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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

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Full Length Research Paper

The extraction and mass transfer process of soluble solids in Russian olive

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In this paper, through the research of mass transfer mechanism of the extraction process of soluble solids (SS) in Russian olive, the kinetic model of concentration change for the SS with the solvent multiple B (mL/g), extraction temperature T (K) and extraction time t (h) during extraction process was established for the first time. Russian olive extract concentration values $\hat{Y}$ (%) can be calculated in different extraction process, and the extraction process can also be predicted and controlled if the extraction temperature, extraction time and a multiple of solvent are provided. Overall, the absolute errors of the kinetic model are less than ± 1% and the χ^2 values are less than $\chi^2_{0.995}$ values also.

Key words: Russian olive, soluble solid, extraction, mass transfer mechanism.

INTRODUCTION

Russian olive (*Elaeagnus angustifolia* L.) is a species of *Elaeagnus* which is drought-resistant, saline-alkali-tolerant and widely distributed in north China, Kazakhstan, Russia, Poland, the United States, Canadian, etc (Ahmadiani et al., 2000). Russian Olive has the same clinical therapeutic effect with Smecta in treating acute infantile watery diarrhea by reducing both severity and durations of diarrhea disease, and superior to Smecta in decreasing volumes of watery diarrhea and ORS intake (Duolikun et al., 2007). It has been shown that the extract of Russian olive play the role of anti-diarrhea and inhibit intestinal propulsive after studying with experimental mice diarrhea and treatment (Keshan and Jianyun, 2007; Hossein and Mohammad, 2003; Field Guide to Plants of China, 1972).

Previous research (Jian and Jianyun, 2008) showed that different extraction temperature, time length and solvent multiples have significant effects on the extract concentration with certain extraction solvent. However, the mathematical relationship among them needs to be established and well explained. The change in regulations of extract concentration needs to be expounded. The established kinetic model of the mass-transfer process of soluble solids (SS) in Russian olive needs to be validated exactly. These problems mentioned above were solved in this paper.

MATERIALS AND METHODS

The raw material of the experiment is complete mature dry Russian-olives. They were purchased from Kashi in Xinjiang. After selection, they were enucleated and crushed up, and the samples were kept in clean and dry bottles.

For each experiment, the samples of Russian-olives were soaked in purified water. According to the multiple solvent, extraction temperature and extraction time, the SS concentration in the solution was detected with the handheld refractometer in the different extraction conditions. Each experiment was carried out in triplicate.

The modeling of the extraction dynamics in Russian Olive

The balance relationship of the extraction system whose mechanism has not been completely removed is complex. But a widely adopted simple model, hypothesizes, is described as follows: an infusibility porous solid contains the amount of solute which cannot be absorbed by the solid. For the amount of solvent,

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solute contents is considered under the saturated solubility. If the solid has sufficiently contacted with the solvent for a long time, solute could completely dissolve and liquid concentration in the lacuna of solid will be equal to the concentration of the surrounding liquid. At this time, the liquid composition will be stable. Theoretically, the extraction process of solid includes three stages: 1) solvent is permeated into the solute and solute begins to dissolve; 2) dissolved solute diffuses across the inside of the solid to the interface; 3) solute continues to diffuse from the interface across the liquid film to the main body of the external solvent.

Among the three stages, stage 1 and 3 which are not the crucial factors can be neglected while the stage 2 is vital for the extraction speed because the solute concentration changed during the extraction process and it decreased with the passing of time, so this process is actually a unstable diffusion according to the Fick's Law. In fact, the extraction operation depends on the control of internal diffusion.

Fick's first law relates the diffusive flux to the concentration field, by postulating that the flux goes from regions of high concentration to regions of low concentration, with a magnitude that is proportional to the concentration gradient (spatial derivative). In one (spatial) dimension, this is:

$$J_A = -D_{AB}\frac{dc_A}{dz} \quad (1)$$

Where J_A is the diffusion flux in dimensions of ((amount of component A) length^{-2} time^{-1}), example (kmol/ m^2·s); c_A is the concentration in dimensions of ((amount of component A) length^{-3}), example (kmol/m^3); z is the position (length), example (m); D_{AB} is the diffusion coefficient or diffusivity in dimensions of (length^2 time^{-1}), example, (m^2/s); is $\frac{dc_A}{dz}$ is the driving force for the one-dimensional diffusion of component t A, example (kmol/m^3·m).

Generally, total concentration c is not constant when main body flow takes place. Fick's law can be described as follows (Jian and Jianyun, 2008):

$$J_A = -cD_{AB}\frac{dx_A}{dz} \quad (2)$$

Where, x_A is the mole fraction of component A, (%), $x_A=c_A/c$; c_A is the the molarity of component A, (kmol/m^3), $c_A=m_A/M_AV$; c is the total molarity of compound (kmol/m^3), $c=\sum_{i=1}^{n} c_i$

In a multicomposition mixture, total molarity equals the sum of every component's molarity. m_A is the quality of component A (kg); M_A is the molecular weight of component A; V is the volume of the compound (m^3)

To the diffusion of the SS in Russian olive in water, for the sake of convenience, considering the diffusion area, the Fick's first law used for describing the extraction process can be derived as follows (Yaping and Weilun, 1997):

$$\frac{dm}{dt} = DS\frac{d\rho}{dz} \quad (3)$$

Where, $\frac{dm}{dt}$ is the leaching rate of SS, example (g/h); D is the diffusion coefficient (m^2/s); S is diffusion area (m^2); $\frac{d\rho}{dz}$ is the volume concentration gradient of solute at the interface (g/m^3.m).

