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Full Length Research Paper

Potential pollinators of *Tamarindus indica* L. (Caesalpinioideae) in Sudanian region of Burkina Faso

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Tamarindus indica **(Tamarind) is a socio-economically important tree species in the Sudanian and Sahelian zone of Africa. Few studies have shown that the species is pollinated by bees. In this article, first we studied the impact of the wind pollination. Secondly, we determined the visitors insect of the flowers of tamarind, studied the production of nectar and pollen. Thirdly, we established relation between nectar production and visit time of insect. The study was undertaken in Sudanian zone of Burkina Faso. The insects were captured using a net on the flowers. The determination of the insects visiting flowers is made using the Chenery key. The results show that wind pollination of tamarind is very low. We determined two long distance pollinators of Hymenoptera group (*Xylocopa olivacea* and *Megachille* sp.) and five short distance pollinators represented by *Apis mellifera* and *Trigona* sp. (Hymenoptera group), *Syrphida* sp. and *Bombylius* sp. (Diptera group). The wasp visitor, *Polistes fastidiosus* (Hymenoptera group)'s role in pollination is badly established. Production of nectar and pollen at the flower level occurs over short periods. We identified two major guilds of plants: one guild of plant for *A. mellifera* and one for *Xylocopa violacae*.**

Key words: *Tamarindus indica*, pollen, nectar, plants visitors, pollination.

INTRODUCTION

Sahelian smallholder farmers depend on many trees and shrubs, primarily indigenous species, for a range of essential products, and for environmental services that help improve food security and crop production. They are harvested by rural population for local consumption and commercialisation on a small scale as well as for supplying small industries such as manufacturers of juice (Lamien and Bayala, 1996). With increasing recognition of their importance, the fruit tree species are beginning to attract attention as renewable natural resources that are possibly under threat (Diallo et al., 2008). Managing their populations, and improving the quality and regularity of fruit production are priorities for the economic development of rural populations (Bonkoungou et al.,

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1998). However, the main limiting factor is the insufficiency of knowledge on the population biology of most of these locally important fruit trees. Genetic diversity at local level and population processes such as mating systems, pollination biology, seed disper-sal and establishment of juveniles, are poorly studied particularly in Sahel countries. Thus, little information is available on the factors that limit fruit production, the potential for genetic improvement via selective breeding, the degree of genetic and ecological vulnerability and many other aspects important in the management of these resources. According to Loveless and Hamrick (1984), Hamrick et al. (1992), Schemske and Horwistz (1984) and Levêque (1997), the reproductive success of a population is known to be the primary factor which determines its natural dynamics and the evolution of its genetic diversity. Hamrick and Godt (1989), Hamrick and Murawski (1990) and Diallo et al. (2008) noted that the reproduction system of plants and the pollinators' behavior play a predominant role in the genetic structure of populations.

Tamarind, *Tamarindus indica* L. (Leguminosae: Caesalpinioïdae) ranks fourth on the list of 15 species considered most important by rural populations in the Sudanian and Sahelian countries of Africa (Bonkoungou et al., 1998). The tree has multiple uses, including uses in traditional medicine (Tybirk, 1993). Due to the sweet and acid flavour of fruit pulp of tamarind, it is widely used for food and beverage preparation. Tamarind is an example of an economically important fruit tree to be little studied in Sahel. It is especially important in the semi-arid countries of Africa and South Asia, where it is present long time ago. Its origin remains controversial (Diallo et al., 2007).

In the Sahel countries, all trees appear to result from natural regeneration, and there is little or no management of individual trees apart from keeping them from other destructive uses (Diallo et al., 2010). Any young tree found in farms is systematically spared and allowed to grow. This dependence on natural regeneration further underscores the interest of understanding the tree's reproductive ecology in order to maintain not only fruit production in the short term, but also for the long-term management of the tree's populations.

Despite its economic importance, very little is known about the reproductive ecology of tamarind. In the Sahel countries, the main problem is the low fruit production of many trees. Another concern mentioned by local people is the small size of many fruits containing few seeds and hence little pulp.

Erratic and low productivity are also cited by El-Siddig et al. (1999) as limiting the scope for commercial cropping. Caesalpinioid legume trees are often self-incompatible or at least preferentially allogamous (Gibbs et al., 1999; Lewis and Gibbs, 1999; Arista et al., 1999). Although, at the tamarind, self-incompatibility is partiality and the consequences of selfing both for fruiting and seeds production are known to be low (Diallo et al., 2008).

As in other Caesalpinioid legume trees, tamarind flowers are mainly bee-pollinated (Radhamani et al., 1993; Nagarajan et al., 1997). Despite the fact that little is known about its pollinators and their behaviour on the pollination process, tamarind popula-tions in the Sahel Country of West Africa are usually small and the individual trees are often isolated from each other. This suggests that pollinators' insects play a fundamental role in fructification success. In zoophilous pollination systems plants and pollinators share mutual interest, each being useful to the other (Kearns et al., 1998; Herrera and Pellmyr, 2002; Dafani et al., 2005). Sahli and Conner (2007) highlighted that plant-pollinator interactions are one of the most important and reciprocal variables in nature. Plant-pollinator interactions have a significant effect on reproductive success (Janzen et al., 1980). Thus, Pesson (1984) noted that the relationships between angiosperms and their pollinators have evolved and diversified on the basis of reciprocal benefit, that is, food for the pollinators and pollen dispersal for the plants. Pollinator importance, visits rate and pollinator effective-ness are descriptive parameters of the ecology and evolution of plant-pollinator interactions (Reynolds and Fenster, 2008). The structural organization of mutualism networks, typified by inter-specific positive interactions, is important to maintain community diversity (Bartomeus et al., 2008).

Therefore, in order to understand the evolution of reproductive ecology in Tamarind populations, we must identify the pollinators and their diet to know how these intervene in pollination and in addition examine the interactions between these pollinators and their plants-hosts in the Sudano-Sahelian forest ecosystems. To generate crucial information for management of fruit production and maintenance of viable populations of this valuable and poorly studied tree, we undertook a study of Tamarind pollinators, focusing on the following: (1) what is the importance of wind in tamarind pollination (2) which insects intervene in tamarind pollination (3) Relationship between reward production by flowers for visitors and the visits time (4) if considering Tamaind to be central plant, which is the visitors guilds in the Sudanian ecosystems forest?

MATERIALS AND METHODS

Study site

Potential pollinators' insects were collected on tamarind population. Trees were localised in the agro forestry parkland (10 ha) of Souroukoudinga (11°14'N, 4°26'W), in western Burkina Faso. The climate is Sudanian (Fontès and Guinko, 1995), that is, less arid than the Sahelian climate. There are two well-marked seasons which are: (i) the dry season which lasts approximately 5 months during which there is hot and dry wind. It includes/understands one dry and cold season and a dry and hot season; (ii) the rainy season, 4 months during which there is a wet wind called monsoon. These two great seasons are separated by two one month inter

Table 1. Study site geographical coordinates climatic and soil characteristics.

Geographical coordinates, climatic and soils characteristics		Parameters value
Degree of latitude		11°14'N
Degree of longitude		4°26'W
Altitude (m)		339
Annual rainfall Average (mm)		1028.0
Showery days number average		85
Average of Annual temperature		27.7°C
Maximum temperature (average / year)		28.4°C
Minimum temperature (average / year)		27.1°C
Aubréville index	Showery months	5
	intermediate months	2
	Dry months	5
Soil		Tropical loam-sandy Ferralitique, without presence of laterite slab.

season, each one characterized by an alternation between the two types of wind. The differences between the temperatures of day labourers and seasonal are very high. The grounds ferruginous tropical are strongly washed low in nitrogen and phosphorus. The characteristics of the site are shown in the Table 1.

Field observations of potential pollinators and nectar production

In tamarind, visitors' insects were collected with a net. The insects were sampled all day (6 to 18 h) for the successive 10 days, and then at fixed hours (6, 9, 12 , 14, 18 h) during 30 days. Pollinators were identified by the INERA entomology laboratory using the Delvare and Arbelenc (1985) key for family and Chenery key for the genus level and when possible to the species level. To determine the role of wind in tamarind pollination, we sampled 5 tamarind trees. 20 inflorescences per tree were randomly selected and 10 inflorescences were protected from the insects' visits and the10 others are left without protection. We used the method of Goldingay et al. (1991) which consists of using a mosquito net with sufficiently small mesh to prevent insect penetration but allows ventilation. The observations were made from April 22th to May 22th. Nectar production was examined in flowers at each of the five phenological stages: (1) stage A: flower bud; (2) stage B: elongated flower; (3) stage C: open flower with closed anthers; (4) stage D: open flower with dehiscent anthers; and (5) stage E: fully opened flower at the point of wilting. For stages C, D, and E, we noted absence (0) or presence (1) of nectar within the corolla at regular time intervals (6, 9, 14, 18 h). Nectar production on tamarind flowers was estimated during 7 non rainy days on 25 flowers per stage on three trees. To determine the floral stage at which pollen viability is highest, we performed viability tests using carmine red (Kearns and Inouye, 1993; Diallo, 2001). For each floral stage, these tests were performed on 100 anthers collected on a total of 40 flowers from 3 different individuals (n = 400, that is, 100 x 40 flowers per individual). Anthers were cut with a razor blade. Sections were placed on microscope slides and pollen grains were counted and scored for viability. Cytoplasm of viable pollen grains appeared stained red whereas unviable grains appeared orange as the staining fluid simply filled up the empty cells.

Guilds constitution

Data analysis

The number of flowers that produced nectar at each stage of the flower was recorded at different hours of the day and the numbers were plotted against the observation times. The pollen production was analyzed by the Khi-deux test.

RESULTS

Impact of visit on the flowering and fruiting

Figure 1a and b express respectively the flowering evolution on the non-protected and protected inflorescences for all trees. The number of closed flowers increases over a short period and decreases progressively from April 22th to May 22th both for protected branches and those put in sacks. For initiated fruits, we noticed a difference between inflorescences inside bag and outside of bags. On inflorescences inside bags, there is no initiated fruit.

Visitors/pollinators

Insects belonging to two groups were collected on tamarind flowers. Figures 2 and 3 shows six insects of *T. indica* flowers visitors in the study area.

Hymenoptera group

Apoidea super family composed of 4 families: (i) Apidae family: *Apis mellifera* (Figure 2a), subspecies *Adansonii*, is a honey bee belonging to the sub-family of Apinae. *Trigona* sp. is a small bee devoid of a stinger and belonging to the Meliponinae sub-family; (ii) Megachilidae family: *Megachile* sp. (Figure 2b) is a solitary bee with very vigorous flight belonging to the Megachiles sub-family. It is called "the lazy's bee" in Fulani jargon; (iii) Vespoidea family: It is a wasp belonging to the Vespidea sub-family. We found only one species, *Polistes*

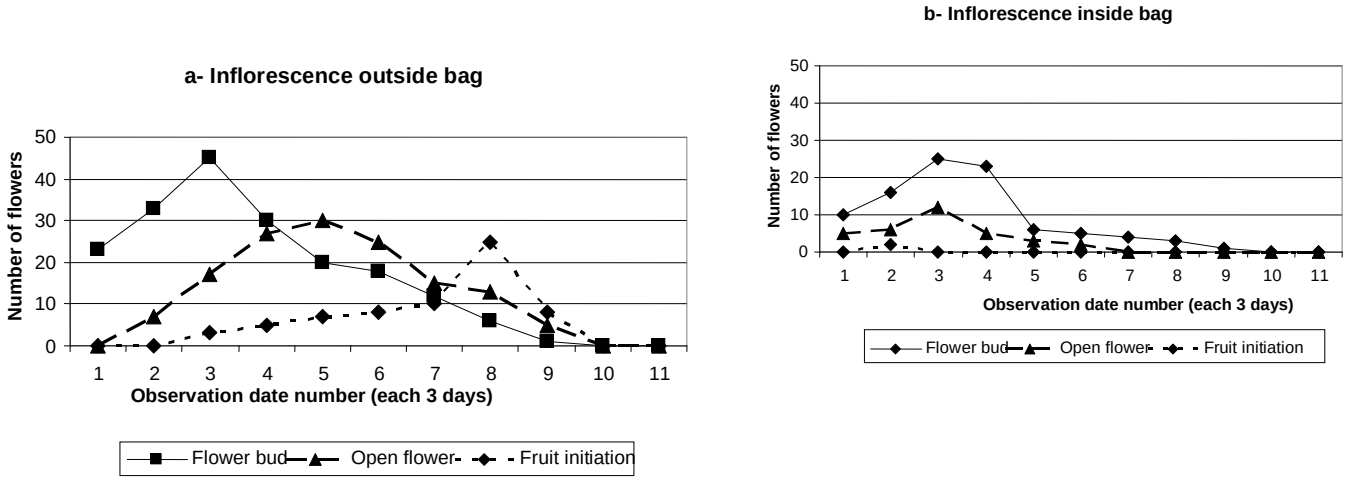

Figure 1. Impact of the visits on the flower evolution: inflorescences outside bag (a) and inflorescences inside bag (b).

fastidiosus (Figure 2c), visiting tamarind flowers within this family; (iv) Anthophoridae family: In this family, we collected *Xylocopa violacea* (Figure 2d). It is a solitary bee in the Anthophorinae sub-family. They make their nest in the trees or in deadwood (carpenter bee). They are known to be pollen eaters.

Diptera group

In this group, we collected two families. (i); the family of the Syrphidae represented by two species *Syrphida* sp. (Figure 3a and b); (ii) the family of Bombyliidae represented by *Bombilius* sp. (Figure 3c).

Nectar and pollen production and insects visit periods

Nectar production

Figure 4 summarizes the periods of nectar production and insect visit time. Most of the flowers at stage C and a small number of flowers at stage D produce nectar early in the morning and later in the afternoon. During the mid-day heat, there is no nectar production during all the floral stage. The Khi-deux Test show there is no statistically significant difference ($P > 0.5$) between the three trees in the number of flowers that produced nectar.

Pollen production

No pollen grains were observed in the anthers at the floral bud stage. At stage B and C, the many intense-red coloration of pollen grains showed that viable pollen grains were abundant (about 80% of all the grains were observed in each anther) (Figure 5).

They are joined together in the shape. At stage D, they become less abundant (40%). They disappear entirely at stage E where the anthers are empty necrosis cavities. Table 2 shows the number of viable pollen grains for each phenological stage.

Visit time of insects

Visits occurred from 6 to 18 h and each species appeared at specific periods of time during the day. The visitors were classified into four groups based on the time of their visit: (i) the “early insects” (6-9 h) composed by *A. mellifera* (Hymenoptera), *Syrphida* sp. and *Bombilius* sp. (Diptera); (ii) the "second hour insects” (8-11 h) dominated by *Polistes fastidiosus*, *Trigona* sp., *Megachile* sp, *A. mellifera*; (Hymenoptera), *Bombilius* sp. (Diptera); (iii) the “warm hour insects” (11-15 h), characterized by the appearance of a species so far absent from the cloud of visitors, *Xylocopa violacea*. We noted again the presence of *A. mellifera* during this period. Around 15 h, *P. fastidiosus*, *Megachile* sp. and *A. mellifera* appeared again; (iv) the “twin light insects” (15-18 h) was dominated by *A. mellifera* until sunset, and then it is the only species present.

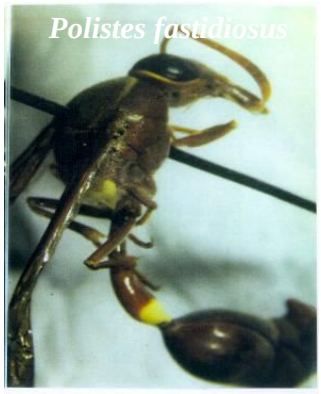

Figure 2. Four visitors of *Tamarindus indica* flowers belonging to the Hymenoptera group. Apidae family (a), Megachilidae family (b), Vespidae family (c) and Anthophoridae family (d). The (a) is *Apis mellifera* short distance pollinator (social bees); The (b) *Megachille* sp. and (d) *Xylocopa violacea* are the long distance pollinators (solitary bees). The (c) *Polistes fastidiosus* is wasp to be a caterpillar larva predator.

