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Centrepont: Science Edition,

Faculty of Physical Science, Department of Industrial Chemistry

University of Ilorin, Ilorin, Kwara State, Nigeria.

Email: fadekola@unilorin.edu.ng

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Selected Biochemical Parameters of Erectile Function in Clonidine-induced Erectile Dysfunction in Wistar Rats Administered Aqueous Root Extract of *Dichrostachys cinerea*

Omoniwa Babasoji P.^{*1}, Adoga Godwin I.², Oladele Kehinde G.^{1,2} and Okpatu Gabriel C.^{1,2}

^{*1}Ethnopharmacology, Reproductive Biochemistry and Biochemical Toxicology Laboratory, Department of Science Laboratory Technology, University of Jos, Nigeria.

²Department of Biochemistry, Faculty of Basic Medical Sciences, University of Jos, Nigeria.

***Corresponding author:** sojiomoniwa@gmail.com

ABSTRACT

Erectile dysfunction is characterized by an inability to obtain and sustain an erection good enough for satisfactory sexual performance. The prevalence is 57.4% in Nigeria. The aim of this research was to ascertain the acclaimed erectogenic potential of aqueous root extract of *Dichrostachys cinerea* (AREDC). Thirty sexually experienced male Wistar rats were randomly shared into 6 groups of 5 rats each (i.e. A - F). Group A served as positive control and received distilled water only. Erectile dysfunction was induced in rats in groups B – F by oral administration of clonidine (0.5 mg/kg body weight [BW]). The rats in groups B, C, D, E and F were respectively treated with distilled water, sildenafil citrate (0.71 mg/kg BW) and AREDC at 50, 100 and 200 mg/kg BW. Animals were treated for 7 d and sacrificed 24 h after. Their penises and blood were collected and prepared for the analysis of biochemical parameters of erectile function. Phytochemical analysis showed the presence of alkaloids, flavonoids, zinc, arginine, tyrosine and tryptophan. Clonidine administration reduced penile nitric oxide (pNO) and increased penile phosphodiesterase V (pPDE5), but did not cause any alteration in penile prostaglandin E1, serum dihydrotestosterone and penile RHO-kinase levels. However, AREDC (200 mg/kg BW) reversed clonidine-induced decrease in pNO to positive control level. Extract (all doses) also reduced clonidine-induced increase in pPDE5. Available results showed that AREDC's pro-erectile ability is owed to its inhibitory effect on pPDE5 and promotion of NO synthesis in corpus cavernosum.

Keywords: Erectile dysfunction, nitric oxide, phosphodiesterase V, extract and clonidine

INTRODUCTION

Reproduction is essential for the continuance of the human species. For reproduction to occur both partners must be sexually competent. Male impotence is often considered as social handicap and a problem of public health. Moreover, this physiological issue is often associated with some chronic pathologies such as diabetes, neuropathy, depression, stroke and cardiovascular diseases

(Gbesso *et al.*, 2016). Impotence in males is defined as the inability to sustain an erection sufficient for the purpose of satisfactory sexual intercourse, or the inability to ejaculate, or both (Shiel, 2018). There has been a worldwide increase in the incidences of male sexual dysfunction, probably due to an aging population (between 40- 60) and other risk factors such as the presence of chronic illnesses, smoking, stress, alcohol, drug abuse and sedentary lifestyles (Yakubu *et al.*, 2005).

Erectile dysfunction (ED) is the second most common sexual dysfunction in men after premature ejaculation and is defined as the persistent inability to achieve or maintain an erection adequate for satisfactory sexual activity (Singh *et al.*, 2005). A study by Singh *et al.* (2005) showed that in an analysis of 1000 consecutive patients with sexual disorders attending a psychosexual clinic, ED was the presenting complaint of 23.6 %. Despite the high prevalence of ED it has largely remained an ignored disorder until recently (Singh *et al.*, 2005).

Erectile dysfunction often represents a multifactorial disease state. In an individual patient, the cause of ED may be neurological, hormonal or psychological. Many biomolecules play important roles in the proper functioning of penile tissues. Any alteration in their optimum concentrations adversely impact on the development and sustenance of an erection. Some of the biomolecules implicated in the physiology of erection has been studied in this work. Nitric oxide (NO) is synthesized from L-arginine by the Ca^{2+} -calmodulin-dependent iron-containing enzyme NO-synthase (NOS). Two isoforms of NOS, the endothelial NOS (eNOS) and neuronal NOS (nNOS) are responsible for the controlled formation of NO, which is physiologically important for erection, with the latter initiating erectile response and the former facilitating full erection (Burnette, 2004). Nitric oxide has a prominent messenger-signaling function, characterized by its release from nerves and endothelium in the cavernosal tissue and binding to soluble guanylyl cyclase in corporal smooth muscle cells, which results in the activation of the enzyme to produce the potent second messenger molecule, 3', 5'-cyclic guanosine monophosphate (cGMP). This product then stimulates a cGMP-dependent protein kinase, protein kinase G (PKG), which critically induces the cavernosal tissue relaxant response (Burnette, 2004). Prostaglandin E1 (PGE1) is a potent vasodilator and activates the prostaglandin E1 (EP) receptor to elicit pharmacological effects which include systemic vasodilatation, prevention of platelet aggregation and stimulation of intestinal activity (Moreland *et al.* 2003). PGE1 elicits its action by binding to G protein coupled PGE1 receptors localized on the surface of smooth muscle cells, thus activating cAMP (cyclic adenosine monophosphate), which in turn induces penile vascular smooth muscle relaxation to provide penile erection (Cuzin, 2016). Phosphodiesterases (PDEs) are classes of enzymes that cleave the phosphodiester bonds in both cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), which are important intracellular messengers that stimulate penile smooth muscle relaxation and generate penile erection (Ghofrani *et al.*, 2004). Phosphodiesterase 5 (PDE5) degrades second messengers, cAMP and cGMP into inactive AMP and GMP (Ghofrani *et al.*, 2004) thus serving as a regulator of penile erection. Dihydrotestosterone (DHT) is produced in the prostate gland, seminal vesicles, epididymides, penis and scrotum among others (Rhoades and Bell, 2012) by irreversible reduction of the C4-5 double bond of testosterone by the action of 5 α -reductase (Lotti and Maggi, 2015). Relative to testosterone, DHT is considerably more potent as an agonist of the androgen receptor (AR). Unlike testosterone, DHT cannot be aromatized into an oestrogen like oestradiol, and for this reason, has no propensity for oestrogenic effects. DHT improves penile erection by encouraging the expression of nitric oxide synthase (Gur *et al.*, 2013; Anawalt, 2017). Rho-Kinase plays a vital role in actualizing the detumescence phase of the male sexual response cycle. In general, Rho-Kinase, along with Ca^{2+} signaling pathways, control smooth muscle contraction (Berridge, 2008). Calcium sensitization is brought about by

agonist activation of heterotrimeric G protein-coupled receptors, leading to the exchange of GDP for GTP on the small monomeric GTPase RhoA. This event elicits activation of RhoA and is catalyzed by the guanine nucleotide exchange factors, which cause dissociation of RhoA from its binding partner, Rho-guanine dissociation inhibitor. As a result, RhoA translocates from the cytosol to the membrane, enabling the downstream activation of various effectors such as Rho kinase. Phosphorylation of the regulatory subunit of MLC phosphatase by Rho kinase causes inhibition of phosphatase activity, which enhances the contractile response at a constant intracellular calcium concentration (Hirano, 2007).

There is a growing market for local herbal and none herbal remedy for sexual dysfunction and aphrodisiacs especially in northern Nigeria (France-Pressé, 2009). Over 80 % of the population in some African and Asian countries depend on traditional medicine which often include herbs for health care (Bello and Isah, 2015). This is due partly to high cost of orthodox therapies (France-Pressé, 2009). Furthermore, use of synthetic remedy is believed to result in unwanted side effects that are harmful to the patients, thus the preference for remedy of herbal origin believed to have lesser side effects (Sabo *et al.*, 2017). However, the efficacy of many of these local herbal remedy have not been scientifically verified. One of such unverified herbal remedy is the root decoction of *Dichrostachys cinerea*.

Dichrostachys cinerea commonly known as Sickle bush, Bell mimosa, Chinese lantern tree or Kalahari Christmas tree, is a semi-deciduous to deciduous fast growing tree of the family mimosaceae, typically grows up to 7 meters in height. It is characterized by strong alternate smooth spines (up to 8 cm long), dark grey-brown fractures on old branches and stems and bark on younger branches (El-Sharawy *et al.*, 2017). It is also characterized by compound and pinnate leaves and bicoloured fragrant flowers (Mudzengi *et al.*, 2014; Sabo *et al.*, 2017). It is native to Africa and Asia, where it is common in the tropics of Africa, the Sahelian and Sudanian ecozones, and the South Arabian Peninsula (Mudzengi *et al.*, 2014). It is identified by different names by the ethnic groups in Nigeria. Hausas call it Dundu, the Yorubas call it Kara, and the Igbos call it Ami-ogwu (Quattrocchi, 2012). Traditionally, the leaves are used as anti-inflammatory, in treatment of pneumonia and leprosy. Dried bark is used to treat diarrhea and postpartum pain. Root is used in the treatment of syphilis and leprosy and as an aphrodisiac (Sabo *et al.*, 2017).

Dichrostachys cinerea roots have been reported as being used traditionally as an aphrodisiac, the boiled roots is used to treat impotence in men and to increase male erection (Quattrocchi, 2012). Oral administration of the bark of *Dichrostachys cinerea* is used in Uganda to treat sexual dysfunction, and in Tanzania the root is crushed and mixed with maize or taken as a decoction (Ajao, 2018). A survey by Sabo *et al.* (2017) in Bauchi Local Government area of Bauchi state, Nigeria showed that 4 % of the respondents surveyed claimed that the root of *Dichrostachys cinerea* has aphrodisiac properties. This study evaluates the effect of AREDC on some biochemical parameters of erectile function as compared to a standard drug (sildenafil citrate) in albino rats in which erectile dysfunction has been previously induced using clonidine. Furthermore, this study attempted to account for some relevant phytoconstituents in AREDC that may be responsible for the observed pharmacological effects.

MATERIALS AND METHODS

Plant Collection and Authentication

Dichrostachys cinerea plant was obtained from NEPA Mile 7, Zaria road Jos, Plateau State, Nigeria. It was identified and authenticated at the herbarium unit of the Department of Plant Science and Biotechnology, Faculty of Natural Sciences, University of Jos, Nigeria where a voucher specimen was deposited and the voucher number (JUNH20000303) obtained.

Experimental Animals

Thirty-five (35) male (average weight 130.32 ± 15.33 g) and 30 female (average weight 100.54 ± 5.25 g) sexually mature and experienced Wistar rats were obtained from the animal house of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos, Jos, Plateau State, Nigeria. They were housed in plastic rat cages and fed standard rat pellets and tap water *ad libitum* during the experiment.

Ethical Approval

The Institutional Animal Ethical Committee of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos, Nigeria approved the study and issued the following approval number (UJ/FPS/F17-00379).

Drugs and Assay Kits

Clonidine (Lot No. 252440) and Viagra (Lot No. B374915) were produced by Sandoz limited, Surrey, England and Pfizer limited, Kent, United Kingdom respectively. Assay kits used in this research and their manufacturers details are as follows: Dihydrotestosterone and RHO-Kinase (Bioassay Technology Laboratory, Shanghai, China); Nitric oxide (Oxford Biomedical Research Inc., United States of America); Prostaglandin E1 (Elabscience Biotechnology Co. Ltd, United States of America) and Phosphodiesterase V (Abcam, United Kingdom).

Plant Preparation and Extraction

The roots of *D. cinerea* were washed, chopped to pieces and then air dried at room temperature (23 ± 1.5 °C) for 21 d. Drying of the roots to constant weight was thereafter achieved using an oven (Carbolite PF 200, Keison Products, Essex, United Kingdom) set at 40 °C. The dried roots were crushed into fine powder using a hammer mill (Model PC 200 x 300, DEWO Machinery Company Ltd, Zhengzhou, Henan, China). The fine root powder (600 g) was thereafter percolated in 2500 ml of boiled distilled water (100 °C) and stirred properly with a glass rod. The mixture was left to stand until temperature dropped to 4 °C before transferring it into the refrigerator and left for 48 h for proper extraction. Mixture was then filtered using clean white muslin fabric followed by Whatman No1 filter paper. The filtrate was concentrated in an oven at 40 °C and weighed. Extract was reconstituted in distilled water to give the doses of 50, 100 and 200 mg/kg BW used in this study.

Secondary Metabolite Analysis

Crude hot AREDC was subjected to qualitative and quantitative secondary metabolite analysis. Extract was screened for the presence of alkaloids, saponins, tannins, flavonoids, steroids, terpenoids, anthraquinones and cardiac glycosides using the methods described by Egbuna *et al.* (2019). The detected secondary metabolites (i.e. alkaloids, tannins, flavonoids, and steroids) were quantified by adopting the procedures outlined by Egbuna *et al.* (2019).

Amino Acid and Mineral Analysis

Amino acids were quantified using the procedures outlined by Benitez (1989) and Maria *et al.* (2004). Minerals were quantified using a hand held Thermo Scientific, Niton XL3t GOLDD+ X-Ray Fluorescence analyzer following procedure given by the manufacturer.

Induction of Erectile Dysfunction

Thirty (30) male and 30 female Wistar rats were used. They were mature and sexually experienced. Erectile dysfunction was induced in the male rats by administering a single oral dose of 0.5 mg/kg BW clonidine (Clark and Smith, 1990). The male rats were then paired in ratio 1:1 with female ones which had previously been made receptive by sequential subcutaneous administration of 10 µg/100 g BW of oestradiol conjugate (48 h before pairing) and intramuscular administration of 0.5 mg/100 g BW of progesterone (4 h before pairing) (Amin *et al.*, 1996). Oestrous phase in female rats was confirmed by vaginal smears examinations according to OECD-106 guideline (OECD, 2009). The following erection-related sexual behaviour parameters were monitored using the methods of Amin *et al.* (1996) and Agmo (1997): (i) Intromission frequency (IF): the number of intromissions by the male rats from the time of introduction of the female rats until ejaculation, (ii). Intromission latency (IL): the time interval from the introduction of the female rats to the first intromission by the male rats. This is usually characterized by pelvic thrusting and springing dismounts and (iii). Post ejaculatory interval (PEI): the time interval from ejaculation until the next intromission. Male rats which showed minimum of 25% reduction in IF as well as minimum increase of 25% in IL and PEI were considered to have erectile dysfunction.

Animal Grouping and Extract Administration

Thirty male Wistar rats were used for the study. Group A (5 rats) represented animals in which erectile dysfunction was not induced and only received distilled water. Twenty-five rats (out of the 30 in which erectile dysfunction has been induced) with the best erection-related physical parameters of male sexual behavior (i.e. IF, IL and PEI) were recruited for the study and randomly assigned into groups B, C, D, E and F, each group containing 5 rats and treated with distilled water only, sildenafil citrate (0.75 mg/kg BW), 50 mg/kg BW of AREDC, 100 mg/kg BW of AREDC and 200 mg/kg BW of AREDC respectively. Treatment administration was done for 7 d and the animals were sacrificed 24 h after the last round of treatments. Their penises and blood were collected for analysis of selected biochemical indices of erectile function.

Preparation and Storage of Serum

Collected blood samples collected into sterile vacutainer bottles were left to clot for 10 min and thereafter centrifuged at 9000 rpm for 5 min. Serum samples obtained as supernatant were then collected into plain tubes using a Pasteur's pipette. Serum was stored in a refrigerator.