The diffusion coefficient D is related to the properties of the materiel and extraction temperature.

The relationship between D and extraction temperature can be described by equation of Arrhniues (Jiufang et al., 1993) in a detailed extraction process:

$$D = Ae^{-E/RT} \quad (4)$$

Where, A is the the pre-exponential factor (m^2/s); E is the diffusion activation energy (J/mol); R is the gas constant (J/mol·K); T is the absoluted temperature (K); E and R are constant, pre-exponential factor A can be regarded as the diffusion coefficient at some basic temperature. Diffusion coefficient D is not only related to temperature but also to the concentration of solute.if (Aikemu and Xinjiang, 2007):

$$A = D_0\rho^n \quad (5)$$

Where, ρ is the volume concentration of solute in the main solvent (g/mL).

The effects of concentration on diffusion flux can be reflect by (5) with the change of index n. In a general way, diffusion coefficient decreases with the increase of the concentration of solute, therefore, n<0.

D_0 which is neither related to the temperature nor to the concentration, is only related to character of material, called natural diffusion coefficient.

The extraction is carried under the stable condition, and the particular concentration of diffusion surface is changing with time during the extraction process, as a result, concentration gradient is the function of not only the space but also the time.

Factually, as to the special diffusion surface, the larger the concentration gradient, the larger the diffusion force and the quicker the diffusion proceeds.

If the changing rate of time of the concentration gradient is indirect proportion to the temporal concentration gradient (Aikemu and Xinjiang, 2007):

$$\frac{d}{dt}\left(\frac{d\rho}{dz}\right) = \alpha\frac{d\rho}{dz} \quad (6)$$

Where, α is the rate constant and concentration gradient decreases with the hoist of concentration of solute and the process. α<0.

if t=0, $\frac{d\rho}{dz} = u_0$, and t=t, $\frac{d\rho}{dz} = u$, the integral of (6) is:

$$\int_{u_0}^{u}\frac{du}{u} = \int_0^t \alpha dt$$

The relationship between concentration gradient and time is:

$$\frac{d\rho}{dz} = u_0 e^{\alpha t} \quad (7)$$

Put (4), (5), (7) into (3), then we get:

$$\frac{dm}{dt} = D_0 u_0 \rho^n S e^{-E/RT} e^{\alpha t} \quad (8)$$

(8) is the amended Fick's first law.

SS exists in the flesh tissue under the skin of the Russian olive which has long olivary shape, and the thickness of the skin is extremely shorter than the length and diameter of the Russian olive itself. Therefore, the extraction process of SS can be simplified as single dimension diffusion under the condition of long flat, and formula (8) can be used to describe the extraction process. At this moment, the diffusion area *S* of SS in Russian olive is in direct proportion to absolutely dry biomass *G*

$$S=kG \quad (9)$$

Where *k* is the surface area of Russian olive in each quality (m^2/g)

Total amounts *m* of SS at T moment has something to do with volume concentration of solute and volume of solvent in the main solvent:

$$m=\rho V \quad (10)$$

where ρ is the volume concentration of solute in main solvent (g/mL); *V* is the volume of solvent (mL)

Put (9), (10) into (8):

$$\frac{d\rho}{dt} = D_0 u_0 \rho^n k \frac{G}{V} e^{-E/RT} e^{\alpha t}$$

We set solvent multiples as $B = \frac{V}{G}$, then:

$$\frac{d\rho}{dt} = D_0 u_0 \rho^n k \frac{1}{B} e^{-E/RT} e^{\alpha t} \quad (11)$$

If t = 0, ρ = 0, the integral of (11) is:

$$\int_0^{\rho} \frac{d\rho}{\rho^n} = D_0 u_0 k \frac{1}{B} e^{-E/RT} \int_0^t e^{\alpha t} dt$$

$$\rho^{1-n} = \frac{1-n}{\alpha B} D_0 u_0 k e^{-E/RT} (e^{\alpha t} - 1) \quad (12)$$

For n<0, α<0, formula (12) can be changed as follows:

$$\rho^{1-n} = \frac{n-1}{\alpha B} D_0 u_0 k e^{-E/RT} (1 - e^{\alpha t}) \quad (13)$$

The calculated value of sugar content $\hat{Y}$ (%), which is also called Brix, can be used to reflect the concentration of SS in Russian olive. In fact, while using handheld sugar meter, refraction of material is determined.

And refraction is the quotient of the sine of angle of incidence and sine of angle of refraction. For the same material, the refraction is in direct proportion to its concentration.