Figure 3. Three visitors of *Tamarindus indica* L. flowers belonging to the Diptera group (short distance pollinator): Syrphidae family (a and b) Bombyliidae family (c)

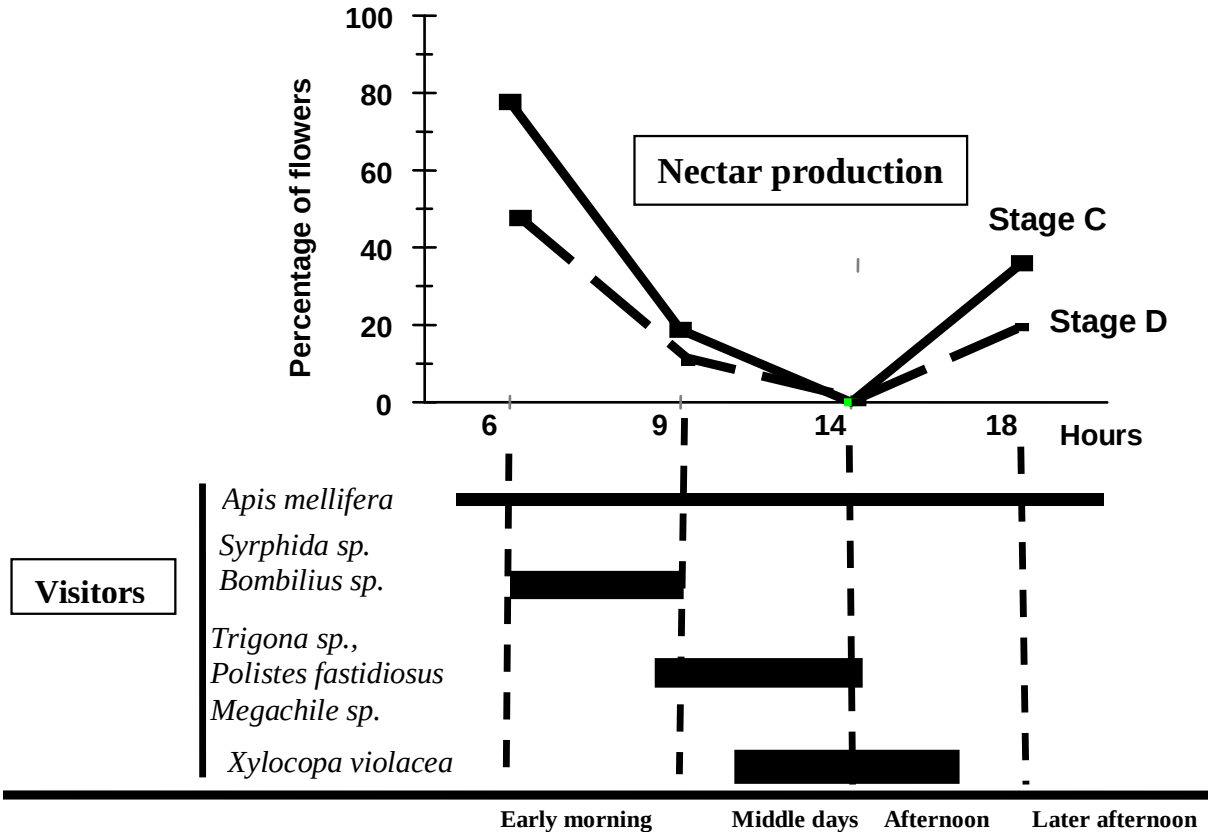

Figure 4. Interactions between plants and pollinators: nectar production periods and times of visits of seven insect species: based on the flowers stage: stage C: open flower with closed anthers; stage D: open flower with dehiscent anthers.

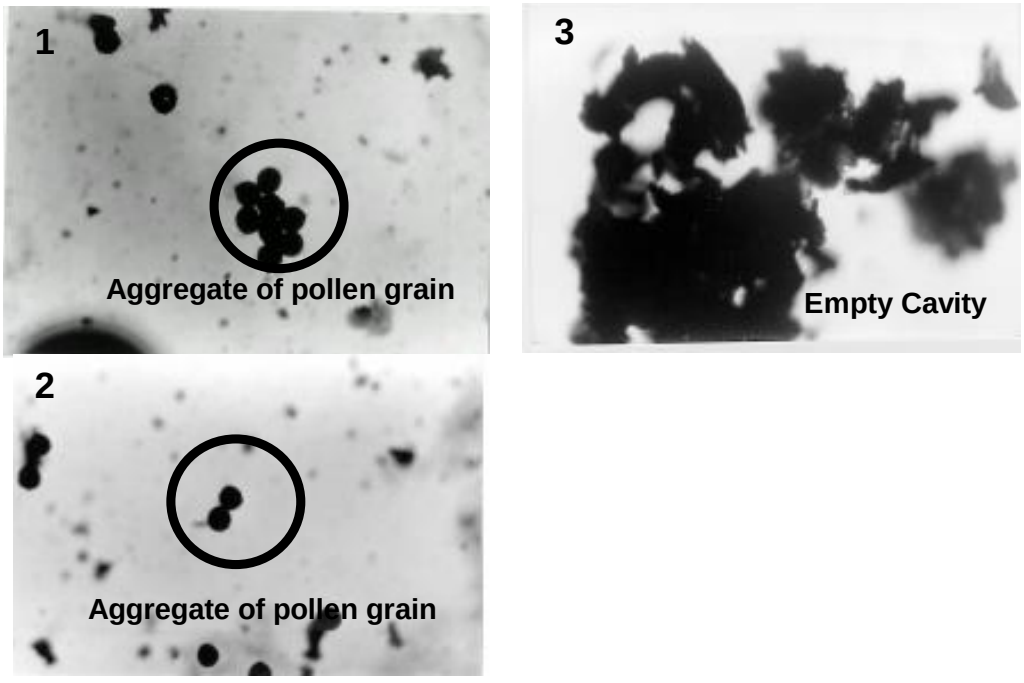

Figure 5. Anthers contain with the 3 stages of flowers 1: Stage c; 2: stage d; 3: Stage e.

Table 2. Viability pollen grains mean number per tree and per phenological stage (100 flowers/tree).

Phenological stage	Tree 1	Tree 2	Tree 3
Stage A	0 (no coloration)	0 (no coloration)	0 (no coloration)
Stage B	0 (no coloration)	0 (no coloration)	0 (no coloration)
Stage C	167.30[a]	70.13[a]	83.3[a]
Stage D	12.53[b]	37.47[b]	32.21[b]
Stage E	3.2[c]	5.93[c]	6.01[c]

Table 3. Some plants guilds of *Apis mellifera and Xylocopa violacea* in Sudanian zone of Burkina Faso.

Visitors/Pollinators	Plants guilds
Apis mellifera	*Tamarindus indica, Piliostigma thonningi, Acacia dudgeoni, Dichrostachys glomerata, Combretum micranthum, Parkia biglobosa, Vitellaria paradoxa.*
Xylocopa violacea	*Tamarindus indica, Cassia sieberiana, Dichrostachys glomerata, Crotalaria micronata*

Plant and visitors guilds

Some insects have several host plants. For example, in the Sudanian eco-zones, *A. mellifera adansoii, X. violacea* and *Megachile* sp. have been captured on other plant species. However, *P. fastidiosus* has only been observed on *T. indica* during our study. Table 3 shows the insect visitors and their host plants throughout the year in Sudanian area.

DISCUSSION

Our study indicates that the wind pollination is low. This put the previous work of Oswald (1984) into perspective by which plants with pollen grains of size less than 20 µ (14-16 µ for tamarind pollen) are mainly pollinated by the wind. However, the lack of visits does not affect the subsequent evolution of flowering, but affect the fruit initiation. This shows that in the tamarind: (i) there are no cleistogamous (no fecondation before the opening of the flowers); (i) pollination by the wind is weak.

The collection and identification of insects on *T. indica* show that the first species group (Hymenoptera) belong to the same group as those identified in India (Rhadamani et al., 1993). This confirms partially Leppik (1956)'s observations that leguminous species are pollinated mainly by Hymenoptera group (especially bees). The second group is represented by the species of Diptera; there is completed information on the tamarind insect's visitors, Frankies et al. (1990) also underlined the dominance of bees as pollinators in most dry tropical areas.

Pollen is available over a short period after the flower's opening. Our results are in conformity with those of Radhamani et al. (1993) which showed that the viability of tamarind-tree pollen lasts for 12 h and the difference between stage C, D and E confirm those of Stone et al. (1995) who noted that generally pollen viability decreases quickly with age of the opening flower. So, it is important that pollinators arrive at good instant to assure an efficient transfer of pollen.

The nectar is produced only during certain times of the day. Therefore, the two main resources which attract visitors to the tamarind tree (nectar and pollen) are not available throughout the entire life of the flower, and this can limit the visitors.

Only *A. mellifera* is present during the period of high nectar production. This insect group is known as short distance pollinators (Diallo, 2001) which are probability attracted by the deposit of honeydew. The other visitors appear when nectar production is low (*Polistes, Trigona* and *Megachile*) or nil (*Xylocopa*). *A. mellifera* is also the only species that visits the flowers throughout the day. The *Polistes* larva's eats a coleopteran larva but their adults consummated pollen.

Despite the successive phenological stage of flower, the *A. mellifera* disperses pollen over short distances resulting in a lot of self-pollination. For self-incompatible (allogamous) plants with low tree densities (like tamarind), *A. mellifera* is not an efficient pollinator. This study also showed that on the G*enista scorpius*, honey bees rarely change trees (Diallo, 1995). Therefore, even if they visit several trees during the day (which is unlikely), only the first flowers visited receive pollen from a different tree whereas all the other flowers have a high chance of being self-pollinated. The worker bees are known to convey the distances, the quality and the food sources to each other through a dance which is a kind of language (Diallo, 2001). In contrast, *X. violacea* and *Megachile* sp. are not as common as *A. mellifera* but appear to disperse pollen over relatively long distances. They spend little time on any tree and can fly tens of meters, so they probably contribute to more cross pollination than self-pollination. The polyphagous characteristic and the vigorous fly of *X. violacea* allows it to reach other

populations by day, thanks to "shift plants" composed by others host plants for other species trees (Da, 2003). In a study of pollination by shelter, Monty et al. (2006) showed that on 47 flowers visitors as potentials pollinators, only two species could be considered as efficient pollinator, among them is *Xylocopa* sp. on the basis of frequency of visits, visiting behaviour and pollen load.

By analyzing the visiting periods, we notice that the insects share the host's resources (pollen and nectar) in a way that reduces inter-specific competition, that is, not all species visit the flowers at the same time of the day. For example, visit time of some insects species show that they are more interested in the pollen, in the sugar secreted on the flowers and leaves, or in the caterpillars that the flowers and leaves shelter. *Polistes* for example hunt Coleoptera larva's or caterpillars to lay their eggs and serve as hosts for their larvae. However, the adults of these parasitic species which eat pollen are potential competitors with the species that disperse the pollen. *A. mellifera* is the only species that uses both pollen and nectar; but it has also diversified its supply sources by gathering pollen from several trees species. So, competition between visitors is probably low because of the diversification of resources used and the diversity of host plants.

Based on our observations, *A. mellifera* is the most generalist species in the Sudanian eco-zones. For this, Le Thomas (1997) noted that in the north of Ivory Coast under Sudanian south tropical climate, taxonomically close bees are attracted by the same flowers and Trigona insects seem to be the most generalist. It is necessary to note that all these species do not flourish at the same period. So, the relationship between plants and the insect visitors occurs through indirect mutualism (flowering at different periods in a year) and competition (same flowering time in a year).

Conclusion

Our data indicates that the wind influence is very restricted in the tamarind pollination. Tamarind potential pollinators are represented by two major groups: short pollinators and long distance pollinators. The honeybee is a short distance pollinator. Its morning arrival as well as its permanent presence disseminates hard on the auto pollen. This situation hampers the success of fruit formation according to pollinators most represented in every population of tamarind. The presence of predators (*Polites*) allows the diminishing rate of parasitism of fruits kernels by the Coleoptera larva. We also noted that the visits time were different in the course of the day. This shows that there is an organization to exploit the host rewards.

Conflict of Interests

The author(s) have declared that there is no conflict of interests.

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Review

Distribution, chemical composition and medicinal importance of saffron (*Crocus sativus* L.)

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***Crocus sativus* L. is native to Iran and Greece, and is now cultivated largely in Southern Europe, Tibet and other countries. In India, it is mainly cultivated in Kashmir. *C. sativus* is an important medicinal plant with aphrodisiac, antispasmodic, expectorant, anti-diabetic, anti-inflammatory, antioxidant, anti-depressant, anticancer and anti-tumor activities. Phytochemical investigations of the species have revealed the presence of a number of important carotenoids especially crocetin and its glycosidic forms such as crocin, picrocrocin and safranal. The genetic origin of *C. sativus* is believed to have occurred by auto-triploidy or by allopolyploidy and *Crocus cartwrightianus* is believed to be its most probable ancestor. World over, saffron shows a declining trend in production and productivity due to high labour cost, lack of variability for major economic traits and poor economic returns. This review focuses on the detailed distribution, chemical composition and the medicinal importance of saffron.**

Key words: *Crocus sativus*, crocin, picrocrocin, safranal, medicinal properties.

INTRODUCTION

Crocus sativus L. (Family Iridaceae) commonly known as saffron is distributed primarily in the Mediterranean Region and South Western Asia. The safranal (for ordor), picrocrocin (for taste) and crocin (for pigment) components of this geophyte constituting the spice "saffron'' are localized in the red stigmatic lobes of the flower (Neghbi et al., 1989; Plessner et al., 1989; Fernandez, 2004). The stigmas (20 - 40 mm) are dark red in color and trumpet shaped, serrated or indented at the distal end and may be isolated or joined in pairs or threes at the end of the style, which is white/yellow in color (Figure 1b, c). It is estimated that, approximately 75,000 crocus blossoms or more than 2,00,000 dried stigmas produce just one 1 kg saffron spice and is thus the most expensive spice in the world at around $500/kg and/or $40-50/gram (Fernandez, 2007; Melnyk et al., 2010). The stigmas must be handpicked from the delicate blossoms upon opening to preserve the desirable volatile compounds which easily degrade in the presence of light and oxidizing agents (Rau, 1969; Hill, 2004). As a result, the best saffron is usually sold as whole stigmas (not powdered) in air tight containers so as to preserve its integrity. The high value of saffron in the international market makes it the object of frequent adulteration

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Figure 1. Corm (1a), flower (1b) and stigma (1c) of *Crocus sativus* L.

and fraud by the growers, traders and other stake holders (Fernandez, 2007; Husaini et al., 2010a). The quality and commercial value of saffron is determined by its coloring power, bitter taste and aroma. These three parameters are certified in the international market following the International Organization for Standardization (ISO 1993). The “Saffron Specifications” and “Saffron Test Methods” issued by the Institute of Standard and Industrial Research Organization of Iran (ISIROI) explain the sampling, packing, labeling and methods of testing of saffron. In India, the Bureau of Indian Standards (BIS) is responsible for setting up guidelines for quality standards of saffron (Husaini et al., 2010a).

Saffron is a geophyte herbaceous plant and is propagated vegetatively by corms (underground, compact, bulb-like, starch-storing organs shrouded in a dense mat of parallel fibres called “corm tunic”). A single corm of 10-15 g weight survives for one season, producing at the end of growing season 6-10 "cormlets" that can grow into new plants in the next season (Figure 1a). The plant is a triploid (X=8; 2n=3X= 24), self and out-sterile, mostly male sterile (Mathew, 1977; Ghaffari, 1986; Grilli Caiola, 2005) and is therefore unable to produce seeds. Archeological and historical sources indicate the saffron cultivation as very old dating back to 2500 - 1500 BC, probably originating in Iran, Asia Minor or Greece which later became wide spread in India, China, the Mediterranean Basin and Eastern Europe (Negbi, 1999; Grilli Caiola et al., 2004). The auto-triploid nature and vegetative mode of reproduction of the species renders improvement by conventional breeding very difficult. As the species has spread by vegetative means, it is believed that saffron exists as a single species all over the world.

