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Highlights

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Discovering Thoughts, Inventing Future

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A Semi-Empirical Model of Winter Wheat Grain Protein Content

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Abstract- Winter wheat grain protein content (GPC) is an important criterion for assessing grain quality. A timely and simple GPC model is urgently required for GPC prediction ahead of maturity. The GPC model included regressional models of dry matter and N accumulation and translocation for anthesis and post-anthesis stages, and incorporated both soil nitrogen (N) supply and meterological factors based on historical as well as current season data, final GPC were calculated as the ratio of N accumulation to dry matter in grain at maturity. This study conducted six field experiments during the 2003–2006 and 2008–2011 growing seasons to establish and validate the model. A three-way factorial arrangement of N fertilization, sowing date, and cultivar was conducted using a split-plot design. Critical growth parameters were determined by field measurements, and historical seasonal meteorological data covering the growing period were collected.

Keywords: triticum aestivum; grain nitrogen content; dry matter; meteorological factor.

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A Semi-Empirical Model of Winter Wheat Grain Protein Content

Qian Wang [α], Cun-jun Li [σ], Yuan-fang Huang [ρ], Wu-de Yang [ω], Wen-jiang Huang [¥] & Ji-hua Wang [§]

Highlights

- Annual wheat grain yield trend could be better captured by the accumulated meteorological factor established here
- The rainfall ratio of total growing season to post-anthesis period were found an influential meterological factor to promote post-anthesis nitrogen and dry matter translocation and assimilation processes, especially for the dry matter
- by merging cultivars data the regressional grain protein content models could achieve acceptable prediction accuracy given the future regional application with variety of cultivars planted

Abstract- Winter wheat grain protein content (GPC) is an important criterion for assessing grain quality. A timely and simple GPC model is urgently required for GPC prediction ahead of maturity. The GPC model included regressional models of dry matter and N accumulation and translocation for anthesis and post-anthesis stages, and incorporated both soil nitrogen (N) supply and meterological factors based on historical as well as current season data, final GPC were calculated as the ratio of N accumulation to dry matter in grain at maturity. This study conducted six field experiments during the 2003–2006 and 2008–2011 growing seasons to establish and validate the model. A three-way factorial arrangement of N fertilization, sowing date, and cultivar was conducted using a split-plot design. Critical growth parameters were determined by field measurements, and historical seasonal meteorological data covering the growing period were collected. The normalized root mean square error (nRMSE, %), which is defined as RMSE divided by the mean of the observed value, multiplied by 100, was adopted to evaluate the model performance. The major results were as follows: (1) The prediction performance of dry matter (DM) and N accumulation (NA), and translocation during the pre-anthesis and post-anthesis periods were different; it was poorer for the former and better for the latter. However, GPC prediction was not significantly affected by the intrinsic ratio-form of the GPC prediction; (2) meteorological factors could capture the overall interannual trends of the corresponding dry matter and N sub-models in an acceptable manner; (3) nRMSE and R^2 of the semi-empirical GPC model (Exp.4 and Exp. 6) were 8.91, 4.50, 0.64, and 0.46, respectively, and that of the simple linear model (Exp.4) were13.3and 0.42, respectively. The established semi-empirical model significantly improved the interannual and intra-annual prediction accuracy compared to the simple linear model.

Keywords: triticum aestivum; grain nitrogen content; dry matter; meteorological factor.

I. Introduction

Wheat (*Triticumaestivum* L.) is an important staple grain, with a global production of 766 million tons in 2019 (FAO, 2020). Sustaining grain quality in dynamic environments has been a research focus because of the growing market requirements for food nutrition, product functionality, and commodity profits. Grain protein concentration (GPC) and composition largely affect the nutritional and end-use properties of dough mixing and rheological characteristics (Nuttall et al., 2017). Numerous studies have been conducted to determine the major factors influencing grain quality, mostly GPC, which includes genetics, management, and the environment.

GPC is the net result of independent starch and protein accumulation in the grain, and applying Nitrogen (N) fertilizer is commonly considered a practical way to improve GPC (Ercoli et al., 2008; Subedi et al., 2007). From agronomical and ecophysiological perspectives, crop nitrogen accumulation is closely related to crop growth rate and biomass accumulation under ample soil availability. It depends on soil mineral N availability, distribution, and root distribution under suboptimal N supply (Gastal and Lemaire, 2002). Because of the critical role of N in wheat growth, the mechanisms of N uptake and redistribution in wheat have been depicted in detail in simulation models, with the simulation results

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prone to be largely affected by the key parameters of crop N demand and supply processes (Jamieson and Semenov, 2000).

In addition to N, climatic conditions often exert notable effects on crop growth and grain quality. Pan et al. (2006a) reported that reliable GPC prediction results based on the stepwise regression method were achieved with climatic factors that mainly covered the grain-filling period as independent variables. With the aid of detailed genotypic parameters acquired by cultivar experiments, the model can explain as much as 94% of GPC variation using validation data from different site-year combinations. Similarly, Li et al. (2020) obtained robust GPC predictions using a hierarchical linear model based on climatic factors and cultivar parameters. As reported by Pan et al., the major difference between the climatic factors and the aforementioned ones is that the latter is before anthesis and covers a period of one month. A recent review (Nuttall et al., 2017) reported that under climate change, elevated atmospheric carbon dioxide (CO_2) consistently reduced the GPC of wheat, and heat stress contributed to a significant weakening of dough properties. Furthermore, rainfall during wheat grain maturation severely reduces grain glutenin polymers, which are intrinsically related to grain functional properties (Koga et al., 2020).

N accumulation (NA), dry matter (DM), and remobilization related to pre- and post-anthesis/head periods have become a research focus. Ercoli et al. (2008) suggested that grain yield (GY), DM, NA, and remobilization were positively affected by N availability and negatively affected by water stress during grain filling and that there was a significant interaction between N rate and water stress for grain N concentration (GNC). Tsukaguchi et al. (2016) observed in another crop belonging to Gramineae that both plant N status before and after heading is sensitive to rice GPC, with the latter being greater. Barbottin et al. (2005) indicated that the main sources of variation in the amount of remobilized N, N uptake during flowering, and N remobilization efficiency were the environment (including site, treatment, and year, respectively).

Due to its large-area coverage, non-damage sampling, and fast acquisition, remote sensing has been widely applied in crop growth monitoring (Thenkabail, 2003). Thus, GPC forecasting can be achieved in advance according to the crop growth conditions obtained by remote sensing based on established models linking GPC with crop growth variables. In addition, such models differ in mechanisms, such as process-based crop growth models, semi-empirical models that mainly consider both pre- and post-anthesis processes, and simple empirical models (Li et al., 2015; Li et al., 2008; Song et al., 2009; Wang et al., 2004). The semi-empirical model appears to be the most promising candidate among the three types of models related to remote sensing data. Moreover, it is easier to compute GPC using a semi-empirical model since it uses fewer processes compared to the complex assimilation algorithms of the growth model. Furthermore, the semi-empirical model can also explain NA and DM and remobilization related to the pre- and post-anthesis periods. Thus, the semi-empirical model appears more applicable toassess medium-to large-scale phenomena (Cichota et al., 2010).

This study conducted multi-year experiments in Beijing, comprising 3factors, including 1–4 N fertilization rates (NF), 12 cultivars, and three planting dates. We aimed to solve the following targets: (1) analyze aboveground DM, NA at anthesis, and GY and grain nitrogen accumulation (GNA) at maturity, and establish new transfer coefficient sub-models that link N and DMat anthesis tothose at the maturity stage; (2) collect weather data such as rainfall, average temperature (T), and solar radiation (SRAD),to establish meteorological factor sub-models that enhance the empirical prediction of NA and DM at anthesis, as well as new transfer coefficients. (3) Soil N mineralization is considered to improve NA prediction, particularly with respect to N fertilization. Provided that the key parameters are fitted to local experimental data beforehand, the approach can be extended to other regions outside of Beijing.

II. Model Description

Mainly focusing on the post-anthesis period, a semi-empirical GPC model was established based on four sub-models and four accompanying meteorological factors involved in DM and N assimilation and their translocation. The basic structure of this model is as follows (the acronyms are listed in Table A1 in the Appendix):

$$GPC = 5.7 \cdot GNA/GY \cdot 100, \quad (1)$$

where 5.7 is the transformation coefficient (Spratt, 1979) used to calculate GPC from GNC.

$$GY_i = \frac{AMF_{\text{tot},i}}{AMF_{\text{tot},r}} \times DM \times \frac{MFR_{R\beta_{C,i}}}{MFR_{R\beta_{C,r}}} \cdot 2 \cdot R_{\beta_{C,i}}, \quad (2)$$

where (1) $AMF_{\text{tot},i}$, $AMF_{\text{tot},r}$ are the accumulated meteorological factors based on data from the whole growing period for growing seasons *i*, *r*, respectively; (2) *i*, *r*are the growing seasons corresponding to model validation and model establishment experiments, respectively; (3) *DM* (kg ha^{-1}) corresponds to the anthesis stage;(4) $R_{\beta C,i}$ is the ratio corresponding to a transformation of $\beta_{C,i}$, which is the DM post-anthesis transfer coefficientin growing season *i*and will be illustrated in detail in the following sections; (5) 2 is the coefficient along with the transformation of $R_{\beta C,i}$; and (6)$MFR_{\beta C,i}$, $MFR_{\beta C,r}$ are the meteorological factors for $\beta_{C,i}$ in growing seasons*i, r,* respectively.

$$GNA_i = NA_i \times \frac{MFR_{R\beta_{N,i}}}{MFR_{R\beta_{N,r}}} \cdot 2 \cdot R_{\beta_{N,i}}, \quad (3)$$

where(1) NA_i(kg ha^{-1}) corresponds to growing season *i*, (2) $R_{\beta N,\,i}$, $MFR_{\beta N,\,i}$, $MFR_{\beta N,\,r}$ are defined similarly to the DM counterparts, and (3) 2 is the coefficient along with the transformation of $R_{\beta N,\,i}$.

a) Accumulation of DM, N

Aboveground DM and NA at anthesis are important because GY and GNA at maturity greatly depend on the translocation of pre-anthesis assimilated to the grain (Papakosta and Gagianas, 1991). Crop biomass production is influenced by a variety of environmental factors, which can be seen in solar-driven CERES(Otter-Nacke et al., 1986), CO_2-driven WOFOST (Supit et al., 1994), and water-driven Aqua Cropmodels (Steduto et al., 2009). For simplification, only four variables were considered in modeling aboveground biomass at anthesis based on a linear regression form: leaf area index (LAI) derived from three key growth stages (jointing, heading, and anthesis stages), seed rate (SR), heat sum (ST, i.e., thermal time), and NF. The original LAI (OLAI) was proposed to represent the effects of soil heterogeneity other than those of SR, ST, and NF,which was derived by dividing the measured LAI by a combined factor (CF)using the following formula:

$$CF = 0.5 \cdot \frac{\mathrm{BNN}+NF}{\mathrm{BNN}+NMAX} + 0.25 \cdot \frac{SR}{\mathrm{BSR}} + 0.25 \cdot \frac{ST}{\mathrm{BST}}, \quad (4)$$

where (1)NMAX (kg ha^{-1}) is the highest NF in the field plots;(2)BNN is the basal N nutrition with 60 kg ha^{-1} mineralized N during the growth stage (Ju et al., 2003);(3)BSR is the basal seed rate with 375 seeds m^{-2};(4)BST is the basal heat sum with 2443 ℃ corresponding to the optimum sowing date treatment of the 2009–2010 field experiment;and (5)0.5,0.25, and 0.25 are the assumed weighting coefficients here.

$$OLAI_{\mathrm{sum}} = \left(LAI_{\mathrm{joint}} + LAI_{\mathrm{head}} + LAI_{\mathrm{anth}}\right)/CF, \quad (5)$$

where (1)$OLAI_{\mathrm{sum}}$ is the sum of the original LAI at the jointing, heading, and anthesis growth stages, (2)LAI_{joint} , LAI_{head} , and LAI_{anth} are the measured LAI at relative stages, and (3)*CF* is the combined factor. By adopting the log-formed DM recommended by Lobell and Burke (2010), itwas calculated as follows:

$$\mathrm{Log}_{10}(DM) = \mathrm{a}_1 + \mathrm{a}_2 \times OLAI_{\mathrm{sum}} + \mathrm{a}_3 \times CF + \varepsilon_{\mathrm{a}}, \quad (6)$$

where a_{1-3} are the model coefficients, and ε_{a} is the error term. The values of a_{1-3}were obtained using the least-squares procedure.

$$DM_i = \frac{AMF_{\mathrm{veg},i}}{AMF_{\mathrm{veg},r}} \times DM, \quad (7)$$

where (1)DM_i(kg ha^{-1}) is aboveground DM at anthesis in growing season *i*; and (2) $AMF_{\mathrm{veg},i}$, $AMF_{\mathrm{veg},r}$are the accumulated meteorological factors based on data before anthesis for growing season *i*, *r*, respectively.

Allometric relationships were used to calculate crop Ndemand based on crop biomass (Gastal and Lemaire, 2002). Actual NA at anthesis was set astheminimum crop N demand (BN, kg N ha^{-1}) and soil N supply (SNS, kg N ha^{-1}), with the latter referring to Gao (2004).

$$BN_i = \mathrm{b}_1 \times DM_i{}^{\mathrm{b}_2} \quad (8)$$

where b_{1-2} are the model coefficients obtainedfrom Eq. (6) after the log transformation of both sides.

b) DM & N post-anthesis transfer coefficients

Parameters related to DM, NA, and remobilization within wheat plants (Ercoli et al., 2008) were calculated as follows:

- Post-anthesis DM and N (PDM, PN) as the difference between DM or N content at anthesis and physiological maturity.
- DM remobilization (DMR) = DM at anthesis (DM)–DM of leaves, culms, and chaff at maturity (SDM)
- Nitrogen remobilization (NR) = N content of aboveground vegetation at anthesis (NA)–Ncontent of leaves, culms, and chaff at maturity (SN);

For the estimation of DMR and NR, it was assumed that all the DM and N lost from vegetative plants were remobilized to develop the grain.

DM and N post-anthesis transfer coefficients were calculated based on the above parameters in the same way: $\beta_{\mathrm{C}} = (\mathrm{PDM\text{-}SDM})/\mathrm{DM}$ and $\beta_{\mathrm{N}} = (\mathrm{PN\text{-}SN})/\mathrm{NA}$. Furthermore, GY and GNA could be derived based on two coefficients: $GY = (1+\beta_C) \times DM$and $GNA = (1+\beta_N) \times NA$. From these definitions, β should be more influenced by post-anthesis growth (PDM and PN) and genetic differences (SDM and SN) rather than pre-anthesis growth (DM and NA) since the pre-anthesis stage has finished considering the model prediction time.Given that the three cultivars were similar in gluten type and a sufficient irrigation regime was applied for all treatments, the β values were believed to be affected by post-anthesis meteorological factors to a larger extent. To avoid negative βvalues in the calculation, which makes the interannual comparison complex when metrological factors are involved, β values were changed into ratios (i.e., R_{β_C} and R_{β_N}) following the transformations $R_{\beta_C} = (\beta_C + 1)/2$ and $R_{\beta_N} = (\beta_N + 1)/2$. R_{β_C}andR_{β_N}were constrained in the range of 0–1, with the calculated values outside the range set as 0 or 1, depending on which was closer.

After definition, $R_{\beta_{\mathrm{C}}}$and $R_{\beta_{\mathrm{N}}}$were predicted using the preferential binary linear regression method. By comparing the two-variable combination results from four potential parameter candidates (i.e., CLND, LAI, SLW, and EWT), LAI and SLW were finally chosen with the following linear equations:

$$R_{\beta_{\mathrm{C}}} = \mathrm{c}_1 + \mathrm{c}_2 \times \mathrm{LAI} + \mathrm{c}_3 \times \mathrm{SLW} + \varepsilon_{\mathrm{c}} \quad (9)$$

$$R_{\beta_N} = d_1 + d_2 \times LAI + d_3 \times SLW + \varepsilon_d \quad (10)$$

where CLND (kg ha^{-1}) is the canopy leaf nitrogen density, SLW (kg m^{-2}) is the specific leaf weight, EWT (mm) is the leaf equivalent water thickness (Yilmaz, 2008), and c_{1-3} and d_{1-3} are the model coefficients.

$$CLND = CLDM \times CLNC, \quad (11)$$

$$SLW = LDM/LAI, \quad (12)$$

$$Log_{10}(LDM) = e_1 + e_2 \times CLND + e_3 \times LAI + \varepsilon_e, \quad (13)$$

where CLDM (kg ha^{-1}) is the top two leaf DM at anthesis, CLNC is the leaf nitrogen content corresponding to CLDM, LDM (kg m^{-2}) is the leaf DM at anthesis, and e_{1-3} are model coefficients.

c) Meteorological factors

The effects of weather conditions on wheat GY have been extensively studied (Ferris et al., 1998; Landau et al., 2000;Sadras et al., 2003;Schillinger et al., 2008). After long-term adaptation to the local environment, high GY should be achieved if the growing season weather is identical to the historical average climate conditions. Based on this assumption, the meteorological factors for DM at anthesis and GY were calculated following the algorithms of Lakatos (1997):

$$\eta(X) = \begin{cases} 1 - (1 - P_n) \times \left|\frac{X - \overline{X}}{\overline{X} - X_n}\right|, X < \overline{X} \\ 1 - (1 - P_x) \times \left|\frac{X - \overline{X}}{X_x - \overline{X}}\right|, X > \overline{X} \end{cases}, \quad (14)$$

where (1) η(*X*) (dimensionless) is the weighting function;(2) *X is* the climate data, including T, SRAD, and standard precipitation index (SPI) (Mckee et al., 1993);(3) $\overline{X}$ is the historical average value of climate indices over the growing season;(4) X_n and X_x are the minimum and maximum values of the historical climate indices over the growing season, respectively; and (5) P_n and P_x are the probable values corresponding to X_n and X_x, respectively, calculated by the probability density function of the standard normal distribution based on the historical long-term data series.

$$AMF = \sum_{t=1}^{j} min[\eta(\text{SRAD}(t)), \eta(T(t)), \eta(\text{SPI}(t))] \; j = 1, 2, 3, \cdots, n \quad (15)$$

where (1) *AMF* (dimensionless) is the accumulated meteorological factor for DM and GY,(2) *t* is the time, and *j* is the number of ten-day periods in the growing season. A month can be divided into three ten-day periods and the rest of the days as the last ten-day period except for the first two ten-day periods. AMF_{veg} and AMF_{tot} can thus be calculated based on Equation 14to determine the aboveground DM at anthesis and GY at maturity, respectively.