According to the definition of the concentration of SS, $\hat{Y}$ (%) is:

$$\hat{Y} = \frac{m \times 100}{m + m_2} = \frac{m \times 100}{m + D_2 V_2} \quad (14)$$

Where, m_2 is the quality of the solvent (water) (g); D_2 is the density of the solvent (water), D_2 =1 (g/mL); V_2 is the volume of the solvent (water), (mL); because $m << m_2$, $m_2 = D_2 V_2 = V_2$ and $V_2 \approx V$, (14) could be recomposed:

$$\hat{Y} = \frac{m \times 100}{V} = 100\rho \quad (15)$$

Put (15) into (13):

$$\hat{Y}^{1-n} = \frac{100^{1-n}(n-1)}{\alpha} D_0 u_0 k \cdot \frac{1}{B} \cdot e^{-E/RT} \cdot (1 - e^{\alpha t})$$

or:

$$\ln \hat{Y} = \frac{1}{1-n} \ln \frac{100^{1-n}(n-1)}{\alpha} D_0 u_0 k - \frac{1}{1-n} \ln B - \frac{E}{R(1-n)} \frac{1}{T} + \frac{1}{1-n} \ln(1 - e^{\alpha t}) \quad (16)$$

In formula (16), *n, D_0, k, α, E, R* are constant. At the time (*t*=0)when extraction begins, the solute concentration is 0，so there is no relationship between concentration gradient u_0 and added solvent and the concentration gradient is only related to primary content of solute in solids, characteristics of solutes and solvents. Therefore, in the process of extraction of SS in Russian olive, u_0 is also a constant.

If

$$\beta_0 = \frac{1}{1-n} \ln \frac{100^{1-n}(n-1)}{\alpha} D_0 u_0 k \quad (17)$$

$$\beta_1 = -\frac{1}{1-n} \quad (18)$$

$$\beta_2 = -\frac{E}{R(1-n)} \quad (19)$$

Then formula (16) could be recomposed:

$$\ln \hat{Y} = \beta_0 + \beta_1 \ln B + \beta_2 \frac{1}{T} - \beta_1 \ln(1 - e^{\alpha t}) \quad (20)$$

Formula (20) is the dynamic pending model which can reflect the relationship between extraction concentration $\hat{Y}$ and extraction multiples *B*, temperature *T* and time *t*. From formula (20) we could get the formulas of extraction concentration and 3 factors respectively.

2) When only the extraction multiplied *B* is taken into account, extraction temperature *T* and time *t* is kept stable, formula (20) could be recomposed:

$$\ln \hat{Y} = \beta_B + \beta_1 \ln B \quad (21)$$

β_B - the constant when extraction multiples *B* is taken into account.

Table 1. Verification of soluble solids concentration in different solvent multiples (T = 353K, t = 1.5 h).

B	**4**	**5**	**6**	**7**	**10**	**13**	**17**	**20**	**30**
Model calculation ($\hat{Y}_i$)	15.5	12.7	10.9	9.5	7.0	5.5	4.4	3.8	2.7
Test value (Y_i)	15.0	12.5	10.5	9.0	7.0	5.0	4	3.5	2.5
Simulated absolute error	0.5	0.2	0.4	0.5	0	0.5	0.4	0.3	0.2
$(\hat{Y}_i - Y_i)^2 / Y_i$	0.016	0.003	0.015	0.028	0.000	0.050	0.040	0.026	0.016

$\chi^2=\sum(\hat{Y}_i - Y_i)^2 / Y_i = 0.194 < \chi^2_{0.995.(6)} = 0.680$, P>0.995

It is related to temperature T and time t. the extraction test of SS in Russian Olive was carried out when keep the temperature T and time t stable and the test value Y_i of the extraction concentration at different extraction multiples was gotten. Then β_B and $\beta_1$can be calculated by the liner regression between $\ln \hat{Y}$ and ln$B$.

2) When only the extraction temperature T is taken into account, extraction multiples B and time t is kept stable, formula (20) could be recomposed:

$$\ln \hat{Y} = \beta_T + \beta_2 \frac{1}{T} \quad (22)$$

β_T- the constant when extraction temperature T is taken into account. It is related to extraction multiplied by B and time t.

the extraction test of SS in Russian Olive was carried out when keeping the extraction multiple B and time t stable and the test value Y_i of the extraction concentration at different extraction temperature gotten. Then β_T and β_2 can be calculated by the liner regression between $\ln \hat{Y}$ and $\frac{1}{T}$.

3) When only the extraction time t is taken into account, extraction multiples B and temperature T is kept stable, formula (20) could be gotten:

$$\ln \hat{Y} = \beta_t - \beta_1 \ln(1 - e^{\alpha t}) \quad (23)$$

β_t - the constant when extraction time t is taken into account. It is related to extraction multiples B and temperature T.

The extraction test of SS in Russian Olive is carried out when keeping the extraction multiples B and temperature T stable and getting the test value Y_i of the extraction concentration at different extraction time.

The value of β_t and α can be calculated by least squares and fitting iterative methods with Y_i. The calculation steps are omitted. Lastly, the calculated results are:

β_1=−0.8701; β_2=−1322.2725; α=−0.6655; β_0= 8.0913

Put the final β_0, β_1, β_2, α into (20), we get the model of extraction dynamics of SS in Russian Olive:

$$\ln \hat{Y} = 8.0913 - 0.8701 \ln B - 1322.2725 \frac{1}{T} + 0.8701 \ln(1 - e^{-0.6655t}) \quad (24)$$

This model can accurately reflect the relationship between the extract concentration and solvent multiples, extraction temperature and extraction time. If the other parameter such as solvents, size of particles and resource of materials are ensured, SS in Russian olive under variety technology could be predicted. However, the veracity of the prediction needs valuing.

RESULTS AND VERIFICATION

A comparison of predicted and experimental SS concentration in different solvent multiples of whole Russian olive during extraction at temperature of 80℃ and time of 1.5 h is shown in Table 1 and Figure 1.