Recent studies have confirmed that saffron exhibits stable biological traits all over the world and there are no genomic differences (Grilli caiola et al., 2004; Zubor et al., 2004; Fernandez, 2007). The stigmas of saffron have been used from ancient times as a spice in food, as a dye in perfumes and cosmetics preparation and for medicinal purposes (Basker and Negbi, 1983). The present review describes the current status of saffron crop and its medicinal properties so as to have insight interest among young researchers for their possible contributions in promoting this precious crop in the world.

Origin and distribution

The name “saffron” is derived from the Arabic word zafaran which means yellow (Winterhalter and Straubinger, 2000), the ancient Greek called it “Koricos” where as Romans used the term “Crocum”. In India, this golden spice is known as “Kum Kum” and “Kesar” in Sanskrit and “Koung” in Kashmiri language. It is believed that saffron is being cultivated for about 3,500 years in Egypt and Middle East and during the Middle Age, saffron crop was extended from Middle East to Europe reaching Great Britain in the 14th Century (Fernandez, 2004). The detailed archaeological and historical records of occurrence and spread of saffron and its allied species have been reviewed by Grilli Caiola (2010). The centre of origin of *C. sativus* according to Vavilov (1951) is the Middle East, while other authors suggest Asia Minor or the South-West Greek Islands as its probable area of origin (Tammaro, 1990). According to Negbi (1999), *C. sativus* was probably selected and domesticated in Crete during the Late Bronze Age.

From here, it spread to India, China and the Middle Eastern countries. From these latter, the Arabs brought saffron to all Mediterranean Europe (Ingram, 1969). Some authors (Alberini, 1990; Winterhalter and Straubinger, 2000) point towards Iran and Kashmir as its origin site from where it has spread to Greek and Roman world. The precise time of introduction of saffron in Kashmir is not known, although evidence from a 12th Century book, “Rajatarangini” written by a Kashmir Poet (kalhana), indicates its presence in Kashmir even before

Table 1. Estimate of saffron world production (Adopted from Gresta et al., 2008).

Country	Area (ha)	Production (Kg)	References
Iran	47,000	160,000	Ehsanzadeh et al., 2004
India	-	8,000-10,000	Fernandez, 2004
Greece	860	4,000-6,000	Fernandez, 2004
Azerbaijan	675	-	Azizbekova and Milyaeva, 1999
Morocco	500	1,000	Ait-Oubahou and El-Otmani, 1999
Spain	200	300-500	Fernandez, 2004
Italy	35	120	NA
France	1	4	Girard and Navarrete, 2005
Turkey	-	10	Thiercelin, 2004
Switzerland	-	0.4	Negbi, 1999

NA - reference not available.

the reign of King Lalitaditya in 750AD (Husaini et al., 2010b). The genetic origin of *C. sativus* is believed to have occurred by auto-triploidy from a wild *Crocus*, probably by fertilization of a diploid unreduced egg cell by a haploid sperm cell or a haploid egg cell by two haploid sperms (Chichiriccò, 1984; Grilli Caiola, 2004, 2005), or by allopolyploidy through the hybridization of *Crocus cartwrightianus* and *Crocus hadriaticus* (Castillo et al., 2005). Brighton (1977) in a kariological study and supported by AFLP analysis (Zubor et al., 2004) suggested that possible ancestors of *C. sativus* are *C. cartwrightianus* or *Crocus thomasii*. Evidences from several other workers suggest *C. cartwrightianus* as the most probable ancestor of *C. sativus* (Mathew, 1999; Brandizzi and Grilli Caiola, 1998; Grilli Caiola et al., 2004).

Saffron is currently being cultivated more or less intensively in Iran, India, Greece, Spain, Italy, Turkey, France, Switzerland, Israel, Pakistan, Azerbaijan, China, Egypt, Japan, Afghanistan, Iraq and recently in Australia (Table 1). While the world's total annual saffron production is estimated at 205t per year, Iran with more than 47,000 ha, of land under saffron cultivation produces 80% (160t) of this total. Khorasan province in Iran alone accounts for 46,000 ha land and 137t of the total production in Iran (Parviz et al., 2004). The traditional cultivated areas in Europe (Spain, Italy and Greece) are showing a severe declining trend while an enormous increase has been registered in Iran in the last 30 years (Skubris, 1990; Fernandez, 2004). In India saffron is exclusively cultivated in Kashmir division of Jammu and Kashmir State.

Locally known as "Koung" and generally grown on uplands (Karewas), this crop covers about 4% of the total cultivated area of the Kashmir valley and produces 5-6t annually (Husaini et al., 2010a, b). Saffron export from India declined from 9.7t (1998-99) to 8.7 tons (2000-01) associated with a decline in spot price of saffron from Rupees (Rs.) 32,936/kg ($ 866) in 1997-98 to Rs.17, 500 ($ 374) in 2004-05 (Nehvi et al., 2007). The total area under this crop and annual production in the state is showing a declining trend over the past more than one decade (Table 2). According to Husaini et al. (2010b), the saffron crop has shown a decrease of 83% in area, 215% in production and 72% in productivity. The major reasons for decline in saffron cultivation and production constraints in the world as well as in J&K state of India are high labour cost, lack of variability for major economic traits, low corm yield, disease susceptibility, low yield of biochemical like safranin, picrocrocin and crocin and above all poor economic returns. The cultivation practices of saffron in Kashmir and the factors responsible for decline in saffron production have been reviewed by Husaini et al. (2010b).

Chemical constituents of saffron

Apart from the primary metabolites such as carbo-hydrates, minerals, fats and vitamins, the *Crocus sativus* L. contains four major bioactive compounds viz., crocin (Mono-glycosyl polytene esters), crocetin (a natural carotenoid dicarboxylic acid precursor of crocin), picrocrocin (monoterpene glycoside precursor of safranal and product of xeaxanthin degradation) and safranal (Figure 2), all contributing to colour, taste and aroma respectively (Melnyk et al., 2010). According to Sobolev et al. (2014), presence of biologically active compounds such as crocetin, picrocrocin and safranal makes this spice a promising candidate for being a functional food. The hydrophilic carotenoids of saffron which includes crocins constitute about 6-16% of saffron's dry matter depending upon the variety, growing conditions and processing methods (Gregory et al., 2005). The highly water soluble crocins are widely used as a natural food colourant and also act as an antioxidant by quenching free radicals, thus protecting cells and tissues against oxidation (Assimopolou et al., 2005; Soeda et al., 2007; Melnyk et al., 2010). Amongst the other minor components belonging to this class, β-crocetin and γ-

Table 2. Area, production and productivity of saffron in Kashmir, India.

Year	Area (ha)	Production (ton)	Yield/productivity (kg ha^{-1})
1996 - 1997	5707	15.96	2.79
1997 - 1998	NA	NA	NA
1998 - 1999	4116	12.88	3.12
1999 - 2000	3997	7.65	1.91
2000 - 2001	2831	3.59	1.26
2001 - 2002	2880	6.52	2.26
2002 - 2003	2742	5.15	1.87
2003 - 2004	3075	4.83	1.57
2004 - 2005	2989	8.85	2.96
2005 - 2006	2928	4.85	1.65
2006 - 2007	2436	9.13	3.74
2007 - 2008	3110	5.06	1.62

Adopted from Husaini et al. (2010) (Sources: Planning Department J&K Government/Directorate of Agriculture Jammu and Kashmir Divisions/Economic Survey 2008 - 09, J&K Government)
NA: Data not available

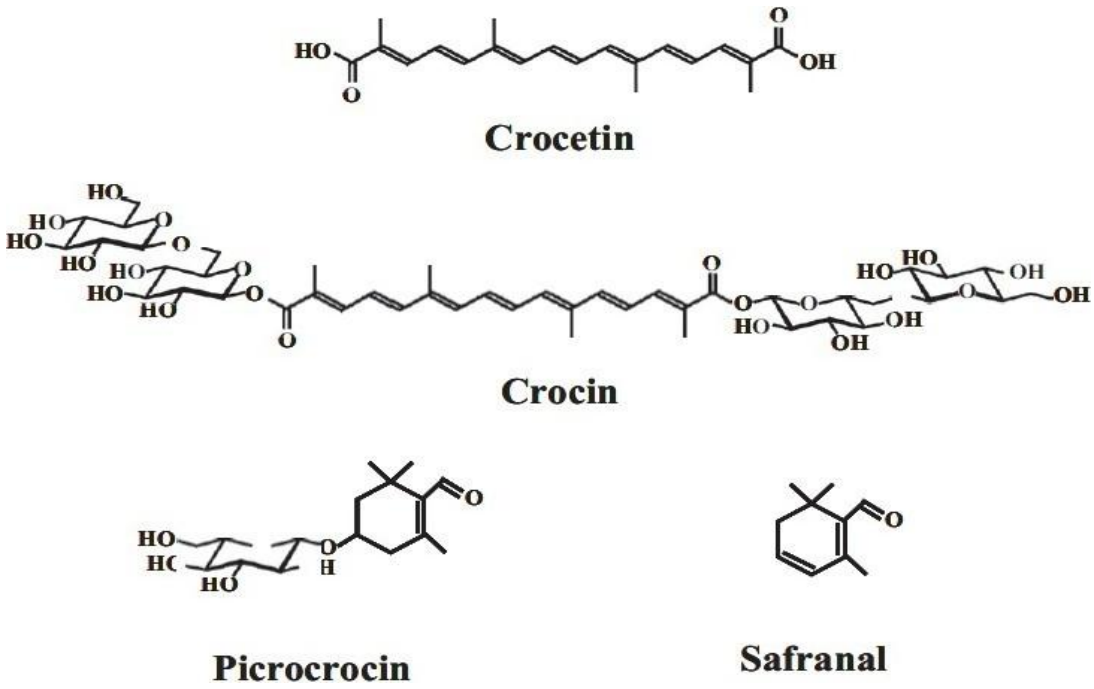

Figure 2. Major chemical constituents of *Crocus sativus* L. (adopted from Gresta et al., 2008).

crocetin, the mono and dimethyl esters of crocetin respectively and mangi-crocin, an unusual xanthone-carotenoid glycosidic conjugate, have also been identified (Sampathu et al., 1984; Ghosal et al., 1989; Fernandez, 2004). The picrocrocin which is the second most abundant component (1-13% of saffrons dry matter) is a colourless glycoside and is considered the main bitter principle of saffron, even though other components, such as flavonoids are also responsible for saffron's bitterness (Alonso et al., 2001; Carmona and Alonoza, 2004). Picrocrocin like other members of the crocin family is derived from the enzymatic degredation of zeaxanthin; in turn, the natural de-glycosylation of picrocrocin gives safranal (Sampathu et al., 1984; Pfander and Schurteberger, 1982) which is the main volatile component of saffron, responsible for the particular aroma of this spice. The safranal represents approximately 30-37% of essential oil and 0.001 to 0.006% of

dry matter (Carmona et al., 2007; Maggi et al., 2009). Besides its aromatic potential, safranal has antioxidant potential (Kanakis et al., 2007) and cytotoxic effect on certain cancer cells (Escribano et al., 1996). There are also other typical volatile components of saffron, all possessing the same skeleton of safranal and like this, are considered to derive from picrocrocin (Melnyk et al., 2010), even though the recent discovery of several new glycosides suggests that picrocrocin is not the soluble glycosidic aroma precursor in saffron (Straubinger et al., 1998; Carmona et al., 2006). The extraction and purification protocol for various chemical and volatile constituents of saffron are available in the literature (Tarantilis et al., 1994; Tarantilis and Polissiou, 1997; Lozano et al., 2000, Zareena et al., 2001).

Several minor components have also been isolated from stigmas and other plant parts, mainly petals and corms. Terpenoids such as crocusatins present in stigmas and petals and showing a significant antityrosinase activity, are among the most recovered components (Li and Wu, 2002, 2004). To the same class of substances namely terpenoids, belong several glycosidic derivatives which are considered as the precursors of volatile saffron components alternative to picrocrocin (Straubinger et al., 1997, 1998). Moreover, a series of flavonoids, all glycosidic derivatives of kaempferol, have recently been characterized in the stigmas of saffron; these polyphenols probably concur together with picrocrocin to produce the bitter taste of saffron (Carmona et al., 2007). Other secondary metabolites from *C. sativus* include anthraquinones and an anthocyanin (Saito et al., 1960; Gao et al., 1999), isolated from corms and petals, respectively.

The chemical composition and concentration of various metabolites in saffron vary from one geographical region to other. Several analytical techniques are available to differentiate saffron samples of different origin based on their chemical composition (Zalacain et al., 2005; Zougagh et al., 2006; Maggi et al., 2009, 2011; Yilmaz et al., 2010). Recently, Sobolev et al. (2014) proposed a microwave assisted NMR based analytical protocol for recovery of metabolites showing significant differences among geographically different saffron extracts.

Medicinal importance of saffron

Saffron (*C. sativus* L.) has been cultivated from time immemorial for its stigmas, which not only comprise a highly valued spice but also have various therapeutic uses (Sampathu et al., 1984). It is used mainly as a dye in industry, as a spice in cooking, as a food colorant and as a component of drugs and perfumes (Mathew, 1982; Basker and Negbi, 1983; Behnia et al., 1999). Saffron has been used as a drug to treat various human health conditions such as coughs, stomach disorders, colic, insomnia, chronic uterine hemorrhage, scarlet fever, smallpox, colds, asthma and cardiovascular disorders (Giaccio, 1990; Winterhalter and Straubinger, 2000; Abdullave, 2003). It has been shown that saffron is a protective agent against chromosomal damage (Prem kumar et al., 2001). Saffron can also be used to help clear up sores and to reduce the discomfort of teething infants (Abdullaev and Espinosa- Agurre, 2004).

Among the secondary metabolites present in saffron, the ester derivatives of crocetin, together with safranal, are nowadays the most studied compounds to evaluate their biological activity. Recent data shows that saffron possesses tyrosinase inhibitory (Li and Wu, 2002, 2004), anticonvulsant (Hosseinzadeh and Younesi Hani, 2002), mutagenic (Abdullave and Espinosa-Aguirre, 2004), cytotoxic and antigenotoxic effects (Abdullaev et al., 2003). It has also anti-amyloidogenic activity against Alzheimer's disease (Papandreou et al., 2006); anti-inflammatory (Hosseinzadeh and Younesi, 2002) and blood pressure reducing (Fatehi et al., 2003) effects. Crocin extracts from saffron have been used for the treatment of nervous, cardiovascular and respiratory systems (Abe and Saito, 2000; Abdullaev, 2002). Components of saffron extract have been found to play a role in management of mental disorders and also act as antidepressant agents (Lechtenberg et al., 2008; Basti et al., 2007). It has been found that the treatment with saffron extracts is not associated with sexual dysfunction in humans, a side effect often encountered with antidepressant drugs (Modabbernia et al., 2012; Kashani et al., 2013).

Recently, saffron extract has been successfully tested as an anticancer agent (Abdullaev, 2007) as well as against mental disorders. Cancer chemopreventive and tumoricidal properties of saffron extracts have been reported by several workers following *in vitro* and *in vivo* assays with encouraging results (Abdullaev, 2002; Ochiai et al., 2004; Ahmad et al., 2005; Hosseinzadeh et al., 2005; Magesh et al., 2006). According to Hartwell (1982) saffron extracts have been used against different kinds of tumors and cancers (liver, spleen, kidney, stomach and uterus tumors) in ancient times. Anti-tumor effect of saffron on different malignant cells in some model animals has also been reported (Abdullaev, 2004). Very recently, De Monte et al. (2014) studied the inhibitory activities of two natural components of *C. sativus* viz. crocin and safranal as well as some newly designed components derived from chemical modifications of safranal on the human monoamine oxidases (hMAO-A and hMAO-B- the two important enzymes which are targets for the treatment of neuropsychiatric and neurodegenerative diseases). Their results confirmed crocin as a relatively weak inhibitor of hMAO, while as safranal was not found as hMAO inhibitor indicating that hMAO are probably not targets of crocin and safranal. The designed chemical derivatives of safranal, however, displayed much improved inhibitory activities against both hMAO enzymes. The synthetic derivatives could thus

Table 3. Major biological functions attributed to saffron and its chemical constituents.