MFR_{β_C} and MFR_{β_N} are meteorological factors for R_{β_C} and R_{β_N}, respectively, and were defined in the same way as R_{β_C} and R_{β_N}. Based on cultivar Jing 9428, MFR_{β_C} and MFR_{β_N} were calculated using four model-establishing experiments. After comparing the correlation coefficients between multiple meteorological factors during anthesis and maturity and MFR_{β_C} and MFR_{β_N}, $Rain_{tot}/Rain_{fill}$ was identified as the best candidate variable for prediction, as follows:

$$MFR_{\beta_C} = f_1 + f_2 \times Rain_{tot}/Rain_{fill} + \varepsilon_f \text{ and} \quad (16)$$

$$MFR_{\beta_N} = g_1 + g_2 \times Rain_{tot}/Rain_{fill} + \varepsilon_g, \quad (17)$$

where $Rain_{tot}/Rain_{fill}$ is the rainfall ratio of the entire growing season to the period during anthesis and maturity, and f_{1-2} and g_{1-2} are model coefficients.

The coefficients of the above equations were based on the experimental data for the four growing seasons, which are listed in Table 1. As shown in the table, except for the nonsignificant sub-model of MFR_{β_N}(P=0.085), all the other sub-models reached significant or even higher levels.

Table 1: Regression coefficients of model parameters

Parameters	Log(DM)	BN	R_{β_C}	R_{β_N}	Log(LDM)	MFR_{β_C}	MFR_{β_N}
Constant	3.228(***)	-0.497(ns)	-0.298(ns)	0.061(ns)	2.576(***)	0.032(ns)	0.151(ns)
$OLAI_{sum}$	0.032(***)	-	-	-	-	-	-
CF	0.249(ns)	-	-	-	-	-	-
DM	-	0.693(0.05)	-	-	-	-	-
LAI	-	-	-0.036(ns)	-0.085(*)	0.137(***)	-	-
SLW	-	-	17.656(*)	14.404(ns)	-	-	-
CLND	-	-	-	-	0.003(**)	-	-
$Rain_{tot}/Rain_{fill}$	-		-	-	-	0.135(*)	0.124(ns)

, **, * indicate the significance at 0.05, 0.01, and 0.001 probability levels, respectively. ns indicates no significance at the 0.05 probability level. — indicates a parameter that is not considered by the model. The same below.*
DM-dry matter at anthesis; BN-crop nitrogen demand at anthesis; -dry matter post-anthesis transfer coefficients; -N post-anthesis transfer coefficients; LDM-leaf dry matter at anthesis; -meteorological factors of dry matter post-anthesis transfer coefficient; -meteorological factors of N post-anthesis transfer coefficient

III. Materials and Methods

a) Treatments

Six growing season experiments were conducted at the National Research and Demonstrating Base of Precision Agriculture, Beijing, China (40°11' N, 116°27' E, 36 m elevation).The experimental design and treatments are summarized in Table 2, and the winter wheat and summer maize rotation systems remained the same for each experiment. During the later period of the growing seasons, the accelerated growth and development produced identical anthesis dates for all treatments, thus showing only one set of meteorological data for the three sowing date treatments. Seeding rates were referenced to local production practices ranging from 375 to 600 seedsm^{-2}. Sprinkler irrigation was adopted after 2005 relative to the previous border irrigation mode. One irrigation before the overwintering period was applied, and another 3–4 irrigations were applied during the re-green, jointing, anthesis, and grain-filling growth stages with an average of 60–75 mm each time.

Four experiments, 2003/2004, 2004/2005/, 2005/2006, and 2009/2010, were used as model establishing experiments (Exp.1–3and Exp.5, respectively), and Exp.5 was the main establishing experiment. Only cultivar Jing 9428 was planted in Exp.1–3. In Exp. 5, three winter wheat cultivars were adopted: Jing 9428, Nongda 195, and Jingdong 13, and the former two were classified as strong-gluten cultivars and the remaining as medium-gluten cultivars. Together with Exp. 5, Exp.1–3 provided data for constructing the meteorological factor sub-models. Two field experiments covering the 2008/2009 and 2010/2011 growing seasons (Exp. 4 and 6, respectively) were used for validation.

Table 2: Experiment design and weather conditions.

Growing Season	Sowing date	Seeding rate (seeds m^{-2})	N fertilization (kg N ha^{-1})					From sowing to anthesis				From anthesis to maturity			
			Sowing	Jointing stage				Water supply (mm)		Global radiation (MJ m^{-2})	Average daily temperature (℃)	Water supply (mm)		Global radiation (MJ m^{-2})	Average daily temperature (℃)
				N1	N2	N3	N4	Rainfall	Irrigation			Rainfall	Irrigation		
Exp.1	S1	460	146		70 (N1-N4)			158	210	2264	6.4	52	38	941	23.3
Exp.2	S1	454	146		105 (N1-N4)			78	240	2402	6.2	81	0	945	21.9
Exp.3	S1	527	146		88 (N1-N4)			26	284	2426	6.8	53	0	672	23.5
Exp.4	S1	450 (S1-S3)	45 (S1-S3)	0	53	105	158	96	270 (S1-S3)	2431	6.8	96 (S1-S3)	68 (S1-S3)	929 (S1-S3)	24.0 (S1-S3)
	S2				105 (N1-N4)			78		2326	6.4				
	S3				105 (N1-N4)			74		2167	5.8				
Exp.5	S1	375	56 (S1-S3)	0	26	53	79	113	270 (S1-S3)	2410	5.3	95 (S1-S3)	68 (S1-S3)	898 (S1-S3)	24.2 (S1-S3)
	S2	525			53 (N1-N4)			108		2293	4.6				
	S3	675			53 (N1-N4)			108		2173	4.0				
Exp.6	S1	600 (S1-S3)	114 (S1-S3)	0 (S1-S3)	59 (S1-S3)	118 (S1-S3)	177 (S1-S3)	74	327 (S1-S3)	2612	5.6	43 (S1-S3)	144 (S1-S3)	893 (S1-S3)	23.7 (S1-S3)
	S2							51		2521	5.3				
	S3							51		2436	5.0				

Exp.1–6 denote 2003/2004, 2004/2005, 2005/2006, 2008/2009, 2009/2010, and 2010/2011 field experiments, respectively. S1–S3 denote the sowing date in a time sequence from the optimal date to the later date. Exp.1–3 had only one sowing date, which corresponded to 10–04, 9–26, and 9–28, respectively.

In Exp.4–6, three sowing date treatments (S1–S3) were designed, corresponding to 9–28, 10–07, and 10–20; 9–25, 10–05, and 10–15; and 9–27, 10–03, and 10–09, respectively. N1–N4 indicate N treatments that received four different top-dressing nitrogen fertilization levels ranging from low to high. S1–S3 or

N1–N4 in parentheses indicate only one sowing date or top-dressing nitrogen fertilization level in the corresponding experimental design. The same below.

Because of the extremely low values presumably caused by sampling or measuring errors, two LAI and two GPC values were deleted from Exp. 5 and 4, respectively. To establish the biomass N sub-model, only13 treatments with top-dressing N fertilization were considered, ignoring the other three nil-top-dressing N fertilization treatments.The cultivars used for Exp.4 and 5 were the same, except for Jingdong 13 in Exp. 5, which was replaced with Jingdong 8 in Exp.4. In Exp. 6, a quasi-four-level orthogonal table design, that is, $L_{16}(4^5)$, was used with three cultivars (Jing 9428, Nongda 195, and Yannong 19), four nitrogen fertilizer rates, and three sowing dates (Table 3). However, the cultivar Jing 9428 was mistakenly replaced by Jing 9843 in plots 3, 4, 7, and 8. Seven additional local popular cultivars planted on the S1 date and with an N3 fertilizer rate were Jing 9843, Jingdong 17, Zhongyou 206, Jingdong 12, Nongda 3432, Nongda 211, and Zhongmai 175. Only 12 treatments from the S1 date, which were far away from the weed-affected treatments, were viewed as suitable for validation because other treatments were affected by weed spread from adjacent freeze-injury treatments in another study. Two of the 12 treatments were removed further for abnormal or missing LAI values.

Table 3: Quasi-four-level orthogonal table design in Exp.6

Num.	Sowingdate	Cultivar	Nfertilization	Num.	Sowingdate	Cultivar	Nfertilization
1	S1	C1	B+N1	9	S2	C1	B+N2
2	S1	C2	B+N3	10	S2	C2	B+N4
3	S1	C3	B+N4	11	S2	C3	B+N3
4	S1	C3	B+N2	12	S2	C3	B+N1
5	S1	C1	B+N4	13	S3	C1	B+N3
6	S1	C2	B+N2	14	S3	C2	B+N1
7	S1	C3	B+N1	15	S3	C3	B+N2
8	S1	C3	B+N3	16	S3	C3	B+N4

C1–C3 denote cultivars Nongda 195, Yannong 19, and Jing 9428, respectively. B+N1 indicates basal nitrogen fertilization when sowing plus N1 level of top-dressing nitrogen fertilization shown in Table 2, while the other N fertilization codes have similar definitions.

b) Sample measurement

Field samples (0.18 m^2 from the center rows) were collected at ground level at the jointing, heading, and anthesis stages in each plot, which was separated into four parts: culm, upper two leaves, lower leaves, and ear. Aboveground NA was calculated by summing the individual organ values obtained by multiplying the organ biomass with the corresponding N concentration. At maturity, two samples of 1 m^2 from the central rows in each plot were cut to measure GY. Each sample was first oven-dried at 105 °Cfor15–20 min,then oven-dried at 70 °Cfor 24 h and weighed. After drying, all samples were ground in a mill to pass through a 1-mm screen.

GPC and grain moisture content were determined by NIT spectroscopy using an Infratec 1241 grain analyzer (FOSS-Tecator, Höganäs, Sweden). Soil organic matter was analyzed by potassium dichromate-sulfuric acid titration using a vario MACRO cube elemental analyzer (Elementar, Hanau, Germany). The total nitrogen content in the soil was analyzed using the Semi-Micro-Kjeldahl method with a KJELTEC 2300 Auto analyzer (FOSS Tecator, Höganäs, Sweden). Soil nitrate-nitrogen was analyzed using the phenol disulfonic acid colorimetric method with a Helios Alpha double-beam ultraviolet spectrophotometer (Thermo Fisher Scientific Inc., MA, USA). All measured and estimated values related to DM, GY, and GPC were based on dry mass.

c) Weather data collection and calculation

Long-term daily sunshine duration (h), T (°C), and precipitation (mm) data covering a 30–60-year period for the Beijing area were obtained from the China Meteorological Data Sharing Service System (China Meteorological Data Service Centre, 2010). SRAD were calculated using the procedures described by Allen et al. (1998). Monthly SPI values were simulated using SPI_SL_6 (National Drought Mitigation Center, 2011) software. Standard values of the SRAD, T, and SPI data series were derived from the standard normal distribution transformation.

d) Model assessment

Model performance was assessed using normalized root mean squared error (nRMSE, %) (Rinaldi et al., 2003):

$$\text{nRMSE} = \sqrt{\frac{\sum_{i=1}^{n}(P_i - O_i)^2}{n}} \times \frac{100}{\overline{O}}$$

where P_i and O_i are the estimated and observed values, respectively, $\overline{O}$ is the mean observed value. The model

performance was considered excellent if the nRMSE was <10%, good if it was 10–20%, fair if it was 20–30%, and poor if the nRMSE was >30%.

III. Results

a) Effects of NF and sowing date

In Exp. 4, the three-factor experimental design of the study was not a complete factorial design; however, two two-factor complete factorial designs could be derived from it (i.e., 4(NF)×3(cultivar) on the S1 date and 3(sowing date)×3(cultivar) under N3 application). Cultivar factors could be viewed as replicates because of their similar gluten types. DM at anthesis, DM post-anthesis transfer coefficients (R_{β_C}), N post-anthesis transfer coefficients (R_{β_N}), and GY were calculated by averaging the values of the three cultivars, and the results were not significant between NF levels ($P>0.05$; Table 4). Generally, DM showed an opposite trend relative to R_{β_C} and R_{β_N} with N rates; with DM rising and R_{β_C} and R_{β_N} falling. As for GY, N2 corresponded to the highest GY (4006 kg ha^{-1}), and GY decreased in cases of higher or lower NF compared to N2.

NA at anthesis increased with N application rates; NA was significantly higher for N4 than for N1 ($P<0.05$), and the former value (138.9 kg ha^{-1}) was double the latter one (68.6 kg ha^{-1}). GPC was significantly higher ($P<0.05$) in N2 and N4 than in N1. For the remaining three N rate treatments, the increasing GPC trend from 12.6 to 17.4% indicated the strong positive effects of NF on GPC. In contrast, the sowing date had no significant effects on any of the five traits ($P>0.05$).

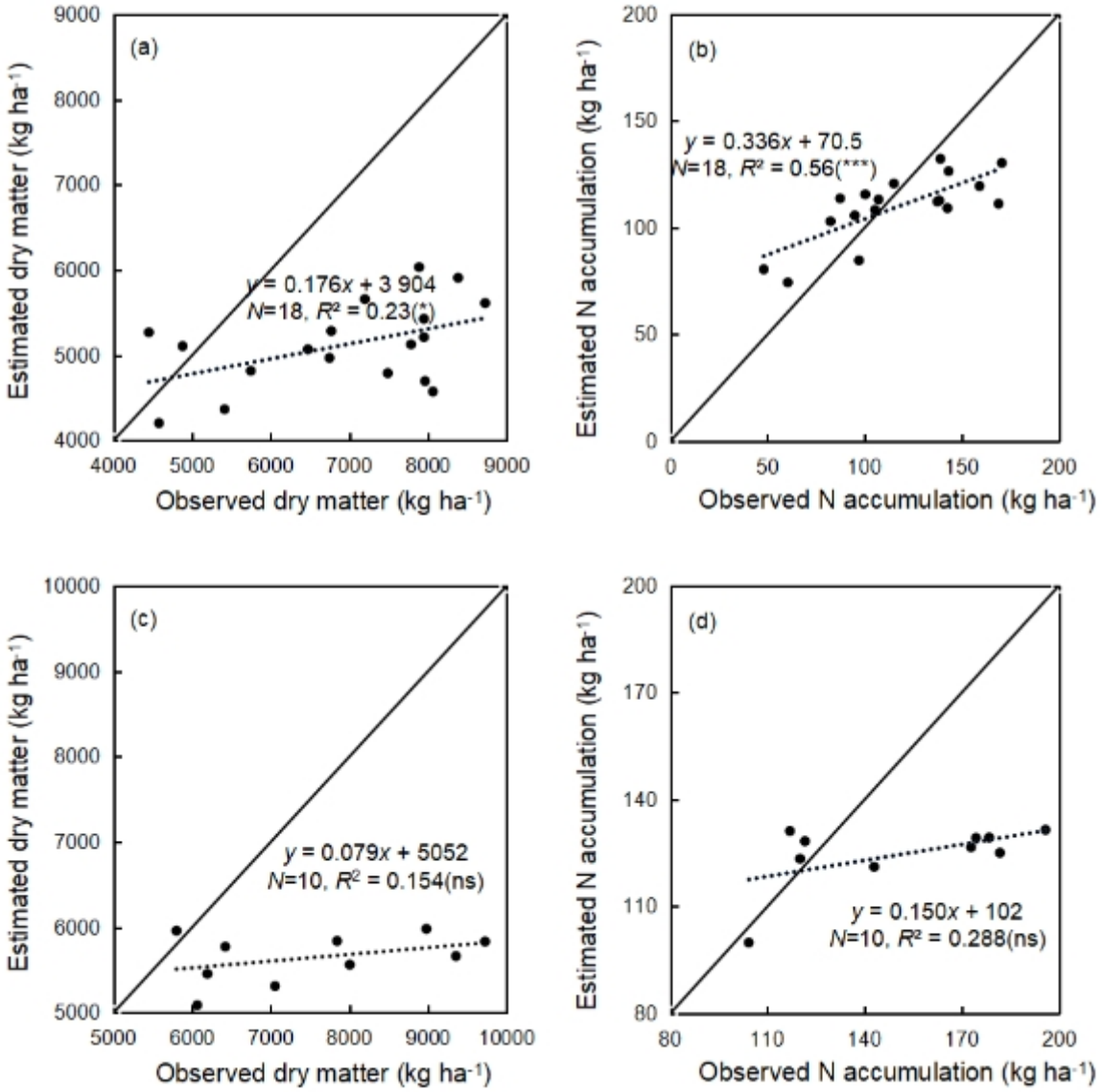
Table 4: Effects of N application rate and sowing date on dry matter (DM) at anthesis, nitrogen accumulation (NA) at anthesis, dry matter post-anthesis transferring coefficient (R_{β_C}), N accumulation post-anthesis transferring coefficient (R_{β_N}), grain yield and GPC in Exp.4

Treatment	Dry matter (kg ha^{-1})	Nitrogen accumulation (kg ha^{-1})	Dry matter post-anthesis transferring coefficient	N accumulation post-anthesis transferring coefficient	Grain yield (kg ha^{-1})	GPC (%)
N application rate						
N1	6431	68.6a	0.299	0.658	3602	12.6a
N2	6985	110.1ab	0.311	0.518	4006	15.7b
N3	6903	119.6ab	0.255	0.398	3525	—
N4	7336	138.9b	0.257	0.419	3700	17.4b
Sowing date						
S1	6903	119.6	0.255	0.398	3525	—
S2	6708	121.2	0.270	0.436	3471	16.4
S3	7106	141.0	0.250	0.370	3467	16.2