Every point in the experiment curve of each figure is the mean of SS concentration that was tested three times and the standard deviation was less than 0.3. It shows that the calculation gotten from formula (21) for SS concentration fits very well with the experiment (χ2=0.68, P>0.995).

A comparison of predicted and experimental of SS concentration in different extraction temperature of whole Russian Olive during extraction at the solvent multiple B of 6 and time of 1.5 h is shown in Table 2 and Figure 2. Every point in the experiment curve of each figure is the mean of SS concentration that was tested three times and the standard deviation was less than 0.3. It shows that the calculation gotten from formula (22) for SS concentration fit very well with the experiment (χ2=0.68, P>0.995).

A comparison of predicted and experimental of SS concentration in different extraction time of whole Russian Olive during extraction at the solvent multiple B of 6 and temperature of 80°C is shown in Table 3 and Figure 3.

Every point in the experiment curve of each figure is the mean of SS concentration that was tested three times and the standard deviation was less than 0.3. It shows that the calculation gotten from formula (23) for SS concentration fit very well with the experiment (χ^2=2.60, P>0.995).

By analyzing the dates from tables and figures, the well simulated results demonstrated that the model was suitable for the extraction and mass transfer process of SS in Russian Olive. Between the model calculations and the test values, the absolute errors of SS concentration are less than ±1% and their χ^2 values are less than

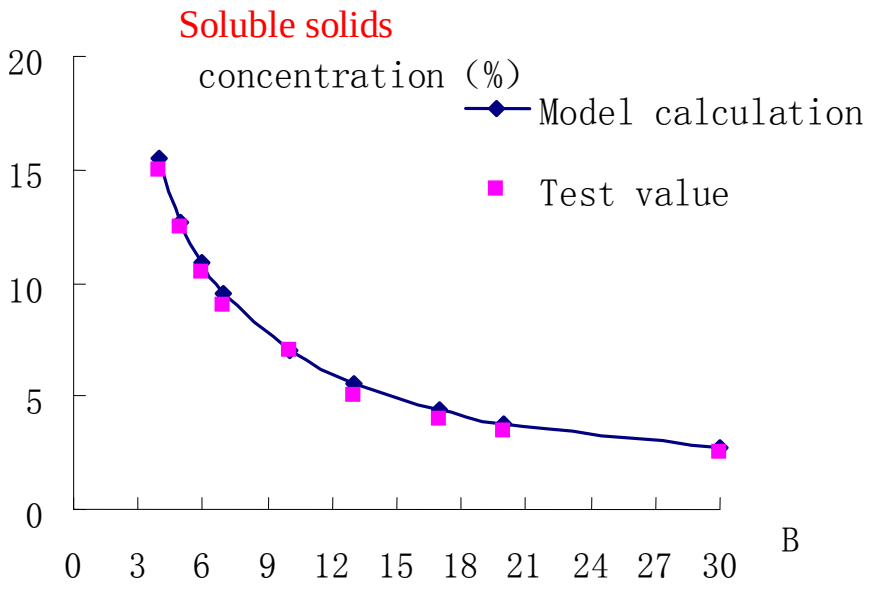

Figure 1. Verification of soluble solids concentration in different solvent multiples (T = 353K, t = 1.5 h).

Table 2. Verification of soluble solids concentration in different extraction temperature (*B* = 6, *t* = 1.5 h).

T	**303K**	**313K**	**323K**	**333K**	**343K**	**353K**	**363K**
Model calculation ($\hat{Y}_i$)	5.9	6.7	7.7	8.7	9.8	10.9	12.1
Test value (Y_i)	6.2	7.2	8.2	9	10.2	11.5	13
Simulated absolute error	-0.3	-0.5	-0.5	-0.3	-0.4	-0.6	-0.9
$(\hat{Y}_i - Y_i)^2 / Y_i$	0.015	0.037	0.032	0.010	0.016	0.033	0.067

$\chi^2=\sum(\hat{Y}_i - Y_i)^2 / Y_i = 0.210 < \chi^2_{0.995.(6)} = 0.680$, $P > 0.995$

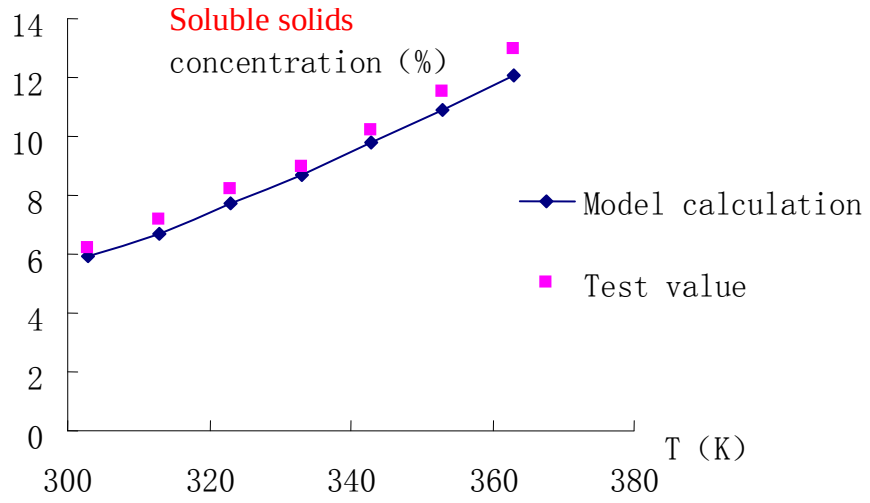

Figure 2. Verification of soluble solids concentration in different extraction temperature (*B* = 6, *t* = 1.5 h).