Activity	Saffron constituents tested	Reference
Prevention of gastric disorder	Saffron crude extract Ethanolic saffron extract	Inoue et al. (2005) Kianbakht and Mozaffari (2009).
Prevention of stomach ulcer	Crocin	Xu et al. (2009)
Digestion enhancement	Aqueous saffron extract	Nabavizadeh et al. (2009)
Anticancer function and cytotoxic effects on tumor cells	Ethanolic saffron extract Crocin, crocetin, safranal and picrocrocin	Tavakkol-Afshari et al (2008) Escribano et al. (1996) Garcia-Olmo et al. (1999) Abdullaev (2002) Mousavi et al. (2009)
Tumor inhibition	Crocin Saffron Crocetin	Garcia-Olmo et al. (1999) Salomi et al. (1991) Wang et al. (1996)
Cardiovascular health promotion Anti-atherosclerosis	Crocin Crocetin	He et al. (2005) He et al. (2007) Sheng et al. (2006)
Prevention of insulin resistance	Crocetin	Xi et al. (2007)
Anti-depression activities	Capsulated ethanolic saffron extract Saffron petal extract Aqueous and ethanolic saffron extract	Akhondzadeh et al. (2005) Akhondzadeh et al. (2007) Moshiri et al. (2006) Hosseinzadeh et al. (2004)
Premenstrual syndrome (PMS) treatment	Capsulated ethanolic saffron extract	Agha-Hosseini et al. (2008)
Detrimental health effects Nausea, vomiting, uterus bleeding, abortion	Saffron	Schimidt et al. (2007) Lucas et al. (2001)

Modified and adopted from Melnyk et al. (2010).

prove novel hMAO inhibitors for clinical management of psychiatric and neurodegenerative disorders. A detailed review by Melnyk et al. (2010) highlights major biological functions attributed to different constituents of saffron (Table 3).

PROSPECTS OF GENETIC IMPROVEMENT

Saffron's high price is due to the much direct labour required for its cultivation, harvesting and handling (Fernandez, 2004). In recent past, saffron cultivation and production has shown a declining trend due to high labour cost, low economic returns and very short and laborious flower picking period. There is an urgent need for increasing saffron production and quality to cope with an increasing demand of this spice in the market. This can be achieved by putting in more efforts on genetic improvement of the crop with main focus on producing more flowers per plant, flowers with a higher number of stigmas, increasing stigmas size or stigmas with an increased amount of dye and aroma (Fernandez, 2004). Due to triploid behavior, the chances of crop improvement by conventional methods like hybridization are not possible (Basker and Negbi, 1983). The utilization of spontaneous variability in the natural population which is due to genetic and environmental factors and other non-conventional approaches of crop improvement offer tremendous scope for saffron improvement (Estilai, 1978; Dhar et al., 1988; Nehvi, 2003). Several workers in recent years have attempted mutation breeding technique for induction of genetic variability followed by selection and multiplication of mutant clones (Nehvi et al., 2010). The use of mutagenesis could enhance the genetic base of the crop species so as to offer chances of selection for elite genotypes particularly with respect to stigmatic and corm characteristics. The preliminary results of induced genetic variability through gamma irradiation and induction of polyploidy through colchinization are, however, not satisfactory and probably would require further work (Akhund-Zade and Mazaferova, 1975; Khan, 2004; Zaffar et al., 2004). Creation of saffron germplasm banks, improvement in cultivation techniques, supply of quality plant material and development of quality evaluation methods are some important measures to be considered while dealing with enhancing saffron productivity and its usage.

CONCLUSION

Saffron cultivation has been neglected for many decades

by farmers, who have relegated it to adverse soil and climate conditions, and by research, which has led to a lack of innovation. The chemical profile of saffron and its medicinal and cultural properties makes it a golden spice and there is an urgent need of attention on scientific community to focus their research on genetic improvement of this precious crop. Increase in saffron production and quality can be achieved by means of plants with more flowers per plant, flowers with a higher number of stigmas, increased stigma size or stigmas with a greater amount of dye and aroma. The sterility of saffron limits the application of conventional breeding approaches for its further improvement. Induced variability by physical and chemical mutagens can be considered a viable option for improvement in saffron yield, even if no significant results have been achieved as yet.

Conflict of Interests

The author(s) have declared that there is no conflict of interests.

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African Journal of Plant Science

Full Length Research Paper

Efficacy of *Moringa oleifera* as a phytoextraction plant in the remediation of heavy metals polluted soil

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The efficacy of *Moringa Oleifera*, for the phytoextraction of heavy metals (Zn, Fe and Pb) in tropical soil was investigated. A randomized complete block design (RCBD) consisting of 3 blocks was used. Each block (A, B and C) was polluted with 100 mg Zn, Pb and Fe, respectively. Each block was subdivided into treatment and control of 5 replicates each. Two seedlings of *M. Oleifera* were planted in each treatment plot (phytoextraction) while no planting was done in the control. Zn, Fe and Pb, were analyzed for the soil and plant parts. Results show that *M. oleifera* was able to extract and accumulate high concentration of these metals (Zn, Fe and Pb) in their roots and shoots. The percentage reductions of the heavy metals in the soil were Zn (20.1%), Fe (22.7%) and Pb (64%) after 3 months of phytoextraction. The transfer factor was in the order of Zn > Fe > Pb while translocation factors indicate that metals were largely retained in roots. On the basis of the result obtained, *M. oleifera*, can be classified as hyperaccumulator of some measured heavy metals and therefore, it is suitable for phytoextraction of heavy metal (Zn, Fe and Pb) contaminated soils.

Key words: Heavy metal, contamination, *Moringa oleifera*, phytoextraction, translocation factor, transfer factor.

INTRODUCTION

Heavy metals are natural constituents of the earth's crust. Heavy metals occur naturally in the ecosystem with large variations in concentrations. In modern times, anthropogenic sources of heavy metals have been introduced to the ecosystem. Heavy metals have an effect on the stability of colloids. Colloids are sensitive to change of ion or heavy metal concentration and it leads to violation of colloid stability and subsequent disintegration (Lane and Morel, 2010).

Heavy metal can occur in any ecosystem- terrestrial or aquatic and when it occurs it affects all components of the ecosystem (biotic and abiotic). The effects range from physical and chemical contaminations of soil, air and water to deleterious impacts on flora and fauna.

Some metals such as Fe, Mn, Zn, Cu, Mg, Mo, and Ni are essential for plant growth in low concentration. However, high concentrations of these heavy metals in soil can negatively affect crop growth, as these metals interfere with metabolic functions in plants such as inhibition of photosynthesis, and respiration and

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degeneration of main cell organelles, even leading to death of plants (Garbisu and Alkorta, 2003; Schmidt, 2003; Schwartz et al., 2003). Soil contamination with heavy metals may also cause changes in the composition of soil microbial community, adversely affecting soil characteristics (Giller, 1998; Kozdrój and Van-Elsas, 2001).

Due to the negative effects of these heavy metals on the ecosystem especially at high concentration, it is imperative to apply some remedial measures. Several methods are already being used to clean up the environment from these kinds of contaminants, but most of them are costly and far away from their optimum performance. Conventionally, remediation of heavy-metal-contaminated soils involves either onsite management or excavation and subsequent disposal to a landfill site. This method of disposal solely shifts the contamination problem elsewhere along with the hazards associated with transportation of contaminated soil and migration of contaminants from landfill into an adjacent environment. Soil washing for removing contaminated soil is an alternative way to excavation and disposal to landfill. This method is very costly and produces a residue rich in heavy metals, which will require further treatment. Moreover, these physico-chemical technologies used for soil remediation render the land usage as a medium for plant growth, as they remove all biological activities (Gaur and Adholeya, 2004).

Recent concerns regarding the environmental contamination have initiated the development of appropriate technologies to assess the presence and mobility of metals in soil (Shtangeeva and Laiho, 2004). Presently, phytoextraction which is a form of phytoremediation has become an effective and affordable technological solution used to remove metal pollutants from contaminated soil. This technology is environmental friendly and potentially cost effective. Many species of plants have been successful in absorbing contaminants such as lead, cadmium, chromium, arsenic and various radionuclides from soils. One of phytoremediation categories, phytoextraction, can be used to remove heavy metals from soil using its ability to uptake metals (Fe, Mn, Zn, Cu, Mg, Mo and Ni) which are essential for plant growth.

This study attempts to determine the uptake and accumulation of heavy metals in different parts of the studied plant. It is expected that the findings obtained from this study will provide us with a clue on the efficacy of *M.oleifera* in the phytoextraction of heavy metal in the tropical soil.

MATERIALS AND METHODS

This study was carried out at the Ecological Centre, University of Port Harcourt. It is located in the Niger Delta area of Nigeria on geographical coordinates: latitude 4° 65 N and longitude 7° 5' E. The climate condition of the area is characterized by high temperature, high rainfall and high relative humidity.

A randomized complete block design (RCBD) was used for the experiment. Weighing balance (Setra 480S USA) calibrated in kilogram, was used to weigh 5 kg of soil into 40 polythene bags. The polythene bags were arranged in three blocks (designated as A, B and C) of 10 replicates each. Each block was polluted with 100 mg of a particular heavy metal solution. That is, block A was polluted with 100 mg of Zn; block B was with polluted 100 mg of Fe and block C was polluted with 100 mg of Pb.

The soil was thoroughly mixed in the bag to enhance the harmonization of the metal solution with the soil. The bags were allowed to stand for two weeks. After the two weeks of post-pollution treatment, each block (treatment) was subdivided into two subsets of five replicates each.

Two seedlings of *M. oleifera* were transplanted from the nursery into each bag of one subset for each block while the other subset acted as the control for each block with no planting. This was done for all the blocks (A, B and C). It was also ensured that the plants were of the same sizes. Watering was done three times a week. The quantity of water used for watering the planting bags was 50 cl per bag. Weeding was done when the need arose. The experiment lasted for 12 weeks.

Soil samples were collected from each bag after pollution (but before the plants were transplanted into them). This was done to ascertain the level of these metals present in the soil before phytoextraction. Soil sample collections were also done at the termination of the experiment. The heavy metals (Zn, Fe and Pb) were analyzed for the soil samples.

At the end of the 12 week period, the plants were carefully harvested from each bag. The shoots were separated from the root by cutting. All samples collected from each treatment were taken to the laboratory immediately for analysis. The plants parts and soil were analyzed for their heavy metal (Pb, Fe and Zn) content. This was done in accordance with their treatment. The plant concentration factor and translocation factor were also calculated.

Soil samples collected were analyzed by air-drying and all clods and clumps removed. Dried soil was sieved using 2 mm sieve to remove coarse particles before analysis. One gram (1 g) of the dried soil sample was placed in 100 ml beaker and; 3 ml of perchloric acid and 5 ml of nitric acid were added. The mixture was allowed to stand for 15 min before digestion by gently heating at low temperature on a hot plate and allowed to cool for 5 min. The digest was then filtered into 50 ml standard flask. The filtrate was analyzed for heavy metal using atomic absorption spectrophotometer (AAS).

Plant samples were analyzed by first rinsing with distilled water and oven-dried at 100°C for 48 h. The plant materials were ground to fine powder. One gram of the powder was digested as described above and analyzed for heavy metals AAS. Transfer factor or plant concentration factor (PCF) using metal concentration in the extracts of soils and plants were calculated. The plants concentration factor (PCF) was calculated as follows:

$$PCF = C_{plant}/C_{soil}$$

Where, $C_{plant\ and}$ C_{soil} represents the heavy metal concentration in extracts of plants and soils respectively (Cui et al., 2005).

Translocation factor (TF) or mobilization ratio (Barman et al., 2000; Gupta et al., 2008) using the formula below.

$$TF = \frac{concentration\ in\ shoot}{concentration\ in\ root}$$

From the data generated, means and standard error mean (SEM) were calculated. The data obtained were also subjected to analysis of variance (ANOVA) using Microsoft Office Excel and least significant difference (LSD) was calculated using SPSS data analysis package (2007 version 9.1).

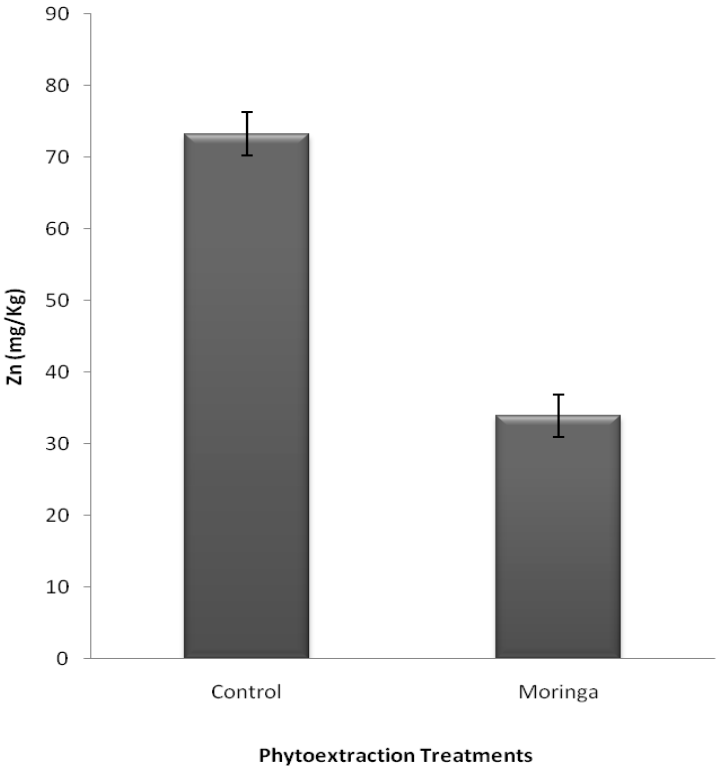

Figure 1. Soil Zn concentrations in phytoextraction treatment.

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RESULTS

Figures 1 to 3 show the efficacy of the test plant (*M. oleifera*) for the phytoextraction of the heavy metal polluted soil. It was observed that there was a significant reduction in soil Zn in the polluted soil phytoextracted with the test plant species as compared to the control soil (no planting) (Figure 1). Similar results were observed in the Fe (Figure 2) and Pb (Figure 3) polluted soil phytoremediated with the test plant. These reductions were found to be significantly different (p = 0.05) from the control.

It was also observed that the reduction of the heavy metals in the soil differed considerably from metal to metal. Results show that the percentage reduction of Pb, Zn and Fe were 64, 20.1 and 22.7%, respectively.

Figures 4-6 showed the concentrations of the metals in both the above-ground and the below-ground parts of the test plant. Figure 4 shows the concentration of Zn in plant parts (roots and shoots) while Figures 5 and 6 shows the concentration of Fe and Pb in the plant parts of the test plant, respectively. It was observed that *M. oleifera* retained more of the heavy metals in its root than in the above-ground part (shoot). In other words, the concentration of Zn in plant's roots was significantly (*P = 0.05*) higher than the shoots. *M. oleifera* accumulation of zinc was significantly higher in root than shoot. Similar results were obtained for the concentration of Fe and Pb in the plant's above-ground (shoot) and below-ground (root) parts.

The calculated result (Figure 7) showed the transfer values from soil to plant. It was observed that the transfer factors for the three (3) heavy metals studied were below one (1). The result indicates that transfer factor for heavy metals in the test plant species were in the orders Zn > Fe >Pb treatments. Reduction in transfer factor was lowest in Pb and highest in Zn polluted soil.

The translocation factor which is an indication of the ratio of heavy metal retention in the shoot over the root showed that the values for the 3 heavy metals studied were below one (1). This also buttress the fact that the phytoextracted plant (*M. oleifera*) accumulated and retained heavy metals (especially Fe, Zn and Pb) more in the root system that the shoot system (Figure 8). The order of translocation factor was: Zn > Pb > Fe.

