaba, Birbof Chole, Hela Tereta, Akaki, Chafe Donsa, Bishoftu, Goro, Ginir, Gololcha, Sirinka, Takusa, Chefa, Dembia and Wolkite from 2016 to 2018. The quadrant to sample the spice population, sample bag to collect the unidentified pest and refrigerator to keep the specimen was used during the survey.

Insect identification

A survey for the insect pest of major seed species of Ethiopia was conducted from October 2016 to November 2016 and October 2018 to November 2018 for two years. Potential seed spices-producing regions of Ethiopia (Oromia, Amhara, and SNNPRS) were surveyed for the occurrence of the insect pests. A stratified random sampling technique was employed to sample regions, woredas, and zones. Each woreda survey field was selected from the farmer's field. From each zone, six small-scale producers were selected after getting the name of the producers and size of production data from the Bureau of Agriculture (BOA), and applying a random sampling technique to sample the field. From each field, a bunch was sampled randomly by using "X" path, when the plantation was sole and followed standard recommended cultivation. Each stand was divided into three strata (near the ground level, at the middle and at the top) to sample the leaves. From each stratum, four leaves were selected. Totally 12 leaves were sampled from each stand to record the severity and incidence of insect damage around the pest foci, in order to collect the insect at any of its growth stages (larva, pupa, or adult). Infected spices were diagnosed for characteristic internal and/or external symptoms of the pest attack and the incidence and severity of the damages it caused was recorded. Besides, information related to the history of the field, such as type of crops and/or cultivars grown, planting year (estimated age), types of cultural practices and previous cropping system of the field was recorded.

RESULTS AND DISCUSSION

From the survey result, we observed that many insects affect seed spices and contribute to yield losses occurring in the field. Oromia, Amhara, and South Nations Nationalities and People Regions were surveyed and the following results were obtained in the following table. This study is in line with Gopa L.

For instance, pod borer, aphids and leaf minor were the serious insects observed in all areas in addition to skeletonizer. From the table below we understand that pod borer, aphids, and cutworms were the major insect pests challenging the production of black cumin and fenugreek spices in the area.

CONCLUSION

On black cumin pod borer and aphid were found the common insect pests as the main production limiting factor and on coriander and fenugreek aphids were found the common insect pests as the main production limiting factor. The results obtained may assist in developing an integrated control program for this insect pest identified. More detailed investigations should be carried out on the biology of insect pests. In the future, integrated pest management should be designed to manage these economically important insect pests.

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Table 1. The average insects observed during the survey on different spices at different locations.

Spice										Order	Family	Seed spice damaged part/s
Region	Study location	Black cumin: Insect pests recorded	Damage (%)	Fenugreek: Insect pests recorded	Damage (%)	Coriander: Insect pests recorded	Damage (%)	White cumin: Insect pests recorded	Damage (%)			
Oromia	Bishoftu	Pod borer	35	Pod borer	11	Pod borer	1	Pod borer	0	Hemiptera	Miridae	Pods
		Aphids	9	Aphids	15	Aphids	2	Aphids	3	Homoptera	Aphididae	Leaves and shoots
		Cutworm	10	Cutworm	4	Cutworm	1	Cutworm	1	Lepidoptera	Noctuidae	Seedlings
		Leaf minor	3	Leaf minor	2	Leaf minor	1	Leaf minor	0	Diptera	Agromyzidae	Leaf
	Chafe Donsa	Pod borer	25	Pod borer	9	Pod borer	0	Pod borer	0	Hemiptera	Miridae	Pods
		Aphids	6	Aphids	14	Aphids	1	Aphids	2	Homoptera	Aphididae	Leaves and shoots
		Cutworm	5	Cutworm	3	Cutworm	1	Cutworm	1	Lepidoptera	Noctuidae	Seedlings
		Leaf minor	2	Leaf minor	1	Leaf minor	1	Leaf minor	0	Diptera	Agromyzidae	Leaf
	Shirka (Hela Zembaba, Hela Tereta)	Pod borer	32	Pod borer	10	Pod borer	0	Pod borer	0	Hemiptera	Miridae	Pods
		Aphids	10	Aphids	17	Aphids	2	Aphids	2	Homoptera	Aphididae	Leaves and shoots
		Cutworm	10	Cutworm	2	Cutworm	0	Cutworm	0	Lepidoptera	Noctuidae	Seedlings
		Leaf minor	3	Leaf minor	1	Leaf minor	0	Leaf minor	0	Diptera	Agromyzidae	Leaf
	Kulumsa	Pod borer	20	Pod borer	7	Pod borer	0	Pod borer	0	Hemiptera	Miridae	Pods
		Aphids	6	Aphids	16	Aphids	3	Aphids	1	Homoptera	Aphididae	Leaves and shoots
		Cutworm	8	Cutworm	2	Cutworm	1	Cutworm	1	Lepidoptera	Noctuidae	Seedlings
		Leaf minor	3	Leaf minor	1	Leaf minor	1	Leaf minor	0	Diptera	Agromyzidae	Leaf
	Sinana	Pod borer	30	Pod borer	6	Pod borer	0	Pod borer	0	Hemiptera	Miridae	Pods
		Aphids	13	Aphids	12	Aphids	3	Aphids	2	Homoptera	Aphididae	Leaves and shoots
		Cutworm	5	Cutworm	2	Cutworm	1	Cutworm	1	Lepidoptera	Noctuidae	Seedlings
		Leaf minor	3	Leaf minor	2	Leaf minor	1	Leaf minor	0	Diptera	Agromyzidae	Leaf
	Bale Robe	Pod borer	15	Pod borer	6	Pod borer	0	Pod borer	0	Hemiptera	Miridae	Pods
		Aphids	8	Aphids	14	Aphids	3	Aphids	2	Homoptera	Aphididae	Leaves and shoots
		Cutworm	7	Cutworm	2	Cutworm	0	Cutworm	1	Lepidoptera	Noctuidae	Seedlings
		Leaf minor	2	Leaf minor	1	Leaf minor	1	Leaf minor	0	Diptera	Agromyzidae	Leaf
		Pod borer	12	Pod borer	5	Pod borer	0	Pod borer	0	Hemiptera	Miridae	Pods