Preparation and Storage of Tissue

Penile tissues were stored in sterile bottles containing ice- cold 0.25 M sucrose solution as soon as they were harvested and were kept refrigerated (4 °C) prior to homogenization. Tissue samples were homogenized mechanically in ice-cold 0.25 M sucrose in the ratio 1:5 (i.e. 1g of tissue to 5 ml sucrose) in an ice bath. Homogenates were then centrifuged at 7000 rpm for 5 min at 4 °C and the supernatant used immediately for biochemical analysis.

Determination of Biochemical Parameters of Erectile Function

All biochemical parameters were analysed using SpectraMax 340 microplate reader by adopting the following previously established methods: Penile nitric oxide concentration (Schmidt, 1995), penile prostaglandin E1 concentration (McCosh *et al.*, 1976), serum dihydrotestosterone concentration (Sartorius *et al.*, 2014), penile phosphodiesterase V activity (Manosroi *et al.*, 2017) and RHO – kinase activity (Fox *et al.*, 2020).

Statistical Analysis

Data obtained from laboratory analysis were subjected to statistical analysis using the IBM® Statistical Package for Social Sciences (SPSS) software version 21 and presented as mean (of 5 replicates) $\pm$ standard error of mean (SEM). All significant differences were determined by one way Analysis of Variance (ANOVA) and Post Hoc multiple comparison was done using Duncan's multiple range test. The significance level was set at $p < 0.05$.

RESULTS

Yield of Extract

Dichrostachys cinerea root (600 g) was extracted in hot water to yield 63.54 g of concentrate. The percentage yield of the crude extract was calculated to be 10.59 %.

Secondary Metabolite Composition of Hot Aqueous Root Extract of *Dichrostachys cinerea*

Secondary metabolite analysis of hot AREDC revealed the presence of alkaloids, tannins, flavonoids and steroids. Flavonoids have the highest concentration (15.10 ± 0.21 %/2 g sample) while steroids have the lowest (3.06 ± 0.11 %/2 g sample). Saponins, terpenoids, anthraquinones and cardiac glycosides were not detected (Table 1).

Mineral Composition of Hot Aqueous Root Extract of *Dichrostachys cinerea*

Mineral analysis of hot AREDC revealed the presence of Zinc (Zn), Rubidium (Rb), Strontium (Sr), Zirconium (Zr), Niobium (Nb), Molybdenum (Mo), Copper (Cu), Thorium (Th), and Uranium (U). Strontium has the highest concentration (58.00 ± 0.80 ppm) while copper, thorium and uranium were lowest (1.00 ppm) (Table 2).

Amino Acid Composition of Hot Aqueous Root Extract of *Dichrostachys cinerea*

Amino acid analysis of hot AREDC revealed the presence of all the standard amino acids except glutamine and asparagine which were converted to glutamic acid and aspartic acid respectively during acid hydrolysis. Glutamic acid had the highest concentration (8.10 ± 0.22 g/100 g protein) while cysteine was present the least (0.36 ± 0.04 g/100 g protein) (Table 3).

Physical markers for ascertaining induction of erectile dysfunction

Administration of 0.5 mg/kg BW of clonidine to male rats reduced IF by 39.80% while IL and PEI were prolonged by 46.76% and 55.43% respectively when compared with distilled water-treated control group (Table 4).

Biochemical Markers of Erectile Function

Clonidine administration caused a significant reduction ($p < 0.05$) in penile NO concentration in the animals when compared with the distilled water only-treated control (Group A). There was

no significant difference ($p > 0.05$) in penile NO concentration of the AREDC-treated ED animals at doses 50 and 100 mg/kg BW when compared with the Clonidine +distilled water-treated and clonidine + sildenafil-treated ED control groups. However, AREDC at 200 mg/kg BW compared favourably with the distilled water only-treated control group (Figure 1).

Clonidine administration did not cause any significant alteration ($p > 0.05$) in penile PGE1 concentration in the animals when compared with the distilled water only-treated control. There was no significant difference ($p > 0.05$) in penile PGE1 concentration of the AREDC-treated ED groups and all the control groups (Figure 2).

Clonidine administration significantly raised ($p < 0.05$) penile PDE5 activity in the clonidine + distilled water treated ED control (Group B) when compared with the distilled water only-treated control (Group A). AREDC at 50 mg/kg BW significantly reduced ($p < 0.05$) PDE5 activity when compared with the clonidine + distilled water-treated ED control but to a level comparable with the distilled water-treated control (Group A) and the sildenafil-treated ED control. AREDC at 100 and 200 mg/kg BW significantly ($p < 0.05$) reduced penile PDE5 activity when compared with the other groups with the 200 mg/kg BW-treated ED group showing a significantly lower ($p < 0.05$) penile PDE5 activity when compared with the 100 mg/kg BW-treated ED group (Figure 3).

Similar to the result recorded for PGE1, clonidine administration did not cause any significant alteration ($p > 0.05$) in serum DHT concentration and penile Rho-kinase activity in the animals when compared with the distilled water only-treated control. There was no significant difference ($p > 0.05$) in serum DHT concentration penile Rho-kinase activity of the AREDC-treated ED groups and all the control groups (Figure 4 and 5).

Table 1: Secondary metabolite composition of hot aqueous root extract of *Dichrostachys cinerea*

Secondary metabolites	Concentration (% / 2g sample)
Alkaloids	7.35 $\pm$ 0.26
Saponins	-
Tannins	13.88 $\pm$ 0.23
Flavonoids	15.10 $\pm$ 0.21
Steroids	3.06 $\pm$ 0.11
Terpenes	-
Anthraquinones	-
Cardiac glycosides	-

Values represent mean of 2 determinations $\pm$ standard deviation (SD)

Table 2: Mineral composition of hot aqueous root extract of *Dichrostachys cinerea*

Minerals	Concentration (ppm)
Zinc (Zn)	15.00 $\pm$ 1.10
Rubidium (Rb)	46.00 $\pm$ 0.56
Strontium (Sr)	58.00 $\pm$ 0.80
Zirconium (Zr)	3.00 $\pm$ 0.22
Niobium (Nb)	5.00 $\pm$ 0.35
Molybdenum (Mo)	6.00 $\pm$ 0.32
Copper (Cu)	1.00 $\pm$ 0.03
Thorium (Th)	1.00 $\pm$ 0.02

Uranium (U)	1.00 ± 0.04
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Values represent mean of 2 determinations ± standard deviation (SD)

Table 3: Amino acid composition of hot aqueous root extract of *Dichrostachys cinerea*

Amino Acid	Concentration (g/100g protein)
Leucine	5.40 ± 0.13
Lysine	4.64 ± 0.11
Isoleucine	2.94 ± 0.09
Phenylalanine	3.01 ± 0.10
Tryptophan	0.37 ± 0.02
Valine	4.30 ± 0.04
Methionine	0.80 ± 0.01
Proline	3.25 ± 0.04
Arginine	2.66 ± 0.23
Tyrosine	2.06 ± 0.17
Histidine	1.66 ± 0.11
Cysteine	0.36 ± 0.04
Alanine	3.60 ± 0.25
Glutamic acid	8.10 ± 1.22
Glycine	2.94 ± 0.28
Threonine	3.00 ± 0.31
Serine	4.00 ± 0.22
Aspartic acid	5.33 ± 0.13

Values represent mean of 2 determinations ± standard deviation (SD)

Table 4: Erection-related sexual behavior parameters in male rats administered clonidine

Parameters	Distilled water (control)	Clonidine (0.5 mg/kg BW)	Change (%)
Intromission Frequency	4.02 ± 0.56	2.42 ± 0.21*	39.80↓
Intromission Latency	18.54 ± 0.71	27.21 ± 0.12*	46.76↑
Post-ejaculatory Interval	12.43 ± 1.46	19.32 ± 0.56*	55.43↑

Data are mean ± SD; n = 25; *p < 0.05, versus control; ↓= Percentage decrease, ↑= Percentage increase.

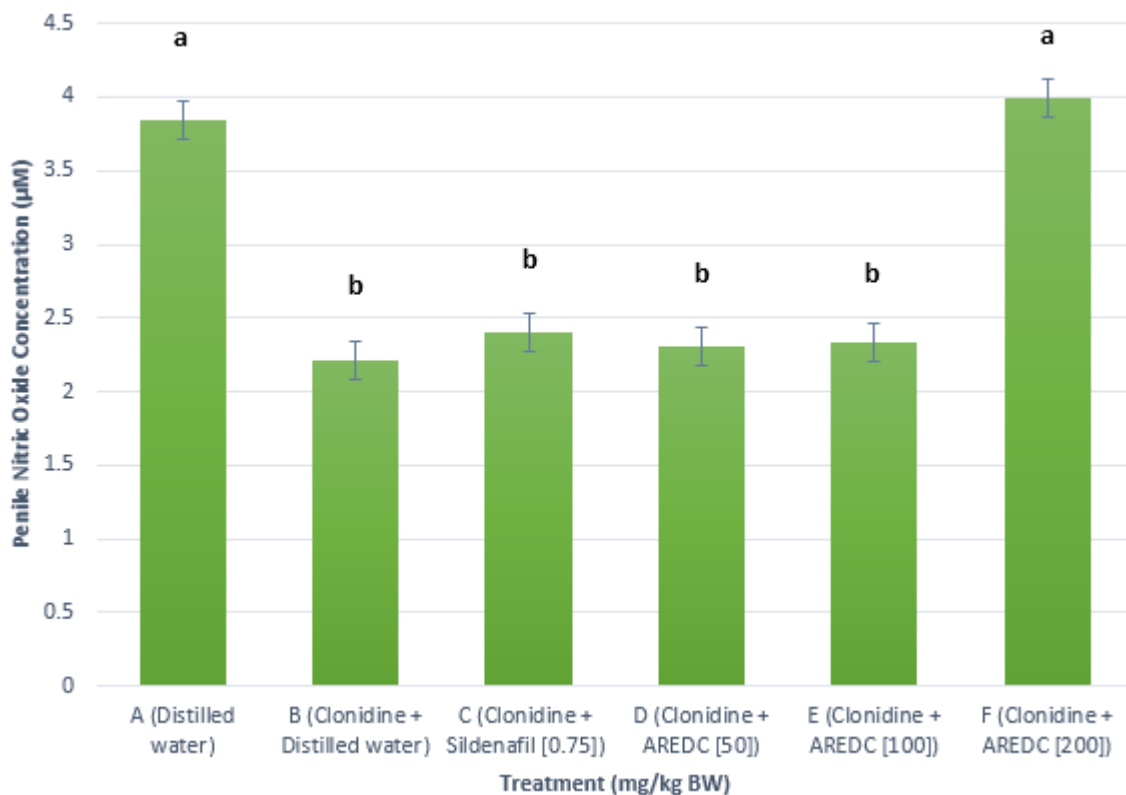


Figure 1: Penile nitric oxide concentration in clonidine-induced erectile dysfunction in Wistar rats administered aqueous root extract of *Dichrostachys cinerea*

Bars represent means (of 5 replicates) $\pm$ SEM. Bars carrying different superscripts are significantly different at $p < 0.05$, AREDC: Aqueous root extract of *Dischrostachys cinerea*

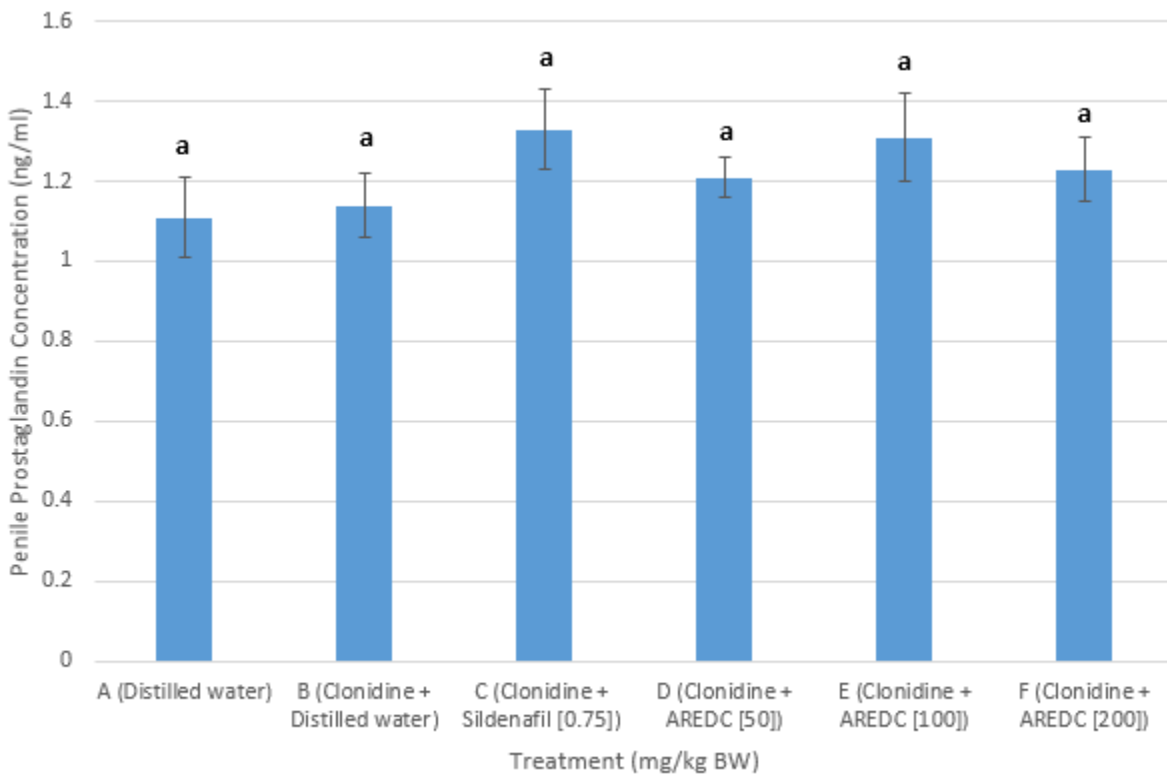


Figure 2: Penile prostaglandin E1 concentration in clonidine-induced erectile dysfunction in Wistar rats administered aqueous root extract of *Dichrostachys cinerea*

Bars represent means (of 5 replicates) $\pm$ SEM. Bars carrying different superscripts are significantly different at $p < 0.05$, AREDC: Aqueous root extract of *Dischrostachys cinerea*