DISCUSSION

It is a well established fact that some plants can extract heavy metals from the soil or water and accumulate or retain them in their body parts (roots, stems or leaves). This uptake and accumulation of metal ions by plants differ from plant to plant and in varying degrees. The bioavailability of the metals can also influence their uptake by plants which are in turn determined by both

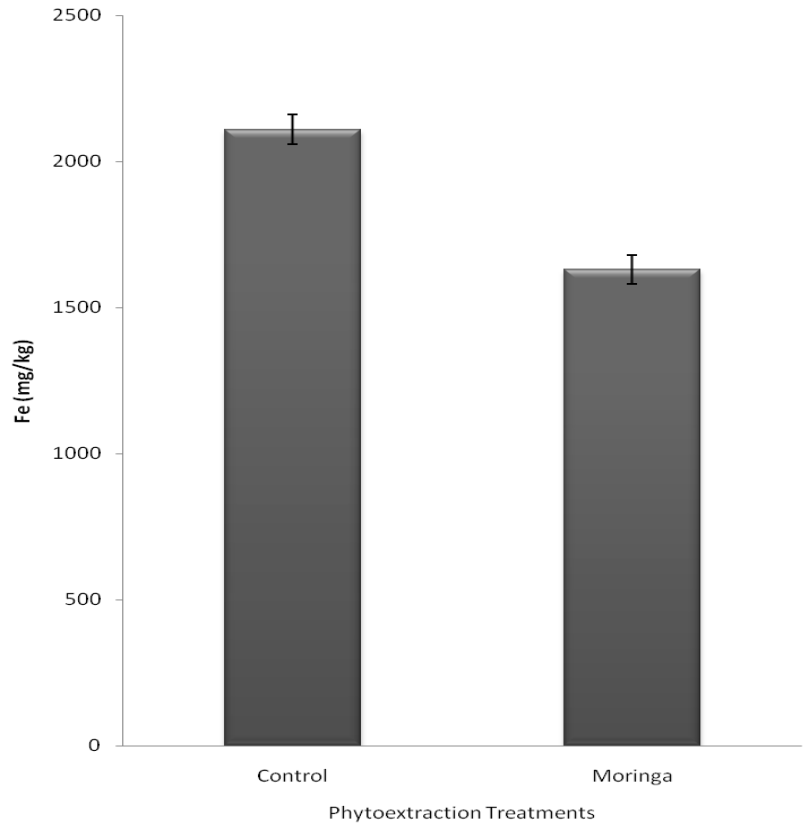

Figure 2. Soil Fe concentrations in phytoextraction treatment.

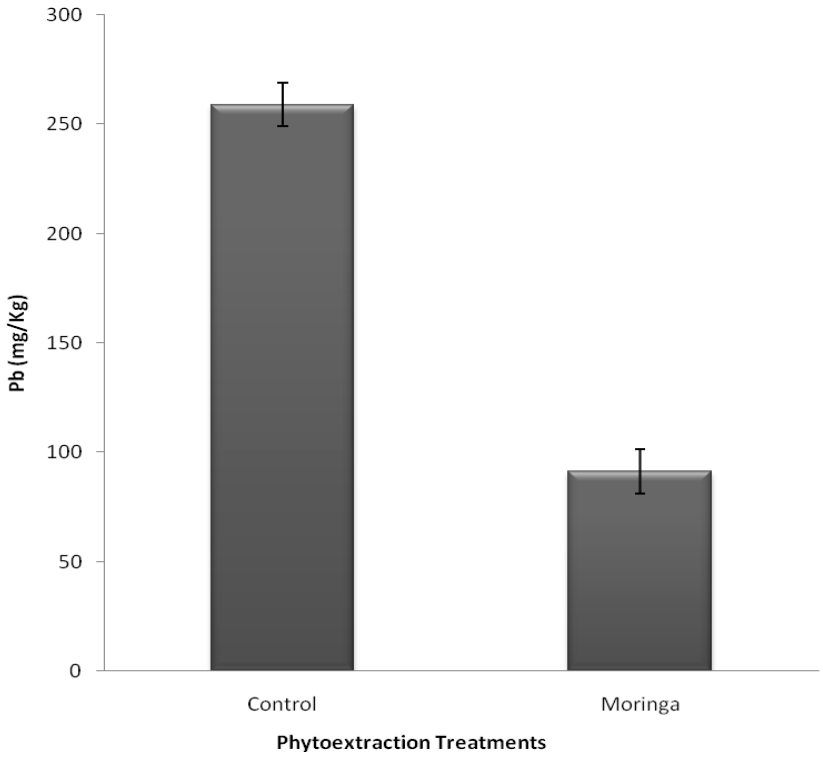

Figure 3. Soil Pb concentrations in phytoextraction treatment.

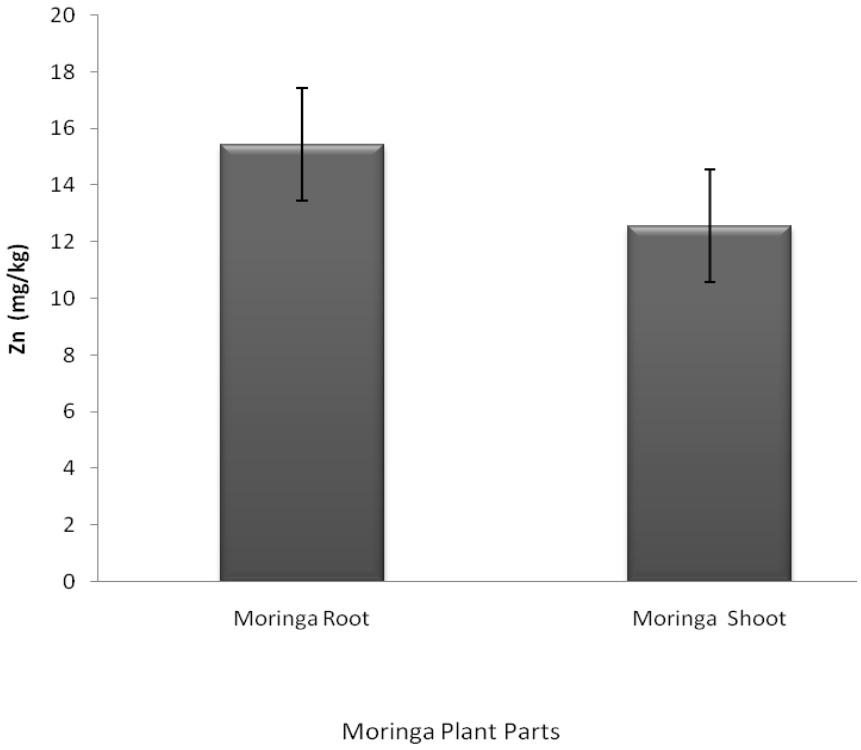

Figure 4. Zn concentration in plants part.

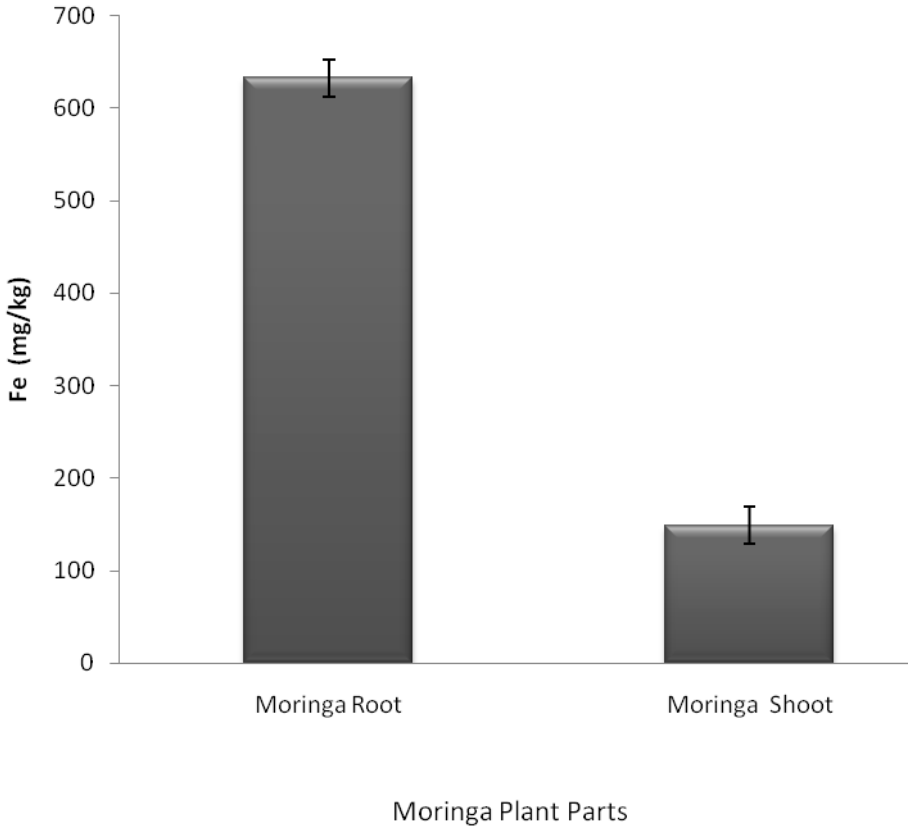

Figure 5. Fe concentration in plants parts.

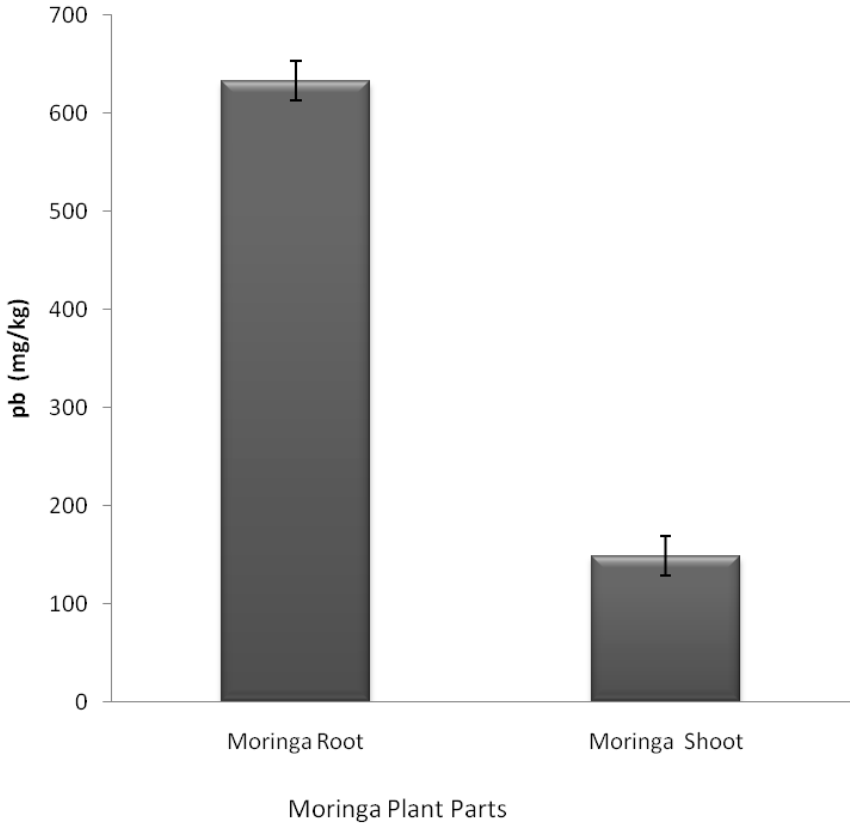

Figure 6. Pb concentration in plants parts.

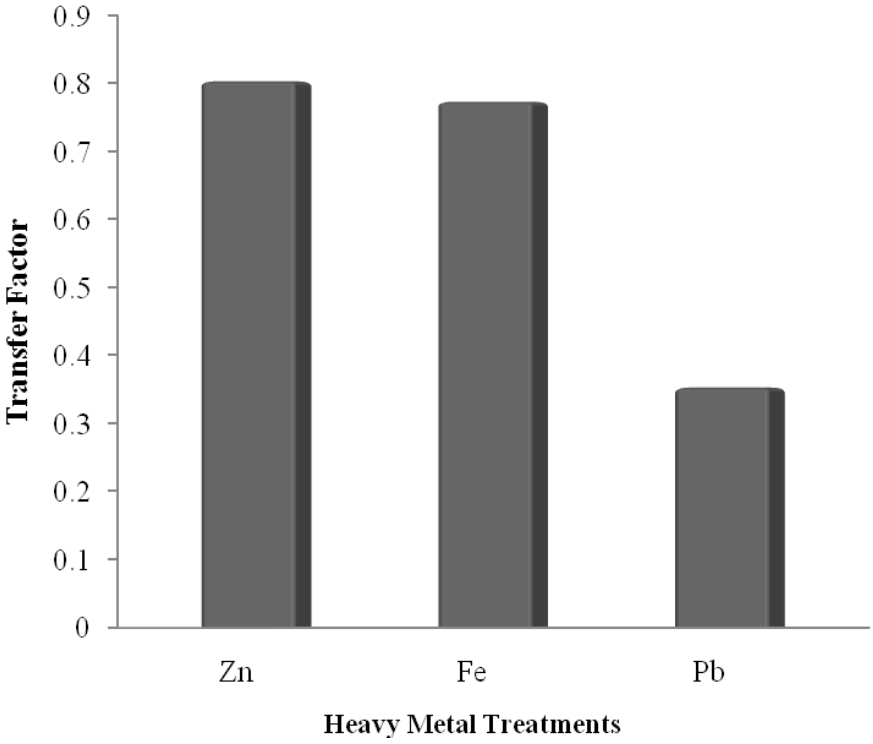

Figure 7. Transfer factor of different metals.

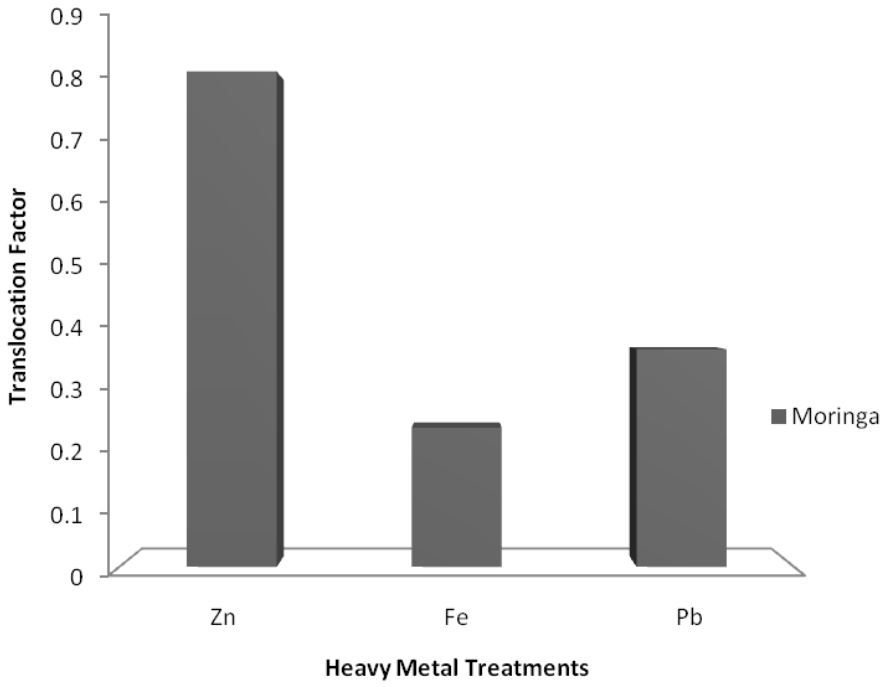

Figure 8. Translocation factors of different metals.

external (soil-associated) and internal (plant-associated) factors.

Results showed that *M. oleifera* plant has the ability to phytoextract heavy metals especially Fe, Zn and Pb and accumulate them in their body parts (below-ground and above-ground). Although, the strategy employed by *M. oleifera* plant for the uptake of heavy metal varies among heavy metal treatments as shown in the result in which the percentage reduction was in the order of Pb (64%) > Fe (22.7%) > Zn (20.1%). This is understandable since some plant exhibit selective absorption of metal ions from soil in which a particular plant may prefer a particular metal ion to another. Fleming and Parle (1977) reported that the uptake of heavy metal varies widely depending on the plant species being studied. It was long recognized that the uptake of metals by plants were plant species dependent (Adriano, 1986). Some plants release specific metal-chelating or reducing compounds into the rhizosphere to aid the absorption when availability of these micronutrients is low (Marschner, 1995).