Table 3. Verification of soluble solids concentration in different extraction time (B = 6, T = 353K).

***t* (min)**	**10**	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**	**110**	**120**	**130**
t/h	0.1667	0.3333	0.5	0.6667	0.8333	1.0	1.1667	1.3333	1.5	1.6667	1.8333	2.0	2.1667
Model calculation ($\hat{Y}_i$)	2.3	4.0	5.4	6.6	7.7	8.7	9.5	10.2	10.9	11.5	12.0	12.4	12.8
Test value (Y_i)	2.1	4.3	5.0	6.2	7.3	8.5	9.0	9.5	10.5	11.3	11.5	12.0	12.2
Simulated absolute error	0.2	-0.3	0.4	0.4	0.4	0.2	0.5	0.7	0.4	0.2	0.5	0.4	0.6
$(\hat{Y}_i - Y_i)^2 / Y_i$	0.019	0.021	0.030	0.024	0.021	0.005	0.026	0.048	0.015	0.003	0.021	0.013	0.028

$\chi^2 = \sum(\hat{Y}_i - Y_i)^2 / Y_i = 0.274 < \chi^2_{0.995,(11)} = 2.60$, $P > 0.995$.

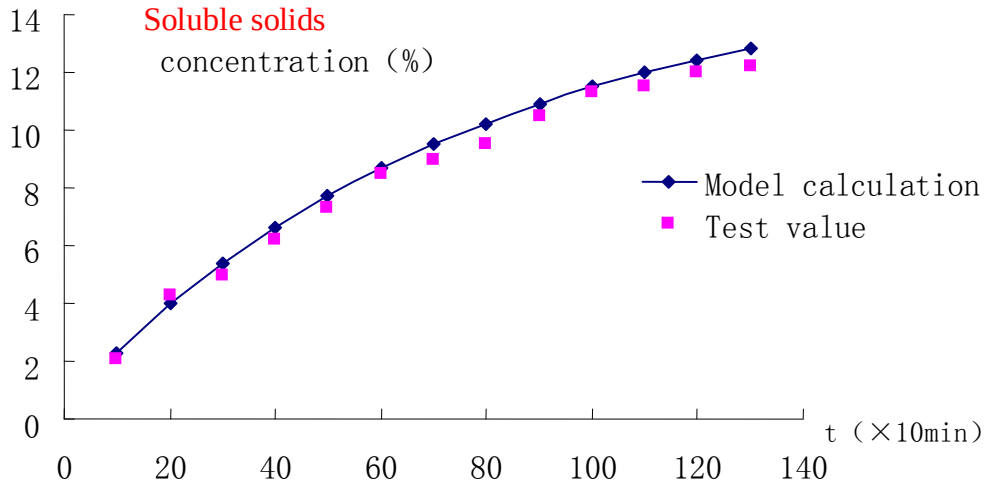

Figure 3. Verification of soluble solids concentration in different extraction time (B = 6, T = 353K).

$\chi^2_{0.995}$ values in different extraction temperature T, extraction time t, and solvent multiples B.

To sum up, the model can predict accurately to a large extent, and describe well the extraction dynamic process of SS concentration of Russian Olive, as well as reflect the correct relationship between SS concentration and extraction temperature, extraction time and solvent multiples.

Conclusion

1) According to the Fick's first law, we assume that the change rate of concentration gradient is in direct proportion to concentration gradient, and the relationship between diffusion coefficient and temperature satisfy the Arrhuiues equation. The model of extraction dynamic of SS in Russian olive has been derived theoretically. The characteristics of Russian olive and its SS are all reflected as constants in this model and such constants can be calculated through the experimental results. Therefore, the model can be applied to describe the other extraction kinetics process in similar dried fruits.

2) Based on the extraction experiments, according

to mathematic formula between the SS concentration and extraction temperature, time, solvent multiples, we get the unknown constant in the model by adopting liner regression analytic method and least square method. The model calculation is a high precision with the test values at various extraction conditions as compared to the traditional orthogonal experiment method. Between the model calculations and the test values, the absolute error of SS concentration is less than ±1% and their χ^2 values are less than $\chi^2_{0.995}$ values in different extraction temperature ***T***, extraction time *t*, and solvent multiples *B*.

3) The effect of extraction temperature, time, solvent multiples on SS concentration has been studied and the corresponding regulation has been gotten. Generally, the lower the multiples, the higher the temperature, the longer the time and the higher the SS concentration. However, there exist limitations if we keep reducing multiples and increasing time to some extent, the increase of SS concentration is limited. So there exist the most economic extraction time and solvent multiples, but the specific level is connected with extraction temperature.

4) When compared with Fick's law, this model is used simple and convenient. Under the same conditions (solvents, origin of materials, material maturity, etc), Russian olive extract concentration values $\hat{Y}$(%) can be calculated in different extraction process, and the production process of extraction can also be predicted and controlled if the extraction temperature *T* (K), extraction time *t* (h) and a multiple of solvent *B* (mL/g) are provided. After all, the model can predict accurately to a large extent, and describe well the extraction dynamic process of SS concentration of Russian olive.