	Jimma (Bashasha)	Aphids	7	Aphids	12	Aphids	2	Aphids	2	Homoptera	Aphididae	Leaves and shoots
		Cutworm	3	Cutworm	1	Cutworm	1	Cutworm	1	Lepidoptera	Noctuidae	Seedlings
		Leaf minor	2	Leaf minor	1	Leaf minor	0	Leaf minor	0	Diptera	Agromyzidae	Leaf
	Ginir	Pod borer	18	Pod borer	7	Pod borer	0	Pod borer	0	Hemiptera	Miridae	Pods
		Aphids	6	Aphids	13	Aphids	3	Aphids	1	Homoptera	Aphididae	Leaves and shoots
		Cutworm	7	Cutworm	2	Cutworm	1	Cutworm	1	Lepidoptera	Noctuidae	Seedlings
		Leaf minor	2	Leaf minor	1	Leaf minor	1	Leaf minor	1	Diptera	Agromyzidae	Leaf
Amhara	Srinka	Pod borer	18	Pod borer	5	Pod borer	0	Pod borer	1	Hemiptera	Miridae	Pods
		Aphids	9	Aphids	10	Aphids	2	Aphids	2	Homoptera	Aphididae	Leaves and shoots
		Cutworm	2	Cutworm	1	Cutworm	0	Cutworm	1	Lepidoptera	Noctuidae	Seedlings
		Leaf minor	1	Leaf minor	1	Leaf minor	1	Leaf minor	0	Diptera	Agromyzidae	Leaf
	Dembia	Pod borer	12	Pod borer	6	Pod borer	0	Pod borer	0	Hemiptera	Miridae	Pods
		Aphids	7	Aphids	10	Aphids	2	Aphids	1	Homoptera	Aphididae	Leaves and shoots
		Cutworm	2	Cutworm	2	Cutworm	1	Cutworm	1	Lepidoptera	Noctuidae	Seedlings
		Leaf minor	1	Leaf minor	1	Leaf minor	1	Leaf minor	0	Diptera	Agromyzidae	Leaf
	Chefa	Pod borer	16	Pod borer	5	Pod borer	0	Pod borer	0	Hemiptera	Miridae	Pods
		Aphids	1o	Aphids	9	Aphids	2	Aphids	2	Homoptera	Aphididae	Leaves and shoots
		Cutworm	2	Cutworm	2	Cutworm	0	Cutworm	1	Lepidoptera	Noctuidae	Seedlings
		Leaf minor	2	Leaf minor	2	Leaf minor	0	Leaf minor	0	Diptera	Agromyzidae	Leaf
	Takusa	Pod borer	19	Pod borer	6	Pod borer	0	Pod borer	0	Hemiptera	Miridae	Pods
		Aphids	8	Aphids	12	Aphids	2	Aphids	2	Homoptera	Aphididae	Leaves and shoots
		Cutworm	2	Cutworm	1	Cutworm	1	Cutworm	1	Lepidoptera	Noctuidae	Seedlings
		Leaf minor	2	Leaf minor	1	Leaf minor	0	Leaf minor	0	Diptera	Agromyzidae	Leaf
SNNPRS	Wolkite	Pod borer	12	Pod borer	5	Pod borer	0	Pod borer	0	Hemiptera	Miridae	Pods
		Aphids	7	Aphids	13	Aphids	2	Aphids	2	Homoptera	Aphididae	Leaves and shoots
		Cutworm	1	Cutworm	2	Cutworm	1	Cutworm	1	Lepidoptera	Noctuidae	Seedlings
		Leaf minor	1	Leaf minor	1	Leaf minor	0	Leaf minor	0	Diptera	Agromyzidae	Leaf

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