Results in Figures 4, 5 and 6 show that the accumulation/retention of heavy metals (Zn, Fe and Pb) was more in root system than shoot system. This suggests that plant have different ability in accumulating particular heavy metals in different parts of their body. The accumulation of heavy metal contents in root and shoot varies from species to species (Salt et al., 1995). This is understandable since the breaking up of soil aggregates is a physical effect of root tips pushing through soil as the root tips grow. Fungi associated with some plant roots (mycorrhizae) can also influence the chemical conditions within the soil, which could account for the accumulation of heavy metal more in root. Similar result has also been reported by Trusby (2003). This also suggests that a number of factors (including anatomical, biochemical and physiological factors) might contribute to heavy metal accumulation in the root than shoot. Although, Salt et al. (1995) reported a contrary observation of high accumulation of heavy metals in the upper vegetative parts of some plants.

The analysis of transfer factor indicates that the uptake capabilities of heavy metals from soil to *M. oleifera* is in the order of Zn > Fe > Pb. This could be attributed to the plant species used. Zurera et al. (1987) have reported that the mobility of metals from soil to plants is a function of the physical and chemical properties of the soil and of plants species, and is altered by innumerable environmental and human factors.

Translocation factor, the ratio of shoot to root metals, indicates internal metal transportation. The data presented indicate that metals accumulated by the test plant species (*M. oleifera*) were largely retained in roots, as shown by TF values (<1). Translocation factor (TF>1) showed that translocation of metals effectively was made to the shoot from root (Baker and Brooks, 1989; Fayiga and Ma, 2006).

Conclusion

It can be concluded that the ability of a plant species to phytoextract and accumulate heavy metals varies among

the heavy metals. There were drastic reductions in the heavy metal contents of soil phytoremediated with *M. oleifera*. Moreover, the level of accumulation of these metals studied (Zn, Fe and Pb) was more pronounced in roots than shoots. Therefore, *M. oleifera* is capable of phytoextracting these metals (Zn, Fe and Pb), but their accumulation rate is dependent on a particular metal.

Conflict of Interests

The author(s) have declared that there is no conflict of interests.

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Full Length Research Paper

Yield and water use efficiency (WUE) responses of forage sorghum ratoon crop under varying salinity and irrigation frequency

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Water stress is associated with low availability of water or with osmotic effects arising from salinity. Besides affecting crop yields, salinity may also influence biochemical composition and nutrient concentration of forage crops. To determine the effect of salinity on biochemical composition and nutrient concentration of forage sorghum [*Sorghum bicolor* (L.) *Moench*], two varieties of sorghum, Speedfeed and KFS4 were grown under rain shelter at salinity levels of 0, 5, 10, 15 dS m^{-1} and irrigated when the leaf water potential reached -1 (control), -1.5 and -2 MPa. The factorial treatment combinations were arranged in a randomized complete block design with three replications. Stem dry matter, leaf dry matter and eventual dry matter yields of ratoon forage sorghum decreased with increasing salinity and irrigation interval. The reduction in plant biomass under stress conditions was found to be associated with increase in water use efficiency (WUE). Since the saline applications continued until the second harvest (first ratoon crop) as soil salinity increased, dry matter yields were reduced dramatically. No viable plants were obtained in the second ratoon crop for KFS4 variety at 15 dS m^{-1} salinity and -2 MPa irrigation frequency treatments. Based on all parameters evaluated in the first ratoon crop, there was no concrete evidence to suggest that Speedfeed was superior in performance, but in the second ratoon, forage yields suggest that this variety can be a good alternative in planning for forage production.

Key words: Salinity, irrigation frequency, ratoon crop, forage sorghum.

INTRODUCTION

The productivity of plants is strongly influenced by environmental stresses. Scientists consider soil salinity as one of the major factors that reduce plant growth in many regions of the world (McCarty and Dudeck, 1993; Murdoch, 1987). Sodium chloride (NaCl) is the predominant component contributing to salinity in soils (Jungklang et al., 2003). To overcome this problem, the search for salt tolerant forage sorghums has increased (Harivandi et al., 1992). Salt tolerant plants have the capability to minimize these detrimental effects by a series of morphological, physiological and biochemical processes (Jacoby, 1999). Under the variable saline

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environments, plants have developed different adaptive mechanisms (Rhodes et al., 2002 and Borsani, et al., 2003).

Sorghum has tillering characteristics that enable it to completely regenerate the above-ground portions of the plant. Individual sorghum plants have been kept alive for as long as 7 years where the climate is mild enough to avoid winterkill and disease and insect protection have been provided. These important features of sorghum have allowed producers to seek a second crop within the same growing season. This decision, however, must be made at or soon after harvest of the main crop, and re-growth should be managed in the same manner as the main crop (Teutsch, 2006). A new generation of salt-tolerant forage varieties would allow for landscape development in saline environments and where salt water usage is a problem, or where fresh water is limited or not available for irrigation. Forage sorghum development in these areas often requires the use of brackish water from affected wells or other secondary sources. The effect of salinity and water deficit on ratoon crops of sorghum is not known. Hence, studies are needed to improve understanding on yield re-growth of forage sorghum under influence of salt and water stresses. In addition, many factors need to be considered when addressing the suitability of irrigation water with respect to salinity. To our knowledge, there are no published studies on drought and salt water tolerance among these forage sorghum varieties. Proper utilization of highly salt tolerant forage sorghum varieties will benefit the growing forage sorghum industry. The specific objectives of this study were: to determine yield, biomass partitioning and water use efficiency (WUE) responses in the first ratoon crop of the two forage sorghum varieties to different levels of salinity and irrigation frequency.

MATERIALS AND METHODS

The factorial experiment was conducted under rain shelter at University Putra Malaysia (02°N 59.476´ 101°ºE 2.867´, 51 m altitude), from January 2009 to December 2009. The climatic conditions recorded under rain shelter were 31°C mean temperature, 88% humidity, 4.5 mm evaporation and 71% light at 12 am. Two selected (Fouman et al., 2003) salt tolerant varieties namely Speedfeed and KFS4, of forage sorghum [*Sorghum bicolor* (L.) Moench] were subjected to the salinity levels of 0, 5, 10 and 15 dS m^{-1} of NaCl concentrations, and irrigated when the leaf water potential reached -1 (control),-1.5 and -2 MPa.

The treatments were arranged in a randomized complete block design with three replications. Polybags (40 × 45 cm) were filled with a mixture of top soil, peat moss and sand at the ratio of 3:2:1 (v/v), respectively. The plants were irrigated with non-saline water for seedling establishment and with saline water starting from the 2nd week after germination according to the treatments. The soil mixture which had a pH of 6.52 was filled into polyethylene bags of size 40 x 45 cm. Each bag of soil was also thoroughly mixed with 62 g of $CaCO_3$, 9 g complete fertilizer (15% N, P_2O_5, K_2O), 1 g of triple super phosphate (45% P_2O_5) and 2.4 g of urea (46% N). Soil field capacity (FC) and permanent wilting point (PWP) were measured before and after completion at the experiment. Soil moisture was determined by gravimetric method (Aslam et al., 2008).

The amount of water required for the irrigation of each treatment was calculated using the following equation: $V = SMD \times A$ where; V= volume of water to be applied (liter); A= polybag area = πr^2; SMD = $(\theta_{FC} - \theta_i)$ D Bd /100: SMD = soil moisture deficit; θ_{FC} = gravimetric soil moisture content at field capacity (%); θ_i = Soil moisture content before irrigation (%); D = rooting depth (cm); Bd = bulk density (in this soil 1.5 g cm^{-3}).

Parameters measured

Determination of shoot dry weight in main crop

After harvesting, main crop plants were allowed to re-grow and 49 days after harvest, plants had grown to approximately 170 cm in height. Shoots were harvested at the cutting height of 15 cm from soil surface the same as for the main crop. The plant samples were carefully washed to remove all soil particles. Samples were then dried in an oven at 70°C for 3 days until constant weight was achieved. The dry weight of shoots (g $plant^{-1}$) was recorded for each treatment.

Determination of shoot dry weight in first ratoon crop

After harvesting main crop plants were re-growth and 49 days later than harvest, plants had reached to 170 cm height approximately, shoots were harvested at a cutting height of 15 cm from soil surface, then dried and weighed the same way as main crop.

Determination of shoot dry weight in second ratoon crop

At the end of the experiment (10% flowering stage) when plants had re-growth that reached 150 cm height approximately, fifty-day-old shoots were harvested, then dried and weighed the same as main crop.

The data were analyzed using Procedure ANOVA in the SAS. Treatment means were compared using least significant differences (LSD) at the 95% (P≤0.05) probability level.

RESULTS

Yield and yield components at harvest in first ratoon crop

Irrigation frequency and salinity, significantly (P<0.01) affected; growth, yield and yield components of forage sorghum (Table 1). Under influence of salinity at 15 dS m^{-1} forage mass was reduced, 33.7% as compared to none saline treatment, while the decreases under salinity at 5 and 10 dS m^{-1} were 13.9 and 22.3%, respectively. Comparison between varieties showed no significant differences between KFS4 and Speedfeed.

The highest forage dry mass was obtained with the more frequent irrigation, but decreased 4.3% with moderate irrigation. The impact of irrigation schedule however was strongly evident in plants when irrigation was delayed from -1.5 to -2 Mpa and as a result the forage yield decreased by 18.5 %.

Table 1. Effect of different levels of irrigation frequency and salinity on leaf, stem and forage dry weight of forage sorghum varieties for the first ratoon crop.

Treatment	Leaf dry weight (g plant^{-1})	Stem dry weight (g plant^{-1})	Forage dry weight (g plant^{-1})
Variety			
KFS4	8.74^{a}	18.67^{a}	27.42^{a}
Speedfeed	8.51^{a}	18.38^{a}	26.85^{a}
LSD $_{0.05}$	0.58	1.68	2.26
Irrigation frequency at			
LWP -1.0 (MPa)	9.38^{a}	20.07^{a}	29.38^{a}
LWP -1.5 (MPa)	8.77^{a}	19.32^{a}	28.09^{a}
LWP -2.0 (MPa)	7.73^{b}	16.19^{b}	23.93^{b}
LSD $_{0.05}$	0.75	2.17	2.76
Salinity (dS m^{-1})			
0	10.25^{a}	22.75^{a}	32.89^{a}
5	8.93^{b}	19.36^{b}	28.30^{b}
10	8.13^{b}	17.40^{b}	25.53^{b}
15	7.20^{c}	14.60^{c}	21.80^{c}
LSD $_{0.05}$	0.90	2.58	3.19
F value			
VxI	2.11**	1.53*	0.61**
VxS	0.13^{ns}	0.15^{ns}	0.10^{ns}
IxS	0.57^{ns}	0.67^{ns}	0.45^{ns}
VxIxS	0.80^{ns}	0.60^{ns}	0.72**
Error and CV			
Error (MS)	216.26	12.62	22.71
CV (%)	14.35	19.17	17.56

**, * and ns are significant at 0.01, 0.05 level and non significant, respectively.

In addition to reduction in yield, stress resulted in changes in composition of leaves and stems of the forage sorghums. The decreases of leaves under leaf water potential of -1.5 to -2 Mpa were 6.5 and 17.5%, respectively, while under salinity treatments the decreases were 12, 20.6 and 29.7%, respectively. Accumulations in dry stem weights under water stress were 3.7 and 19.3%, while under salinity the values were 14.9, 23.5 and 35.8%, respectively. Comparison between yield and yield components of the two varieties showed no significant differences between KFS4 and Speedfeed variety (Table 1).

Water use efficiency at harvest in first ratoon crop

The water use by plants showed very complicated responses; and it was significantly dependent on salinity, variety and interaction of irrigation and variety as well as interaction of salinity and irrigation (Table 2). Unlimited water use lead to higher yields, however this caused some wastage. There was a significant interaction effect of irrigation and salinity levels on water use efficiency (WUE).

WUE in the control treatment was 34.5%, while the WUE with the irrigation frequency at leaf water potential of -2 MPa was 19.3% more than in the control treatment. Irrigation when applied at longer intervals had higher WUE. Irrespective of variety, the more frequently watered plants accumulated greater dry matter to eventually produce higher dry forage yield than other irrigation frequencies (Table 2).

The results derived from the irrigation study showed that despite the possibility of greater surface evaporation with light frequent irrigations, differentials of the sorghum varieties and other indicators of plant water stress were found to be improved with low frequent irrigation. Irrespective of variety, intermediate irrigation regime had

Table 2. Effect of irrigation frequency on yield of first ratoon crop and water use efficiency (WUE)

Irrigation schedule	Days after treatment							Dry forage yield (g plant^{-1})	Total water used (liters)	WUE (g plant^{-1} liter^{-1})
	1	11	19	28	33	38	45			
I1	1	0.98	1.17	1.27	1.09	1.08	1.26	29.38	7.85	3.73
I2	1	0	1.59	0	1.70	0	2.01	28.09	6.30	4.43
I3	1	0	0	2.36	0	0	2.48	23.93	5.84	4.07
LSD $_{0.05}$								2.76	0.61	0.43

I1, I2 and I3 are irrigation frequencies applied when the leaf water potential reached -1, -1.5 and -2 MPa, respectively. * Salinity levels averaged for these data.

Table 3. Mean forage yields in ratoon crops of sorghum varieties at different levels of irrigation frequency and salinity.

Treatment	Main crop (dry forage) (g plant^{-1})	First ratoon crop (dry forage) (g plant^{-1})	Second ratoon crop(dry forage) (g plant^{-1})	Total (dry forage) (g plant^{-1})
Variety				
KFS4	42.25	27.42	2.14	71.81
Speedfeed	39.41	26.85	11.55	78.50
LSD $_{0.05}$	2.66	2.26	0.61	4.52
Irrigation frequency at				
LWP -1.0 (MPa)	45.12	29.38	7.91	81.91
LWP -1.5 (MPa)	38.88	28.09	6.88	74.11
LWP -2.0 (MPa)	38.48	23.93	5.74	69.45
LSD $_{0.05}$	3.25	2.76	0.75	5.54
Salinity (dS m^{-1})				
0	45.73	32.89	8.38	87.23
5	43.61	28.30	7.15	79.46
10	39.79	25.53	6.47	72.36
15	34.17	21.80	5.37	61.57
LSD $_{0.05}$	3.76	3.19	0.87	6.40
F value				
V×I	4.37*	0.61**	16.93**	0.97^{ns}
V×S	0.03^{ns}	0.10^{ns}	8.41**	0.20^{ns}
I×S	0.29^{ns}	0.45^{ns}	1.48^{ns}	0.61^{ns}
V×I×S	0.70^{ns}	0.72**	1.73^{ns}	0.35^{ns}
Error and CV				
Error (MS)	31.43	22.71	2.91	91.05
CV (%)	13.73	17.56	18.94	12.69

**, * and ns are significant at 0.01, 0.05 level and non significant, respectively.

higher WUE than the most frequently irrigated regime. Though, when comparing the two varieties, variety KFS4 significantly produced higher dry forage in the main crop; however, in the second ratoon crop, the total dry weight (submission of three cutting) of Speedfeed was higher than KFS4 (Table 3 and Figure 1).