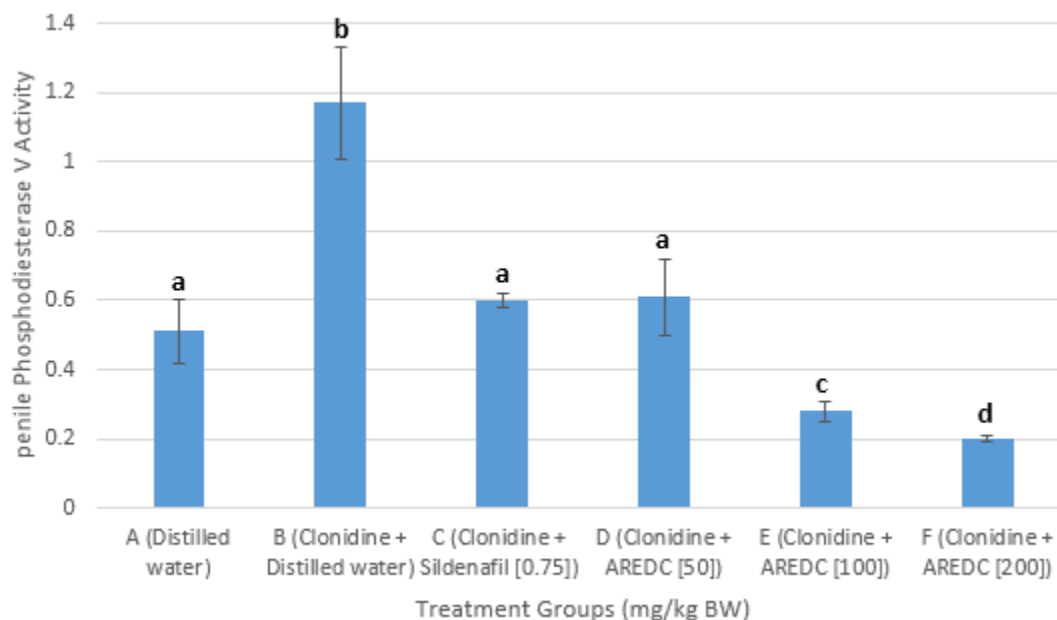


Figure 3: Penile phosphodiesterase V activity in clonidine-induced erectile dysfunction in Wistar rats administered aqueous root extract of *Dichrostachys cinerea*
 Bars represent means (of 5 replicates) ± SEM. Bars carrying different superscripts are significantly different at $p < 0.05$, AREDC: Aqueous root extract of *Dischrostachys cinerea*

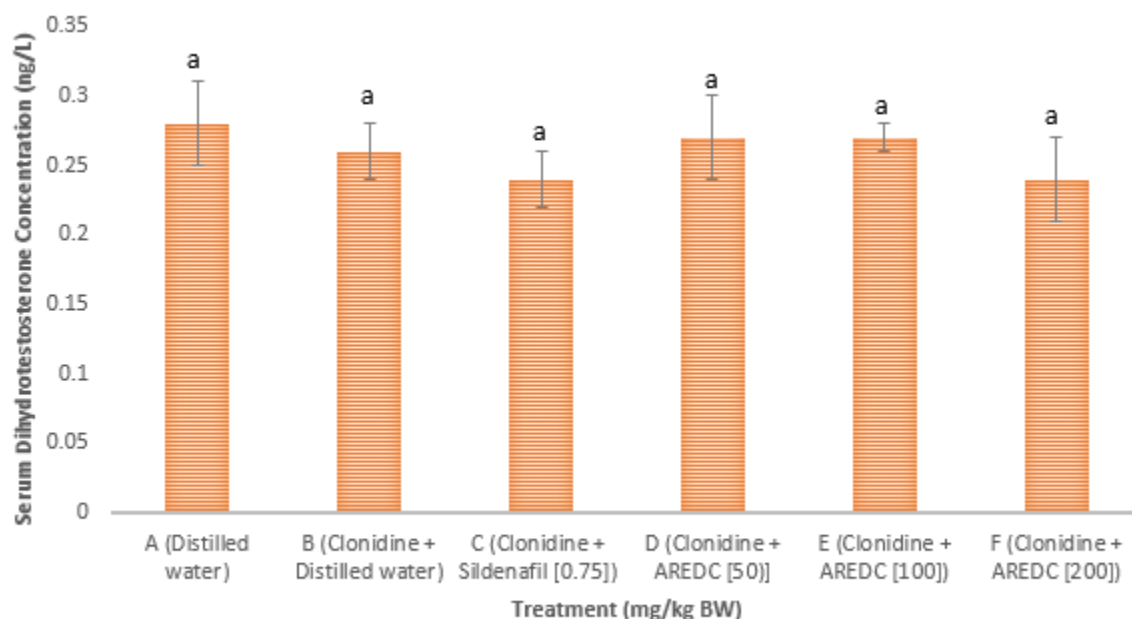


Figure 4: Serum dihydrotestosterone concentration in clonidine-induced erectile dysfunction in Wistar rats administered aqueous root extract of *Dichrostachys cinerea*

Bars represent means (of 5 replicates) $\pm$ SEM. Bars carrying different superscripts are significantly different at $p < 0.05$, AREDC: Aqueous root extract of *Dischrostachys cinerea*

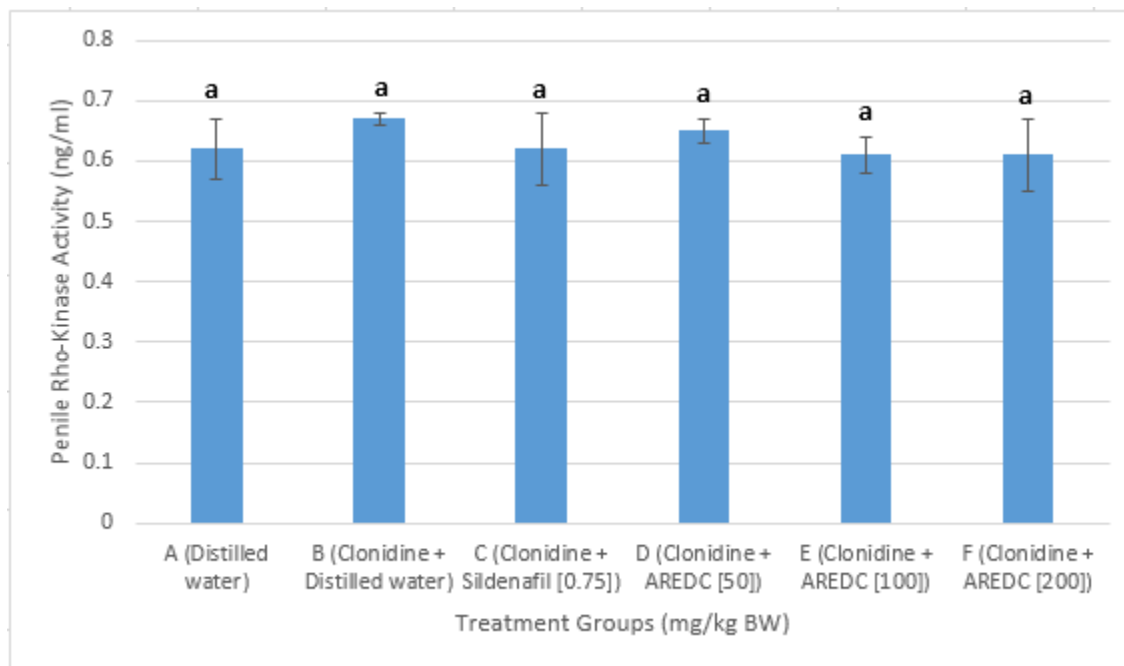


Figure 5: Penile Rho-Kinase activity in clonidine-induced erectile dysfunction in Wistar rats administered aqueous root extract of *Dichrostachys cinerea*

Bars represent means (of 5 replicates) $\pm$ SEM. Bars carrying different superscripts are significantly different at $p < 0.05$, AREDC: Aqueous root extract of *Dischrostachys cinerea*

Discussion

Clonidine is an imidazole antihypertensive compound that acts as an alpha-adrenergic agonist with selectivity for alpha-2 receptors (Yusoff *et al.*, 2013; Fernandes *et al.*, 2018). Experimental induction of erectile dysfunction in male rats using clonidine has been reported by previous researchers (Clark and Smith, 1990; Clark 1991; Lin *et al.*, 2015).

In this research the secondary metabolites detected in the hot AREDC partly agrees with that of Johnstone *et al.* (2012) which also identified the presence of alkaloids and flavonoids in hot water extract of *Dichrostachys cinerea* root. These metabolites may be responsible for the recorded increase in NO synthesis and inhibition of PDE5 activity. Studies have found that increased habitual intakes of several dietary flavonoids are associated with improved erectile function (Cassidy *et al.*, 2016). Certain flavonoid compounds and their derivatives such as catechin, epicatechin, and quercetin have been shown to strongly inhibit arginase activity (Oboh *et al.*, 2015), thus reducing its competition with NO synthase for the available arginine in the penile tissue and as a result facilitating penile erection (Laurentius *et al.*, 2018). Also, alkaloids rich extracts of many plants like bitter leaf (*Vernonia amygdalina*) and black nightshade (*Solanum nigrum*) have been found to exhibit pro-erectile activities via the inhibition of PDE5 and arginase (Omojokun *et al.*, 2019).

Studies have found a clear relationship between zinc and testosterone levels, zinc is involved in the biosynthesis of testosterone, testosterone is essential for normal erection at very low levels, although high level of testosterone does not appear to have a major impact on erectile function (Mikhail, 2006; Case-Lo and Watson, 2016; Kayode and Yakubu, 2017). Thus zinc may be helpful in treating erectile dysfunction if the underlying cause is hypogonadism relating to testosterone or zinc deficiency (Mikhail, 2006).

Arginine, tyrosine and phenylalanine are known to play various roles in penile erection. Hot AREDC may improve erectile function by serving as a reservoir for arginine, which may then be released to support the synthesis of nitric oxide (Kayode and Yakubu, 2017). Arginine intake increases blood circulation throughout the body, including the Penis (Kayode and Yakubu, 2017). A study by Barassi *et al.* (2016), showed that a significant proportion of erectile dysfunction patients have low L-arginine levels. Arginine serves as substrate for nitric oxide synthase required to make nitric oxide. Nitric oxide (NO) from arginine increases arterial elasticity, reduces blood pressure and improves erectile processes (Kayode and Yakubu, 2017). Tyrosine and phenylalanine are both precursors for the synthesis of dopamine, norepinephrine, and epinephrine, making these chemical compounds candidates for enhancing libido (Kayode and Yakubu, 2017). It has been suggested that L-tyrosine may help reduce stress and depression due to its role in dopamine synthesis, thereby encouraging erection. A study found that L-tyrosine seemed to reverse effect of neurological erectile dysfunction in mice (Wu, 2017). Glycine, cysteine and glutamic acid in hot AREDC could serve as precursors of glutathione, an antioxidant that safeguards cells against the pathogenesis of many diseases including those of sexual origin (Kayode and Yakubu, 2017).

Various doses of hot AREDC was found to affect some biochemical index of erectile function, these include: nitric oxide, phosphodiesterase V, and Rho- kinase. Nitric oxide level was found to be lower in groups induced with erectile dysfunction using clonidine. However, hot AREDC showed ability to restore nitric oxide level to normal especially at a dose of 200 mg/kg. This suggests that extract of *Dichrostachys cinerea* root may contain compounds that induce penile nitric oxide synthase.

Phosphodiesterase V activity was inhibited by hot AREDC in a dose dependent manner and compared well with viagra, suggesting that hot AREDC contains substances that inhibit phosphodiesterase activity or repress its synthesis. The most potent dose of the extract (i.e. the dose with the highest phosphodiesterase V activity inhibition) was the 200 mg/kg. The 50 mg/kg dose of the extract elicited similar level of phosphodiesterase V activity inhibition as 0.75 mg/kg BW of sildenafil citrate. Phosphodiesterase V (PDE5) degrades cAMP and cGMP into inactive 5'-AMP and 5'-GMP (Ghofrani et al., 2004). Inhibition of PDE5 translates to accumulation of cAMP and cGMP, which relax vascular smooth muscles, causing vasodilation and increased blood flow to the penis, ultimately resulting in penile erection. A number of plants from the family fabaceae to which *Dichrostachys cinerea* belongs, have PDE-inhibiting properties (Rahimi *et al.*, 2010). Also, it has been discovered that the main plant compounds with this inhibitory activity are alkaloids, flavonoids, saponins, lignans, and coumarins (Rahimi *et al.*, 2010). Interestingly, alkaloids and flavonoids are present in substantial quantity in AREDC. In addition, Prapapan *et al.* (2011) also reported that *Kaempfeira parviflora* rhizome extracts exhibited PDE5-inhibitory activity, with 5,7-dimethoxyflavone, one of its flavonoid extracts, being tipped to serve as a lead compound for the development of clinically efficacious PDE5 inhibitor.

In this study, clonidine lack of effect on the levels of DHT and rho-kinase means that clonidine induces erectile dysfunction by altering the concentrations of biomolecules other than DHT and

rho-kinase. In a report by Kunelius *et al.* (2002), transdermal application of DHT to andropausal men improved early morning erections after three months which was followed by a corresponding significant improvement in the ability to maintain erections at six months. Dihydrotestosterone is produced from testosterone by the action of 5 α –reductase (Gur *et al.*, 2013). Clonidine did not inhibit or activate 5 α –reductase, which means that testosterone was converted to DHT normally. Similarly, the extract (at all the doses considered) supported normal conversion of testosterone to DHT and compared favourably with sildenafil. Rho-kinase facilitates detumescence and its inhibition supports penile erection. Clonidine and the extracts did not activate or inhibit Rho-kinase activity. This means that clonidine did not exhibit any negative impact on the detumescence phase of male sexual response cycle. In addition, extract at the doses considered encourages normal regulation of erection.

CONCLUSION

Hot AREDC possesses erectogenic properties. Its ability to promote penile erection is owed to its inhibitory effect on penile PDE5 activity and promotion of NO synthesis in rat penile tissue. The most potent dose was 200 mg/kg. Results from this study lend credence to *Dichrostachys cinerea* root decoction local use as erectile function restorer and male aphrodisiac.

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Aqueous Stem Extract of *Erythrina senegalensis* Reverses Clonidine-induced Alterations in Selected Biochemical Parameters of Male Sexual Functions and Modulates Liver and Kidney Function Parameters in Rats

Omoniwa Babasoji P.^{1*}, Olorunyomi Olumuyiwa A.², Ayodele Caleb S.¹, Lohor Tongdyen S.¹
Barry Peace¹ and Charles Ada¹

¹Ethnopharmacology, Reproductive Biochemistry and Biochemical Toxicology Laboratory, Department of Science Laboratory Technology, University of Jos, Nigeria.

²Department of Biochemistry, Faculty of Basic Medical Sciences, University of Jos, Nigeria.

***Corresponding Author:** sojiomoniwa@gmail.com

ABSTRACT

This study evaluated the ameliorative effect of aqueous stem extract of *Erythrina senegalensis* (ASEES) on clonidine-mediated alterations of some biochemical parameters that are associated to male sexual function (MSF) and its modulatory effects on liver and kidney function parameters in Wistar rats. To determine extract effect on sexual function parameters, 25 male rats were randomly allocated into 5 groups (A -E). Group A received distilled water only and represented the sham control. Groups B - E received 0.5 mg/kg body weight (BW) of clonidine and were thereafter treated with distilled water, 13.3 mg/kg BW of Adam's desire (standard drug), 500 mg/kg BW of ASEES and 1000 mg/kg BW of ASEES respectively. For the assessment of the modulatory effect of ASEES on rat liver and kidney functions, 15 male rats were grouped into 3 (M – O) and administered distilled water, 500 mg/kg BW ASEES and 1000 mg/kg BW ASEES respectively. For both experiments, animals were sacrificed 24 hours after treatment which lasted for 7 days. Phytochemical analysis revealed the presence of phenolics, steroids, saponins, flavonoids, calcium, potassium, selenium, copper, phenylalanine, tyrosine, glutamic acid and arginine among others. Clonidine reduced the concentrations of testicular and serum testosterone, serum dihydrotestosterone (DHT), luteinizing hormone (LH) and nitric oxide (NO). However, administration of ASEES (500 and 1000 mg/kg BW) reversed the clonidine-induced alterations in the aforementioned biochemical parameters. ASEES also increased the levels of liver aspartate aminotransferase (AST) and alanine aminotransferase (ALT), kidney gammaglutamyl transferase (GGT) and serum total protein and sodium ion while serum potassium ion and urea levels were reduced. ASEES at 500 and 1000 mg/kg BW reversed clonidine-mediated alterations in the concentrations of the considered MSF-related biochemical parameters but also altered liver and kidney function parameters at the doses examined.