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Full Length Research Paper

Short-term responses of shrub layer communities to dry season fires and tree thinning in semi-arid miombo woodlands of north-western Zimbabwe

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Short-term responses of shrub layer communities to dry season fires and tree-thinning were investigated in semi-arid miombo woodlands in North-western Zimbabwe. Fifty-four (54) plots, 50 x 50 m each, were demarcated at three miombo sites. Treatments applied were: no burn, no tree thinning; no burn, thinned; early burn, no thinning; early burn, thinned; late burn, no thinning; late burn, thinned. After three years, height structure of the shrub layer communities had significantly changed, with increases in proportions of plants below 10 cm in burnt plots due to height reductions of burnt plants and additions from natural recruitment. Non-burnt plants significantly increased in heights while frequent burning kept plants in a fire-trap. Reductions in heights were further exacerbated by herbivore browsing of new resprouts. Numbers of stems significantly increased in late burnt plants. This is a survival strategy inherent in the life history characteristics of plants in fire-prone environments to ensure quick re-establishment and regain their above-ground biomass for survival. Changes in heights of selected individual samplings did not differ among sites or between thinning treatments while burning led to increases in stems. *Monotes glaber* and *Pseudolachnostylis maprouneifolia* had above-average height increases. Mortality was low with only 5.1% in late-burnt, thinned plots, indicating good tolerance to fire.

Key words: Fire, miombo woodland, shrub dynamics, Sengwa, tree-thinning, Zimbabwe.

INTRODUCTION

The structure and dynamics of savanna ecosystems are influenced by a number of primary and secondary determinants including fire. Fire has been a common phenomenon in these ecosystems such that it has significantly influenced their functioning for millennia. The fire regime of a particular region, defined by its intensity, frequency and timing, is largely determined by grass productivity (which itself is a function of rainfall) and local grazing regimes. Frequent fires are detrimental to the development of woody plants while long fire-free periods and complete fire protection tend to promote woody plant development. Short fire-free intervals in tree and shrub communities usually result in low severity fires due to low grass production (Govender et al., 2006). Where fires are less frequent and/or of low intensity, there is often an increase in shrub development (Kruger, 1984; Smit et al., 2010). Thickets have developed in areas that either experienced less or no fires, or were overgrazed (Lock, 1993), a situation often referred to as 'bush encroachment' (Skowno et al., 1999; Archer, 1990; van Wilgen et

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al.,1990).

Fire causes various forms of damage to plants and its effects are more pronounced in lower vegetation strata such as shrubs, saplings and small trees. The ability of plants to survive the effects of fire is therefore an important attribute. Inherent abilities of plants to survive a fire depend on their tolerance to heat and resistance to fire (De Bano et al., 1998). Severe or more frequent fires can cause plant mortality, particularly of seedlings, shrubs and saplings whose fire tolerance levels may be generally low. Plant responses to fires are varied and largely depend on the intensity, timing and frequency of fires and the plant's life history characteristics. Re-sprouting is one of the common responses of plants to burning and this can take place from rootstocks, lignotubers, stems and/or branches.

Whilst the effects of fire on floristic composition may be difficult to interpret due to the complex interactions with rainfall, drought, herbivory and differences in species sensitivities (Frost and Robertson, 1987; Levick et al., 2012), effects of tree thinning are expected to trigger systematic responses (Thomas et al., 1999). Removal of trees affects the understorey layer by increasing light availability and may lead to increases in water and nutrient availability to the understorey plants. It is widely accepted that understorey cover increases with canopy openness (Thomas et al., 1999). By increasing available resources, tree thinning could allow a greater number of understorey species to persist. However, Alaback and Herman (1988) argued that thinning may also result in increased dominance by one or a few understorey species thereby reducing diversity.

In Sengwa Wildlife Research Area (SWRA) in north-western Zimbabwe, annual hazard-reduction burning of peripheral areas of the park has been undertaken for more than three decades for reasons of fire protection. Burnt areas were mainly parts of miombo woodland which forms about 25% of the vegetation cover. The objective of peripheral burning was to reduce cases of fire entering the area from adjacent communal lands. Whilst this objective was largely achieved (Mapaure et al., 2009), no data exist on the current or likely future ecological impacts of maintaining frequent fires within the periphery of the area, particularly on species composition, richness, diversity and structure of the lower vegetation strata. Implications of woodland thinning by elephants on lower vegetation strata need to be further explored.

This study therefore, aimed to investigate short-term responses of saplings to early and late dry season fires and tree thinning (simulating elephant impacts). It was hypothesised that dry season fires curtail recruitment of woody plants resulting in changes in the structure of the shrub/sapling layer, with more severe undesirable consequences in thinned-late burnt plots and non-thinned, non-burnt plots.

MATERIALS AND METHODS

Description of study area

The study was carried out in Sengwa Wildlife Research Area (SWRA) in north-western Zimbabwe (Figure 1). SWRA lies between 28°03′ and 28°20′E and 18°0′and 18°13′S, covering an area of 373 km^2. It is bounded by communal lands on all but the northern side, where it shares a boundary with Chirisa Safari Area, a state protected hunting area. The area experiences three climatic seasons: a hot wet period from November to April, a cool dry period from May to July and a hot dry period from August to October. Mean annual rainfall was 642 mm while mean annual temperature was 23.6°C. October is the hottest month and July is the coldest. Altitude varies from 808 to 1043 m. The area is drained by three major rivers, the Sengwa, Manyoni and Lutope. Two main soil types occur, one formed on sandstones of the Escarpment Grits and another formed on mudstones (Selibas, 1974; Bennett et al., 1983). The vegetation is generally deciduous *Brachystegia-Julbernardia* (miombo) woodland on sandy soils and dry early deciduous woodland dominated by *Colophospermum mopane* on the lower-lying heavier soils. Other vegetation types include riverine *Acacia* woodlands and mixed *Combretum* thickets on sands. These habitats are home to a diverse large mammal community of seven species of large carnivores and 18 species of large herbivores.