The results of this study showed that the yield of forage sorghum was significantly increased with more frequent irrigation. More frequent irrigation is usually associated with increase in salt concentrations in the soil as water is extracted by the crop. Typically, salt concentrations are lowest following irrigation and higher just before the next

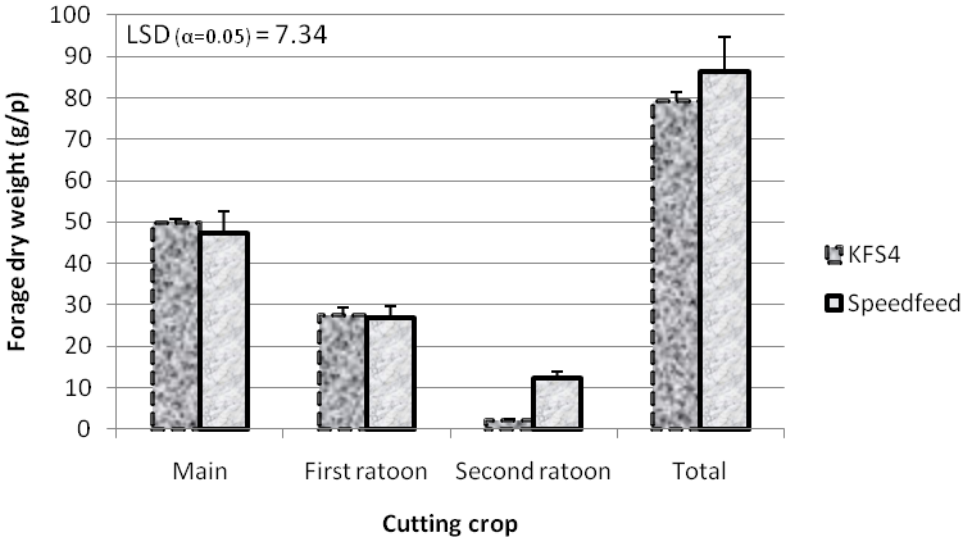

Figure 1. Forage yield in ratoon crops of the two sorghum varieties.

irrigation. Increasing irrigation frequency maintains higher constant moisture content in the soil, and thus more of the salts are kept in solution which aids the leaching process.

DISCUSSION

The effect of salinity and irrigation frequency as reflected by a lower yield may be the result of a combination of osmotic and specific ion effects of Cl and Na. As the salt concentration and water stress increased, dry matter yield diminished the first ratoon with reductions of 13.9, 22.3 and 33.7% as compared to none saline treatment, respectively (Table 1). The highest reduction (33.7%) was from the most salt stressed (15 dS m^{-1}) against 18.6% for the most water stressed plants, and significant decreases were also recorded for other salt levels. This is consistent with reports in other monocots including rice, wheat and maize (Krishna et al., 1993; Shabala, et al., 1998). KFS4 variety which in the main crop study exhibited a higher yield in terms of dry matter production did not show any significant differences with Speedfeed variety in the first ratoon crop. As the saline applications continued into the first ratoon crop, there were significant losses in yield under the stress treatments, however with three cuttings characteristic of Speedfeed led to higher total production of Speedfeed (Figure 1 and Table 3). No viable plants were obtained in the second ratoon crop for KFS4 variety at 15 dS m^{-1} salinity and -2 MPa irrigation frequency treatments, because KFS4 was genetically known to be a one ratoon crop.

Salinity stressed plants certainly faced osmotic challenges. This is in agreement with several previous reports (Munns and Tester, 2008; Lee et al., 2004), which concur that osmotic adjustment is the main response for survival and growth of plants under salinity stress. Also under saline conditions a positive association between photosynthetic rate and yield has been reported in sorghum (Faville et al., 1999).

When plants are under water stress, they firstly reduce and then stop leaf expansion (Hsiao and Jing, 1997). In this study, the WUE obtained for the infrequently watered plants were higher than earlier reports on five forage sorghum cultivars grown on dry land in Texas (Gulzar et al., 2003) or values previously reported for the same crop in the northern part of Sudan (Mustafa and Abdel Magid, 1982). Although larger volumes of water were used in this investigation as compared to the two previous reports, the higher WUE obtained here could be attributed to the reduction in irrigation with the infrequent watering.

Conclusion

There were significant losses in yield in the second ratoon crop under the stress treatments. Irrigation can overcome effect of salinity as increasing irrigation frequency maintains constant moisture content in the soil. Thus, more of the salts are then kept in solution which aids in the leaching process. Irrigation may be intensified in saline soils to mitigate the effect of salinity on plant growth. However, there is a critical level of salinity after which irrigation cannot mitigate the effect of salinity. The critical level of salinity for KFS4 was 15 dS m^{-1}, while for Speedfeed it was 10 dS m^{-1}. The results obtained in this study would serve as a useful guide for managing forage sorghum in saline and water stressed field conditions. On the other hand, WUE in forage sorghums can be increased with reduced irrigation frequency, and this will

enable larger field areas to be irrigated with the savings in water used. The present findings suggest that in semiarid environments (where saving water is very important), sorghums should be irrigated infrequently but heavily, if the aim is to get high WUE forage sorghum.

The final concluding remarks describe characteristics, advantages and limitations of the varieties studied: KFS4 variety under stress condition gave better vegetative growth performance especially in main crop dry matter production as compared to Speedfeed. While considering second ratoon crop characteristics, Speedfeed was superior to KFS4 variety.

Conflict of Interests

The author(s) have declared that there is no conflict of interests.

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Conservation of tree genetic resources of North-Eastern Lagos Nigeria

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This study investigates the rate of concurrent depletion on the remnant flora growing in the North-eastern part of Lagos, which lies in the South-western part of Nigeria. Tree species growing in this area are not spared from advancing civilization, which has resulted in inevitable loss of genetic resources. Hence, molecular technique is adopted in an effort to conserve the genetic resources of the tree species. Samples were collected at random from various sites in north eastern part of Lagos and identified. A total of 66 tree species was recorded. Genomic DNA was extracted from fresh leaves samples following modified cetyltrimethyl ammonium bromide (CTAB) DNA extraction protocol. The DNA when viewed on 1% agarose revealed bands of high molecular weight. Also, spectrophotometric check on the genomic DNA showed a good quality DNA samples with absorbance ratio of 1.7 to 1.8. The purified DNA was dissolved in buffer and stored at -80°C in the established DNA Bank at the University of Lagos, Akoka, Lagos, Nigeria. This can be used for further investigations including understanding genetic and evolutionary relationships between taxa, functional analysis of genes, comparative genomics, DNA barcoding and plant breeding amongst others.

Key words: Bio-conservation, cetyltrimethyl ammonium bromide (CTAB), Lagos, trees, genetic resources.

INTRODUCTION

Globally, the removal or destruction of significant areas of forest cover is moving apace, where every year an integral part of the nation's forest is destroyed through industrialization, urbanization, road construction, commercial agriculture amongst others (Okafor et al., 2009). These cumulative anthropogenic activities have resulted in a degraded environment with reduced biodiversity. The effects of these impacts are mostly evident in the developing countries, with highest rate of notoriety in Nigeria, where almost all the ancestral forest is lost with an alarming rate of disappearance of the remnant vegetation (Batta et al., 2013; Pelemo et al., 2011; Ladipo, 2010; FAO, 2010; Kabiru, 2008). This massive incessant deforestation is shaping climate and geography of several plants species.

Of all the species of plants exploited, the trees are mostly targeted (Elsiddig, 2003; Alamu and Agbeja, 2011) owing to their vast values ranging from economic, social to spiritual paraphernalia amongst others (Seth, 2002). In fact, several authors, including Okafor et al.

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(2009), Ihenyen (2009), Keay (1989) and Redhead (1971) lamented that out of about 565 species of trees existing in Nigeria, over 60 species are faced with extinction and various forms of risk. Despite studying trees for centuries and chronicling their vital importance to humans, there still exists lack of reliable information on where and when the indigenous trees are disappearing. In fact, the tree species growing in the study area, situated in the commercial and most urbanized state in Nigeria and which accommodates about 10% of the entire population of the country (Pelemo et al., 2011), are not spared from the above aforementioned threats. The influx of human population in search of white collar jobs in the study area has necessitated the development of several infrastructural facilities so as to provide comfort to the populace and this has led to the destruction of almost all the ancestral and proximate vegetation in the study. This is a socio-economic problem which seems to be too difficult to be controlled (Pelemo et al., 2011). As a result of massive loss of valuable plant species and adverse impact on environmental and socio-economic values, policies have been formulated for proper conservation and management of the genetic diversity through establishment of several nature reserves and botanical gardens amongst others in ensuring *in situ* conservation strategy. Despite these, it is very evident that *in situ* conservation is no longer effective given the global socio-economic problems aforementioned. The need to adopt molecular technique is appreciable given its advantage of providing a less laborious means for assigning known and unknown plant taxa. Molecular techniques such as DNA barcoding, random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), microsatellites and single nucleotide polymorphisms (SNP) have recently been used for plant diversity studies and are referred to as easy way of conserving biodiversity (Arif et al., 2010; Pagnotta, 2009).

According to Arif et al. (2010), appropriate identification and characterization of plant materials are essential for the successful conservation of plant resources and to ensure their sustainable use. Hence, in other to conserve the trees species growing in the study area for posterity, attempts have been made by several researchers (Adekanmbi and Ogundipe, 2009; Shonubi and Okusanya, 2007; Orebamjo and Njoku, 1970) to list and highlight the existing species in the study area. Since effective conservation of plant genetic resources requires a complementary approach which makes use of both *ex situ* and *in situ* conservation methods to maximize the genetic diversity available for use, this study aims at conserving the tree genetic resources in northeastern Lagos using DNA banking techniques.

Description of study area

The study was conducted in the University of Lagos campus at Akoka, Yaba, Lagos, Nigeria. The area which is largely surrounded by the scenic view of the Lagos lagoon comprising a total of 802 acres (3.25 km^2) of land. It is located on longitude 3° 24' E and latitude 6° 30' N and on elevation of 40-90 m, which makes flooding difficult. The vegetation in this area is half cleared and developed and the remainder is represented by mangrove vegetation and most of the species recorded by Orebamjo and Njoku (1970) have diminished in number and density. It has an undulating terrain, half of which represents buildings, with various fresh water channels and creeks passing across at different location of this area. A large area of mangrove swamps, roughly 50%, dominates the vegetation. In the north and south east lies the brackish water lagoon which supports a typical terrestrial habitat, and experiences less human disturbance while in the south and south west lies the fresh water, where the soil is highly rich and supports a rich flora which is highly favored by the climate type much disturbed by human activities (Figure 1).

MATERIALS AND METHODS

Sample collection and identification

This study is based on extensive field surveys conducted in the North-eastern Lagos, Akoka Yaba, Lagos. A Global Position System (GPS) was used during the sampling period. For sample collection, the study area was divided into four sampling plots. Trees were enumerated in 50 x 20 m plots, whereas 0.5 x 2 m quadrat was used to study herbs and grasses. Samples were collected at random within each plot and identified. The assessment of native versus introduced status of the trees was done following Keay (1989) Keay et al. (1964) Hutchinson and Dalziel (1954) and Dalziel (1937). Voucher specimens of all plants have been collected and deposited at the University of Lagos Herbarium, Lagos, Nigeria. For DNA analysis, fresh young leaves, fruit, seed and flower samples were collected and silica-gel was added to the each sample in a zip lock bag and preserved in freezer for molecular analysis.

DNA extraction and purification

Genomic DNA was extracted from fresh leaf samples using the modified cetyltrimethyl ammonium bromide (CTAB) protocol (Doyle and Doyle, 1989). The phenolic compounds were removed by passing the extracted DNA through the vacuum cleaner.

Gel electrophoresis

This involved quality check of the DNA samples on 1% agarose gel. The gel was run on 0.5x tris Borate EDTA (TBE) buffer at 75 V for 1 h 30 min. The gel was visualized by staining with 10 mg/ml ethidium bromide under ultra violet (UV) light and photographed with the gel documentation system (UVitec).

Quantification of DNA samples

This involved the determination of the concentration and

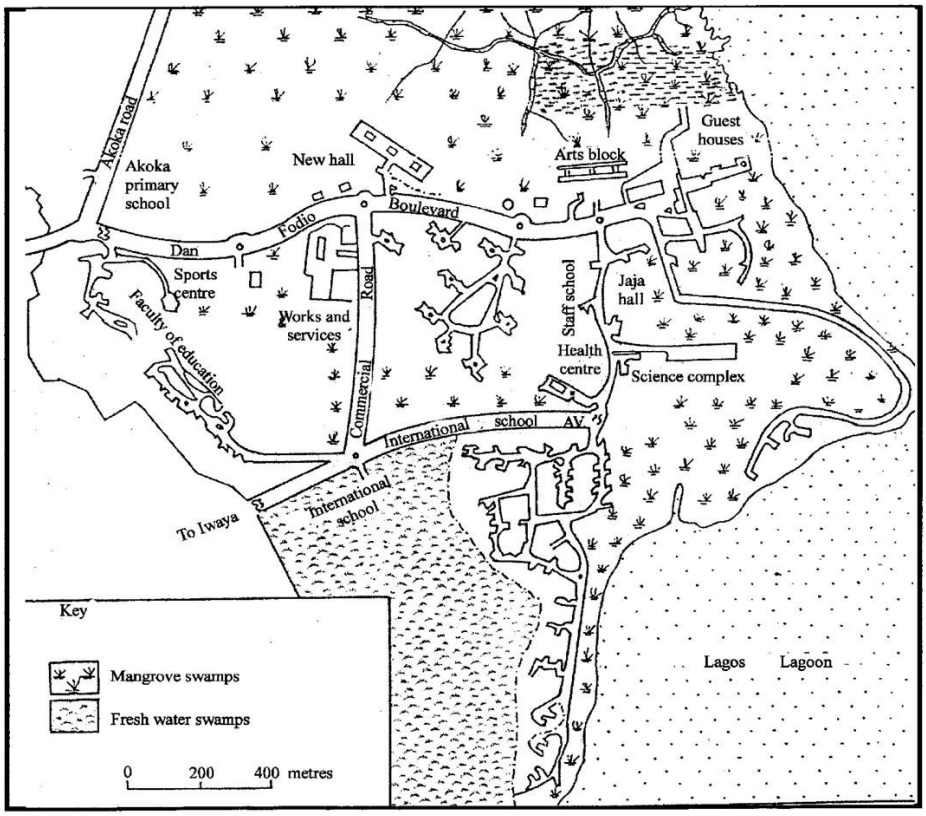

Figure 1. Map showing study area (fresh and mangrove swamp of University of Lagos). Source: Curled from Shonubi and Okusanya (2007).

relative absorbance of each DNA samples using an Eppendorf biophotometer. It was achieved by mixing 55 µL of sterile water with 2 µL of the DNA sample in a cuvette. The cuvette was then placed in an Eppendorf Biophotometer Plus, and readings were documented at 260 and 280 nm, respectively.

RESULTS

Our sampling showed a total of 66 woody tree species capable of attaining a maximum height of 12 m and girth of 60 cm (Table 1). They are made up of 58 genera which can be grouped into 27 families. Of these, only 42 species are indigenous to the environment (Plate 1) while 24 species are categorized as introduced (Plate 2). Most of the species encountered belong to the legume family Fabaceae (Plate 3) representing 20.89% of total number of species recorded. The vegetation of the study area is undulating, as some species were found existing in higher elevated areas, whereas some were found to exist in low areas.

All the samples yielded good quality DNA with high level of purity. The absorbance ratio of the extracted DNA samples as recorded from the spectrophotometric analysis ranging from 1.74 to 1.84 (Figure 2). Four of the samples however had absorbance ratio of > 1.9 (*Casuarina equisetifolia, Callophyllum innophylum, Spondias mombin* and *Tabebuia rosea*) showing that impurities might be present in the samples. The concentration of the DNA samples obtained ranges from123 to 1670 ng/µl (Figure 3). All the samples have been deposited in the DNA bank at the University of Lagos, Akoka, Lagos, Nigeria for conservation purposes.

DISCUSSION

The tree species encountered in this study have a maximum height of 12 m and girth of 60 cm and this conforms to the definition of trees as stated by Redhead (1971). However, continuous existence of these species is doubtful owing to the fact that nearly all the ancestral vegetation in the study area has been degraded mainly as a result of clearing secondary vegetation and Mangrove forest to build public houses or infrastructures. Also, the drainage patterns are drastically changed as streams are straightened, redirected and made into concretized canals and ditches. When compared with the report given by Orebamjo and Njoku (1970), many species were found to be missing (especially species such as *Anogeissus leiocarpus, Triplochyton scleroxylon,*

Table 1. List of trees in North-Eastern Lagos, their location and conservation status.