Values represent the means of the sub-plots. Values followed by the same letter are not significantly different at a probability level of 0.05. Only treatments with significant differences are indicated by the letters.

b) Model simulations

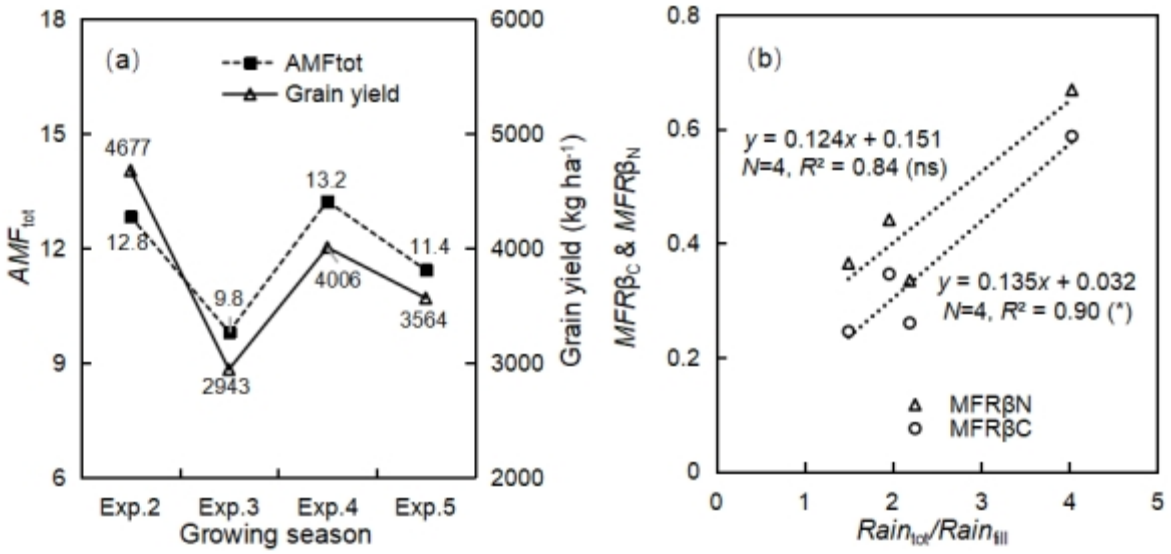
In Exp. 4, for DM and NA at anthesis, R^2 of correlation between observation and prediction were 0.23 and 0.56, reaching a significant level ($P<0.05$) and extremely significant level ($P<0.001$), respectively (Figure 1 (a), (b)). However, the majority of the DM was underestimated, with a larger deviation toward higher DM. In comparison, a higher consistency existed between the estimation and observation of NA. A similar phenomenon was observed for DM and NA simulations in Exp. 6 compared with those in Exp. 4, while neither of the R^2 values reached a significant level ($P>0.05$) (Figure 1 (c), (d)).



where N indicates the number of samples. The solid lines represent y=x. The dashed lines are the fitted simple linear regression models with estimation and observed values as dependent and independent variables, respectively. The same as below.

Figure 1: Comparison of estimation and observation values for dry matter (a) and N accumulation (b) in Exp. 4 and dry matter (c) and N accumulation (d) in Exp. 6 at anthesis

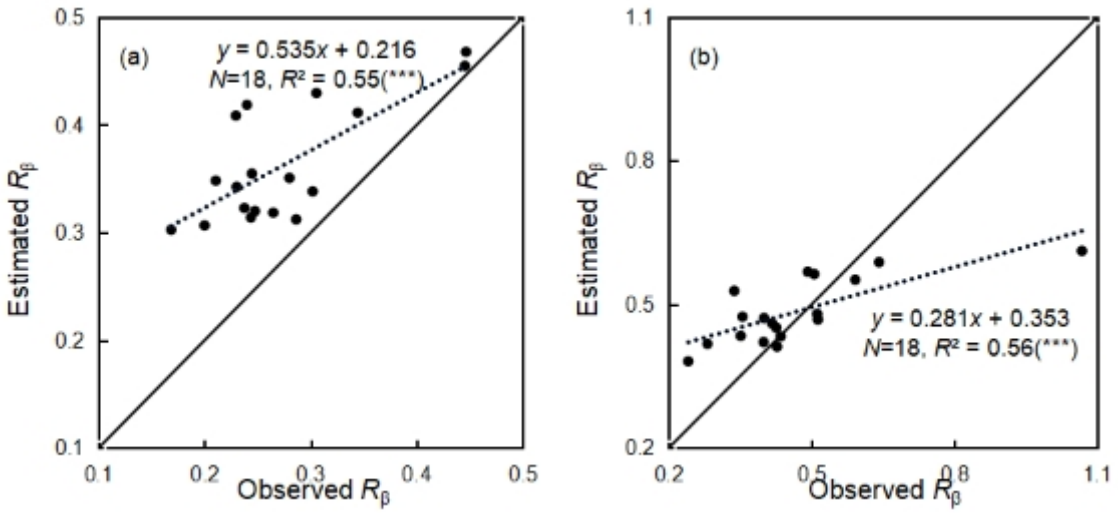
The average GY of Exp. 2 and 3 and identical NF of N2 in Exp. 4 and N3 in Exp. 5 were compared with the accumulated meteorological factor of GY (AMF_{tot}) (Figure 2(a)). Exp. 2 and 3 had the highest and lowest GYof 4677 and 2943 kg ha^{-1}, respectively, and Exp. 4 and 5 had GY of 4006 and 3564 kg ha^{-1}, respectively. Except for an obvious underestimation in Exp. 2, AMF_{tot} perfectly captured the GY trend of Exp. 3–5. The underestimation was attributed to higher N rates in Exp. 2–3 than in Exp. 4–5, producinga high GY inExp. 2–3. The lowest global radiation during the grain-filling period in Exp. 3 among the four experiments corresponded to higher GYloss compared to the other three experiments (Table 2). Both meteorological factors of post-anthesis transfer coefficients (MFR_{β_C}, MFR_{β_N}) were positively correlated to $Rain_{tot}/Rain_{fill}$ (Fig.2(b)). MFR_{β_C}had a higher R^2 than MFR_{β_N} at 0.90 and 0.84, reaching significant ($P<0.05$) and nonsignificant levels ($P>0.05$), respectively.



AMF tot indicates accumulated meteorological factors for grain yield; Exp. 2–5 indicate 2004/2005, 2005/2006, 2008/2009, and 2009/2010 growing seasons, respectively; MFR βC and MFR βN are the meteorological factors of dry matter and N post-anthesis transfer coefficients, respectively; Rain tot /Rain fill , the rainfall ratio of the whole growing season to the period during anthesis and maturity.

Figure 2: Meteorological factors of grain yield (a) and for post-anthesis transferring coefficients (b)

In Exp. 4, for DM and N post-anthesis transfer coefficients, R^2 values were similar at approximately 0.56, reaching an extremely significant level ($P<0.001$) (Figure 3 (a), (b)). All 18 treatments overestimated the DM post-anthesis transfer coefficients. The N post-anthesis transfer coefficient performed much better, except for one apparent underestimation possibly caused by sampling errors. In Exp. 6, neither of the R^2 values were significant ($P>0.05$) (Figure 3 (c), (d)).



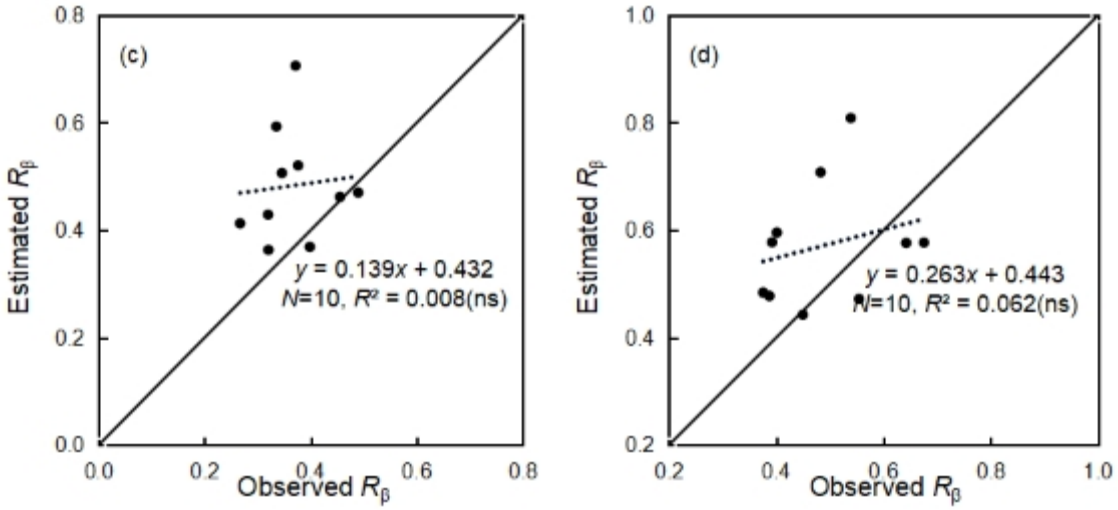
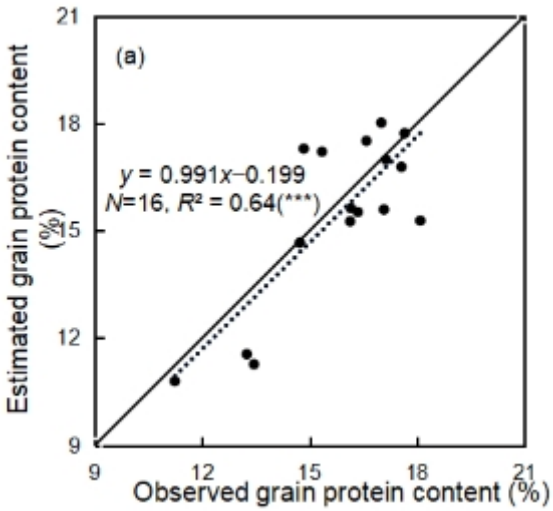


Figure 3: Comparison of estimation and observation values for R_{β_C}(a) and R_{β_N} (b) in Exp. 4 and R_{β_C} (c) and R_{β_N} (d) in Exp. 6 at anthesis)

A simple linear model has been widely applied to GPC forecasting because of its convenient application in remote sensing; thus, a simple linear model was established for comparison with leaf nitrogen content at anthesis as an independent variable. In Exp. 6, only 10 treatments of the S1 date, free from weed invasion, were selected as validation data. The R^2 and nRMSE of the semi-empirical model for Exp. 4 and 6 and the simple linear model for Exp. 4 were 0.64 and 8.91, 0.45 and 4.50,and 0.42 and13.3, respectively (Figure 4). The semi-empirical model had higher interannual prediction stability than the linear model, with average deviations of – 1.7 and – 7.6%, respectively. However, under the optimal sowing date and late sowing date conditions in Exp. 4, the GPC tended to be underestimated and overestimated by the semi-empirical model to an extent as high as – 16.2 and 16.6%, respectively.



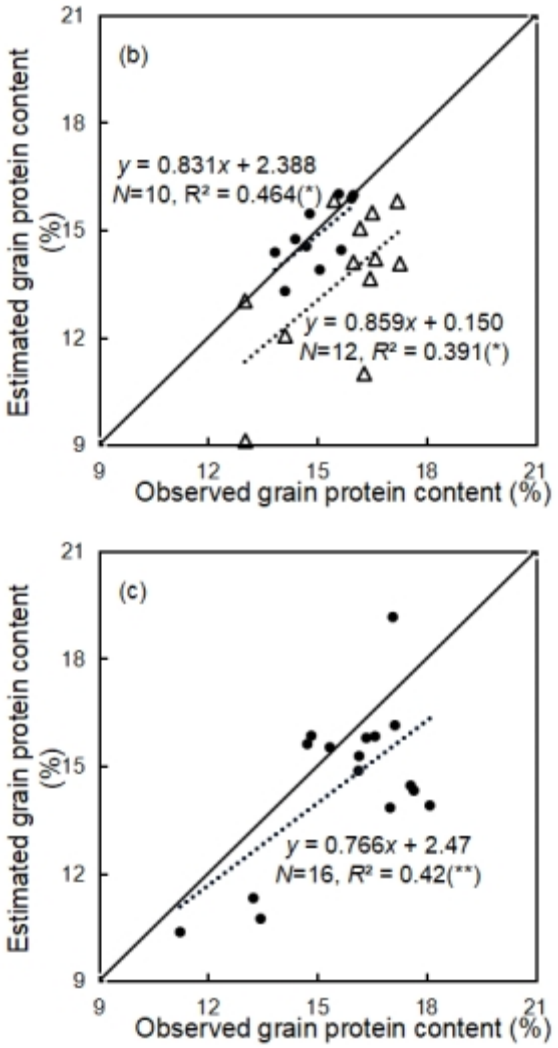


Figure 4: Comparison of grain protein content estimation and observation for new semi-empirical model with Exp. 4 (a) and Exp. 6 (b) and for simple linear model (c).

V. Discussion

By conducting multi-year field experiments and introducing the climate and soil N effects, the semi-empirical GPC prediction model established here fulfilled its intended role of demonstrating superiority over the simple linear model regarding the intra-annual GPC prediction. However, the inner ratio form and empirical method of the modeling also constrained further improvement of GPC prediction accuracy.

a) GPC simulations

The GPC was generally underestimated by both the semi-empirical and the simple linear models. This could be a result of the different climate conditions during the pre-anthesis period for the establishment and validation experiments. A higher precipitation and lower average temperature were observed in the establishing experiment than in the validation experiment. Similar results were obtained in a study conducted in England during 1975–1995 (Smith and Gooding, 1999): GPC was negatively correlated with the rainfall from 31 Dec.–3 Feb. (winter) and 4 Mar.–26 May. (spring). The negative effects of rainfall before anthesis were attributed to the following two aspects: soil nitrogen reserve dilution by vegetative proliferation and soil N loss, and leaf life extension during grain growth favoring carbohydrate assimilation and translocation more than N. Subedi et al. (2007) showed that GPC increased by 6–17% for all late planting dates, consistent with the sowing date trend effects,as simulated by the semi-empirical model (i.e., overestimation for later sowing conditions and underestimation for optimum sowing conditions).

The semi-empirical model proposed here has a limited dataset in terms of cultivar parameters and growing season experiments, which could bepartly compensated by long-term historical climate data to overcome interannual GPC fluctuations with a relatively satisfactory nRMSE below 9%. In comparison, Weiss and Moreno-Sotomayer (2006) reported an nRMSE range of 9–14% with GPC simulation results of the CERES-Wheat crop model. As illustrated by Pan et al.

(2006a), the meteorological factors affecting GPC were incorporated by genotypic parameters, including a number of traits such as characteristic GPC, physiological vernalization time, temperature sensitivity, photoperiod sensitivity, and rainfall sensitivity. Li et al. (2020) found that the regression coefficients of first-layer models could be used to construct second-layer models and proposed a hierarchical linear modeling method for GPC.The first-layer model was a multilinear model with vegetation growth indices as independent variables. The fitted coefficients, such as intercept and slopes, became the dependent variables for the second-layer model, which is also a multilinear model with otherwise meteorological factors as independent variables.

b) Post-anthesis transfer coefficients and corresponding meteorological factors

Post-anthesis DM and the N transfer coefficient (R_{β_C}) were significantly correlated with SLW and LAI at anthesis, respectively. R_{β_C} positively correlated with SLW. In comparison, the correlations between R_{β_C} andLAIwere negative (Table 1). These results agree with the findings of Hodáňová (1975) and Marini and Barden (1981), who reported that SLW is an important indicator of leaf photosynthetic rate. In addition, the post-anthesis photosynthetic rate is an important factor for GY, as the assimilate contributesat least 60% of the GY at maturity (Bidinger et al., 1977; Wang and Shangguan, 2015). Thus, SLW plays an important role in GY by affecting the intermediate DMpost-anthesis transfer coefficient. In contrast, a higher LAI at anthesis decreased the post-anthesis N-transfer coefficient (R_{β_N}). In parallel with the findings of Pan et al. (2006b) and Xu et al. (2009), N remobilization from leaves was assumed to decrease with increasing LAI for both wheat and barley. Przulj and Momcilovic (2001) reported that 60–92% of the N accumulated in wheat grain originates from the translocation in vegetative tissue after anthesis. Halloran (1981) suggested that nitrogen translocation from leaf tissue is more difficult than that from culm or glume tissue. As a result,a larger LAI at anthesis indicates greater nitrogen loss with the senesced leaves at maturity. In contrast, it delays maturity owing to staying green effects.