Experimental design

Three miombo woodland sites were selected for demarcation of plots, one in the north, one in the east and another in the south of SWRA. These sites are respectively referred to as Samapakwa (K), Airstrip (C) and Rongaronga (D) (Figure 1). They were chosen on the basis of being typical miombo woodlands among all patches of miombo in the area. At each site, 18 plots were demarcated, each measuring 50 m x 50 m, arranged in three blocks of six plots each. The blocks were arranged across a gradient and blocking was based on slope. Blocks of plots were separated by fire guards measuring 5 m wide, and the plots within each block were separated by a fire guard of the same width. All woody plants were cut just above ground level and removed from the fire guards. Fire guards were cleared of woody re-growth and herbaceous plants every year over a period of three years.

Combinations of treatments applied to plots were two fire timings (early and late dry season burns done in May and October, respectively) and two tree density levels, a 50% reduction of the original density (thinned) and a non-thinned treatment. Treatment combinations were: no burn and no thinning (NBNT - the control); no burn and thinned (NBT); early burn and no thinning (EBNT); early burn and thinned (EBT); late burn and no thinning (LBNT), and late burn and thinned (LBT). Thinning was done by evenly cutting and removing 50% of the trees form the affected plots. Treatments were randomly assigned to plots at the start of the study period and were replicated three times at each site. The burning treatments were repeated in the same plots annually but thinning was only done once at the beginning of the experiment. Plots were not protected from herbivores.

Monitoring and assessment of plants

General assessments of the structure of the shrub layer (before and after treatments) were done in belt transects each measuring 2 m x 50 m demarcated at random positions along the long axes of each plot. Within each belt transect all shrubs and saplings were assessed. The height of each shrub/sapling was measured and the

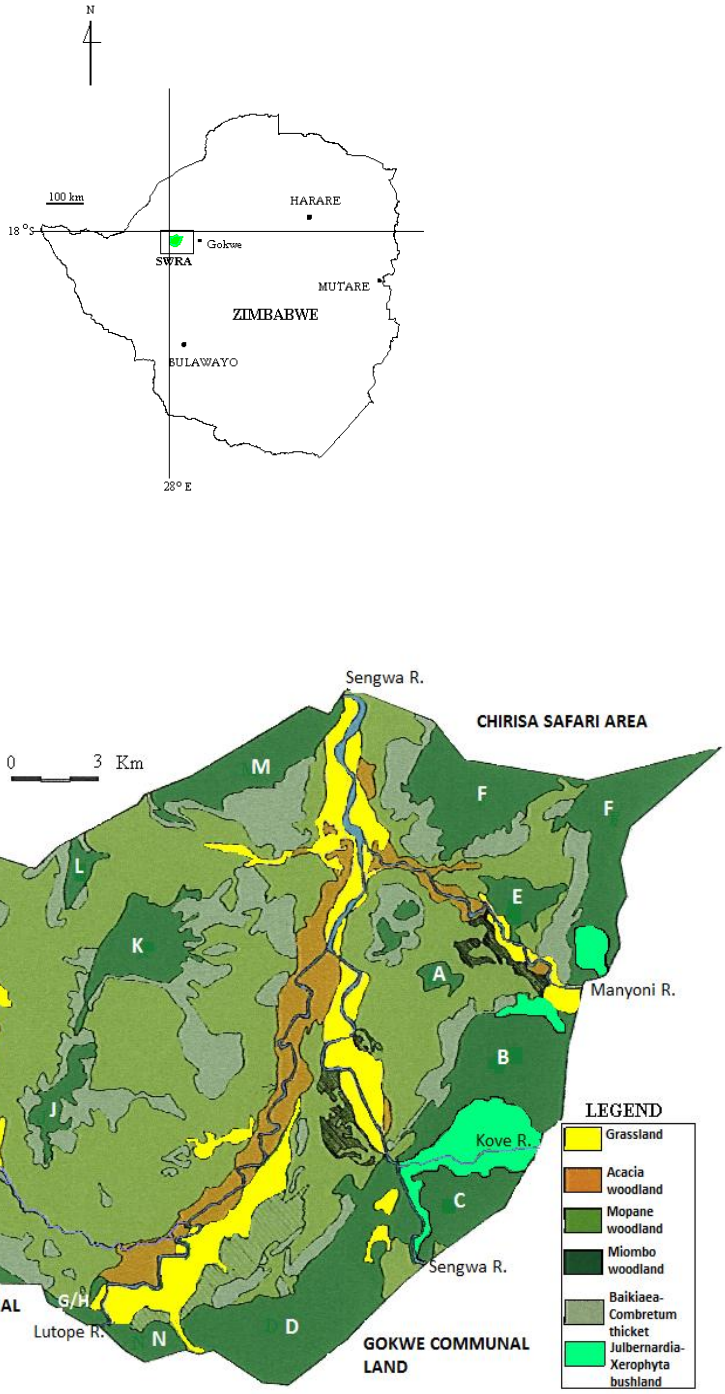

Figure 1. Location of Sengwa Wildlife Research Area (SWRA) in north-western Zimbabwe and a detailed vegetation map of the area (Patches of miombo woodland are labelled A - M).