Keywords: *Erythrina*, clonidine, sexual dysfunction, dihydrotestosterone, aminotransferases

INTRODUCTION

Clonidine is a centrally acting α_2 -adrenergic agonist and imidazoline receptor agonist which was patented in 1961 and came into medical use in 1966 (Khan *et al.*, 1999; Stahle, 2000). It is sold under trade names catapres, clonidine, clonidine hydrochloride and clonigen among others. Clonidine is given for the treatment of a wide range of disorders which include high blood pressure, menopausal flushing, attention deficit hyperactivity disorder (ADHD) and drug withdrawal and in 2019, was the 64th most commonly prescribed medication in the United States, with more than 11 million prescriptions to over 2 million patients (Kane, 2021). It has also found a lot of off-label applications as it is being employed to manage psychiatric disorders like stress, sleep disorders and hyperarousal caused by post-traumatic stress disorder, borderline personality disorder and other anxiety disorders (Boehnlein and Kinzie, 2007; Huffman and Stern, 2007; Strawn and Geraciotti, 2008; Najjar *et al.*, 2008; Ziegenhorn *et al.*, 2009). Clonidine also has a lenient sedative activity, and can therefore be administered to patients before undergoing a surgery (Fazi *et al.*, 2001).

Drugs secondary effects is a crucial determinant of the willingness of users to adhere to treatment. Very common unwanted effects of clonidine which occur in above 10% of users include dizziness, headache, fatigue, hypotension and dry mouth. Others, which are categorized as common unwanted effects and which have been reported in 1 – 10% of users include sedation, anxiety, constipation, rash, nausea/vomiting, weight gain/loss and erectile dysfunction among others. Clonidine sexual side effect profile is inseparable from its hypotensive action (Giovannitti *et al.*, 2015). Clonidine induces sexual dysfunction (SD) by facilitating neuropeptide Y-mediated activity and delaying bulbocavernous reflex (Crenshaw and Goldberg, 1996). It also causes cavernosal vasoconstriction thus causing changes in the intracavernosal pressure (ICP), which aggravates erectile failure (Hedlund and Andersson, 1985). In addition clonidine inhibits adenylate cyclase which leads to inactivation of cAMP (Breuiller *et al.*, 1990). Clonidine dose-dependently inhibited erectile function in rats (Clark and Smith, 1990). Clark (1991) also reported dose-dependent reductions in the number of males that mounted, intromitted and ejaculated with administration of clonidine into the 3rd ventricle less effective than into the medial preoptic area. Clonidine significantly prolonged the latencies of mounting and intromission and reduced the number of mount and intromission as well as intracavernosal pressure (ICP) in male rats (Srilatha *et al.*, 1999).

Drugs with side effects are usually combined with other agents that can alleviate the severity of those side effects. This drugs combination can encourage patients' adherence to treatment. For example, although clonidine produces lots of side effects, it offers numerous benefits in the treatment of Attention-deficit Hyperactivity Disorder (ADHD), especially when combined with stimulant medications (Palumbo *et al.*, 2008; Kollins *et al.*, 2011). Clonidine has been marketed as a combination drug with chlortalidone as Arkamin-H, Bemplas, Catapres-DIU, and Clorpres, and in combination with bendroflumethiazide as Pertenso.

Medicinal plants contain secondary metabolites that exhibit impressive therapeutic effects against various diseases and illnesses. Many medicinal plants have been reported to possess aphrodisiac and sexual performance-enhancing activities, and as such may be used to alleviate the sexual function-dampening effects of clonidine. One of such plants with acclaimed aphrodisiac activity is *Erythrina senegalensis*.

Erythrina senegalensis DC is known as coral tree in English while in Nigeria it is called Ologun-sese (Yoruba), Minjirya (Hausa) and Echichi (Igbo) (Kayode and Kayode, 2008; Doughari, 2010; Obidah *et al.*, 2014). It is a thorny shrub used as vegetable, growing up to 5 - 7 m tall. The

leaves are composed of triple leaflets, each measuring 5 - 15 cm long and have thorny stalk. It is commonly distributed in Nigeria through Sierra Leone to Ivory Coast. Phytochemical constituents of the stem bark, root and leaf extracts of *E. senegalensis* include several bioactive compounds such as simple aromatic natural products and some phytochemicals (Bunu *et al.*, 2018). Across Africa, *E. senegalensis* extracts have been used to treat wounds, female secondary bareness, asthma, liver disorders, gastrointestinal disorders, fever, jaundice, nose bleeding, urinary bilharziasis, eye infections and body pain (Doughari, 2010; Adamu *et al.*, 2005).

Musa *et al.* (2016) reported the analgesic, sedative and anxiolytic properties of ethanol extract of the root bark of *E. senegalensis* while Nnama *et al.* (2017) documented that alkaloids, saponins, flavonoids, tannins and terpenoids were present in the leaf ethanol extract. Musa *et al.* (2016) also reported the intraperitoneal median lethal dose of the ethanol root bark extract of *E. senegalensis* to be 1,137 mg/kg in mice. However, the acute oral lethal dose in Wistar rats was put at greater than 5000 mg/kg for the ethanol extract of the leaf. Wakawa and Hauwa (2013) reported the hepatoprotective activity of aqueous extract of the leaf of *E. senegalensis* against liver injury induced by carbon tetrachloride in rats.

E. senegalensis is employed traditionally for the treatment of a wide range of diseases, it is therefore very necessary to evaluate its safety, especially on the liver and kidney, because of the involvements of these organs in xenobiotic metabolism. This study also evaluated the ability of *E. senegalensis* aqueous stem extract to reverse clonidine-related alterations in selected biochemical parameters associated with MSF, with the aim to ascertain its acclaimed folkloric use as a male aphrodisiac and sexual function enhancer.

MATERIALS AND METHODS

Plant material collection and authentication

Erythrina senegalensis was collected at Shere hills, Jos, Plateau State, Nigeria. It was identified and authenticated at Herbarium of Federal College of Forestry, Jos, Nigeria, where a specimen was deposited and voucher number FHJ274 obtained.

Experimental animals

Forty (40) male adult Wistar rats with average weight of 150.55 ± 10.05 g were obtained from the Animal Housing Section of National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria. The rats were moved to the Animal House Section of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos, where they were kept in well ventilated cages and given a period of 14 days to acclimatize to animal house conditions before commencement of experiment. They were fed normal rat pellet (Top Feeds Ltd., Sapele, Nigeria) and had unrestricted access to clean water.

Ethical approval

Animal handling was conducted according to laid down guidelines by University of Jos committee on animal use for research. The research was approved and allocated reference number UJ/FPS/F17-00379.

Drugs, assay kits and reagents

Clonidine hydrochloride tablet (Batch No. 252440) and Adam's desire (Lot No.1746198) were products of Sandoz Limited, Surrey, England and Now Foods, United States of America respectively. Assay kits for DHT (catalog No. E-EL-0031), alkaline phosphatase (ALP) (catalog

No. E-BC-K091-S), phosphate (catalog No. E-BC-K245-S) and sodium ion (catalog No. E-BC-K207-S) were products of Elabsience Biotechnology Co., Ltd, Houston, Texas, USA while that of NO (product No. NB98) and testosterone (catalog No. EA78) were produced by Oxford Biomedical Research Inc., United States of America. Kits for total protein (catalog No. BXC0173), urea (catalog No. BXC0123), potassium ion (catalog No. BXC0132), ALT (catalog No. BXC0213), AST (catalog No. BXC0203), GGT (catalog No. BXC0362) and creatinine (catalog No. BXC0111) were products of Fortress Diagnostics, United Kingdom. Kits for LH (catalog No. LH231F) and follicle stimulating hormone (FSH) (catalog No. 11-FSHHU-E01) were products of CalBiotech Inc., El Cajon, California, USA and Alpco, Salem, New Hampshire, United States of America respectively. Kit for albumin (catalog No. A7502-500) was a product of Pointe Scientific, Inc. Canton, USA while that for calcium (catalog No. REF:227 003) was a product of Egyptian Company for Biotechnology, Cairo, Egypt. All other chemicals used in this study were of research grade and were purchased from reputable chemical vendors.

Methods

Plant preparation and extraction

Erythrina senegalensis stems first reduced to bits, air-dried to constant weight and then pulverized. A known weight (1000 g) of the powdered plant material was percolated in 4000 ml of distilled water and kept in the refrigerator for 48 hours with intermittent stirring. The mixture was first filtered using a white muslin fabric and the resulting filtrate further filtered using Whatman No.1 filter paper. The resulting filtrate was thereafter concentrated on a water-bath at 50 °C. The concentrate was reconstituted to the required doses employed in this experiment (i.e. 500 and 1000 mg/kg BW).

Secondary metabolite analysis

E. senegalensis aqueous extract was screened for secondary metabolites such as coumarins, glycosides, phenolics, terpenoids, saponins, flavonoids, steroids, tannins, triterpenes, anthocyanins, phlobatannins and alkaloids using the methods described by Egbuna *et al.* (2019). The present secondary metabolites (i.e. coumarins, glycosides, phenolics, terpenoids, saponins, flavonoids and steroids) were quantified using the methods described by Egbuna *et al.* (2019).

Mineral and amino acid analysis

Mineral composition of aqueous stem extract of *E. senegalensis* was determined using a handheld X-Ray fluorescence analyser (Thermo Scientific, Niton XL3t GOLDD+) according to Jenkins (1988) while the method of Moore *et al.* (1958) was employed for the determination of amino acid composition of the extract.

Animal grouping and extract administration for determination of sexual function biochemical parameters

Twenty-five (25) male rats were assigned into 5 groups (A -E). Group A was administered distilled water only and represented the sham control. Groups B - E received 0.5 mg/kg body weight (BW) of clonidine orally and were thereafter treated with distilled water, 13.3 mg/kg BW of Adam's desire, 500 mg/kg BW of ASEES and 100 mg/kg BW of ASEES respectively. Animals were treated for 7 days and sacrificed 24 hours after. Their testes and blood were collected and prepared for the assay of testosterone, DHT, FSH, LH and NO.

Animal grouping and extract administration for toxicity test

Fifteen (15) male Wistar rats were allocated into 3 groups of 5 rats each (i. e. M, N and O). Group M received distilled water (Control) while groups N and O received 500 mg/kg BW and 1000 mg/kg BW ASEES respectively. Rats were treated for 7 days and sacrificed 24 hours after the last dose was administered. Their blood, livers and kidneys were collected and prepared for determination of indices of liver and kidney function.

Determination of biochemical indices of male sexual function

Testosterone and DHT were assayed for by the methods of Tietz (1995) and Sartorius *et al.* (2014) respectively. FSH and LH were determined using the methods of Kjeld (1976) and Cumming *et al.* (1985) respectively, while NO was assayed for by the method of Schmidt (1995).

Determination of indices of liver and kidney function

AST, ALT, total protein, creatinine, calcium and phosphate were determined by the methods of Tietz (1995). Albumin, ALP and GGT were assayed for by the methods of Hinton *et al.* (1990), Wright *et al.* (1972) and Szasz (1974) respectively. Urea, sodium ion and potassium ion were analysed using the methods of Fawcett and Scott (1960), Trinder (1951) and Henry (2001) respectively.

Statistical analysis

Data are presented as mean (of 5 replicates) $\pm$ SEM. Statistical analysis was carried out on laboratory-generated raw data using the IBM[®] Statistical Package for Social Sciences (SPSS) Software Version 22. All significant differences were figured out by one way Analysis of Variance (ANOVA) and Post Hoc multiple comparisons was done using Duncan's multiple range test. The level of significance was set at $p < 0.05$.

RESULTS

Secondary metabolite constituents of aqueous stem extract of *Erythrina senegalensis*

Secondary metabolite analysis detected coumarins, glycosides, phenolics, terpenoids, saponins, flavonoids and steroids in ASEES while tannins, triterpenes, anthocyanins, phlobatannins and alkaloids were not detected. Steroids had the highest concentration (293.69 mg/100 g) while coumarins had the lowest (0.00649 mg/100 g) (Table 1).

Mineral constituents of aqueous stem extract of *Erythrina senegalensis*

Mineral analysis of ASEES detected thirteen minerals which include molybdenum, potassium, calcium, copper, zinc and selenium among others. Calcium was highest in concentration (1173.00 ppm) while zinc had the lowest concentration (1.01 ppm) (Table 2).

Amino acid constituents of aqueous stem extract of *Erythrina senegalensis*

Eighteen of the twenty standard amino acids were detected in ASEES. These include leucine, phenylalanine, tryptophan, arginine, tyrosine, glutamic acid and glycine among others. Glutamic acid had the highest concentration (12.26 g/100 g) while glycine was lowest in concentration (0.42 g/100 g). Asparagine and glutamine were not detected as they were converted to aspartic acid and glutamic acid respectively during acid hydrolysis (Table 3).

Biochemical indices of male sexual functions in clonidine-treated rats administered aqueous stem extract of *Erythrina senegalensis*

Clonidine administration significantly reduced ($p < 0.05$) the levels of testicular testosterone, serum testosterone, serum DHT, serum LH and serum NO while serum FSH was not altered (Table 4).

Administration of ASEES at both doses (500 and 1000 mg/kg) significantly elevated ($p < 0.05$) testicular testosterone level in comparison to the clonidine and distilled water-treated group. Animals treated with ASEES at 1000 mg/kg showed a significantly elevated ($p < 0.05$) testicular testosterone level than those treated with ASEES at 500 mg/kg. There was no significant difference ($p > 0.05$) in the level of testicular testosterone of the clonidine and Adam's desire-treated group and the clonidine and distilled water-treated ones. The distilled water only-treated control group showed a significantly elevated ($p < 0.05$) testicular testosterone concentration in comparison to all the clonidine-treated groups (Table 4).

The clonidine-treated groups that received Adam's desire and ASEES (500 and 1000 mg/kg) all showed a significantly elevated ($p < 0.05$) serum testosterone and DHT levels in comparison to the clonidine and distilled water-treated group. There was no significant difference ($p > 0.05$) in serum testosterone and DHT levels of clonidine-treated groups that received Adam's desire and ASEES (500 and 100 mg/kg). The distilled water only-treated control group showed a significantly elevated ($p < 0.05$) serum testosterone concentration in comparison to all the clonidine-treated groups (Table 4).

The ASEES (at both doses) induced a significant rise ($p < 0.05$) in serum LH level of the clonidine-treated animals in comparison to the other clonidine-treated groups and the distilled water only-treated control (Table 4).

The ASEES (at both doses) similarly raised the serum level of NO in the clonidine-treated animals in comparison to the clonidine and distilled water-treated group. Clonidine-treated animals that received ASEES at 1000 mg/kg showed a significantly elevated ($p < 0.05$) serum NO concentration than the group that received ASEES at 500 mg/kg. The ASEES at 500 mg/kg compared favourably with Adam's desire while the distilled water only-treated control group showed serum NO level significantly higher ($p < 0.05$) than all the ASEES and Adam's desire-treated animals (Table 4).

Liver and kidney function indices in rats administered aqueous stem extract of *E. senegalensis*

Administration of ASEES (at both doses) significantly elevated ($p < 0.05$) liver AST activity in comparison to the control group. There was also a simultaneous significant reduction ($p < 0.05$) in the serum AST activity in the ASEES-treated groups in comparison to the control group (Table 5).