The relative rainfall portion with regard to pre-anthesis and post-anthesis ($Rain_{\text{tot}}/Rain_{\text{fill}}$) can considerably determine post-anthesis DM and N assimilation and translocation in the study, which were chosen to establish meteorological factors of post-anthesis transfer coefficients (MFR_{β_C}, MFR_{β_N}). As shown in Table 1, $Rain_{\text{tot}}/Rain_{\text{fill}}$ was both positively and significantly correlated with MFR_{β_C}, and positively correlated with MFR_{β_N}. Identical to the results of Nakagami et al. (2004), who observed relatively low soil moisture conditions during the later growth cycle, heavier wheat DM and GY could be achieved because of the high photosynthesis rate and leaf area during leaf senescence and enhanced root system. Similarly, Soon et al. (2008) showed that the ratio of rainfall in May and June. (the pre-anthesis period for wheat in Canada) compared to the average in history was highly correlated with the amount of remobilized nitrogen. Palta and Fillery (1995) also demonstrated that N remobilization within the plant can provide most of the grain N required to synthesize grain protein under post-anthesis water deficit. However, under severe post-anthesis water stress, N remobilization is reduced by approximately 15% (Ercoli et al., 2008).

c) Further model improvement

Ideal GPC and yield usually occur under favorable environmental and management conditions, and in most cases, an inverse relationship, known as the "dilution phenomenon," exists between GPC and yield (Soon et al., 2008; Stewart et al., 1990). For some genotypes, high GPC and GY can be achieved, which is called grain protein deviation (Monaghan et al., 2001; Bogard et al., 2010). GPC mostly depends on the relative fluctuations in NA and DM to a greater extent than the corresponding absolute values. Thus, key processes around critical periods are crucial for GPC modeling (Mcmullan et al., 1988). The inaccuracies related to DM estimation were partly correlated with the simplified modules of DM and N uptake and translocation. The current prediction accuracy could be accepted given that the model is used to predict regional GPC before harvest and assist graded purchases for processing enterprises. Particularly, this holds true for Exp. 6 where the majority of different cultivars were introduced but with good model performance, suggesting a sound theoretical basis and regional application prospect. However, more field experiments should be carried out to improve the DM and N flow modules by incorporating specific meteorological factors for critical stages or adopting multi-factor regression. Comparing the prediction nRMSE of 6.87 by Li et al. (2020) with two-layer multi-factor regression models and considering the cultivar effects, the semi-empirical model showed a larger annual prediction nRMSE at 8.91 and 4.50 and needs further improvement.

VI. Conclusion

The priority task of establishing the semi-empirical GPC model was to realize prediction ahead of maturity with higher accuracy. Anthesis was deemed suitable for the ahead-of-time prediction stage, which ends vegetative growth and launches the grain filling period, whereby the whole growth period was divided into pre-anthesis and post-anthesis periods. The DM and NA and translocation involved in the two periods were separately modeled based on the experimental

data. Parameters such as LAI, SLW, and CLND, mainly acquired at the anthesis stage, were adopted as independent variables for the sub-model establishment. Meteorological factors were defined and calculated for prediction and reference growing seasons, and the ratio of meteorological factors involved in the two growing seasons was assumed to be climate effects, which were incorporated into relevant modeling. With independent evaluation data from two growing seasons, the semi-empirical GPC model performed better with normalized nRMS Evalues of 8.91 and 4.50. Interannual uncertainty accompanied by a simple linear model was overcome with the semi-empirical model, which shows a promising future when combined with remote sensing technology. However, complex physiological processes involved with DM and NA and translocation were simplified with empirical equations by the study, which constrains the model prediction accuracy. More experiments should be conducted to determine critical parameters for key growth processes affecting GPC.

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Competing interests

The authors declare there are no competing interests.

Author contribution

Q. Wang: Conceptualization, Formal analysis, Investigation, Methodology, Writing-original draft. Cun-jun Li: Funding acquisition, Supervision, Investigation. Yuan-fang Huang: Supervision, Writing-review & editing. Wu-de Yang: Methodology, Writing-review & editing. Wen-jiang Huang: Project administration, Investigation. Ji-hua Wang: Supervision, Funding acquisition, Writing-review & editing.

Data availability statement

Primary data were stored in the database of the institute if they agreed that they could be accessed.

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Ethno-Botanical Study at the Nabiganj Upazila of Habiganj District, Bangladesh

By Jontu Chandra Deb

Abstract- Between August 2019 and October 2020, an ethnobotanical research was carried out to look at how the inhabitants of Nabiganj Upazila of Bangladesh's Habiganj district was noted. The information regarding the ethnobotanical data was obtained by interviewing residents of different age groups, most of them were in the 15 to 55 age range, including medicinal herbalists and hakims. The present paper reported that 68 medicinal plants belonging to 41 families, and 60 genera which have been proven to be helpful in the treatment of 78 diseases. Out of these plant species, 24 belonged to herbs, 27 trees, eight shrubs, and nine climbers. However, other parts of medicinal plants, including the stem, bark, latex, leaf bud, pulp, petiole, fruits, and rhizome, Seed, root, calyx, and peduncle were also shown to be helpful. For each species, scientific name, local name, habit, family, ailments to be treated, mode of treatment, and part(s) used are provided.Local residents of gathered data on medicinal plants, including Nabiganj Upazila of Habiganj district.

Keywords: ethnobotany, medicinal plants, rural people, disorders, local communities, nabiganj upozila.

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Keywords: ethnobotany, medicinal plants, rural people, disorders, local communities, nabiganj upozila.

I. Introduction

Ethno botanists scour the globe in search of the world's many indigenous peoples, who practice a wide variety of culturally specific rituals and performances and have developed deep ties to the flora and fauna of their respective environments. The word "ethnobotany" was created by John William Hershberger in the 1890s. Ethno-botany, a combination of the terms "ethnographic" and "botanical," refers to the study of both people and plants (Tree, Shrubs, and Herbs). As a subfield of Ethnobiology, "Ethno-botany" studies plant life in indigenous communities. In ethnobotany, researchers compile all available data on plants and their therapeutic use. Humans have used wild plants for thousands of years to provide for their most fundamental requirements: food, shelter, and clothing. Some plants have medicinal properties that are used to cure both internal and exterior ailments. In rural places, wild plants constitute a significant economic driver. States, Canada, Germany, Australia, and New Zealand are examples of developed nations. Even though fast-developing countries like China, India, Brazil, Indonesia, and Russia provide 80-85% of the world's medicinal plant medications, only 20-25% of the world's pharmaceuticals are derived from plants. More than 85,000 plant species are used for medical purposes, out of a total of 250,000 higher plant species known on Earth. Being a Mega-diversity country, Bangladesh has an abundance of useful plants for medicine. These plants have been used for a very long time. in Ayurvedic medicine, dating back thousands of years. Ethno-botanical research in Bangladesh has only recently begun. Studies of both medicinal plants and ethnobotanical practices in Bangladesh have been conducted.

Objectives:

- To record ethnobotanical knowledge of medicinal plants used by the local people living in Nabiganjupazila, Habiganj district of Bangladesh;
- To explain Ethno-medicinal Uses;
- To find out the origin of the plant;
- To investigate and collect knowledge about medicinal plants' therapeutic properties collected from traditional medicine practitioners and indigenous populations.

II. Materials and Methods

68 species were gathered and identified in this ethnomedicinal study, with the samples representing 60 different genera and 41 different families. Local herbalists, community leaders, and elders were interviewed using a semi-structured questionnaire to compile information on the medical uses of plants in their respective areas. Data collected by one individual was double-checked by asking similar questions to another person. Most medicinal plants were recognized in the wild, and when this was not possible, plant specimens were gathered. Herbarium specimens were compared, and their identities were confirmed, using this process. The field observations also highlighted the dangers threatening medicinal plants and their ecosystems. The verified identification of the plants to which these individuals referred Hooker (1961), Prain (1963), Khan and Huq (1975), Kirtikar and Basu (1987), Rahman et al. (2012, 2013), and Ahmad et al. (2010), Ahmed et al. (2007).

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III. Ethno-Botanical Enumeration

All species have been sorted alphabetically by their botanical name, their common name, and their family. The components, conventional applications, and application methods have all been described.

Albiziaprocera Benth.
Local name: Koroi
Family: Mimosaceae
Habit: Tree
Part(s) used: Leaves, Bark

Ethno-medicinal Uses: The leaves can be used as an insecticide and as a poultice for skin ulcers. To get rid of threadworms, a doctor may recommend a bath in water infused with bark and table salt, and the same treatment may be used for scabies. The use of barks as a remedy for tooth pain is well documented.

Alternanthera philoxeroides(Mart.) Griseb.
Local name: Helena
Family: Amaranthaceae
Habit: Herb
Part(s) used: The whole plant

Ethno-medicinal Uses: Foggy vision, night blindness, malaria, postpartum symptoms, diarrhea, dysentery, and puerperal fever are all treated with the whole plant.

Annona squamosa L.
Local name: Ata
Family: Annonaceae
Habit: Tree
Part(s) used: Leaves, Bark, Fruits, Seeds

Ethno-medicinal Uses: One teaspoon of bark juice is commonly used to cure diarrhea. Fruit fully ripened has several medicinal uses, including as a sedative for the heart, a pain reliever, a laxative, a maturing, and a tonic.

Artocarpusheterophyllus Lamk.
Local name: Kathal
Family: Moraceae
Habit: Tree
Part(s) used: Young leaves, Seeds, Roots

Ethno-medicinal Uses: Treatment of skin disorders often begins with fresh leaves. Seeds have diuretic, aphrodisiac, and laxative properties. Diarrhea treatment with roots

Artocarpuslacucha Buch.-Ham.
Local name: Deua
Family: Moraceae
Habit: Tree Part(s)
used: Seeds, Bark

Ethno-medicinal Uses: Purging with seeds is common. To treat constipation in breast-feeding babies infants, a paste formed from three to four roots is added to the mother's milk. To treat cracked and dry skin, an infusion of the bark is applied. The powdered bark is then put into the wound to help remove the pus.

Azadirachtaindica A. Juss
Local name: Neem
Family: Meliaceae
Habit: Tree
Part(s) used: Leaves, Fruits, Dry Nuts, Kernels

Ethno-medicinal Uses: In cases of ulcers and eczema, a strong decoction of the fresh leaves is employed due to theirmildly antibacterial properties. The fruit may be used as a laxative and as an anthelmintic, and it can also treat urinary tract infections, skin conditions, tumors, piles, and toothaches. When used after being crushed and mixed with water or another liquid, dried nuts have nearly the same therapeutic characteristics as the oil. To treat swollen gums, discomfort, and pyorrhea boils around 250 g of leaves in 1 liter of water until you get 250 ml to use as a gargle. You may treat scabies by taking a bath in water that has been cooked with the leaves, or you can take pills produced from the paste of the leaves. Jaundice treatment includes drinking the juice of the leaves.

Averrhoa carambola L.
Local name: Kamranga
Family: Oxalidaceae
Habit: Tree
Part(s) used: Fruits

Ethno-medicinal Uses: Eaten to treat jaundice, fruits are tonic, cooling, and antiscorbutic. The digestive tract can be soothed by eating green foods. Bloody piles, especially inside, respond well to the ripe fruit.

Aegle marmelos(L.) Correa
Local name: Bel
Family: Rutaceae
Habit: Tree
Part(s) used: Ripe Fruits, Unripe Fruits

Ethno-medicinal Uses: Fruits aid digestion, stimulate the appetite, and have long been touted as a miracle cure for intractable digestive issues, including persistent diarrhea, dysentery, and nausea. Both diarrhea and dysentery can be treated with unripe fruit, whereas ripe fruit can be used to treat constipation. An astringent, digestive, and stomachic, dried unripe fruit slice is recommended for cases of diarrhea and dysentery.

Allium cepa L.
Local name: Piaj
Family: Liliaceae
Habit: Herb
Part(s) used: Bulbs, Leaves

Ethno-medicinal Uses: A diabetic person's daily insulin needs can be cut in half if they consume 50 grams of onion daily day. Consuming 80 grams of onion daily for five months reduced serum cholesterol below normal in healthy humans.

Allium sativum L.
Local name: Rosen
Family: Liliaceae
Habit: Herb
Part(s) used: Bulbs, Leaves

Ethno-medicinal Uses: For relief from stomach gas, try taking anbulbs extract. Headaches can be treated by applying a paste made from the bulb to the forehead. Cough in children can be treated by giving them a paste made from leaves and cow or goat's milk and taking one teaspoonful twice a day for four or five days.

Asparagus racemosus L.
Local name: Stimuli
Family: Liliaceae
Habit: Herb
Part(s) used: the whole plant

Ethno-medicinal Uses: Child appetites are stimulated as a result. Sesame oil is a cooling and tonic for the hair as well as a remedy for acidity and impotence.

Aloe vera (L) Burm. f.
Local name: Gritakumari
Family: Aloeaceae
Habit: Herb
Part(s) used: the whole plant

Ethno-medicinal Uses: Rheumatism, edema, and paralysis can all be alleviated by applying a warm leaf directly to the afflicted region. When a leaf is cooked, the extract is used to cure paralysis.

Ananassativus Schult. f.
Local name: Aneros
Family: Bromeliaceae
Habit: Herb
Part(s) used: Leaves, Flowers, Fruits

Ethno-medicinal Uses: The ingestion of a young leaf extract is used to prevent nausea and vomiting. Women seeking an abortion may ingest an extract made from young flowers.

Basella alba L.
Local name: Puishak
Family: Basellaceae
Habit: Herb
Part(s) used: Leaves, Roots

Ethno-medicinal Uses: Constipation, especially in young children and pregnant women, can be treated using the juice extracted from the leaves. An effective remedy for tooth pain that involves chewing on a robot

Benincasahispida(Thunb.) Cogn.
Local name: Chalkumra
Family: Cucurbitaceae
Habit: Climber
Part(s) used: Fruits, Seeds

Ethno-medicinal Uses: Tonic, nutritive, diuretic, antiperiodic, constipation, heart disease, TB, colic discomfort, and aphrodisiac are among the conditionsthat benefit from eating fruit curry. The fried seeds are helpful fortreating tapeworms, lumbrical, and as a diuretic.

Bauhinia acuminate L.
Local name: Kanchan
Family: Caesalpiniaceae
Habit: Tree
Part(s) used: Leaves, Barks

Ethno-medicinal Uses: A decoction made from the bark or leaves is administered to patients suffering from biliousness, bladder stones, leprosy, and asthma. In other words, it aids digestion. A decoction from boiling the root in oil is used to treat burns. Herbal treatments for dropsy often use barks and leaves.

Borassusflabellifer L.
Local name: Tal
Family: Arecaceae
Habit: Tree
Part(s) used: Leaves

Ethno-medicinal Uses: In situations of dysentery, the young leaves' juice is administered in conjunction with water.

Bambusaarundinacea(Retz.) Willd.
Local name: Bansh
Family: Poaceae
Habit: Herb
Part(s) used: Barks, Leaf bud

Ethno-medicinal Uses: Stopping bleeding by applying a thin green covering of bark. As a remedy for aching joints and overall weakness, the roots are often used topically. To promote the smooth passage of menstruation or lochia after childbirth, a decoction of the leaf bud is given.

Carica papaya L.
Local name: Pepe
Family: Caricaceae
Habit: Shrub
Part(s) used: Latex

Ethno-medicinal Uses: The milky juice of both the fruit and the plant includes a digestive and anthelmintic enzyme called papain, which is used to treat digestive disorders, intestinal inflammation, and ringworm. Wounds, ulcers, boils, warts, and malignant tumors can benefit from an external application of latex, which speeds up the healing process. Abortion is caused by the latex of green fruits.

Coccinia cordifolia(L.) Cogn.
Local name: Telakucha
Family: Cucurbitaceae
Habit: Climber
Part(s) used: Whole plant, Fruit, Leaves, Roots, Stem

Ethno-medicinal Uses: Diabetes, aphrodisiac, biliousness, and blood diseases are all treated with a young fruit curry. Diabetes, anorexia, asthma, fever, dropsy, catarrh, epilepsy, and gonorrhea can all be treated using the juice of the entire plant. Patients with diabetes mellitus benefit from the entire plant because of its well-known ability to lower sugar levels in their urine. Patients with glycosuria do not see a decrease in sugar levels in their blood or urine after consuming fresh juice from leaves, stems, and roots. In the event that a snake bit you, you should consume the fruit and, use the leaves as medicine.

Cucumis melon L.
Local name: Bangi
Family: Cucurbitaceae
Habit: Climber
Part(s) used: Seeds and Fruits.

Ethno-medicinal Uses: The mature fruit has several ethnomedical applications, including treatment for renal ailments, cooling, flattening, toning, laxative, aphrodisiac, biliousness, diuretic, and severe eczema. When consumed, the seeds have diuretic, cooling, nutritional, and enlarging effects on the prostate gland.

Cucumis sativus L.
Local name: Sasha
Family: Cucurbitaceae
Habit: Climber
Part(s) used: Leaves, Fruits, Seeds

Ethno-medicinal Uses: Demulcents can be made directly from the fruit. These fried seeds have several medicinal uses, including as a diuretic, a laxative, and even an anthelmintic. leaves and cumin seeds for throat problems.

Cassia fistula L.
Local name: Badarlathi
Family: Caesalpiniaceae
Habit: Tree
Part(s) used: Leaves, Pulps, Barks

Ethno-medicinal Uses: The ringworm-curing properties of the juice extracted from the young leaves. the fruit pulp is administered for liver problem and is a pleasant laxative that may be used safely by pregnant women and young children. The pulp, when used topically, is effective against gout, rheumatism, and ringworm.

Cajanuscajan(L.) Huth.
Local name: Arhar
Family: Fabaceae
Habit: Shrub
Part(s) used: Leaves, Seeds

Ethno-medicinal Uses: Mouth and piles ailments are treated with leaves. The laxative effects of the leaf juice make it a standard treatment for jaundice and pneumonia. Coughs benefit from the leaves and seeds, and so does regulating breast milk production. Pectoral infusions includebuds, flowers, and green pods. Jaundice patients are often administered leaf juice.

Clitoriatarnetea L.
Local name: Aprajita
Family: Fabaceae
Habit: Climber
Part(s) used: Leaves, Flowers, Roots

Ethno-medicinal Uses: Colliquative perspiration during a frantic fever can be treated with a mixture of leaf juice and fresh ginger juice, earaches can be alleviated by applying warm saltwater compresses to the outer ear. Children with coughs are treated with flower juice combined with their mother's milk or honey for three days. An effective method of birth control involves the use of roots (White Flowered Variety).

Chenopodium album L.
Local name: Batuashak
Family: Chenopodiaceae
Habit: Herb
Part(s) used: Leaves, Flowers

Ethno-medicinal Uses: The infusion of the leaves is used to treat intestinal ulcers, while the leaves themselves are used as an anthelmintic to treat hepatic diseases and splenic enlargement. Stomach issues, kid weakness, and weight gain are all treated with flowers and buds.

Cinnamomumtamala Nees.
Local name: Tejpata
Family: Lauraceae
Habit: Small tree
Part(s) used: Leaves, Barks

Ethno-medicinal Uses: Coughs and colds can be treated by brewing tea from the leaves and bark. Insulin resistance can be reversed by taking a tablet containing leaf paste twice a day for an extended period. In cases of gonorrhea, bark can be used as a carminative.