Species	Family	Conservation status	Location	Elevation (m)
Adansonia digitata A.L	Bombacaceae	LC *	N06°31.139° E003°24.033°	69
Albizzia lebbeck Benth	Fabaceae	LC	N06°30.666° E003°23.799°	54
Albizzia zygia (DC.) Macbor.	Fabaceae	LC	N06°31.032° E003°23.976°	32
Alstonia boonei De Wild.	Apocynaceae	LC	N06°30.803° E003°23.642°	54
Anacardium occidentale De Wild.	Anacardiaceae	LC	N06°31.116° E003°24.043°	27
Annona muricata L	Annonaceae	LC *	N06°31.078° E003°24.052°	59
Anthocleista djalonensis A Chev	Loganiaceae	LC	N06°30.987° E003°23.925°	32
Anthocleista vogelii Planch	Loganiaceae	LC	N06°30.610° E003°23.708°	43
Artocarpus communis J.R Forst. & G. Forst	Moraceae	LC	N06°31.292° E003°23.933°	28
Avicennia germinans (L.) L	Avicennaceae	LC	N06°31.292° E003°23.933°	28
Azadirachta indica A Juss	Meliaceae	LC	N06°30.608° E003°23.743°	57
Bauhinia monandra Kurz	Fabaceae	LC	N06°30.064° E003°23.078°	33
Blighia sapida K. Koenig	Sapindaceae	LC	N06°31.066° E003°24.043°	55
Bombax buonopozense P Beauv	Bombacaceae	LC	N06°31.086° E003°24.052°	99
Bridelia micrantha (Hochst) Baill	Euphorbiaceae	LC	N06°31.097° E003°24.048°	26
Calophyllum inophyllum L	Calophyllaceae	LR *	N06°31.088° E003°24.050°	43
Carica papaya L.	Caricaceae	LC	N06°30.380° E003°23.800°	55
Cassia siamea (Lamarck) Irwin et. Barneby	Fabaceae	LC	N06°31.201° E003°23.823°	40
Casuarina equisetifolia L	Casuarinaceae	LC *	N06°30.461° E003°23.812°	60
Ceiba pentandra (L.) Gaertn	Bombacaceae	LC *	N06°31.082° E003°24.055°	83
Chrysophyllum albidum G. Don	Sapotaceae	LC *	N06°31.116° E003°24.047°	27
Citrus sinensis Osbeck	Rubiaceae	LC	N06°30.506° E003°23.823°	47
Cocos nucifera G. Don	Arecaceae	LC	N06°30.455° E003°23.809°	73
Cola gigantea L	Sterculiaceae	LC	N06°31.042° E003°24.047°	21

Table 1. Contd.

Cola nitida et. Endl. Schot	Sterculiaceae	LC	N06°31.070° E003°24.052°	83
Cordia abyssinica Lam	Boraginaceae	LC *	N06°31.112° E003°24.043°	62
Delonix regia (Hook)Raf	Fabaceae	VU	N06°30.445° E003°23.779°	45
Dialium guineensis Willd	Fabaceae	LC	N06°30.476° E003°23.795°	25
Elaeis guineensis Jacq	Arecaceae	LC	N06°30.605° E003°23.763°	58
Erythrina senegalensis DC	Fabaceae	LC	N06°30.596° E003°23.743°	44
Eugenia malaccensis L.	Myrtaceae	LC *	N06°30.613° E003°23.743°	45
Ficus congoensis	Moraceae	LC	N06°30.334° E003°23.788°	46
Ficus exasperata L.	Moraceae	LC	N06°31.037° E003°23.903°	32
Ficus sycomorus L	Moraceae	LC	N06°31.064° E003°24.111°	32
Ficus vallis-chaudae L	Moraceae	LC	N06°30.529° E003°23.825°	66
Gliricidia sepium (Jacq) Kunth	Fabaceae	LC	N06°30.348° E003°23.783°	71
Gmelina arborea Roxb	Lamiaceae	LC	N06°31.066° E003°24.058°	86
Holarrhena floribunda (G. Don) T. Durand& Schinz	Apocynaceae	LC	N06°30.599° E003°23,765°	40
Hildegardia barteri Roxb	Malvaceae	LC	N06°31.225° E003°23.961°	94
Hura crepitans L	Euphorbiaceae	LC *	N06°31.088° E003°24.063°	29
Jacaranda mimosifolia G. Don	Bignonaceae	VU	N06°30.870° E003°23.875°	34
Khaya grandifoliola C.DC	Meliaceae	VU	N06°31.063° E003°24.036°	61
Lagerstroemia speciosa (L.) Pers	Lythraceae	LC	N06°31.113° E003°23.925°	38
Mangifera indica L	Anacardiaceae	LC	N06°30.296° E003°23.785°	45
Milicia excelsa (Welw.) C.Berg	Moraceae	EN*	N06°31.080° E003°24.052°	52
Millettia thonningii (Schum. &Thonn.)Baker	Fabaceae	LC	N06°30.424° E003°23.821°	42
Morinda lucida Benth	Rubiaceae	LC	N06°30.367° E003°23.783°	54
Newbouldia laevis (P.Beauv.) Seeman ex Heyne	Bignonaceae	LC	N06°30.330° E003°23.793°	51
Peltophorum pterocarpum (DC.) Baker ex Heyne	Fabaceae	LC *	N06°30.870° E003°23.852°	34

Table 1. Contd.

Persea americana Mill	Lauraceae	LC	N06°30.511° E003°23.828°	34
Phoenix reclinata Jacq	Arecaceae	LC	N06°30.464° E003°23.784°	46
Pithecelobium dulce (Roxb.)Benth	Fabaceae	LC *	N06°31.070° E003°24.114°	60
Psidium guajava L	Myrtaceae	LC	N06°30.296° E003°23.814°	48
Raphia hookeri Marm Wendland	Arecaceae	LC	N06°30.429° E003°23.828°	54
Rauvolfia vomitora Afzel	Apocynaceae	LC	N06°31.054° E003°24.036°	75
Roystonea oleraceae O.F. Cook	Arecaceae	LC	N06°31.114° E003°23.927°	33
Senna alata	Fabaceae	LC	N06°31.201° E003°23.823°	40
Senna fistula	Fabaceae	LC	N06°31.201° E003°23.823°	40
Spondias mombin L	Anacardiaceae	LC*	N06°31.088° E003°24.037°	61
Sterculia tragacantha Lindl	Malvaceae	LC	N06°31.054° E003°24.034°	57
Tabebuia rosea (Bertol.)DC.	Bignonaceae	LC	N06°31.112° E003°23.894°	75
Tectona grandis L	Verbenaceae	LC *	N06°30.467° E003°23.784°	51
Terminalia catappa L	Combretaceae	LC	N06°30.466° E003°23.819°	69
Terminalia randii Baker. f	Combretaceae	LC	N06°31.336° E003°24.406°	71
Terminalia superba Engl.et Diels	Combretaceae	LC *	N06°31.089° E003°24.054°	58
Treculia africana Decne	Moraceae	LC *	N06°31.083° E003°24.052°	56

LC - Least concerned, LR - local risk, VU - vulnerable, EN - endangered, *protected by cites.

Daniellia ogea, Celtis spp. *and Entandrophragma* spp., *Daiella olivieri, Pterocarpous* spp*., Diosporos* spp. and *Pychanthus* spp*. Lovoa* spp*. and Vitex doniana,* among others) suggesting that these trees species are under the threat of anthropogenic human activities, as most of the trees species were probably replaced by buildings after deforestation.

Although DNA extraction is the first step in every molecular studies research (Qi-Xing et al., 2013), the selection of suitable protocol for DNA extraction from a specific plant species has always been problematic, given that some plants are rich in cellulose, polysaccharides, polyphenols, proteins and lipids, which are responsible for the complication of the nucleic acid separation and purification, and this is mostly associated with tropical plants (Li et al., 2011; Mohammad et al., 2008; Tan and Yiap, 2009; Sharma et al., 2008; Wang et al., 2008; de la Cruz et al., 1997; Porebski et al., 1997; John, 1992). As a result, wide variety of DNA extraction techniques has been developed (Mohammad et al., 2008) however, this study adopted the CTAB protocol methods of Doyle and Doyle (1987) for the extraction of genomic DNA from the tree species, given that the samples studied are of tropical origin (Qi-Xing et al., 2013; Sahu et al., 2012; Oboh et al., 2009; Ogunkanmi et al., 2008). The study also highlights how rapid and reliable the CTAB protocol is specifically for extracting DNA from plants which are rich in polysaccharides and

Plate 1. Some indigenous species encountered. a) *Adansonia digitata*; b) *Athrocarpus sp;* c) *Bridelia micrantha;* d) *Cola nitida* e) *Khaya grandifofolia*; f) *Hildegardia barteri*; g) *Holarrhena floribunda;* h) *Raphia hookeri*; i) *Treculia africana.*

secondary metabolites, and the protocol also excludes the use of expensive liquid nitrogen and toxic phenols. Purity of extracted DNA was excellent as evident in the absorbance ratio recorded (1.74 to 1.84). This suggests that the preparations were sufficiently free of proteins and polyphenolics/polysaccharide compounds, with minimal contamination (Clark and Christopher, 2000). Hence, the purified DNA was dissolved in buffer and stored at -80°C in the established DNA Bank at the University of Lagos, Akoka, Lagos, Nigeria. The pure DNA extracted is a prerequisite to reliable molecular biology research (Maltas et al., 2011; Pagnotta, 2009; Savolainen et al., 2007; Mace, 2003) including molecular marker study such as AFLP, RAPD or any other PCR, based analysis or research. It could also be used in forensic research, understanding genetic and evolutionary relationships between taxa, functional analysis of genes, comparative genomics research, DNA barcoding and plant breeding. Furthermore, it can be used for studying DNA structure and chemistry, examining DNA-protein interactions, carrying out DNA hybridizations, and for cloning and sequencing; which provide additional option for conservation of biodiversity.

Conclusion

This study is probably the first attempt at using molecular techniques in conserving the flora of northeastern Lagos. Hence, this study has contributed to the genomic conservation of the tree species in Nigeria and the geno-

Plate 2. Some Introduced species encountered. a) *Casuarina equisetiifolia;* b) *Cocos nucifera;* c) *Delonix regia;* d) *Eugenia malaccensis;* e-f) *Lagerstroemia speciosa*.

Plate 3. Some members of the family Fabaceae encountered. a) *Albizzia lebbeck;* b) *Albizzia zygia;* c) *Senna siamea;* d) *Millettia thonningii;* e) *Bauhinia monandra;* f) *Peltophorum pterocarpum.*

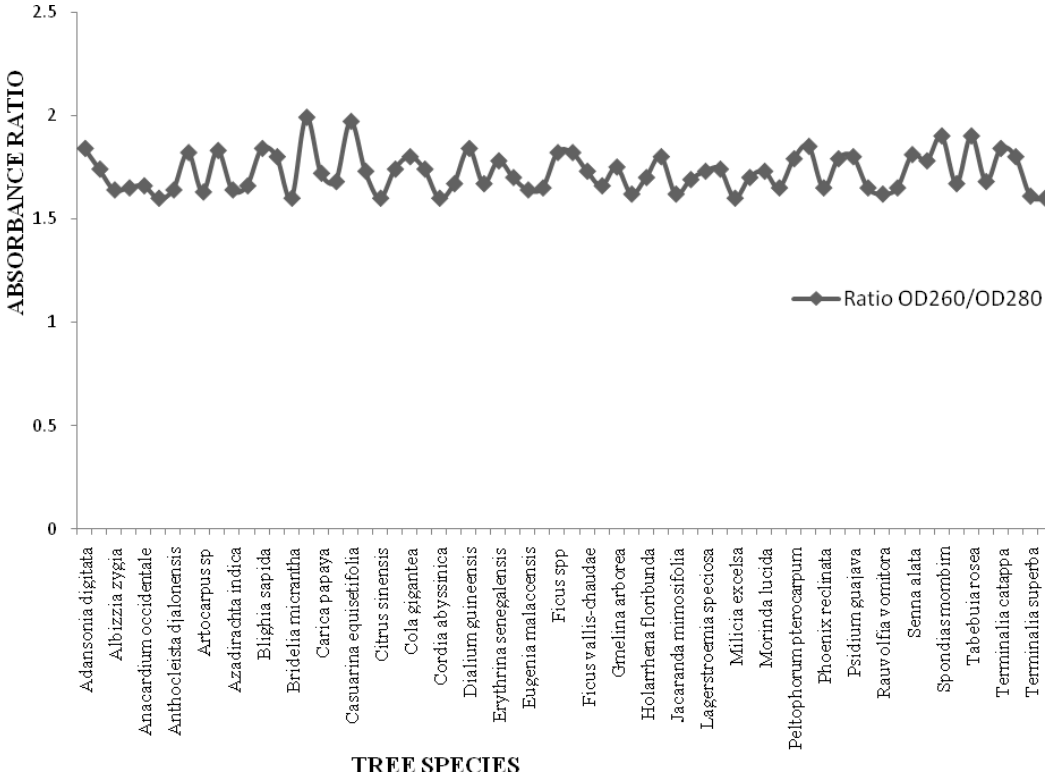

Figure 2. Absorbance ratio of DNA samples of the tree species.

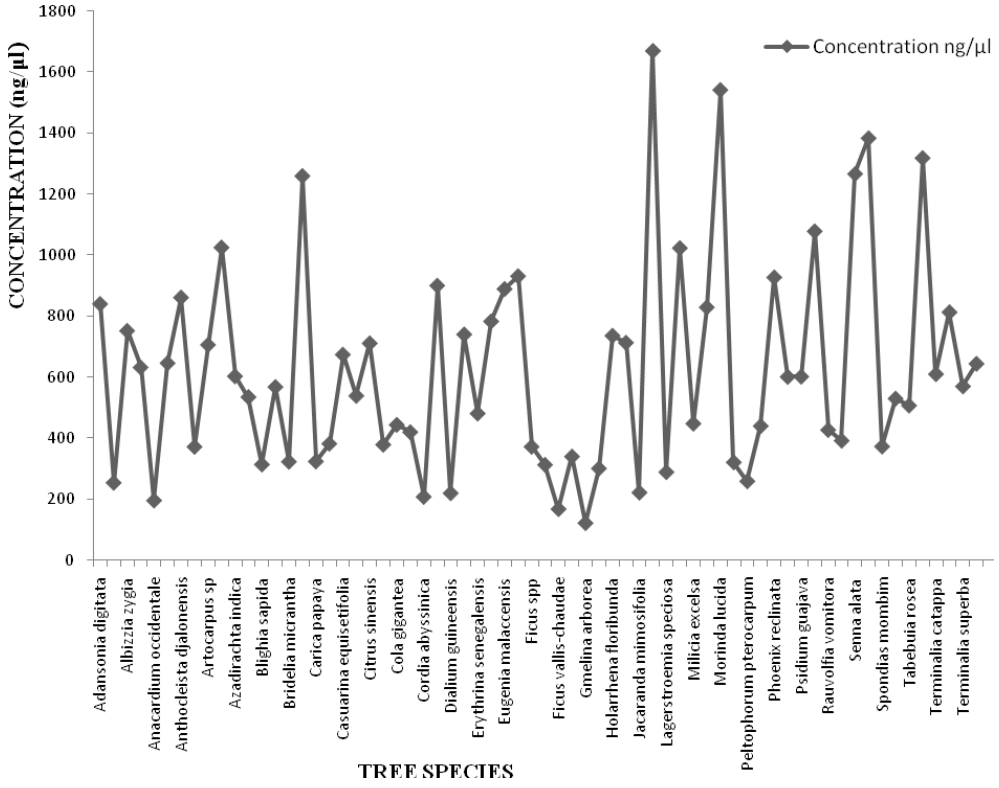

Figure 3. Concentration of DNA samples of the tree species in ng/µl.

mic DNA extracted would serve as a bench mark for further researches.

Conflict of Interests

The author(s) have declared that there is no conflict of interests.

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