Animals that received ASEES at 500 mg/kg showed a significant elevation ($p < 0.05$) in liver ALT activity in comparison to the control group. There was however no significant difference ($p > 0.05$) in liver ALT activity of the group administered ASEES at 1000 mg/kg and the distilled water-treated control group. In the serum, there was no alteration of ALT activity in the ASEES-treated groups in comparison to the control (Table 5).

Both groups that received ASEES showed a significant rise ($p < 0.05$) in serum level of total protein in comparison to the control. Nevertheless, the extract did not induce any alteration in serum albumin concentration of the treated animals (Table 5).

Administration of ASEES elicited no alteration to kidney and serum ALP activities as well as serum GGT activity. However, the kidney GGT activity significantly elevated ($p < 0.05$) in the ASEES-treated animals in comparison to the control (Table 6).

Serum concentrations of creatinine, calcium ion and phosphate ion were not altered following ASEES administration (Table 7). Serum urea and potassium ion levels were significantly reduced ($p < 0.05$) by ASEES at both doses (Table 7). In contrast, serum sodium ion level was significantly raised ($p < 0.05$) by ASEES at both doses (Table 7).

Table 1: Secondary metabolite constituents of aqueous stem extract of *Erythrina senegalensis*

S/No	Secondary Metabolites	Concentration (mg/100g of Sample)
1	Coumarins	0.00649 ± 0.002
2	Glycosides	4.94 ± 0.30
3	Phenolics	44.16 ± 0.09
4	Terpenoids	17.13 ± 0.55
5	Saponins	16.86 ± 0.11
6	Flavonoids	18.64 ± 1.27
7	Steroids	293.69 ± 0.23
8	Tannins	ND
9	Triterpenes	ND
10	Anthocyanins	ND
11	Phlobatannins	ND
12	Alkaloids	ND

Values are means $\pm$ SD, n=2; ND = Not detected

Table 2: Mineral composition of aqueous stem extract of *Erythrina senegalensis*

S/No	Minerals	Concentration (ppm)
1	Rubidium	25.00 ± 1.21
2	Strontium	17.02 ± 0.22
3	Zirconium	12.01 ± 1.31
4	Niobium	17.00 ± 2.12
5	Molybdenum	14.21 ± 1.31
6	Potassium	17.43 ± 1.22
7	Calcium	1173.00 ± 5.21
8	Titanium	9.23 ± 0.32
9	Copper	24.22 ± 1.43
10	Zinc	1.01±1.14
11	Selenium	1.11 ± 0.02
12	Lead	2.01 ± 1.43
13	Mercury	2.21 ± 0.34

Values are means ±SD, n=2

Table 3: Amino acid composition of aqueous stem extract of *Erythrina senegalensis*

S/No	Amino acids	Concentration (g/100 g protein)
1	Leucine	8.35 ± 0.21
2	Lysine	2.62 ± 0.06
3	Isoleucine	4.52 ± 0.13
4	Phenylalanine	5.14 ± 0.23
5	Tryptophan	1.42 ± 0.17
6	Valine	4.62 ± 0.33
7	Methionine	3.02 ± 0.07
8	Proline	3.45 ± 0.10
9	Arginine	4.30 ± 0.21
10	Tyrosine	3.81 ± 0.32
11	Histidine	2.11 ± 0.11
12	Cystine	1.70 ± 0.03
13	Alanine	3.50 ± 0.22
14	Glutamic acid	12.26 ± 1.21
15	Glycine	0.42 ± 0.22
16	Threonine	3.36 ± 0.16
17	Serine	3.13 ± 0.21
18	Aspartic acid	7.13 ± 1.03

Values are means ±SD, n=2

Table 4: Selected biochemical indices of male sexual functions in clonidine-induced sexual dysfunction in male rats administered aqueous stem extract of *Erythrina senegalensis*

Treatment Groups	TT (pg/mL)	ST (pg/mL)	SDHT (ng/L)	SFSH (mIU/mL)	SLH (mIU/mL)	SNO (μM)
Distilled water	35.80 ± 1.39 ^a	12.71 ± 0.33 ^a	3.07 ± 1.87 ^a	1.27 ± 0.07 ^a	1.37 ± 0.11 ^a	23.72 ± 1.61 ^a
Clonidine (0.5 mg/kg BW)	14.94 ± 1.09 ^b	8.45 ± 0.91 ^b	0.34 ± 0.10 ^b	1.29 ± 0.08 ^a	0.79 ± 0.28 ^b	7.12 ± 0.55 ^b
Clonidine + Adam's desire (13.3 mg/kg BW)	15.44 ± 1.29 ^b	10.78 ± 0.33 ^c	2.87 ± 0.41 ^c	1.33 ± 0.04 ^a	1.94 ± 0.14 ^c	9.81 ± 0.27 ^c
Clonidine + ASEES (500mg/kg BW)	18.87 ± 0.91 ^c	11.67 ± 0.69 ^c	2.73 ± 0.39 ^c	1.65 ± 0.43 ^a	2.64 ± 0.20 ^d	9.73 ± 0.77 ^c
Clonidine + ASEES (1000mg/kg BW)	25.38 ± 2.46 ^d	11.95 ± 0.65 ^c	2.87 ± 0.39 ^c	1.14 ± 0.38 ^a	2.29 ± 0.14 ^d	11.85 ± 0.95 ^d

Data are mean (of 5 replicates) ± SEM; Values carrying different superscripts down the column are significantly different at $p < 0.05$; ASEES: Aqueous stem extract of *Erythrina senegalensis*; TT: Testicular testosterone, ST: Serum testosterone, SDHT: Serum Dihydrotestosterone, SFSH: Serum Follicle-stimulating hormone, SLH: Serum Luteinizing hormone, SNO: Serum Nitric oxide

Table 5: Selected liver function indices in Wister rats administered aqueous stem extract of *Erythrina senegalensis*

Treatment Groups	Liver AST (U/L)	Serum AST (U/L)	Liver ALT (U/L)	Serum ALT (U/L)	Serum Total protein (g/dL)	Serum Albumin (g/dL)
Distilled water	273.29 ± 2.86 ^a	204.47 ± 17.29 ^a	329.60 ± 21.16 ^a	73.42 ± 7.59 ^a	5.26 ± 0.31 ^a	4.09 ± 0.19 ^a
ASEES (500mg/kg BW)	285.92 ± 4.32 ^b	197.89 ± 10.11 ^a	467.63 ± 28.62 ^b	72.45 ± 5.49 ^a	6.97 ± 0.26 ^b	4.46 ± 0.11 ^a
ASEES (1000mg/kg BW)	296.58 ± 3.76 ^c	194.08 ± 17.99 ^a	311.90 ± 4.79 ^a	72.34 ± 8.47 ^a	7.19 ± 0.20 ^b	4.61 ± 0.03 ^a

Data are mean (of 5 replicates) ± SEM; Values carrying different superscripts down the column are significantly different at $p < 0.05$; ASEES: Aqueous stem extract of *Erythrina senegalensis*; AST: Aspartate transaminase; ALT: Alanine transaminase

Table 6: Kidney and serum alkaline phosphatase and gammaglutamyl transferase activities in Wister rats administered aqueous stem extract of *Erythrina senegalensis*

Treatment Groups	Kidney ALP (U/L)	Serum ALP (U/L)	Kidney GGT (U/L)	Serum GGT (U/L)
Distilled water	71.28 ± 1.65 ^a	52.58 ± 0.89 ^a	42.56 ± 4.93 ^a	13.34 ± 0.86 ^a
ASEES (500mg/kg BW)	72.25 ± 4.24 ^a	50.82 ± 0.56 ^a	53.06 ± 2.20 ^b	12.56 ± 4.94 ^a
ASEES (1000mg/kg BW)	70.03 ± 1.48 ^a	54.23 ± 0.88 ^a	65.35 ± 7.50 ^c	14.35 ± 2.50 ^a

Data are mean (of 5 replicates) ± SEM; Values carrying different superscripts down the column are significantly different at p < 0.05; ASEES: Aqueous stem extract of *Erythrina senegalensis*; ALP: Alkaline phosphatase; GGT: Gammaglutamyl transferase

Table 7: Selected kidney function indices in Wister rats administered aqueous stem extract of *Erythrina senegalensis*

Treatment Groups	Creatinine (μmol/L)	Urea (mg/dL)	Na⁺ (mmol/L)	K⁺ (mmol/L)	Ca²⁺ (mmol/L)	Phosphate ion (mmol/L)
Distilled water	159.30 ± 18.29 ^a	59.05 ± 7.26 ^a	97.83 ± 4.12 ^a	11.66 ± 3.84 ^a	3.23 ± 0.17 ^a	2.91 ± 0.54 ^a
ASEES (500mg/kg BW)	153.92 ± 13.37 ^a	35.96 ± 2.93 ^b	112.53 ± 3.91 ^b	5.12 ± 0.69 ^b	3.72 ± 0.13 ^a	2.29 ± 0.29 ^a
ASEES (1000mg/kg BW)	148.82 ± 12.27 ^a	44.84 ± 3.75 ^c	114.32 ± 3.42 ^b	5.75 ± 3.46 ^b	3.25 ± 0.17 ^a	2.68 ± 0.07 ^a

Data are mean (of 5 replicates) ± SEM; Values carrying different superscripts down the column are significantly different at p < 0.05; ASEES: Aqueous stem extract of *Erythrina senegalensis*

DISCUSSION

Male sexual dysfunction, which can present as any of lack of sexual desire, erectile dysfunction, ejaculation disorder, detumescence failure or orgasmic failure, can leave a man frustrated and inadequate. Certain medications, like clonidine, dampen sexual functions as a secondary effect, and as such patients to whom they are prescribed may find it hard to adhere to their use. Some negative impacts of clonidine on MSF documented in literature include suppression of copulatory behavior, induction of erectile dysfunction and reduction of conception rate (Clark and Smith, 1990; Clark, 1991; Lin *et al.*, 2015). The recorded drop in the concentrations of testosterone, DHT, LH and NO in this study following clonidine administration to male rats corroborate previous findings on clonidine sexual side effects.

To take full advantage of the numerous benefits that clonidine offers, it is necessary to consider its combination with sexual function enhancers. Medicinal plants have been used since ancient times for the treatment of diseases including those involving men's sexual challenges. The ability of these plants to perform diverse therapeutic functions has been linked to their constituent phytochemicals. Musa *et al.* (2016) previously detected alkaloids, saponins, tannins and flavonoids in the ethanol root bark extract of *E. senegalensis* while Nnama *et al.* (2017), further reported the presence of terpenoids in the ethanolic leaf extract of the plant. In our study, ASEES did not contain alkaloids and tannins, but rather included coumarins, glycosides, phenolics and steroids, which were not reported in the two previous studies. The difference in these results is attributable to difference in plant parts used, harvest time of plant, extraction solvents and even the soil on which the plants grew.

Many of plants secondary metabolites, minerals and amino acids reportedly exhibit sexual function-enhancement activity. The presence of coumarins, saponins, phenolics and flavonoids in ASEES might be responsible for the recorded elevation of the studied sexual function-associated biochemical parameters. Coumarins improve sexual function by exhibiting vasorelaxant activity on penile smooth muscle leading to improved penile erection due to enhanced blood flow into the cavernosum (Wen-Fei *et al.*, 2001; Coung *et al.*, 2014; Kim *et al.*, 2017). This may explain why the level of NO, which plays crucial role in penile erection, was elevated in this study. The presence of saponins in the extract could facilitate the repair of damaged endothelial function of the cavernosum while flavonoids could improve libido and sperm quality. For example saponins from *Tribulus terrestris* and *Panax notoginseng* were reported to repair damaged endothelial function of the penile cavernosum of type 2 diabetic rats, thus ameliorating erectile dysfunction (Huan *et al.*, 2014; Zhang *et al.*, 2019). Similarly, flavonoids-rich fractions of *Rosmarinus officinalis* were reported to have improved sperm motility and sexual behavior (i.e. mount frequency and male orientation towards female) in streptozotocin-induced diabetic male mice (Messaoud *et al.*, 2020).

Minerals perform important roles in metabolic processes, including those related to MSF and fertility (Beigi *et al.*, 2019). Potassium, calcium, copper, zinc and selenium (which are present in ASEES) have all been implicated to influence MSF. Zinc boosts androgen plasma concentrations, improves sperm count, reverses testicular dysfunction, prolongs ejaculation latency and increases the frequency of penile thrusting (Suescun *et al.*, 1981; Dissanayak *et al.*, 2009). Copper acts as cofactor for enzymes like cytochrome oxidase, catalase, tyrosinase, monoamino oxidase, ascorbic acid oxidase and uricase (Karunanithi *et al.*, 2019) and is positively correlated with testosterone (Miska-Schramm *et al.*, 2018). Selenium is essential to spermatogenesis and male fertility, probably due to the vital role it plays in modulation of antioxidant defense mechanisms and other important biological pathways (Hallinan, 2011; Qazi *et al.*, 2019). Potassium plays a crucial role in negating the detrimental impact of sodium ion concentration and thus helping to control blood

pressure. Also, owing to its vasoactive property, it can encourage the flow of blood to the penis and other organs (Haddy *et al.*, 2006). Effective blood flow to the penis is essential for erection and intromission. Calcium, which is relatively abundant in ASEES, stimulates steroidogenesis in testicular leydig cells. Increased testosterone enhances overall MSF. In addition, calcium influences other aspects of MSF like spermatogenesis, sperm motility and chemotaxis, capacitation and acrosome reaction as well as fertilization (Beigi *et al.*, 2019).

Quite a number of amino acids have been reported to influence positively different aspects of MSF. These amino acids which include phenylalanine, tryptophan, arginine and tyrosine are present in ASEES. Arginine is a substrate for NO synthase (NOS), an enzyme that catalyzes the conversion of arginine to NO and citrulline. NO aids penile erection through a cascade of reactions involving guanylate cyclase which ultimately culminates into the relaxation of cavernosal tissues, leading to erection (Burnett, 2004). Phenylalanine and tyrosine are both precursors to dopamine, norepinephrine, and epinephrine (adrenaline). Dopamine activates motivated behaviors like copulation, controls genital reflexes, copulatory patterns and specifically sexual motivation (Hull *et al.*, 2004), while norepinephrine, and epinephrine have been mooted as libido enhancers (Kayode and Yakubu, 2017).

The reduction in the levels of testosterone and LH following clonidine administration in this research did not agree with previous studies. In one study, clonidine administration to five adolescent males at closely monitored doses, for 4 weeks did not alter the levels of testosterone, LH, FSH, prolactin, growth hormone, cortisol and aldosterone (Boyar *et al.*, 1980). Similar results of clonidine dampening MSF without altering androgens and gonadotropic hormones have been previously reported (Lin *et al.*, 2015). In this study, clonidine did not alter FSH, which agrees with aforementioned previous reports. A reduction in testosterone level will attenuate sexual desire and penile erection and also decrease bone and body mass. DHT is formed from testosterone by the action of NADPH-dependent enzyme, 5 α –reductase. It is a biologically more effective androgen than testosterone exhibiting about two – three times higher affinity for the androgen receptor. The conversion of testosterone to DHT is believed to be a very imperative step in the expression of androgenic responses (Gur *et al.*, 2013). Since DHT is synthesized directly from testosterone, its concomitant reduction along with testosterone in the clonidine-treated animals is natural. LH which is produced by the gonadotropin-releasing hormone and released into general circulation binds to receptors on the leydig cells in the testes to facilitate testosterone synthesis. Therefore, clonidine-induced inhibition of LH production can be associated with the recorded reduction in testosterone level in this study. NO plays very pivotal role in the facilitation of penile erection. Clonidine might have induced a reduction of NO by inhibiting NO synthase activity. Administration of ASEES reversed clonidine-mediated reductions in the levels of testicular/serum testosterone, DHT, LH and NO and performed better than Adams desire, a MSF-enhancing polyherbal drug, employed as standard drug in this study.