Croton bonplandianum Bail.
Local name: Croton
Family: Euphorbiaceae
Habit: Herb
Part(s) used: Leaves, Seeds, Latex

Ethno-medicinal Uses: Coughs can be alleviated by ingesting the juice from three to four leaves for three to four days. Eczema and ringworm are cured by applying a paste from ground seeds directly to the affected area. Wounds and cuts can be treated with latex.

Citrus aurantifolia Sw.
Local name: Kagochilebu
Family: Rutaceae
Habit: Shrub
Part(s) used: Fruits, Unripe Fruits

Ethno-medicinal Uses: In cases of skin irritation and nausea, eating fruit can help. Indigestion can be alleviated by eating the salted peel. Onestandard

treatment for catarrhal fever is to drink a warm water mixed with two teaspoons of honey and some of the fruit juice.

Citrus grandis(L.) Osbeck.
Local name: Jambura
Family: Rutaceae
Habit: Tree
Part(s) used: Leaves, Fruits, Seeds

Ethno-medicinal Uses: A fruit juice preparation is used to treat jaundice and fever. To prevent nausea and vomiting, leave the fragrance where it is. Those who suffer from nausea and vomiting might benefit from taking seeds.

Centellaasiatica(L.) Urban.
Local name: Thankuni
Family: Apiaceae
Habit: Herb
Part(s) used: Leaves, Whole plants

Ethno-medicinal Uses: Leaf extract from boiling water is used to cure conjunctivitis. For diarrhea, flatulence, and TB, take four teaspoonfuls of whole plant extract twice daily for two days. To treat gastrointestinal issues, including diarrhea, dysentery, and stomach ache, the entire plant is ground into a paste and eaten with boiling rice. A memory tonic derived from the leaves.

Colocasia esculenta (L.) Schott.
Local name: Kachu
Family: Araceae
Habit: Herb
Part(s) used: Petioles, Leaves

Ethno-medicinal Uses: It is common practice to treat an athlete's foot and halt bleeding from cuts using the styptic, stimulant, and rube facient juice extracted from the petioles. The juice made from the leaves is used to treat malignant growths, polyps with ulcers, nasal cancer, and warts. The laxative properties of corm juice make it helpful in treating piles, portal system congestion, and alopecia.

Cynodondactylon Pers.
Local name: Durba
Family: Poaceae
Habit: Herb
Part(s) used: whole plants, Roots

Ethno-medicinal Uses: To staunch bleeding, fresh plant juice is applied to new cuts and wounds. To treat vesicle calculus and secondary syphilis, a decoction of the roots is helpful. It's also beneficial for soothing irritated urinary organs.

Canna indica L.
Local name: Kolaboti
Family: Cannaceae
Habit: Herb
Part(s) used: Seed, Rhizome, Root

Ethno-medicinal Uses: Seed juice relieves earaches. The Rhizome is used in ringworm.

Curcuma longa L.
Local name: Holud
Family: Zingiberaceae
Habit: Herb
Part(s) used: Rhizome, Flowers

Ethno-medicinal Uses: Dysentery can be cured by eating a diet of rhizome paste or powder combined with wild rice, mustard oil, and table salt for three to four days. When applied as a poultice, a mixture of ground rhizome and lime can help alleviate sprain pain. Intestinal worms are treated with a saline solution of freshly squeezed rhizome juice. Oil from the rhizome can be used as an antacid, carminative, stomachic, and tonic. Gonorrhea, ringworm, and other parasitic skin illnesses are treated using flower paste.

Curcuma zeoderia Rosc.
Local name: Shakthi
Family: Zingiberaceae
Habit: Herb
Part(s) used: Rhizome, Leaves

Ethno-medicinal Uses: Diarrhea is treated using a decoction made from the rhizome. Dropsy treatment includes ingesting the leaf juice. Cough, cold, fever, and bronchitis are all treated with a combination of long pepper, cinnamon, and honey. New rhizome inhibits the spread of gonorrhea and leucorrhea.

Dilleniaindica L.
Local name: Chalta
Family: Dilleniaceae
Habit: Tree
Part(s) used: Fruits

Ethno-medicinal Uses: Fruit juice is an expectorant and cooling drink for fevers and coughs.

Dyospyrosperigrina(Gaertn.) Gur.
Local name: Gab
Family: Ebenaceae
Habit: Tree
Part(s) used: Fruits, Seeds, Clayx, Peduncle

Ethno-medicinal Uses: Injuries, ulcers, and diarrhea can all benefit from the application of the fruit's juice, while aphthae and sore throats can be gargled with an infusion. In cases of diarrhea and dysentery, the seeds are used as an astringent. Coughs and dyspnea can be alleviated with the help of fruit peduncles and calyx.

Eichhorniacrassipes(Mart.) Sol.-Lau.
Local name: Kochuripana
Family: Pontederiaceae
Habit: Herb
Part(s) used: The whole plant

Ethno-medicinal Uses: Half a cup of a mixture of totri and dorearfena paste and poser sap twice a day till

asthma is gone. A three-month course of therapy for goiter involving oral administration of plant juice and topical application of a poultice made from the plant pulp is recommended.

Ficusbenghalensis L.
Local name: Bot
Family: Moraceae
Habit: Tree
Part(s) used: Young buds, Arial Roots.

Ethno-medicinal Uses: In cases of diarrhea and dysentery, an infusion of the young buds might be helpful. For persistent vomiting, try giving your child a taste of the dangling roots.

Ficus religiosa L.
Local name: Pakur
Family: Moraceae
Habit: Tree

Part(s) used: Fruits, Ethno-medicinal Uses: By consuming powdered dried fruit in water over two weeks, asthma can be cured.

Feronia limonia(L.) Sw.
Local name: Kothbel
Family: Rutaceae
Habit: Tree
Part(s) used: Leaves, Fruits, Seeds

Ethno-medicinal Uses: Unripe fruit treats diarrhea and dysentery due to its astringent properties. Therapeutically, seeds have been utilized to treat cardiac conditions. You can induce vomiting with the leaves since they are astringent and carminative.

Hibiscus esculentus L.
Local name: Bhandari
Family: Malvaceae
Habit: Herb Part(s) used: Fruits

Ethno-medicinal Uses: The fruits are effective in treating various medical conditions, including gonorrhea, urinary discharges, strangury, and diarrhea; chronic dysentery; and gastrointestinal upset.

Hibiscus rosa-sinensis L.
Local name:Joba
Family: Malvaceae
Habit: Shrub
Part(s) used: Flowers

Ethno-medicinal Uses: The flower buds are astringent and cooling, relieving burning sensations and eliminating urinary discharges, impotence, and impotence. Acute dysentery can be cured by combining flower juice with banana inflorescence juice.

Kalanchoe laciniate L.
Local name: Himsagor
Family: Crassulaceae
Habit: Herb

Part(s) used: Leaves Ethno-medicinal Uses: Patients with jaundice can benefit from ingesting leaf juice. Indigestion and abdominal discomfort are two other common indications for its use. Blood dysentery can be treated by drinking a mixture of mucilaginous water made from soaking pounded leaves in water overnight. In cases of gonorrhea, the leaves juice is combined with sugar.

Lagenariasiceraria(Mol.) Stan.
Local name: Lau
Family: Cucurbitaceae
Habit: Climber
Part(s) used: Leaves, Fruits, Roots, Stem, Seeds

Ethno-medicinal Uses: The fruit's white flesh has a yellowish tint. Earaches can be alleviated by the soothing warmth of the sensitive stem. Fruit curry has antibilious, cooling, emetic, purgative, diuretic, and diuretic properties and is used to treat cholera. The chilly oil extracted from the sources is used to treat headaches. It is well known that seeds provide both dietary and diuretic benefits. A sugary leaf decoction is served.

Lablab purpureus(L.) Sweet.
Local name: Sim
Family: Fabaceae
Habit: Climber
Part(s) used: Leaves

Ethno-medicinal Uses: The ringworms are cured by rubbing fresh leaves that have been mashed and combined with lime.

Lawsoniainermis L.
Local name: Mehedi
Family: Lythraceae
Habit: Shrub
Part(s) used: Leaves

Ethno-medicinal Uses: Headaches, eczema, leprosy, dandruff, and burred feet are only some of the conditions where an emollient poultice like the paste made from the leaves may be helpful. The spermatorrhea treatment involves ingesting a mixture leaf juice, water, and sugar.

Litchi chinensis Sonn.
Local name: Litchi
Family: Sapindaceae
Habit: Tree
Part(s) used: Roots, Barks, Flowers, Leaves

Ethno-medicinal Uses: A decoction from the plant's root, bark, or blossoms is gargled for sore throats. Animal bites can be treated using leaves.

Microcospaniculata L.
Local name: Pisa
Family: Tiliaceae
Habit: Shrub

Part(s) used: Leaves

Ethno-medicinal Uses: Leave is helpful for treating various of medical issues, including indigestion, eczema, itching, smallpox, typhoid fever, dysentery, and syphilitic ulcers of the mouth.

Momordica charantia L.
Local name: Korola
Family: Cucurbitaceae
Habit: Climber
Part(s) used: Fruit, Root, Leaves, Seeds, Whole plant

Ethno-medicinal Uses: The fruits are used to treat rheumatism, gout, and liver and spleen diseases, and they are also tonic, stomachic, febrifuge, carminative, and cooling. Seeds are utilized as an anthelmintic. Colic and fever can be treated with a stomachic made from an alcoholic extract of the entire plant. In treating diabetes, the plant's whole-leaf juice is employed. Its fruits, leaves, and roots have been used traditionally as a home treatment for diabetes. The berries and greens are used medicinally as an anthelmintic and in the treatment of piles, leprosy, jaundice; and scurvy.

Manilkaraachras(Mill.) Per.
Local name: Sofa
Family: Sapotaceae
Habit: Tree
Part(s) used: Leaves, Fruits, Whole plant

Ethno-medicinal Uses: Diarrhea can be treated by ingesting a mixture of guava (Pisdiumguajava) leaf juice, sofa's leaf juice, and nutmeg (Myristicafragrans) for seven days. Asthma and coughs can be alleviated by consuming a paste made from the entire plant and a two cups of hot water per day for three days.

Mimosa pudica L.
Local name: Lajjaboti
Family: Mimosaceae
Habit: Herb
Part(s) used: Leaves, Roots, Whole plants

Ethno-medicinal Uses: When the entire plant is cooked in water, the extract is consumed to reduce inflammation and alleviate urinating discomfort. To cure diarrhea, a root paste is made and consumed. Boils may be treated with a paste made from the plant's roots, In contrast, root extract can be used to alleviate symptoms of dysentery, bug bites can be alleviated with a paste made from the plant's leaves, and leucoderma can be helped with a paste made from the entire plant and Limnophilarepens. A root extract is taken twice daily for 30 days to help with piles.

Mangiferaindica L.
Local name: Am
Family: Anacardiaceae
Habit: Tree
Part(s) used: Leaves, Ripe Fruits

Ethno-medicinal Uses: You may use a decoction made from the leaves to treat things like a fever, diarrhea, or toothache. Diarrhea treatment that includes young leaves. The mature fruit is beneficial for chronic constipation due to its astringent and laxative properties.

Musa paradisiacal L.
Local name: Kola
Family: Musaceae
Habit: Herb
Part(s) used: Fruits

Ethno-medicinal Uses: Children with splenomegaly can be healed by taking one teaspoonful of ash made from the dried banana fruit shell cleaved, along with a few drops of lemon essence.

Nelumbo nucifera Gaertn.
Local name: Padma
Family: Nelumbonaceae
Habit: Aquatic Herb
Part(s) used: Roots, Leaves, Seeds

Ethno-medicinal Uses: Ringworms and other skin irritations can be treated using a paste made from the roots of several plants. The young leaves are soothing to the body while it is on fire. Seeds are used as a diuretic and to prevent vomiting in youngsters.

Nymphaea nouchali Burm.f.
Local name: Sapla
Family: Nymphaeaceae
Habit: Aquatic Herb
Part(s) used: Flowers, Seeds

Ethno-medicinal Uses: Medicinal uses for flowers include treating nausea, dizziness, worms, and skin burns. In cases of skin problems, the seeds are utilized as a cooling medication.

Phyllanthus emblica L.
Local name: Amloki
Family: Euphorbiaceae
Habit: Tree
Part(s) used: Fruits

Ethno-medicinal Uses: Insomnia, skin issues, gall discomfort, leucorrhea, and tympanites are some of the other ailments supposedly helped by eating fruits. A sherbet made from the fruit and some lemon juice can be used to stop acute bacillary dysentery. Triphala, an essential ingredient in most Ayurvedic remedies, includes fruit as one of its many benefits.

Psidium guajava(L.) Bat.
Local name: Payara
Family: Myrtaceae
Habit: Tree
Part(s) used: Roots, Leaves

Ethno-medicinal Uses: Diarrhea and dysentery can be treated with an astringent decoction made from the bark of the root, and a paste made from the heart and water

can be used for the same purpose. For example, bronchitis and eye sores are conditions where flowers are utilized medicinally. Fruits are beneficial for treating everything from colic to bleeding gums to a need for a laxative. Chewing on the leaves is supposed to alleviate toothache, and a decoction is used to treat cholera. The leaves can also treat wounds, ulcers, worms, and loose bowels. The diarrhea-curing effects of the young leaves' juice are drunk.

Punicagranatum L.
Local name: Dalim
Family: Punicaceae
Habit: Shrubs
Part(s) used: Fruits, Root barks

Ethno-medicinal Uses: To treat diarrhea and stomach pain, ingest a decoction made from the dried fruit rind. The decoction made from the root bark is used to treat tapeworms due to its strong astringent properties.

Quisqualisindica L.
Local name: Madhabilata
Family: Combretaceae.
Habit: Climber
Part(s) used: Seeds.

Ethno-medicinal Uses: Seeds are anthelmintic; given to children to expel the worms.

Ricinus communis L.
Local name: Bherenda
Family: Euphorbiaceae
Habit: Shrub
Part(s) used: Seed, Root, Leaves

Ethno-medicinal Uses: Strong in its ability to relieve constipation, the seed oil is also massaged into the skin to alleviate rheumatic aches, joint discomfort, and paralysis. The decoction of the root bark and leaves is used to treat rheumatism, inflammations, and neurological problems. Dysentery treatment sometimes involves administering the juice of young leaves together with sugar or sugar candies.

Syzygiumcumini(L.) Skeel.
Local name: Jam
Family: Myrtaceae
Habit: Tree

Part(s) used: Leaves, Barks, Seeds, Ripe Fruits Ethno-medicinal Uses: Drinking a mixture of equal parts fresh milk and bark juice first thing in the morning for three days will help treat dysentery. Fresh bark juice is utilized to treat gastrointestinal distress. Dysentery can be cured by drinking the juice of this plant, which contains astringent leaves. The mature fruit juice has several health benefits, including general tonic, liver tonic, stomachic, carminative, and diuretic. The seeds have been used to treat Jaundice; diabetes, and constipation.

Tamarindusindica L.
Local name: Tetul
Family: Caesalpiniaceae
Habit: Tree
Part(s) used: Leaves, Barks, Pulps

Ethno-medicinal Uses: Cholesterol levels can be lowered by consuming pulp. For a soothing effect on a sore throat, try gargling with tamarind water. The astringent seeds can be used to treat diarrhea. Inflammatory swellings, sprains, tumors, and ringworm can benefit from a poultice made from the mashed leaves. The conjunctiva may be poulticed with the flowers to relieve inflammation, and the juice can be taken orally to stop bleeding from piles. Asthma, amenorrhea, fever, diarrhea, and topical use for loss of feeling in paralysis are among conditions that benefit from the astringent and tonic properties of the stem bark.

Terminalia arjuna (Roxb.) Wt. & Arn.
Local name: Arjun
Family: Combretaceae
Habit: Tree
Parts used: Barks.

Ethno-medicinal Uses: Heart conditions, anemia, hyperhidrosis, dysentery, asthma, hypertension, wounds, eruptive skin conditions, menstrual cramps, and leucorrhea can all benefit from the bark's anti-inflammatory, antiseptic, antibacterial, antiviral, and antiviral properties. Redness and swelling of the lips, tongue, and gums can also be relieved, by reducinggum bleeding and the risk of pus development.

Terminalia chebula(Gaertn.) Retz.
Local name: Haritaki
Family: Combretaceae
Habit: Tree
Part(s) used: Fruits

Ethno-medicinal Uses: When combined with honey, powdered seeds are an effective antiemetic for nausea and vomiting. Adding powder to your toothpaste can give it a more granular texture. Trifla Churna, which contains powdered amla and behera, treats digestive problems. Dysentery sufferers might benefit from powdered seed, ghee, and honey.

Zizyphusmauritiana Lam.
Local name: Boroi
Family: Rhamnaceae
Habit: Tree
Part(s) used: Bark, Roots

Ethno-medicinal Uses: The bark is astringent and is used as a treatment for diarrhea, while the powdered bark is used as a home cure for wounds and ulcers. Fever can be treated using roots.

Zingiber officinale Rosc.
Local name: Ada
Family: Zingiberaceae
Habit: Herb

Part(s) used: Rhizome Ethno-medicinal Uses: Zinger may be extracted by boiling its pieces in water. For symptomatic relief of indigestion and cough, consume half a cup of this extract combined with honey twice daily for five to seven days. Rhizome juice is used as a carminative and digestive, as well as for treating flatulence and cold fever when taken with salt. Rhizomes can also be used as a mouthwash when dried.

IV. Results and Discussion

People in the Nabiganj Upozila, Habiganj district, were found to have in-depth knowledge of ethnobotany, according to the current study. This list of medicinal plants is organized alphabetically by plant name, then by the plant's local name, then by its family name, then by the portion of the plant utilized and its therapeutic capabilities. Of the 41 plant families, 68 were used by the inhabitants of Nabiganj Upozila. The primary medicinal plant species were found to consist of 39.70% tree species, 35.29% herb species, 11.76% shrub species, and 13.23% climbers, according to an analysis of the data based on growth habits. There is much variety in the medicinal use of plants. Most medicinal plants employ their leaves first, then their fruits, roots, bark, stem, latex, bulb, rhizomes, seed, pulp, leaf bud, petiole, flower, calyx, and peduncle. Mother plants are impacted by any herbal preparation that uses roots, rhizomes, bulbs, barks, stems, or the entire plant (Dawit and Ahadu, 1993). Mostly vegetation Amaranthaceae, Apocynaceae, Araceae, Acanthaceae, Asclepiadaceae, Asteraceae, Caricaceae, Combretaceae, Cucurbitaceae, Liliaceae, Meliaceae, Moringaceae, Moraceae, Rutaceae, and Solanaceae were found to be the most prevalent families of medicinal plants in the research region. Results from this study corroborate those from Yusuf et al. (2009) and Ghani (2011) on the families of plants most commonly used in therapeutic contexts (1998). These people employ a variety of plant components, but the leaves are the most common for treating wounds on the skin's surface. Most of the time, the plant's new parts are best for making medication. Dried leaves or roots can be substituted with fresh ones if necessary. The people of Nabiganj Upozila, according to the findings of this study, continue to rely on traditional plant medicines for the treatment of a wide variety of ailments, including but not limited to: cough, dysentery, diarrhea, dyspepsia, eczema, eye disease, fever, glaucoma, gonorrhea, headache, high blood pressure, insomnia, intestinal infections, jaundice; low blood pressure, laxative, liver disorders, menstru. It was also hypothesized in the study that future botanical and pharmacological research might benefit from the existing data on the therapeutic usage of plants by local and ethnic communities to find new drug sources.

V. Conclusions

Recent research has revealed that the region is abundant in useful plants for medicine. There are 68 plant species known to cure various ailments, classified into 60 genera and 41 families. The elders in a community typically have more information about local medicinal herbs. Young people in the research region exhibited a decreased interest in traditional techniques, perhaps because traditional healers are not as widely respected and modern therapies are more accessible. That's why fewer people than ever before are turning to nature for their health care needs in the form of plants and plant components. Since it was discovered that ethno medicine is still practiced among the Nabiganj Upozila in the Habiganj district of Bangladesh, it is clear that appropriate regulations are needed to conserve this expertise.

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A Comparative Study on the Proximate Composition and Protein Quality of Soya Bean Flour and Smoked Crayfish Sold in Dschang, Cameroon

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Abstract- A study was carried out to compare the proximate composition and protein quality of soya beans flour and smoked crayfish. Proximate compositions of soya beans flour and smoked crayfish were determined and diets composed for rat growth and balance experimentation. Eighteen (18) weanling male wistar rats were distributed in three groups of six elements. Test groups received respectively soya beans-based and smoked crayfish-based diets. Each protein product was fed as the sole source of 10% dietary protein. The evaluated biological parameters were protein efficiency ratio (PER) and net protein ratio (NPR). Smoked crayfish had significantly ($p < 0.05$) higher values of crude protein (77.31±0.63% DM) and ash (5.89±0.08%) while soya beans flour contained higher values of crude lipid (18.03±0.11%DM), carbohydrates (37.2±0.01% DM) and crude fibre (5.50±0.15% DM). Smoked crayfish showed significantly ($p < 0.05$) higher PER (3.83) and NPR (3.96) values compared to soya bean (3.20) and (3.39) respectively. Smoked crayfish could be a better source of dietary protein especially in weaning diet.

Keywords: *proximate composition, soya bean flour, smoked crayfish, protein quality, PER, NPR.*

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Strictly as per the compliance and regulations of:

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I. Introduction

Weaning is described as the transitional period starting from 4 months onwards till the end of second or even third year in certain cultures so that the infant's diet progressively changes from milk alone to semi-solids and finally to the adult family food (1). WHO recommends the introduction of complementary foods (traditional or commercial weaning foods) around the sixth month of life, instead of between the fourth and sixth month, as previously recommended (2). During this period, children need nutritionally balanced supplementary foods in addition to breast milk because of the increasing nutritional demands of the growing body (3, 4).

Several commercial weaning foods are marketed in developed and developing countries, but they are too expensive for people of low socio-economic status, especially those in the rural areas.

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In developing countries, Cameroon inclusive, the low- income mothers use traditional weaning foods formulated from local inexpensive and readily available raw materials such as cereals (maize and rice) and legumes (soya beans flour). 70% of the traditional weaning foods are supplied by cereals which are relatively poor source of protein (5). Cereals are deficient in essential amino acids like lysine and tryptophan while, legumes are deficient in sulphur containing amino acids, that is methionine and cystine, but rich in tryptophan and lysine(6).The traditional weaning foods formulated by mothers in the rural areas contain high levels of carbohydrate with little or no protein due to the high cost of animal protein rich foods. This could be the cause of high prevalence of protein-energy malnutrition (PEM) during the weaning period (7, 8).

Many researchers have proposed traditional weaning foods formulated from maize, rice, soya bean, potatoes, vegetable, fishmeal, and crayfish in varying proportions (9, 8, 10). Unfortunately, in Cameroon, the rural mothers do not use these food formulas because of time constraint. They formulate traditional weaning food predominantly made up of maize flour (source of carbohydrate) and small amount of soya bean flour (source of protein). Mothers exclude crayfish (*Euastacusspp)* in the traditional weaning food which is highly recommended by researchers (8). Crayfish, classified as an animal polypeptide and a freshwater crustacean, is relatively cheap, affordable and readily available throughout the year. Crayfish is a good source of protein (36 -45%) with a superior biological value, true digestibility, net protein utilization, high content of essential amino acid, and protein efficiency compared to casein (11, 12). It is very low in carbohydrate but rich in vitamin D, A and mineral elements such as calcium, potassium, copper, zinc and iodine, (13, 14).

In Dschang, locally processed soya bean flour and smoked crayfish are sold in the market. This work was therefore designed to compare the nutritional value of these foods which are consumed by the population without any quality control.

II. Materials and Methods

a) Source of food samples

Smoked crayfish and soya beans flour were brought from Dschang market, West Region of Cameroon. The smoked crayfish sample was sorted for stones, disposed of all kinds of waste and ground into powder using an electric blender (Sinbo Multifonction Blender Robots) to obtain a meal. The soya beans flour and the ground smoked crayfish were separately stored in identified tightly corked stainless containers awaiting proximate analysis and formulation of test diets.

b) Proximate composition

Soya beans flour and the ground smoked crayfish samples were separately analyzed for proximate composition. The method of AOAC (15) was adopted for the estimation of crudeproteins, crude fiber, ash and crude lipid. All analyses were carried out in triplicate. These analyses were necessary for the formulation of test diets. Total carbohydrate content was determined by subtracting the sum of crude fat, crude proteins, ash and crude fibre contents from 100g of the food (16).

c) Formulation of experimental diets

A total of 3 diets were prepared as per ICN (17) protocol. Table 1 gives the composition of the experimental diets. The 2 test diets were formulated on the basis of their proximate compositions with all the diets containing 10% protein. The protein free diet provided an estimate of weight loss (protein used) due to metabolic processes while the soya beans flour-based, and smoked crayfish-based served as the two test diets.

Table 1: Composition of the experimental diets used in biological assay (g/100 g complete diet)

Ingredients (g/100g complete diet)	Protein free diet	*Soya bean diet*	Smokedcrayfishdiet
Corn starch	15	15	15
Pure soybeanoil	5	5	5
Minera l Complex	4	4	4
Vitamin Complex	1	1	1
Cellulose	7,9	7,9	7,9
Sucrose	67,1	38,1	52,1
Protein	0	0	0
Soya beans flour	0	29	0
Smokedcrayfish	0	0	15
Total	100	100	100

d) Experimental design

A rat feeding study was carried out to determine the protein quality of the test diets based on rat growth. Twenty four weanling male wistar rats (Ratusno-vergicers) aged 21-23 days with an average weight of 28.5 ± 2.7g, bred in the Biochemistry Departmental Animal House, University of Dschang, Cameroon, were used. The animals (rats) were divided into 3 groups (n = 8), with differences in average group weight not more than 3 as recommended by AOAC (18). Protein-free group was placed on protein-free diet and the 2 test groups received respectively soy bean-based and smoked crayfish-based diets.

The animal cages used were designed as described by Sarwar and Estatira (19). The animal were housed individually in labeled stainless steel screening bottom plasticcages, to permit free dropping of feces and stainless steel mesh tops to ease ventilation. Highly absorbent paperwas placed under the cages to catch spilled food and to minimize contamination of feces with urine. All the cages were placed away from direct sunlight in a cage rack, thoroughly cleaned daily and maintained at room temperature with 12 hours light/dark cycle. The rats were given the corresponding diets and water ad libitum for 14 days and records of daily food consumption and body weights were kept from the fifth day. The first 4 days were considered acclimatization period.

e) Protein quality determination

Records of dailyfood consumption and body weights of individual rats were kept during the collection period. Protein efficiency ratio(PER) and Net protein ratio (NPR) values (10days), were calculated using the following equations (20).

$$\text{PER} = \frac{\text{Weightgainoftestrat (g)}}{\text{Total protein consumed by test rat (g)}}$$

$$\text{NPR (g/g)} = \frac{\text{Weightgain of test rat + Weight loss of protein free rat}}{\text{Total protein consumed by test rat}}$$

f) Data analysis

Statistical analyses were performed with the aid of SPSS for windows software programme (Release 11.0). Data were submitted to analysis of variance (ANOVA), and the test of Duncan was used to compare treatment means at a 5% significance level.

III. Results and Discussion

a) Proximate composition

Table 2 presents the proximate composition of the smoked crayfish and soya bean flour. Chemical

composition (lipids, proteins, carbohydrates and ashes) varied with source of protein. The crayfish showed significantly ($p < 0.05$) higher values crude protein content (77.305±0.63%DM). The soja bean has more fat (18.025±0.11%DM), crude fibre (5.495±0.15%DM) and carbohydrate (37.2%DM).

Table 2: Proximate composition of the crayfish and soya bean

Sample	Dry matters	Lipids (%DM)	Proteins (%DM)	Ash (%DM)	Carbohydrates (%DM)	Crude fiber (%DM)
Crayfish	87.55 ± 0.10b	4.23 ± 0.16b	77.30 ± 0,63a	5.89 ± 0,08a	8.93± 0.10b	3.66 ± 0.16b
Soya bean	96.52 ± 0.11a	18.02 ± 0.11a	35.67 ± 0,06b	3.61 ± 0,27b	37.2± 0.12a	5.50 ± 0.15a

Values are means (n =3). Within a column, values with different superscripts are significantly different ($p < 0.05$). DM: Dry Matter.

Proximate composition was carried out to have an idea of the nutrient contents of soya beans flour and smoked crayfish. The results of this study revealed different values of nutrients in soya beans flour and smoked crayfish with smoked crayfish having higher value of crude protein and lower value of crude lipid. This agrees with the findings of other researchers (13, 12, 14).However, the values gotten in this study were higher than those presented by other studies. The reason being that our results were presented as percentage dry matter.

b) Protein quality

The protein efficiency ratio (PER) and net protein ratio (NPR)of test diets are showed in table 3. Crayfish showed significantly ($p < 0.05$) the higher PER value (3.83) compared to soya bean (3.20). The results showed that crayfish had significantly ($p < 0.05$) the higher NPR (3.96) and soya bean had the lower (3.39). The rat growth and food intake data needed to determine PER and NPR of the diets are also showed in table 3. The rats fed crayfish (8.36g/day) registered the higher food consumption, and soya bean diet (5.73g/day) had the lower.

The weight gain followed approximately the same trend as the food consumption. The crayfish diet showed the higher weight gain (28.75g/10days), and soya bean diet (16.43g/10days) had the lower.

Table 3: Growth of rats and protein quality of experimental diets*

Diets	Growth of rats		Protein quality	
	Weight gain(g/10 days)	Food consumed (g/10 days)	PER	NPR
Crayfish	28.75±0.02a	8.36 ± 0.01a	3.83 ± 0.02 a	3.96 ± 0.02 a
Soya bean	16.43 ± 0.05b	5.73 ± 0.03b	3.20 ± 0.03 b	3.39 ± 0.03 b

**values are means (n =8). Within a column, values with different superscripts are significantly different ($p < 0.05$). PER: Protein Efficiency Ratios, NPR: Net protein ratio.*

The smoked crayfish-based diet had a better protein quality than the soya bean flour-based diet. This followed a trend similar to the findings of Ibironke, *et al*.(12) and Ibironke, *et a*l. (14) though higher. The lower protein quality observed with the soya bean flour-based diet agrees with the report of Hertzler et al.(21) which indicates that the nutritional quality of plant proteins may be inferior in some respects relative to animal proteins.NPR values of all the protein sources (soya bean flour and smoked crayfish) were higher than the PER values. The reason being that NPR method unlike the PER method credits protein used for both growth and maintenance (22). The protein required to prevent weight loss of rats fed the protein-free diet is assumed to be equivalent to the protein needed for maintenance. PER measures only growth and not maintenance, hence NPR is more reliable than PER to determine the protein quality of a food (23, 24).

IV. Conclusion

This study has demonstrated that rats fed smoked crayfish-based diet had significantly higher NPR value than those fed soya bean-based diet. This suggests that the proteins in smoked crayfish were more biologically available than those in soya bean flour. Hence, smoked crayfish could be a better source of dietary protein especially in weaning diet. Dietary protein sources intraditional weaning foods could be either the smoked crayfish alone or a mixture of smoked crayfish and soya bean flour.

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Author Contributions

All the authors collaborated during the execution of this research work. Author EEA designed study, wrote the protocol and the first draft. Authors GTY and BT participated in sample collection, proximate analysis and animal experimentation. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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A Comparative Study on the Larvicidal Effect of Ethanol Leaf Extracts of *Cymbopogon Citratus* (Lemongrass) and *Ximenia Americana* (Sea Lemon) on *Anopheles* and *Culex* Larva

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Abstract- *Aim:* Synthetic insecticides are widely being used for the control of mosquitoes. However this is faced with many challenges among which are development of insecticide resistance by the mosquitoes, damage to the environment, effect on human health and non-target organisms. This study thus aimed at comparing the larvicidal effect of leaves extracts of Cymbopogoncitratus (Lemon grass) and Xymenia-americana(Sea lemon) against anopheles and culex larvae.

Method: The leaves components were extracted and phytochemical analyses of the extracts were also done. Anopheles and culex larva were collected and identified using morphological features. Larvicidal susceptibility test were carried out using WHO standard method and the mortalities were observed after 24 hours and 48 hours of exposure.

Keywords: *cymbopogon citratus, xymenia americana, anopheles, culex, laevae.*

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Strictly as per the compliance and regulations of:

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Abdullahi Hasan Amoto [α], Muhammad Yusha'u [σ], Umar Sani Inuwa [ρ], Salawudeen Shuaibu Omeiza [ω] & Abdulrahman Itopa Suleiman [¥]

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Method: The leaves components were extracted and phytochemical analyses of the extracts were also done. Anopheles and culex larva were collected and identified using morphological features. Larvicidal susceptibility test were carried out using WHO standard method and the mortalities were observed after 24 hours and 48 hours of exposure.

Results: Higher mortality was observed in *anopheles* larvae exposed to ethanol leaf extracts of *C. citrates* after 24 hours of exposure (LC50 5.905mg/ml and LC90 16.241mg/ml) compared to the ethanol leaf extract of *X.americana* with the lower motality after 24 hours of exposure (LC50 7.617mg/ml and LC90 43.471mg/ml). Against *culex* larvae, higher motality was also observed in ethanol leaf extract of *C. citrates* after 24 hours of exposure (LC50 6.851mg/ml and LC90 25.678mg/ml) compared to ethanol leaf extract *X. americana* with the lower motality after 24 hours of exposure(LC50 10.626mg/ml and LC90 54.434mg/ml).

Conclusion: The result suggest that the ethanol leaf extract of the plants used possessed significant larvicidal activities against the *anopheles* and *culex* laeva. However, the *C. citratus* extract is more potent than that of the *X. Americana* extract. These show that they may be considered as natural sources for the production of natural larvicides.

Keywords: cymbopogon citratus, xymenia americana, anopheles, culex, laevae.

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I. Introduction

Mosquitoes are the most important single group of insects in terms of public health, which transmit a number of diseases such as malaria, filariasis, dengue and zika virus, causing millions of deaths every year [28, 4]. Malaria is transmitted by female anopheles mosquito from person to person. Various species have been found to be the vectors in different parts of the world. *Anopheles gambiae* complex is the principal vector in Africa [7]. Vector control strategies have traditionally focused on killing mosquitoes using a variety of insecticides. Environmental management (through reduction or removal of mosquito breeding sites) has often been used alongside chemicals or microbiological ovicides, larvicides and pupicides [31] in areas where endemics mosquito-borne diseases occur. The use of synthetic insecticides has to be regulated given that the development of insecticide resistance is widespread and that there is concern regarding the damage of the environment, effect on human health and non-target organisms [21]. Hence there is a need to develop and incorporate new alternative insecticidal agent.