The rise in liver AST (at both doses) and ALT (only at 100 mg/kg) activities recorded in this experiment following ASEES treatment may be ascribed to increased synthesis or activation of the transaminases. Since there was no simultaneous rise of the enzymes in the serum, one can conclude that ASEES and its constituent phytochemicals did not engender any damage to hepatocytes membranes which could have led to leakage of transaminases into the serum. However, an upsurge in the activities of AST and ALT above control levels will translate to increased inter-conversion or degradation of amino acids. Transamination is known to increase during fasting, so as to channel amino acids into the TCA cycle for energy generation. However, since the ASEES-treated animals had access to food *ad libitum* just like the control animals, it is possible that ASEES may prevent normal absorption of carbohydrates in the small intestine.

Incidentally, ASEES ability to reduce intestinal absorption of glucose has been documented by Ekissi *et al.* (2021).

Serum total protein along with albumin and globulin are used to assess the soundness of liver's synthetic functions. In this experiment, the recorded rise in serum total protein with unaltered albumin level suggests an elevation in globulin level. Increase in total protein associated with increased globulin can be caused by dehydration among other causes like acute infections and chronic inflammatory conditions (Colin, 2020).

ALP and GGT are membrane enzymes which are present in kidney cell membranes among other tissue membranes. A rise in serum concentrations of these enzymes suggests a loss of membrane integrity resulting in their leakage into extracellular space. Kidney and serum concentrations of ALP were unaltered by ASEES in this experiment. However, kidney GGT was elevated without an alteration in serum levels. This suggests that ASEES did not disrupt kidney cell membranes. The recorded elevation of GGT in the kidney may be ascribed to ASEES ability to induce excessive production or increase in activity of the enzyme. Similar inductive effect of plant extracts on kidney enzymes have been reported in literature and has been ascribed to the presence of nephrotoxic secondary metabolites (Yakubu and Omoniwa, 2012; Omoniwa and Yakubu, 2017). A higher than normal GGT level will cause excessive transfer of amino acids across cellular membranes and distort glutathione homeostasis (Zhang *et al.*, 2005).

Part of the functions of the kidneys is to excrete metabolic wastes and maintain electrolyte homeostasis. Creatinine and urea are reliable indices for measuring the integrity of renal function (Adeneye *et al.*, 2008; Chinnappan *et al.*, 2019). Creatinine, calcium ion and phosphate ion levels were not altered following ASEES administration. The recorded reduction in urea level suggests decreased protein catabolism. The reduction is attributable to the relatively high level of steroids in the plant extract. Steroids have long been reported to impact either positively or negatively on blood urea concentration (Sigler, 1967). Many plants with nephroprotective activity have been documented to contain steroids (Yadav *et al.*, 2016; Chinnappan *et al.*, 2019). In one instance, herbal extract of *Eurycoma longifolia* reversed paracetamol-induced elevation of serum urea concentration (Chinnappan *et al.*, 2019). It is likely that steroids reduced protein catabolism in the experimental animals leading to the recorded reduced serum urea level. This result corroborate the work of Esposito *et al.* (2011) who reported the ability of plant steroids (brassinosteroids) to activate protein biosynthesis and inhibit protein catabolism in L6 rat skeletal muscle cells. Sodium ion was increased by ASEES in a dose-dependent manner, which resulted in a simultaneous drop in the serum level of potassium ion. A rise in aldosterone concentration induces re-absorption of sodium and loss of potassium, an imbalance that can cause the body to hold too much water, leading to elevated blood volume and pressure. It is possible that the steroids and other secondary metabolites present in ASEES may mimic the activity of aldosterone or induce its synthesis, thus causing the recorded imbalance in sodium and potassium homeostasis.

CONCLUSION

ASEES improved the selected biochemical parameters associated with MSF in clonidine-treated male Wistar rats but altered liver and kidney enzymes, liver protein synthesis function, kidney excretory functions and kidney ability to maintain electrolyte homeostasis.

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Identification, Nomination and Release of Early Maturing Stress Tolerant Maize Variety in Nigeria

¹Bankole, F., ¹Olaoye, G., ²Oyekunle, M., ³Olakojo, S., ^{1*}Takim, F. O.,
¹Omotesho, K. and ¹Ayinde, O. E.

¹Faculty of Agriculture, University of Ilorin, Ilorin.

²Institute for Agricultural Research, Ahmadu Bello University Zaria.

³Institute of Agricultural Research and Training, Moor Plantation, Ibadan.

***Corresponding Author:** fotakimson@unilorin.edu.ng

Abstract

Identifying promising early maize varieties that combine high-yielding properties with tolerance/resistance to the prevailing abiotic/biotic stress factors is one of the steps in improving maize production in Nigeria's savannah. This study was conducted to identify and nominate an early maturing yellow open-pollinated maize variety with combined resistance/tolerance to *Striga*, drought, and low-N for registration and release in Nigeria. Twenty-five open-pollinated maize varieties including the local check varieties were evaluated at Ilorin (Kwara State) in an on-station trial in 2017. Eleven varieties were selected and reevaluated under *Striga* and non-*Striga*-infested conditions (Mokwa and Abuja), drought and non-drought conditions (Ikenne). The outstanding varieties were later evaluated in on-farm trials at Apata in Oyo State, Mokwa and Lapai in Niger State, Ballah and Omu Aran in Kwara State, in 2020. The experimental design was a Randomized Complete Block Design with three replications per location. The trials were planted in two-row plots, 5 m in length, with 0.75 m spacing between rows, 0.25 m spacing between hills, and an intra-row spacing of 0.40 m. Data were collected for days to anthesis /silking, ear height, plant height, ear aspect, plant aspect, grain yield, and foliar diseases. All data were subjected to analyses of variance using SAS software. The stability of the varieties across the locations was done with genotype plus genotype by environment (GGE) biplot analysis. The evaluation results in 2018 and 2019 show that DTE STR-Y Syn Pop C4 F2 had a yield advantage of 35%, 9%, and 15% over the reference check and 7%, 6%, and 6% over the local check variety, respectively, under *Striga*, non-*Striga*, and both conditions. Under drought and well-watered conditions, DTE STR-Y Syn Pop C4 F2 out yielded 28 as well as 5% higher than the reference check, respectively, while the local check out yielded 6% more. DTE STR-Y Syn Pop C4 F2 had a yield advantage of 7 and 14% over the local check in low and high soil nitrogen, respectively, while in high soil N, DTE STR-Y Syn Pop C4 F2 had a yield advantage of 18% over the local check. The results of three states' on-farm trials revealed that the variety DTE STR-Y Syn Pop C4 F2 (4.6 t/ha) out-yielded the farmer's variety and the reference check by 15% and 19%, respectively. The GGE biplot analysis revealed that DTE STR-Y Syn Pop C4 F2 was the most stabilised with high grain yield values in the test environments. The cost-benefit ratio of DTE STR-Y Syn Pop C4 F2 was higher than that of the reference check and the farmer's varieties, implying that the resource-constrained farmer will see higher returns on his investment. DTE STR-Y Syn Pop C4 F2 (ILO-MAZ 1) an open-pollinated

variety with tolerance to drought and low N, resistant to Striga performed well in terms of yield and agronomic features in both on-farm and on-station trials, it is therefore proposed for registration and release in Nigeria.

Keywords: Maize, Grain yield stability, open-pollinated, Striga resistance, On-farm trial

Introduction

Maize (*Zea mays* L.) is Africa's most important food crop (Wossen *et al.*, 2017). It is an annual crop grown in Sub-Saharan African (SSA) countries for use as a staple food as well as raw materials for industrial purposes. However, the sub-maize region's potential is typically not realized due to the negative effects of some biotic and abiotic stress factors, combined with the use of non-improved varieties, cultivation under sub-optimal conditions, application of fertilizers at sub-optimal levels, and ineffective weed control methods (Olaniyan, 2015). To achieve high grain yield and stability through maize improvement programs, maize breeders must develop varieties that combine high yields with improved levels of tolerance to multiple stresses for the benefit of resource-limited farmers in Africa (Cairns *et al.*, 2013). This will ensure stable and high yields under production conditions while mitigating the negative effects of climate change. As a result, there is an urgent need to make maize varieties with a high level of resistance/tolerance to multiple stresses available for reception and commercialization in Nigeria.

Several varieties of maize with different maturity groups (extra-early, early, intermediate/late), and varying endosperm colours (Yellow and white), from different source populations (OPVs and hybrids) with tolerance to the prevailing biotic and abiotic stress factors, have been developed under the Drought Tolerant Maize for Africa through the collaborative efforts of Maize breeders from International Institute of Tropical Agriculture and National partners such as the Institute of Agricultural Research, Zaria and University of Ilorin, Nigeria. Early varieties that are resistant or tolerant to multiple stresses are required to encourage the rapid adoption and commercialization of maize in Nigeria's savanna zones. Farmers with limited resources should have access to improved maize varieties with resistance to Striga and Streak, as well as tolerance to both drought and low levels of soil nitrogen, to minimize the rapid yield reduction that threatens food security and leads to malnutrition and poverty.

The ability to identify genotypes that have high yield potential and are stable across a wide range of environments is an important goal of any maize improvement programme (Mendes *et al.*, 2012). Therefore, newly improved varieties of maize that possess tolerance to abiotic stress factors that are developed from different germplasm are usually assessed for their yield potential, adaptability, stability, and even for acceptability by the producers who are often resource-limited farmers. Maize breeders at the International Institute of Tropical Agriculture (IITA) developed potential maize varieties that can be released and promoted for commercial use, and these varieties are carefully evaluated at multiple locations representing various agroecological zones over multiple years. These varieties are also tested across farmers' ecologies under typical growing conditions.

The availability of early maturing maize varieties in marginal regions with low rainfall or areas with sandy or shallow soil can reduce scarcity and greatly contribute to food security in Nigeria because they can complete their life cycle before the drought period begins, thereby avoiding drought. Drought-tolerant genes can also be incorporated into some varieties thereby combining drought escape with drought-tolerant ability. Farmers have a high demand for early maturing varieties because they respond well to fertilizer application, mature earlier than other cereal crops such as sorghum and millet adapted to the region, and allow farmers to be more flexible with their

planting dates. When the rainfall distribution is normal or delayed, the varieties can also be planted (Badu-Apraku *et al.*, 2012).

Scientists at IITA in partnership with scientists at the University of Ilorin with the support of the Stress Tolerant Maize for Africa Project have been involved in testing several maize varieties including the early maturing maize varieties in southern Guinea savannah of Nigeria for over a decade, we been able to identify promising early maize varieties that combine high yield with tolerance/resistance to the prevailing abiotic/biotic stress factors. Therefore, the objective was to identify and nominate an early maturing yellow open-pollinated variety with combined resistance/tolerance to *Striga*, drought and low-N for registration and release in Nigeria.

Materials and Methods

Multi-location evaluation of maize varieties

A total of twenty-five open-pollinated maize varieties including the reference and local check varieties were evaluated in an on-station trial in 2017 in Ilorin (Kwara state) a typical Southern Guinea savannah ecology. The experimental design was a randomized complete block design with three replications with two rows per location. At all locations, entries were planted in two-row plots, 5 m in length, with 0.75 m spacing between rows, 0.25 m spacing between hills and an intra-row spacing of 0.40 m. Based on the results of the first-year evaluation, 11 varieties were selected and evaluated under multiple locations and various growing conditions at various locations representing the various agroecological zones in Nigeria in 2018 and 2019. They have also evaluated under *Striga* and non-*Striga*-infested conditions (Mokwa and Abuja) in 2018 and 2019, drought and non-drought conditions (Ikenne) in 2018 and 2019, and under low and high N conditions (Ile-Ife and Mokwa) in 2018 and 2019. The experimental design was a randomized complete block design with three replications with two rows per location. At all locations, entries were planted in two-row plots, 5 m in length, with 0.75 m spacing between rows, 0.25 m spacing between hills and an intra-row spacing of 0.40 m. Three seeds /hill were sown and the seedlings were thinned to two / stand about 2 weeks after emergence to give a final plant population density of about 66,666 plants ha⁻¹. Fertilizer (NPK 15:15:15) was applied at the rate of 60 kg N and 60 kg P₂O₅, with nitrogen fertilizer applied at planting and six weeks after emergence. The fields were kept free of weeds by using pre-emergence and post-emergence herbicides applied immediately after sowing and at about 5 weeks after emergence respectively, this was supplemented with hand weeding.

Data were recorded for days to anthesis (PS) days to silking (DS), ear height (EH), plant height (PH), ear aspect (EA), and plant aspect (PA). The anthesis silking interval was estimated as the difference between the days of silk extrusion and pollen shed per plot. Ears per plant were estimated as the ratio of the ears harvested per plot to the plants harvested while grain yield was computed from the field weight of the ears harvested per plot with an assumed shelling percentage of 80% after adjustment for grain moisture at harvest. In addition, data was collected on foliar diseases (Maize streak, Southern leaf blight, Southern rust and *Curvularia* leaf spot) by scoring on a scale of 1 to 5, where 1 is the minimal expression of symptoms and 5 where symptoms were fully expressed. All data were subjected to analyses of variance using SAS version 9.3 (SAS Institute 2009) . The stability of the varieties across the locations was done using a GGE biplot.

The outstanding varieties were evaluated in on-farm trials at Oyo (Apata and Ballah), Kwara (Omu aran) and Niger (Mokwa and Lapai) states. The spacing was 20 m by 20 m with an intra-row spacing of 0.40 m. The management practices and data collection did not vary from those adopted for the multilocation trial. The total cost for production of the seed included the cost of fertilizer, insecticide, herbicide, and variable costs like labour, land preparation, management cost

etc. The gross return is the income generated from the sales of the seed while the profit is the Gross return less the total cost of production. The benefit-cost ratio is the Total field benefit (per hectare)/Total investment (per hectare) (Quandeel *et al.*,2020).

Results and Discussion

The mean grain yield of the early maturing stress-tolerant maize variety under the rainfed condition in 2017 at Ilorin was 2.8 t/ha (Table 2). Variety DTE STR-Y Syn Pop C4 F2 had the highest grain yield (3.87 t/ha) with yield advantages of 24 and 87% over both the local and reference (TZE Comp 3 DT C2F2) check varieties respectively. The local check variety had the highest plant height value (169 cm) followed by 2014 TZE-Y DT STR (164 cm) and DTE STR-Y Syn Pop C4 F2 (161 cm). The days to flowering were quite similar for all tested varieties with the earliest varieties shedding pollen after 54 days. The combined analysis of variance for yield and yield-related traits of the early yellow maize varieties and checks evaluated across eight environments in 2018 and 2019 are shown in Table 3-5. The result revealed that the genotypes differed significantly from each other for grain yield. The grain yield of the early maturing maize varieties ranged from 2479 kg/ha (2015 TZE-Y DT STR Syn C0)- 4484 kg/ha (DTE STR-Y Syn Pop C4 F2) with a mean of 3541 kg/ha. DTE STR-Y Syn Pop C4 F2 out-yielded the reference and local checks by 16 and 26% respectively. The local check had the highest plant height values (208 cm) followed by DTE STR-Y Syn Pop C4 F2 with a plant height of 176 cm. The days to silking ranged between 52 days to 56 days only. Under Striga, non-Striga and across both conditions, DTE STR-Y Syn Pop C4 F2 had a yield advantage of 35%, 9% and 15% over the reference check and 7%, 6% and 6% over the local check variety respectively (Table 6). Under drought and well-watered conditions (Table 7), the mean of DTE STR-Y Syn Pop C4 F2 out yielded that of the reference check with 28 and 5% respectively while under well-watered conditions the local check was outyielded by 6%. Under varying conditions of soil nitrogen (N), low and high N (Table 8) the variety DTE STR-Y Syn Pop C4 F2 had a yield advantage of 7 and 14% over the local check while under high soil N, DTE STR-Y Syn Pop C4 F2 had a yield advantage of 18% over the reference check.

Plant breeding aims at the selection of genotypes that have superior yields while eliminating those with inferior performances (Kaiser *et al.*, 2020) therefore, screening of genotypes in environments that are similar to those under the farmer's environment which are prone to biotic and abiotic stress factors becomes necessary. The performance of DTE STR-Y Syn Pop C4 F2 under varying conditions compared with the local and reference checks is notable because the checks are among the best open-pollinated varieties available to farmers in the region. Also, despite the differences across the test environments, which may have been due to varying weather conditions, and edaphic factors amongst others, a variety y DTE STR-Y Syn Pop C4 F2 performed relatively well across the environments.

Consequent upon the fact that the genotypes and genotype by environment interaction for grain yield were significant, the genotype plus genotype by environment (GGE) biplot analysis was used to identify the most stable variety across the test environments. DTE STR-Y Syn Pop C4 F2 was the variety located at the vertex for Minna, Mokwa and Ilorin (Fig 1). which is an indication that the variety is the most adapted to the environment as reflected by its performance in the environment (Yan *et al.*, 2010) The ranking of the varieties along the average tester axis with their average performance shown on the horizontal axis across the environment is represented in fig 2. The line with the single arrow is referred to as the average environment coordination view of the biplot which points to the variety with the highest yield pooled across the environments. The vertical line separates the varieties with high grain yields (above average) and low yields (below average). However, the projection of varieties from the middle horizontal line determines their stability (Haruna *et al.*, 2017). The mean performance and stability of the early varieties as shown from the average-environment coordination (AEC) revealed that variety DTE STR-Y Syn Pop C4 F2 designated as 3 had an outstanding performance in terms of yield and also an appreciable level of stability across the test environment.

The results of the on-farm trials across Kwara, Niger and Oyo States revealed that the highest grain yield was recorded for variety DTE STR-Y Syn Pop C4 F2 (4.6 t/ha), followed by TZE

Comp 3 DT C2F2 which is the reference check (4.1 t/ha) (Tables 9 and 10). DTE STR-Y Syn Pop C4 F2 out-yielded the reference check and the farmer's variety by 19% and 15%, respectively. The on-farm trials were also scored for agronomic traits of importance, armyworm and termite infestations and DTE STR-Y Syn Pop C4 F2 had the highest score compared with the other two varieties. The results of the cost and returns per hectare of the maize varieties used for on-farm trials (Table 11) revealed that the cost of producing the maize varieties did not vary however the returns differed. The cost-benefit ratio of DTE STR-Y Syn Pop C4 F2 was higher than those of the reference check and the farmer's varieties therefore the resource-limited farmer will have more returns on his investment because of the higher yield obtained from this variety compared to the others.

Conclusion

Based on the high grain yield of variety DTE STR-Y Syn Pop C4 F2 designated as ILOMAZ 1 in multi-location and on-farm trials as well as their outstanding performance under *Striga* infestation, drought and low-N conditions, ILOMAZ-1 is hereby nominated for registration and release in Nigeria. The release of the early maturing variety will ensure the sustainability of maize production and productivity and revolutionize agriculture in Nigeria.

Acknowledgement

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Table 1: List of the experimental materials used for the study

GENOTYPES	SOURCE
2010 TZE-W DT STR	IITA
2011 TZE-W DT STR Syn	IITA
2011 TZE-Y DT STR	IITA
2012 TZE-W DT C4 STR C5	IITA
2013 DTE STR-Y Syn	IITA
2014 TZE-W DT STR	IITA
2014 TZE-Y DT STR	IITA
2015 DTE STR-W Syn	IITA
2015 DTE STR-Y Syn	IITA
2015 TZE-W DT STR SynC0	IITA
2015 TZE-Y DT STR Syn C0	IITA
2016 TZE-W DT STR QPM SynF2	IITA
2016 TZE-W DT STR SynF2	IITA
2016 TZE-Y DT STR QPM Syn F2	IITA
2016 TZE-Y DT STR Syn F2	IITA
DTE STR-W Syn Pop C3	IITA
DTE STR-W Syn Pop C4 F2	IITA
DTE STR-Y Syn Pop C3	IITA
DTE STR-Y Syn Pop C4 F2	IITA
Local Check	FARMERS
TZE Comp 3 DT C2F2 (RE)	IITA
TZE-W POP DT C4 STR C5	IITA
TZE-W POP DT C5 STR C5	IITA
TZE-Y POP DT C4 STR C5	IITA
TZE-Y POP DT C5 STR C5	IITA

IITA-International Institute of Tropical Agriculture, Ibadan, Nigeria

Table 2: Grain yield and related traits in 25 Early maturing ST varieties in Ilorin in the Southern Guinea Savannah of Nigeria (2017).

GENOTYPES	GY (t/ha)	Anthesis (days)	Silking (days)	ASI (days)	PH (cm)	EH (cm)	EPP (no)
2010 TZE-W DT STR	2.81	54	57	3	151	72	1
2011 TZE-W DT STR Syn	3.25	54	57	3	155	71	1
2011 TZE-Y DT STR	2.76	55	58	3	153	77	1
2012 TZE-W DT C4 STR C5	3.11	54	57	3	148	72	1
2013 DTE STR-Y Syn	3.24	54	57	3	153	73	1
2014 TZE-W DT STR	3.70	55	58	3	155	77	1
2014 TZE-Y DT STR	3.25	55	59	3	164	81	1
2015 DTE STR-W Syn	3.03	54	57	3	145	68	1
2015 DTE STR-Y Syn	2.30	55	58	3	148	73	1
2015 TZE-W DT STR SynC0	2.61	56	60	3	141	65	1
2015 TZE-Y DT STR Syn C0	1.60	54	57	3	129	59	1
2016 TZE-W DT STR QPM SynF2	2.64	54	57	3	142	62	1
2016 TZE-W DT STR SynF2	2.60	56	59	3	129	60	1
2016 TZE-Y DT STR QPM Syn F2	1.85	55	58	3	136	63	1
2016 TZE-Y DT STR Syn F2	1.44	56	59	3	134	60	1
DTE STR-W Syn Pop C3	2.27	55	58	3	138	73	1
DTE STR-W Syn Pop C4 F2	2.79	55	57	3	145	66	1
DTE STR-Y Syn Pop C3	3.76	55	58	3	145	69	1
DTE STR-Y Syn Pop C4 F2	3.87	54	57	3	161	78	1
Local Check	3.11	56	59	3	169	82	1
TZE Comp 3 DT C2F2 (RE)	2.07	54	57	3	153	76	1
TZE-W POP DT C4 STR C5	2.81	55	57	3	154	71	1
TZE-W POP DT C5 STR C5	3.25	54	57	3	151	72	1
TZE-Y POP DT C4 STR C5	2.76	54	57	4	155	71	1
TZE-Y POP DT C5 STR C5	3.11	55	58	3	153	77	1
MEANS	2.80	55	58	3	149	71	1
LSD(0.05)	0.8	1.4	1.5	0.7	18.1	10.7	0.1
CV	32	3	3	25	13	16	13

GY (Grain yield), ASI (Anthesis silking interval), PH (Plant Height), EH (Ear height), EPP (Ears per plant)

Table 3: Grain yield and other agronomic traits of early maturing ST variety evaluated across eight locations in Nigeria during the 2018 and 2019 growing seasons

Entry	Variety	Grain yield(kg/ha)	Days to silking(days)	Anthesis silking interval	Plant height(cm)	Ear aspect (1-5)	Ears per plant	Maize streak (1-5)	Curvularia (1-5)	Rust (1-5)	Blight (1-5)
1	2016 TZE-W DT STR SynF2	3767	56	2	165	2	1	2	2	2	1
2	DTE STR-W Syn Pop C4 F2	4054	54	2	162	3	1	1	2.5	2	1
3	DTE STR-Y Syn Pop C4 F2	4484	55	2	176	2	1	2	2	2	1
4	2016 TZE-W DT STR QPM SynF2	3496	56	2	150	3	1	1	2	2	1
5	2016 TZE-Y DT STR QPM Syn F2	2734	53	2	161	2	1	2	2	2	1
6	2015 TZE-W DT STR SynC0	3450	56	2	170	2	1	2	3	2	1
7	2015 TZE-Y DT STR Syn C0	2479	52	1	137	3	1	2	2	2	1
8	DTE STR-W Syn Pop C3	3330	54	2	162	3	1	1	3	2	1
9	2011 TZE-W DT STR Syn	4000	55	2	159	3	1	2	2.5	2	1
10	TZE Comp 3 DT C2F2 (RE)	3562	55	2	169	3	1	1	2	2	1
11	Local Check	3861	53	2	208	3	1	2	3	2	1
	MEAN	3541	54	2	165	3	1	2	2	2	1
	LSD	1207	1	1	40	0.4	0.1	0.4	0.6	0.4	0.1
	CV	22	3	51	33	15	12	23	19	25	12

*1 represents well filled uniform ear while represents an undesirable or rotten ear

** 1 represents plants with no disease expression while 5 represents plants with disease expression

Table 4: Grain yield of early maturing stress tolerant maize varieties evaluated in eight locations in Nigeria during the 2018 and 2019 growing seasons.

Entry	Variety	ILORIN		IKENNE		MOKWA		BAGAUDA	MINNA		ABUJA	IFE		ORIN
		2018	2019	2018	2019	2018	2019	2018	2018	2019	2019	2018	2019	2019
1	2016 TZE-W DT STR SynF2	3353	1871	5346	6792	1836	6453	3293	1910	2644	7032	4021	2542	1876
2	DTE STR-W Syn Pop C4 F2	4115	2313	5679	6617	2798	6478	4090	3232	2403	6651	2878	3180	2264
3	DTE STR-Y Syn Pop C4 F2	4768	3300	5835	6384	3156	6636	3433	4142	3384	6415	3571	3353	3914
4	2016 TZE-W DT STR QPM SynF2	3240	2021	5509	4974	2961	5520	3591	2650	2475	4997	3064	2241	2200
5	2016 TZE-Y DT STR QPM Syn F2	3070	1823	3241	3911	871	4891	2732	2131	2607	4013	1681	2839	1733
6	2015 TZE-W DT STR SynC0	3248	2234	5679	5354	1270	4831	3178	2200	2206	5538	2909	3480	2721
7	2015 TZE-Y DT STR Syn C0	2786	1802	2623	3627	1855	4499	1580	1944	2452	3583	1968	2612	894
8	DTE STR-W Syn Pop C3	2623	2434	4513	4736	2626	4935	3587	3073	2565	5033	1661	3414	2094
9	2011 TZE-W DT STR Syn	4220	2450	4256	6738	1956	6120	3581	4300	2819	6858	3979	3254	1464
10	TZE Comp 3 DT C2F2 (RE)	2746	2022	6276	6186	2912	4743	3699	2323	2605	6175	1870	2781	1962
11	Local Check	4173	1796	5872	5078	2874	6459	4224	3332	2906	5158	3083	2283	2950
	MEAN	3486	2187.8	4948	5361	2328	5511	3370	2932.7	2642.2	5442	2666.4	2943.7	2219.6
	LSD	875	589	1569	876	1121	1411	1952	1638	612	1802	1270	1358	614
	CV	22	24	24	22	23	15	29	24	19	20	20	22	17

Table 5: Mean Grain yield (kg/ha) of early maturing ST varieties evaluated in eight locations in Nigeria during the 2018 and 2019 growing seasons.

Entry	Variety	ILORIN	IKENNE	MOKWA	BAGAUDA	MINNA	ABUJA	IFE	ORIN
1	2016 TZE-W DT STR SynF2	2612	6069	4144	3293	2277	7032	3282	1876
2	DTE STR-W Syn Pop C4 F2	3214	6148	4638	4090	2818	6651	3029	2264
3	DTE STR-Y Syn Pop C4 F2	4034	6109	4896	3433	3763	6415	3462	3914
4	2016 TZE-W DT STR QPM SynF2	2630	5241	4240	3591	2563	4997	2653	2200
5	2016 TZE-Y DT STR QPM Syn F2	2446	3576	2881	2732	2369	4013	2260	1733
6	2015 TZE-W DT STR SynC0	2741	5516	3051	3178	2203	5538	3195	2721
7	2015 TZE-Y DT STR Syn C0	2294	3125	3177	1580	2198	3583	2290	894
8	DTE STR-W Syn Pop C3	2528	4624	3780	3587	2819	5033	2538	2094
9	2011 TZE-W DT STR Syn	3335	5497	4038	3581	3560	6858	3617	1464
10	TZE Comp 3 DT C2F2 (RE)	2384	6231	3827	3699	2464	6175	2326	1962
11	Local Check	2985	5475	4666	4224	3119	5158	2683	2950
	MEAN	2837	5238	3919	3370	2788	5442	2806	2220
	LSD	1251	1409	985	1026	1128	972	1003	1217
	CV	11	18	23	16	20	22	11	15

Table 6: Grain yield and other agronomic traits of early maturing ST varieties evaluated under *Striga*-infested and non-infested conditions at Abuja and Mokwa in Nigeria during the 2018 and 2019 growing seasons.

Entry	Variety	Grain yield, (kg/ha)			Days to silk(days)			Anthesis silking interval			Plant height (cm)			Ear aspect(1-5)			Ears/plant		
		STR	NSTR	Across	STR	NSTR	Across	STR	NSTR	Across	STR	NSTR	Across	STR	NSTR	Across	STR	NSTR	Across
1	2016 TZE-W DT STR Syn F2	2082	4854	3745	56	56	56	2	1	1	158	171	166	5	5	5	0.7	0.9	0.8
2	DTE STR-W Syn Pop C4 F2	2444	5094	4034	54	54	54	2	1	2	141	168	157	5	5	5	0.7	0.9	0.8
3	DTE STR-Y Syn Pop C4 F2	2564	5210	4152	55	55	55	2	1	1	167	182	176	4	4	4	0.7	0.9	0.8
4	2016 TZE-Y DT STR QPM Syn F2	1863	3269	2706	56	57	56	3	2	2	138	153	147	5	5	5	0.6	0.8	0.7
5	2016 TZE-W DT STR QPM Syn F2	2100	4338	3443	54	54	54	2	1	1	147	163	157	5	5	5	0.6	0.9	0.8
6	2015 TZE –W DT STR Syn C0	2015	4146	3293	55	57	56	2	2	2	148	169	161	5	5	5	0.7	0.8	0.8
7	2015 TZE –Y DT STR Syn C0	1908	3002	2565	53	53	53	2	1	1	126	138	133	5	6	5	0.8	0.9	0.9
8	DTE STR-W Syn Pop C3	1760	4167	3204	54	54	54	2	1	1	144	167	158	5	5	5	0.7	0.9	0.8
9	2011 TZE-W DT STR Synthetic	2440	4697	3794	54	55	55	2	1	1	153	163	159	4	5	5	0.8	0.9	0.9
10	TZE Comp 3 DT C2F2 (RE)	1901	4769	3622	55	54	54	2	1	1	158	173	167	5	5	5	0.7	0.8	0.8
11	Local Check - EVDT-Y 2000 STR	2387	4922	3908	54	54	54	2	1	1	160	230	202	4	4	4	0.8	0.9	0.8
MEAN		2133	4406	3497	55	55	55	2	1	2	149	171	162	5	5	5	0.7	0.9	0.8
LSD		459	473	336	1	1	1	1	0	0	10	51	30	1	0	0	0.1	0.1	0.1
CV		29	17	20	3	3	3	61	76	68	10	53	43	20	19	19	20	15	17

Table 7: Grain yield (kg/ha) and other agronomic traits of early maturing ST variety evaluated under drought stress at Ikenne under well-watered conditions at 7 research locations in Nigeria during the 2018 and 2019 seasons.