In recent years, the emphasis on control of the mosquito population has shifted steadily from the use of conventional chemicals towards more specific and environmentally friendly materials, which are generally of botanical origin. Plant products have been used traditionally by human communities in many part of the world against the vector and pest species of insects [25]. The plant derived natural products as larvicides have the advantage of being harmless to beneficial non-target organisms and environment when compared to synthetic insecticides [27]. The demand for plant-based insecticides is that they are non-toxic, easily available at affordable prices, biodegradable and show broad spectrum, target specific activities against various species of mosquitoes. A lot of phytochemicals extracted from various plant species have been tested for their larvicidal actions against mosquitoes [25]. The use of active toxic agent from plant extract as an alternative mosquito control strategy was dated back to

ancient time as such many studies on plant extract and their active constituent compounds against mosquito larvae have been carried out in different parts of the world [16, 18]. Although, there are several reports on the antibacterial effect of *Ximenia Americana,* there is dearth of information on the larvicidal effect of the plant, thus this work is aimed atcomparing the larvicidal effect of ethanol leaf extracts of *Cymbopogon ctratus* and *Ximenia americana.*

II. Materials and Methods

a) Plant identification and harvesting

The plant samples of *C.citratus and X. americana leave* were collected from the botanical garden behind Aminu Kano Teaching Hospital (AKTH) and Jibga Town in Bebeji Local Government Area (LGA) Kano respectively. Each plant specimen was submitted to the Herbarium division of Plant Science Department, Bayero University Kano (BUK) and then identification was confirmed in the laboratory according to Delziel [9].

b) Processing of samples to remove pesticides residues

Harvested plant samples were washed with tap water, a commercial detergent solution and rinsed again with distilled water to remove all traces of pesticides [33]. The leaves were then shade dried and electric blender was used to grind the dried leaves of the plant into powder at pharmacology laboratory, department of Pharmacology, Bayero University Kano.

c) Extraction of leaves component

The plant powder was extracted according to the method of Anees [2], using soxhlet extraction device. Fifty grams each of the plant fine powder of *Cymbopogon citratus* and *Ximenia americana* were extracted using the soxhlet extraction with ethanol as a solvent. The solvent ethanol was then evaporated to obtain ethanolic extract of the leaves using rotary evaporator.

d) Phytochemical Screening

The plant extracts were analyzed for the presence of Alkaloids, flavonoids, saponins, phytosteroids and tannins as described by Ngbede *et al*. [22] and Amzad *et al*. [1].

i. *Test for alkaloids*

About 1g powder sample was mixed with 3ml of ammonia solution in a conical flask. It was then allowed to stand for 3 minutes to evaluate for free alkaloids. Chloroform (10ml) was added to the conical flask, shaken and then filtered. The chloroform was evaporated from the crude extract by water bath and Mayer's reagent (3ml) was added. Observation for cream precipitate was done and result was recorded.

ii. *Test for flavonoids*

About 1ml of stock solution was taken in a test tube, four drop of dilute NaOH solution was added. An intense yellow colour appeared in the test tube. It became colorless upon addition of few drop of dilute acid which indicate the presence of flavonoids.

iii. *Test for saponins*

About 1ml of stock solution was taken in a test tube and diluted with 20ml of distilled water. It was shaken by hand for 15minutes. Observation for foam layer was done and the result recorded.

iv. *Test for phytosteroids*

About1ml of the crude plant extract was taken and mixed with chloroform (10ml) and then equal volume of concentrated sulphuric acid was added to the mixture. Observation for colour change was done and result recorded.

v. *Test for tannins*

About 3ml of the crude extract mixed with chloroform and 1ml of acetic anhydride was added. Finally, 1ml of sulphuric acid was added carefully by the side of test tube to the solution. A green color shows the presence of tannins.

vi. *Test for resins*

About 5ml of petroleum ether was added to 1g of the powdered extract. Equal volume of copper acetate solution was added and shaken vigorously, then allow to separate. A green color indicates the presence of resins.

e) Mosquito larval collection and identification

Anopheles larvae were collected from rice field, and small temporary rain pools in Doko town Garki L.G.A, Jigawa State, Northern Nigeria while *Culex* larvae were collected from drainage behind provost office of college of health sciences, Aminu Kano Teaching Hospital, Kano. Larvae were collected by dipping method using entomological larval spoons, plastic cups and suitable containers. The larvae were processed at site of collection, worms and other insects were removed according to the method described by Cheah *et al.,* [6].

Morphological features such as presence or absence of siphons, resting position to water surface contained in taxonomic keys were used to identify the *Anopheles* and *Culex* larvae as described by Gillies and Coetzee [13].

f) Transportation of mosquito larvae

All specimen collected from a particular breeding place was kept in a plastic bucket and labeled. (Date and site of collection). The buckets were not filled to the brim in other to allow air space for the larvae to breath. The buckets were well covered before they were transported to the laboratory [29].

g) Rearing of mosquito larvae

The collected larvae were kept inside in a rearing bowl, at 270C. The larvae were fed daily with yeast according to WHO [34].

h) Larvicidal effect of the plant extracts

The efficacy of the plants extracts as larvicides against the *Anopheles* and *culex* mosquito larvae were evaluated in accordance with guidelines of World Health Organization (WHO) standard method [34]. Ten milliliter (10 ml) of the solution for each concentration 30mg/ml, 20mg/ml, 10mg/ml and 5mg/ml was placed in a small plastic container, mixed thoroughly with 90 ml of distilled water. Batches of 25 matured larvae in four replicates were exposed to each of test concentrations. During the period of exposure larvae were fed with yeast [34]. The control contains 100 ml of distilled water with 0.2 ml of ethanol.

i) Determination of mortality

The effect of each plant extract was carefully monitored. Larvae were considered dead if they were unable to move after touching with a needle. Moribund larvae were unable to rise to the surface when the water was disturbed. The mortality was observed counted and recorded after 24 and 48 hours of exposure and the percentage mortality was computed using the expression below according to WHO [34].

$$Percentage\ Motality = \frac{Number\ of\ dead\ larvae}{Numberof\ larvae\ introduced}\ x\ 100$$

j) Statistical analysis

The statistical tools used in this study include; Arithmetic mean to get the average number of dead larvae and percentage mortality as well Probit Analysis using SPSS 20 software to calculate LC_{50} and LC_{90} values to determine lethal concentrations of the plant extracts on mosquito larvae at 24 and 48 hours of treatment. Analysis of variance (ANOVA) and a two tailed type one t- test was used to determine whether there exists a significant difference in the mean mortality over the period of observation with the 5% confidence interval.

III. Results

a) Phytochemicals constituents of the plants extract

The result shows the phytochemical constituents of the *C. citratus* and *X. americana* leave extracts. As shown on table 3.1, *C. citratus* contains alkaloids, saponins, flavanoids, phytosteroids, tannins and resins while *X. americana* contains saponnins, flavanoids, tannins and resins only, it does not contain alkaloids and phytosteroids.

Table 3.1: Phytochemical constituents of the plants extracts

Plants	Alkaloids	Saponins	Flavonoids	Steroids	Tannins	Resins
C. citratus	+	+	+	+	+	+
X.americana	-	+	+	-	+	+

+: Phytochemical presence,

-: Phytochemical absent.

b) Larvicidal activities of plants extracts against Anopheles larvae

The result shows the larvicidal effects of *C. citratus* and *X. americana* against *Anopheles* larvae. At concentration of 5mg/ml *C. citratus* shows 45% mortality after 24 hours and 65% mortality after 48 hours of exposure. At concentration 10mg/ml it shows 73% mortality after 24 hours and 87% after 48 hours. At concentration of 20mg/ml it shows 90% mortality after 24 hours and 100% after 48 hours. At concentration of 30mg/ml it shows 99% mortality after 24 hours and 100% after 48 hours while *X. americana* on the other hand shows 38% mortality after 24 hours and 53% after 48 hours at concentration of 5mg/ml. At concentration of 10mg/ml it shows 54%% mortality after 24 hours and 69% after 48 hours. At concentration of 20mg/ml it shows 72% mortality after 24 hours and 92% after 48 hours. At concentration of 30mg/ml it shows 85% mortality after 24 hours and 100% after 48 hours. There is significant association between *Anopheles* larvae mortality and the concentration of the extracts at $P<0.05$.

Table 3.2: Larvicidal effects of plants extracts of against *Anopheles* larvae

Plant type	Concentration (mg/ml)	n	Percentage Mean 24 hours	Mortality 48 hours
Control		25	0	0
C. citratus	5	25	45.00±2.00	65.00±3.83
	10	25	73.00±2.00	87.00±2.00
	20	25	90.00±2.31	100.00±0.00
	30	25	99.00±2.00	100.00±0.00
P- value			0.0000	0.0000
X. americana	5	25	38.00±2.31	53.00±2.00
	10	25	54.00±2.31	69.00±3.83
	20	25	72.00±3.27	92.00±0.00
	30	25	85.00±2.00	100.00±0.00
P- value			0.0000	0.0000

c) *Larvicidal activities of plants extracts against Culex larvae*

The result shows the larvicidal effects of *C. citratus* and *X. americana* against *Culex* larvae. At concentration of 5mg/ml *C. citratus* shows 37% mortality after 24 hours and 67% mortality after 48 hours of exposure. At concentration 10mg/ml it shows 68% mortality after 24 hours and 90% after 48 hours. At concentration of 20mg/ml it shows 83% mortality after 24 hours and 100% after 48 hours. At concentration of 30mg/ml it shows 92% mortality after 24 hours and 100% after 48 hours while *X. americana* on the other hand shows 28% mortality after 24 hours and 40% after 48 hours at concentration of 5mg/ml. At concentration of 10mg/ml it shows 47% mortality after 24 hours and 67% after 48 hours. At concentration of 20mg/ml it shows 67% mortality after 24 hours and 84% after 48 hours. At concentration of 30mg/ml it shows 81% mortality after 24 hours and 100% after 48 hours. There is significant association between *Anopheles* larvae mortality and the concentration of the extracts at $P<0.05$.

Table 3.3: Larvicidal effects of plants extracts of against *Culex* larvae

Plant type	Concentration (mg/ml)	n	Percentage Mean 24 hours	Mortality 48 hours
Control		25	0	0
C. citratus	5	25	37.00±2.00	67.00±2.00
	10	25	68.00±4.62	90.00±2.31
	20	25	83.00±2.00	100.00±0.00
	30	25	92.00±0.00	100.00±0.00
P- value			0.0000	0.0000
X. americana	5	25	28.00±0.00	40.00±0.00
	10	25	47.00±2.00	67.00±2.00
	20	25	67.00±2.00	84.00±0.00
	30	25	81.00± 2.00	100.00±0.00
P-value			0.0000	0.0000

d) *Comparative larvicidal effects of plants extracts against Anopheles larvae*

At concentration of 5mg/ml *C. citratus* shows 45% mortality while *X. americana* shows 38% with a p-value of 0.8184 after 24 hours of exposure and after 48 hours *C. citratus* shows 65% while *X. americana* shows 53% with a p-value of 0.0000.At concentration of 10mg/ml *C. citratus* shows 73% mortality while *X. americana* shows 54% with a p-value of 0.8184 after 24 hours of exposure and after 48 hours *C. citratus* shows 87% while *X. americana* shows 69% with a p-value of 0.8184. At concentration of 20mg/ml *C. citratus* shows 90% mortality while *X. americana* shows 72% with a p-value of 0.5825 after 24 hours of exposure and after 48 hours *C. citratus* shows 100% while *X. americana* shows 92% with an undefined p-value. At concentration of 30mg/ml *C. citratus* shows 99% mortality while *X. americana* shows 85% with a p-value of 1.0000 after 24 hours of exposure and after 48 hours *C. citratus* shows 100% while *X. americana* shows 100% with an undefined p-value.

Table 3.4: Comparative larvicidal effects of plants extracts against *Anopheles* larvae Concentration of plant extract (mg/ml)

		Concentration of plant extract (mg/ml)			
Plant type	Time (hrs)	5	10	20	30
C. citratus	24	45.00±2.00	73.00±2.00	90.00±2.31	99.00±2.00
X. americana		38.00±2.31	54.00±2.31	72.00±3.27	85.00±2.00
P-value		0.8184	0.8184	0.5825	1.0000
C. citratus	48	65.00±3.83	87.00±2.00	100.00±0.00	100.00±0.00
X. americana		53.00±2.00	69.00±3.83	92.00±0.00	100.00±0.00
P- value		0.0000	0.8184	-	-

e) *Comparative larvicidal effects of plants extracts on Culex larvae*

The result shows the comparative larvicidal effects of *C. citratus* and *X. americana* against *Culex* larvae. At concentration of 5mg/ml *C. citratus* shows 37% mortality while *X. americana* shows 28% with a p-value of 0.0000 after 24 hours of exposure and after 48 hours *C. citratus* shows 67% while *X. americana* shows 40% with a p-value of 0.0000.At concentration of 10mg/ml *C. citratus* shows 68% mortality while *X. americana* shows 47% with a p-value of 0.2025 after 24 hours of exposure and after 48 hours *C. citratus* shows 90% while *X. americana* shows 67% with a p-value of 0.8184. At concentration of 20mg/ml *C. citratus* shows 83% mortality while *X. americana* shows 67% with a p-value of 1.0000 after 24 hours of exposure and after 48 hours *C. citratus* shows 100% while *X. americana* shows 84% with an undefined p-value. At concentration of 30mg/ml *C. citratus* shows 92% mortality while *X. americana* shows 81% with a p-value of 0.0000 after 24 hours of exposure and after 48 hours *C. citratus* shows 100% while *X. americana* shows 100% with an undefined p-value.

Table 3.5: Comparative larvicidal effects of plant extractagainst *Culex* larvae

		Concentration of plants extract (mg/ml)				
Plant type	Time (hrs)	5	10	20	30	
C. citratus	24	37.00±2.00	68.00±4.62	83.00±2.00	92.00±0.00	
28.00±0.00		47.00±2.00	67.00±2.00	81.00±2.00		*X. americana*
P-value		0.0000	0.2025	1.0000	0.0000	
C. citratus		4867.00±2.00	90.00±2.31	100.00±0.00	100.00±0.00	
X. americana		40.00±0.00	67.00±2.00	84.00±0.00	100.00±0.00	
P- value		0.0000	0.8184	-	-	

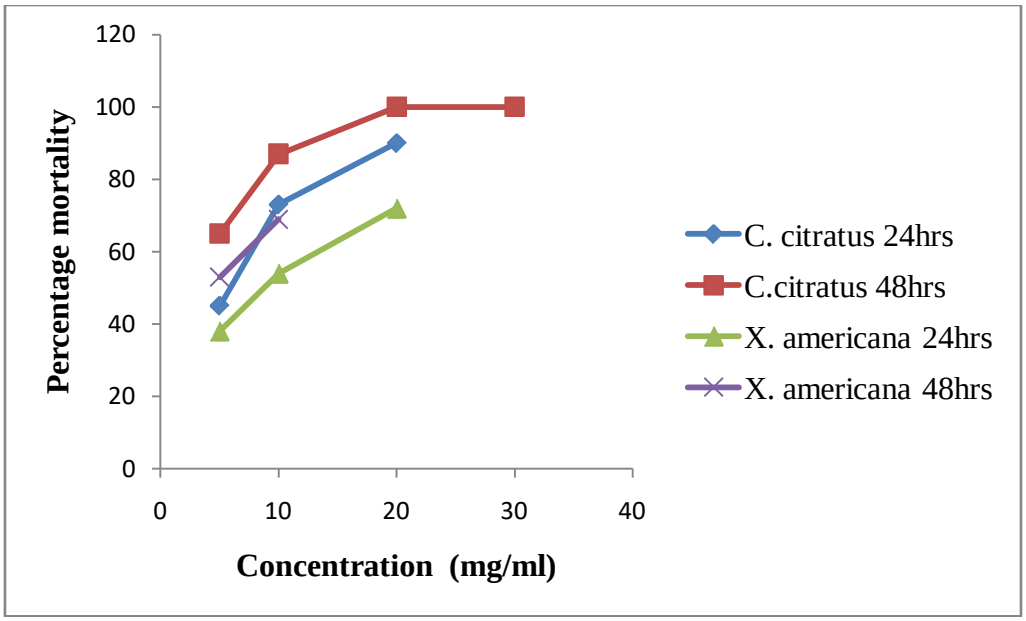

Figure 3.1: Percentage mortality of anopheles in plants extracts

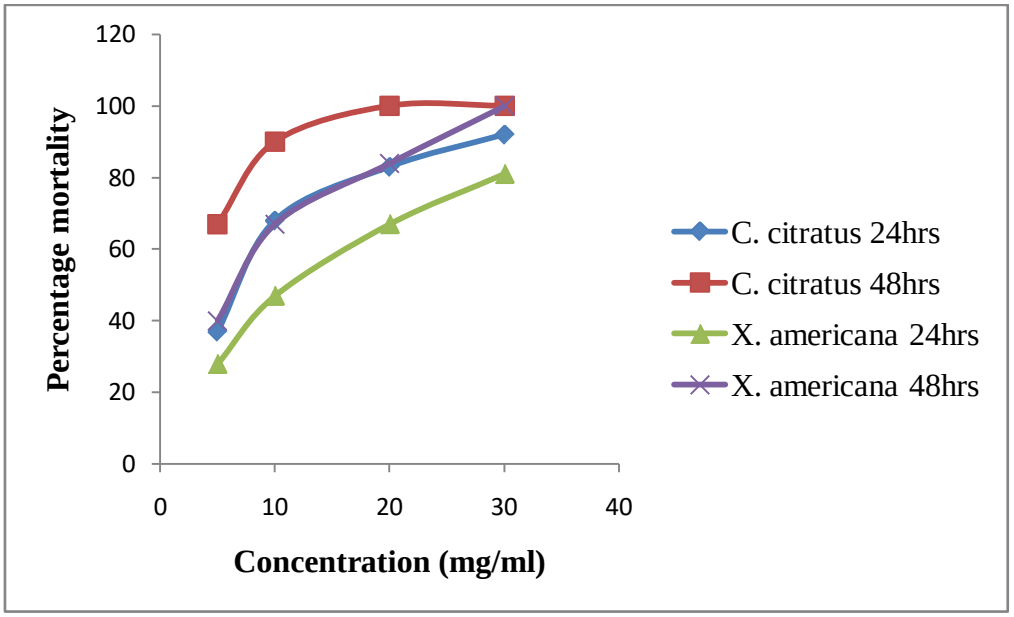

Figure 3.2: Percentage mortality of culex in plants extracts

f) LC_{50} and LC_{90} of plants extracts on Anopheles larvae

The result of probit analysis on *Anopheles* larvae to different concentration of *C. citratus* after the period of exposure showed that the lethal concentration capable of killing 50% of the larvae LC_{50} after 24 and 48 hours were 5.095 and 3.731mg/ml and LC_{90} were 16.241 and 9.391mg/ml respectively. While for *X. americana* LC_{50}were 7.617 and 5.377mg/ml and LC_{90}were 43.471 and 16.911mg/ml after 24 and 48 hours respectively.