Entry	Variety	Grain yield, (kg/ha)		Days to silk(days)		Anthesis silking interval		Plant height (cm)		Ear height (cm)		Ear aspect		Leaf death score(1- 9)	Ears/plant	
		DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	DS	WW
1	2016 TZE-W DT STR Syn F2	1471	5057	56	56	2	1	152	177	74	82	5	5	4	0.7	0.9
2	DTE STR-W Syn Pop C4 F2	1787	5225	54	54	3	1	151	173	73	82	5	5	4	0.7	0.9
3	DTE STR-Y Syn Pop C4 F2	1647	5300	55	55	3	1	167	186	85	88	5	4	3	0.6	0.8
4	2016 TZE-Y DT STR QPM Syn F2	1056	3379	56	57	3	2	142	159	73	76	5	6	4	0.5	0.8
5	2016 TZE-W DT STR QPM Syn F2	1620	4366	53	54	3	1	152	170	66	81	5	5	5	0.6	0.9
6	2015 TZE –W DT STR Syn C0	1943	4459	55	56	2	1	165	176	78	83	5	5	4	0.7	0.9
7	2015 TZE –Y DT STR Syn C0	1022	2953	52	53	2	1	134	140	67	65	5	6	4	0.7	0.9
8	DTE STR-W Syn Pop C3	1277	4277	54	54	3	1	148	175	72	85	5	5	4	0.5	0.9
9	2011 TZE-W DT STR Synthetic	1850	4885	55	55	2	1	155	164	68	84	4	5	4	0.7	0.9
10	TZE Comp 3 DT C2F2 (RE)	1291	5038	55	54	2	1	161	176	72	83	5	5	4	0.7	0.9
11	Local Check - EVDT-Y 2000 STR	1872	4995	53	54	3	1	167	250	80	82	5	5	4	0.7	0.9
GRAND MEAN		1531	4539	54	55	2	1	154	177	73	81	5	5	4	0.7	0.9
LSD		649	534	1	1	1	1	13	66	10	8	1	0	1	0.1	0.1
CV		32	17	2	3	35	66	7	58	11	16	12	16	23	16	14

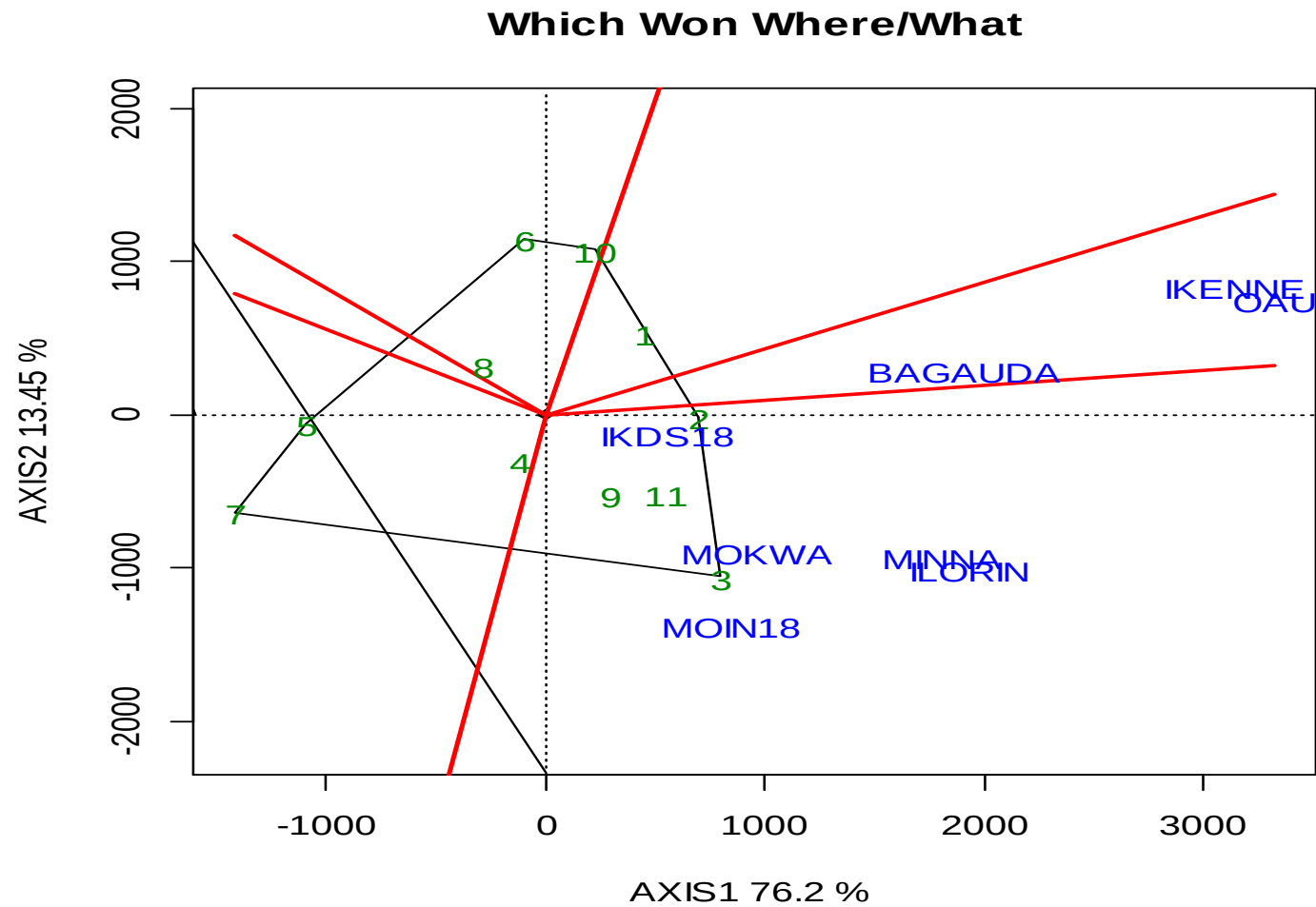
DS- Drought Stress Conditions, WW- Well watered condition,

*1-9 represents plants stay green properties- 9 represents plants whose leaves have senesced

Table 8: Grain yield and other agronomic traits of early maturing ST varieties evaluated under low-N and high-N conditions at Mokwa in Nigeria during the 2018 and 2019 growing seasons.

Entry	Variety	Grain yield, (kg/ha)		Days to silk		Anthesis silking interval		Plant height (cm)		Ear aspect		Stay green	Ears/plant	
		LN	HN	LN	HN	LN	HN	LN	HN	LN	HN	10wk	LN	HN
1	2016 TZE-W DT STR Syn F2	2476	3494	56	55	2	2	189	160	4	5	5	0.9	0.8
2	DTE STR-W Syn Pop C4 F2	2546	3661	55	54	3	2	147	157	5	5	4	0.8	0.8
3	DTE STR-Y Syn Pop C4 F2	2226	4291	54	53	2	2	173	176	4	4	4	0.8	0.8
4	2016 TZE-Y DT STR QPM Syn F2	2221	3099	54	55	2	2	147	139	5	5	4	0.7	0.7
5	2016 TZE-W DT STR QPM Syn F2	2099	3652	57	53	2	2	145	158	6	5	5	0.7	0.8
6	2015 TZE –W DT STR Syn C0	1848	2639	53	55	2	2	153	165	5	6	4	0.7	0.7
7	2015 TZE –Y DT STR Syn C0	2062	3127	52	52	1	1	126	139	5	6	5	0.9	0.9
8	DTE STR-W Syn Pop C3	1829	3381	54	54	2	2	152	158	5	5	4	0.8	0.8
9	2011 TZE-W DT STR Synthetic	2388	4164	53	55	2	2	156	146	5	5	4	0.9	0.9
10	TZE Comp 3 DT C2F2 (RE)	2291	3623	54	53	2	2	158	161	5	5	4	0.7	0.8
11	Local Check - EVDT-Y 2000 STR	2086	3772	55	54	2	2	168	166	5	5	4	0.8	0.8
GRAND MEAN		2188	3537	54	54	2	2	156	157	5	5	4	0.8	0.8
LSD		657	944	2	2	1	1	19	25	1	1	1	0.2	0.2
CV		24	21	3	3	51	54	10	13	19	16	20	16	16

*LN= low soil Nitrogen, HN = high soil nitrogen



*IKDS- Ikenne under drought stress, MOIN- Mokwa in 2018, OAU-Obafemi Awolowo University, Ile-Ife

Fig. 1: Performance of early-maturing varieties in terms of grain yield as measured by principal components across eight environments

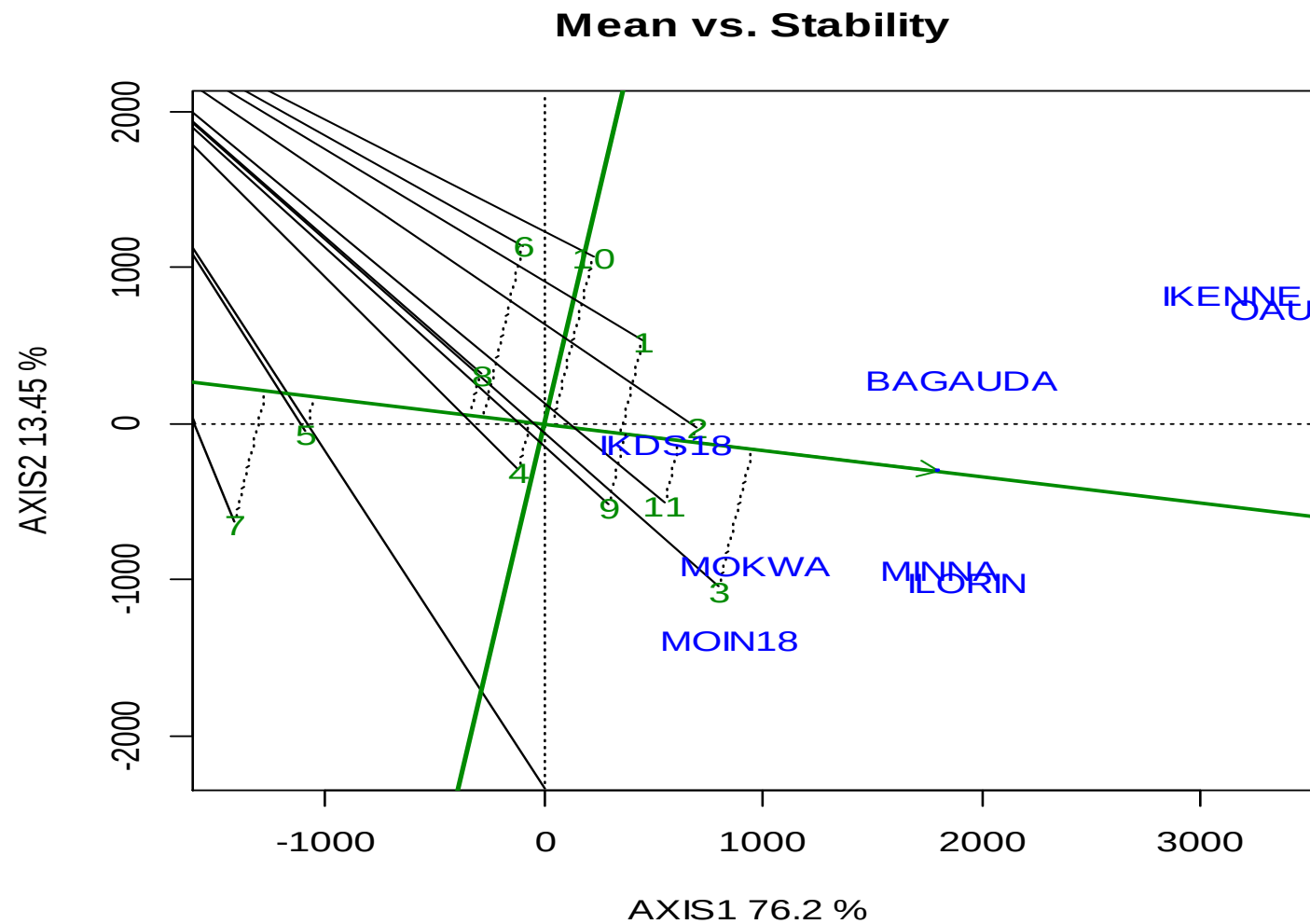


Fig. 2: Mean performance and stability of early-maturing varieties in terms of grain yield as measured by principal components across eight environments

Table 9: Mean grain yield and other agronomic traits of varieties evaluated in on-farm trials at three locations in Kwara, Niger and Oyo States in 2020.

Variety	Grain yield kg/ha	Days to Anthesis	Days to Silking	ASI	Plant height (cm)	Ear height (cm)	Plant Aspect	Termite score/count (0-4)*
DTE STR-Y Syn Pop C4 F2	4616	52	54	2	170	78	1.8	0
TZE Comp 3 DT C2F2	4050	52	54	2	168	74	1.8	2
Farmers variety	3867	54	56	2	168	78	2.0	4
Mean	4177	53.0	55.3	2	169	76	1.9	3
CV	21	1.6	1.7	29	13	20	26	26
LSD	765	1.1	1.2	0.6	23	16	1.1	2.1

***0- No attack, 1 -attempted attack, 2- slight attack, 3- average attack, 4- strong attack, ASI-Anthesis silking interval**

Table 10: Mean grain yield (kg/ha) and other agronomic traits of varieties evaluated in on-farm trials at three locations in Kwara, Niger and Oyo States in 2020.

GENOTYPE	No of Farmer	Mokwa (Niger State)	Kishi (Oyo State)	Omu-Aran (Kwara State)	Across locations
DTE STR-Y Syn Pop C4 F2	4	4085	5090	4715	4616
TZE Comp 3 DT C2F2	4	3450	4200	4300	4050
Farmers variety	4	3500	4350	4000	3867
Mean		3678	4546	4146	4177
CV		18	19	19	22
LSD		615	893	779	656

Table 11: Cost and returns per hectare for the maize varieties and check in Kwara, Niger and Oyo States in 2020.

Variety	Grain yield, kg/ha	Fertilizer cost (₦)	Labour/other costs(₦)	Total cost (₦)	Gross Return (₦)	Profit (₦)	Benefit- Cost ratio
DTE STR-Y Syn Pop C4 F2	4616	80,000	64,000	144,000	553,920	443,420	3.1
TZE Comp 3 DT C2F2	4050	80,000	64,000	144,000	486,000	375,500	2.6
Farmers variety	3867	80,000	64,000	144,000	464,040	353,540	2.5

Prevailing market price of maize = ₦120/kg