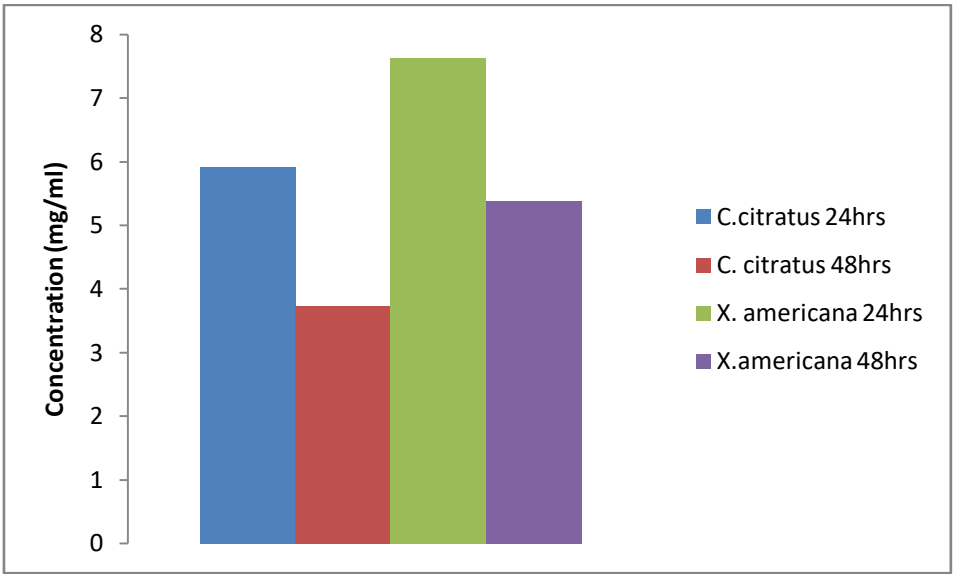

Figure 3.3: LC_{50} of extract of the plants against anopheles larvae

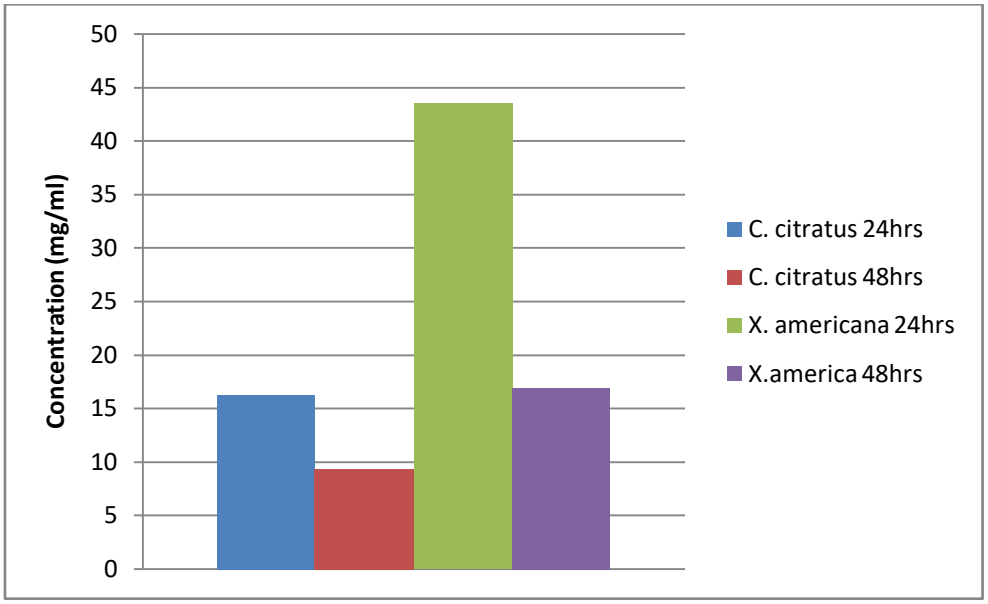

Figure 3.4: LC_{90} of extract of the plants against anopheles larvae

g) LC_{50} and LC_{90} of plants extracts on Culex larvae

The result of probit analysis on *Anopheles* larvae to different concentration of *C. citratus* after the period of exposure showed that the lethal concentration capable of killing 50% of the larvae LC_{50} after 24 and 48 hours were 6.851 and 3.7741mg/ml and LC_{90} were 25.678 and 8.584mg/ml respectively. While for *X. americana* LC_{50}were 10.626 and 6.534mg/ml and LC_{90}were 54.434 and 20.130mg/ml after 24 and 48 hours respectively.

Table 3.8: LC_{50} and LC_{90} of plants extracts on *Culex* larvae

Plants	Times (hrs)	LC_{50} (mg/ml)	LC_{90} (mg/ml)
C. citrates	24	6.851	25.678
	48	3.774	8.584
X. Americana	24	10.626	54.434
	48	6.534	20.130

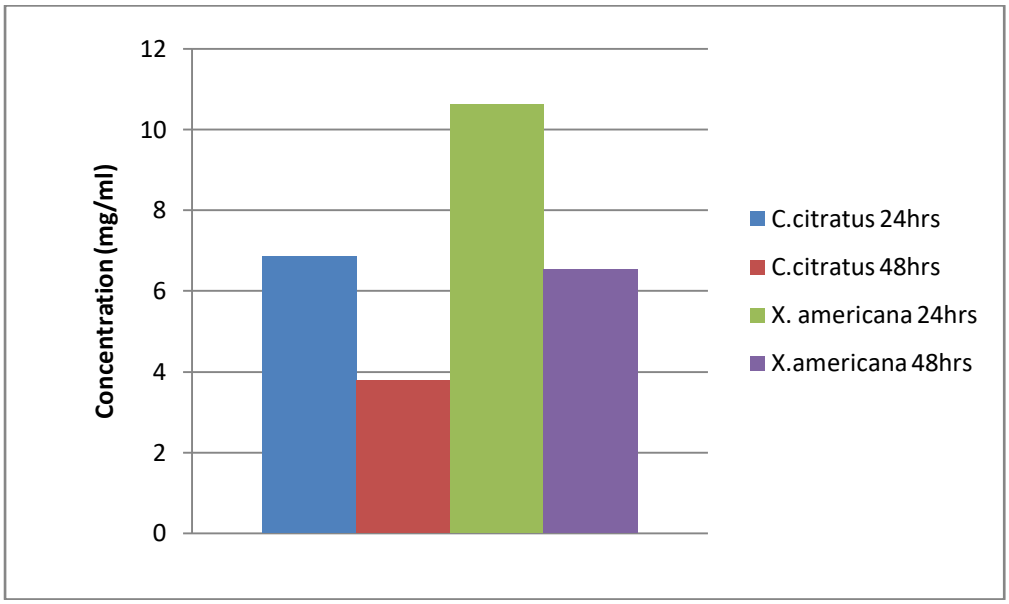

Figure 3.5: LC_{50} of extract of the plants against culex larvae

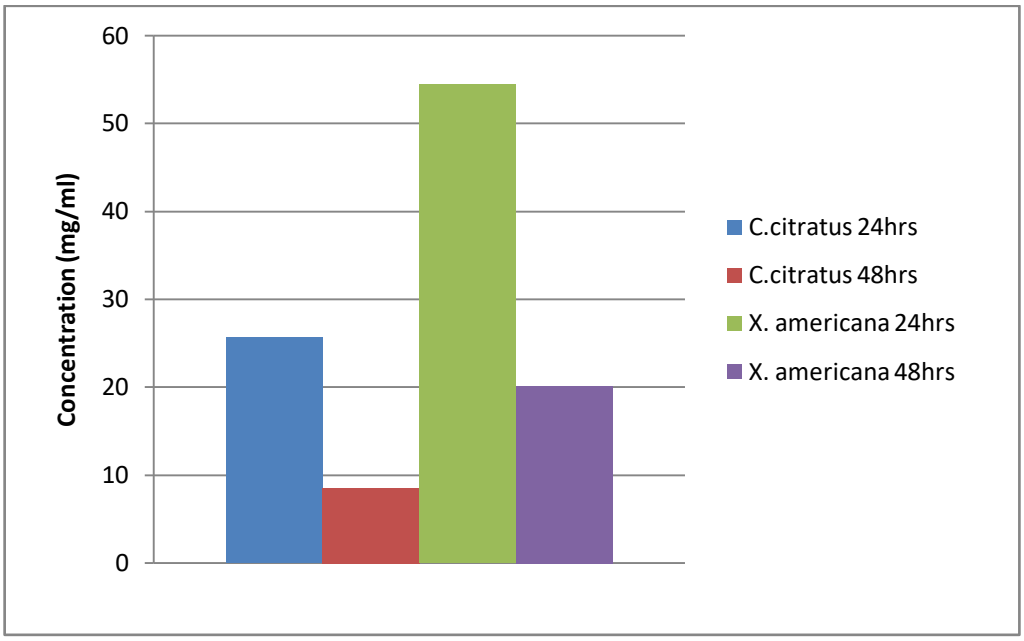

Figure 3.6: LC_{90} of extract of the plants against culex larvae

IV. Discussion

Result of the phytochemical consitituents *C. citratus* show that it contains alkaloids, saponins, flavanoids, phytosteroids, tannins and resins. Researchers such as Hasim *et al.*[14], identified the same phytochemicals in *C. citratus*. Egunyomi *et al.* [11] worked on the phytochemical component of Nigerian medicinal plants and found the above mentioned active components in *Citrus sinensis* and *C. citratus*. Otabor *et*

al.[24]also reported the presence of the above components in the methanolic extract of *C. citratus*.

However, *X. americana* contains saponnins, flavanoids, tannins and resins only, it does not contain alkaloids and phytosteroids which is in line with the findings of Shagal *et al.* [32], Ogunleye and Ibiotoye [23] also reported the absence of alkaloid in *X. americana.* This variation may be due to geographical location and soil type. More so, Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, they protect plants from diseases, damage and also contribute to the plants colour, aroma and flavor [30]. Insecticidal effects of plants extracts vary not only according to plant species, mosquito species, geographical variation and plant part used, but also due to method of extraction followed and the polarity of the solvent used during extraction, Phytochemicals are extracted either from the whole body of little herbs or from various parts like fruits, leaves, stem bark and roots etc of larger plants or trees. In all cases where the most toxic substances are concentrated are usually extracted for mosquito control [3].

From the mortality bioassay of the ethanolic leaves extract of *C. citratus* and *X. americana* shows a proven larvicidal effect against both the mosquito larvae tested (*Anopheles* and *Culex*) at different concentrations. The highest concentration of 30mg/ml from *C. citratus* shows 99% mortality within 24 hours of exposure against *Anopheles* larvae while against *Culex l*arvae the same extract at same concentration shows 92% mortality. Similarly, other concentrations, 20mg/ml and 10mg/ml shows almost 100% mortality on both *Anopheles* and *Culex* mosquito larvae after 48 hours of exposure with the same plant extract. The 5mg/ml concentration shows 65% mortality against *Anopheles* larvae and 67% mortality against *Culex* larvae after 48 hours of exposure with the same plant extract.

In contrast, the highest concentration (30mg/ml) of *X. americana* leave extract shows 85% mortality within 24 hours of exposure against *Anopheles* larvae and 81% against *Culex* larvae. Other concentrations, 20mg/ml and 10mg/ml shows more than 50% mortality against both *Anopheles* and *Culex* mosquito larvae after 48 hours of exposure with the same plant extract.

The leaves extract of *C. citratus* and *X. americana* were found to be effective in killing the mosquito larvae, but *C. citratus* extract have more effect on the larvae compared to *X. americana* extract. The results of larvicidal activity showed that the percentage mortality of larvae increases with the increase in concentration of the extract with prolong exposure to treatment. However, in all the experiments no mortality was recorded in the control group which shows that the mortality observed in treating the larvae with extract is due to the presence of some active ingredients that are proven to have some larvicidal properties against mosquito larvae as described by Anupam *et al.,* [3]. Higher percentage mortality was observed on *anopheles* larvae than on *culex* larvae which could be as a result of their breeding habitat, *Anopheles* larvae breed in a fresh water that are free of pollutant, therefore find the plant extracts more toxic than *Culex* larvae which breed in a stagnant water containing pollutants and other toxic substances, therefore they are more resistant to the plant extracts. The comparison of percentage mean mortalityof larvae in extract of *C. citratus* and that of *X. americana* showed significant difference at various concentrations and hours of exposure while some showed no significant difference at all concentration and hours of exposure. This could be due to the possession of various phytochemicals constituents that are responsible for the larval mortality, some of these constituents are common within the plants while some are not, therefore the variation in the percentage mortality of the larvae could be as a result of the phytochemical constituents present in the plants. The result of this research shows that leave extracts of *X. americana* has larvicidal effect on mosquito larvae and it could be due to the presence of secondary metabolites present in the plant such as flavonoids, saponins, tannins, and resins. Flavonoids have been reported to has insecticidal properties acting as a mitochondrial poison, which blocks the electron transport chain and prevent energy production (Musau et al., 2016). Flavonoids have also been reported to inhibit Acetylcholinesterase, act as an insect growth regulator and antifeedant (Jagruti *et al.,* 2014). Saponins on the other hand are freely soluble and can be extracted in both aqueous and organic solvents and perform their action by attacking the cuticle membrane of the larvae, eventually disturbing the membrane which is the main cause for larval death [15].

However, in this study probit analysis shows that *C. citratus* has the highest effects against *Anopheles* and *Culex* larvae with LC_{50} 5.905mg/ml and LC_{90} 16.241mg/ml while against *Culex* larvae with LC_{50} 6.851mg/ml and LC_{90} 25.678mg/ml. The result shows that *C. citratus* has the least concentration required to kill 50% and 90% of the larvae within 24 hours of exposure, compared to *X. americana* which has the least effect by having the highest concentration required to kill 50% and 90% of the larvae within the time of exposure with LC_{50} 7.617mg/ml and LC_{90} 43.471mg/ml against *Anopheles* larvae while LC_{50} 10.626mg/ml and LC_{90} 16.241mg/ml against *Culex* larvae. Ebe *et al.* [10] in their findings also revealed that *C. citratus* have some larvicidal effects when tested against *Anopheles gambiae, Aedes eagypti* and *Culex quinquefaciatus.* Musa *et al.*[19] also revealed that *C.citratus* have some larvicidal and insecticidal effect when tested against *Anopheles* mosquitoes. Several researchers studied the components of essentials part of plants such as bark [15] oil from the leaves [19] etc against different species

of insects which were proven to have some insecticidal and larvicidal properties.

However, the nature of the bioactive components of plants activities depend on the nature of the solvent used during the extraction. In this study ethanol was used which is among the good solvent for extraction of polar organic compounds. Different compounds have been found to be present in the plants used such as flavonoid, alkaloid, saponin, tannin, resin and phytosteroid which have some insecticidal properties. Furthermore, Eliman *et al.,*[12] suggest that the use of plants available stand a better option when compared to chemicals for the control of mosquito larvae as the affect non-target organisms and environmental hazards.

V. Conclusion

Plants derived natural products possess a number of phytochemicals that have been proven for larvicidal effect against mosquitoes and that they are non-toxic, easily available at affordable prices, biodegradable and show broad spectrum, targets specific activities. The result for phytochemical screening indicated that alkaloid, flavonoid, saponin, tannin, steroid and resin were present in *C.citratus* while alkanoid and steroid were absent in *X. americana.* The leaves extract of *C. citrates* kills more mosquito larvae than that of *X. americana.* Therefore, in this study it was concluded that the leaves extract of *C. citrates* was found to be more effective against both species of mosquitoes exposed (*Anopheles* and *Culex*) compared to the leaves extract of *X. americana.* The higher mortality of the mosquito larvae recorded with *C. citrates* maybe due to the presence of more bioactive compounds found in it compared to *X. americana.*

This study shows that treating the larvae with plant extracts prevent them from molting to pupae while in the control group the larvae successfully molted and emerged into larvae.

Competing Interest

The authors of this article declare no conflict of interest through the processes of this work.

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Acknowledgments

Contributors to the research other than authors credited should be mentioned in Acknowledgments. The source of funding for the research can be included. Suppliers of resources may be mentioned along with their addresses.

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The following is the official style and template developed for publication of a research paper. Authors are not required to follow this style during the submission of the paper. It is just for reference purposes.

Manuscript Style Instruction (Optional)

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27" x 11'", left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word "Abstract" in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
- Line spacing of 1 pt.
- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

Structure and Format of Manuscript

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

a) A title which should be relevant to the theme of the paper.
b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
c) Up to 10 keywords that precisely identify the paper's subject, purpose, and focus.
d) An introduction, giving fundamental background objectives.
e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.
f) Results which should be presented concisely by well-designed tables and figures.
g) Suitable statistical data should also be given.
h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
j) There should be brief acknowledgments.
k) There ought to be references in the conventional format. Global Journals recommends APA format.

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The Editorial Board reserves the right to make literary corrections and suggestions to improve brevity.

Format Structure

It is necessary that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

All manuscripts submitted to Global Journals should include:

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The title page must carry an informative title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) where the work was carried out.

Author details

The full postal address of any related author(s) must be specified.

Abstract

The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Keywords

A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy: planning of a list of possible keywords and phrases to try.

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, “What words would a source have to include to be truly valuable in a research paper?” Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

Numerical Methods

Numerical methods used should be transparent and, where appropriate, supported by references.

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Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

Formulas and equations

Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

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Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.

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Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

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Tips for Writing a Good Quality Science Frontier Research Paper

Techniques for writing a good quality Science Frontier Research paper:

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

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7. Revise what you wrote: When you write anything, always read it, summarize it, and then finalize it.

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

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10. Use proper verb tense: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. Upon conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

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- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
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- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
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Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

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This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."

Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

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- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

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Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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