number of stems counted. In addition, between 20 and 25 randomly selected saplings of common woody species were tagged in each plot at each site for monitoring. A total of 1164 saplings were tagged for monitoring. Tagging was done by means of metal strips engraved with identification numbers and attached to pieces of thick wire, 20-30 cm long, which were thrust into the ground next to bases of individual plants. The height of each plant was measured and the number of stems was counted. The presence of any browsing was noted and dead saplings were counted during re-assessments. Species monitored were *Brachystegia boehmii*, *Brachystegia spiciformis*, *Burkea africana*, *Erythrophleum africanum*, *Julbernardia globiflora*, *Monotes glaber*, *Ochna pulchra*,

Pseudolachnostylis maprouneifolia, and *Terminalia sericea*. Re-assessments were done three years after initial baseline assessments.

Data analyses

Differences in the responses of the plant species to the treatments were tested using General Linear Model (GLM) ANOVA, with thinning, early burning and late burning entered as nominal continuous variables using integer scores of 0, 1 and 2 (0 - no thinning, no burning; 1- thinned, early burnt; 2 - late burnt). Response variables were changes in sapling heights and numbers of stems. Interactive effects of the treatments were included for testing in the GLM ANOVA model. Changes in height class distributions (of all shrubs/saplings) over the experimental period were tested using a χ^2 test. Height classes used were: <10, 10-20, 20-50, 50-100, 100-150, 150-200, 200-250, and >250 cm. For comparisons of individual species responses, GLM ANOVA was used for five species only (*B. boehmii*, *B. africana*, *J. globiflora*, *M. glaber* and *P. maprouneifolia*) because these were common to all sites. Sapling mortality was calculated in terms of proportions of dead plants relative to the original number of live plants in the respective treatments.

RESULTS

Changes in numbers of stems and heights

Overall changes in numbers of stems of the whole shrub/sapling community significantly differed among sites ($F = 414.39$, $df = 2$, $p < 0.001$), with significantly higher changes at Samapakwa than at the other two sites. Burning led to significant increases in numbers of stems ($F = 65.46$, $df = 2$, $p < 0.001$), and thinning had a similar effect on stems ($F = 30.32$, $df = 1$, $p < 0.001$) (Figure 2a). Changes in numbers of stems were significantly influenced by the following interactions: site x burn ($F = 12.29$, $df = 4$, $p < 0.001$; higher increases at Samapakwa with burning), site x thin ($F = 15.99$, $df = 2$, $p < 0.001$; higher increases at Samapakwa with thinning), and burn x thin ($F = 18.35$, $df = 2$, $p < 0.001$; higher increases in LBT plots) (Figure 2b). Burning significantly reduced plant heights ($F = 87.59$, $df = 2$, $p < 0.001$) while thinning led to significant increases in heights ($F = 150.28$, $df = 1$, $p < 0.001$) (Figure 3a). Interactive effects of site x burn ($F = 35.22$, $df = 4$, $p < 0.001$), site x thin ($F = 24.24$, $df = 2$, $p < 0.001$) and burn x thin ($F = 58.21$, $df = 2$, $p < 0.001$) all significantly influenced plant height responses (Figure 3b).

Since changes in overall plant heights significantly differed among sites, changes in height class distributions are presented on a per site basis. At Airstrip (Figure 4a), there were significant changes in the height class distribution in late-burnt (LB) plots, with higher changes in thinned (LBT) plots ($\chi^2 = 25.66$, $df = 5$, $p < 0.001$), non-thinned plots (LBNT) ($\chi^2 = 21.00$, $df = 5$, $p < 0.01$), with notable increases in proportions of plants <10 cm in height over the three years. Changes in height class distribution were also significant in early burnt, non-thinned (EBNT) plots ($\chi^2 = 18.21$, $df = 4$, $p < 0.01$) and in non-burnt, non-thinned (NBNT) plots ($\chi^2 = 16.28$, $df = 5$, $p < 0.01$). There was also an increase in proportions of plants <10 cm in height. The height class distribution of shrubs at Rongaronga changed significantly in EBT ($\chi^2 = 20.30$, $df = 4$, $p < 0.001$) and in LBNT ($\chi^2 = 19.40$, $df = 4$, $p < 0.001$) plots (Figure 4b), with notable increases in proportions of plants <10 cm in height and decreases in proportions of plants in the 50 to 200 cm range in burnt plots. Changes in NBT plots were also significant ($\chi^2 = 15.26$, $df = 6$, $p < 0.05$) with notable recruitment of plants into higher classes. At Samapakwa, changes in height class distributions were not significant in all treatments (Figure 4c). There were, however, decreases in proportions of plants in the 100 to 150 cm range in burnt plots.

Sapling mortality

Of the 977 saplings positively identified after 3 years, 30 had died, all of which were in burnt plots except one. The majority (66.7%) of dead saplings were at Airstrip site, while 13.3and 20% were at Rongaronga and Samapakwa, respectively. Sapling mortality was generally low, ranging from zero in non-burnt plots to 5.1% in late-burnt thinned plots (Table 1). *J. globiflora* suffered the heaviest mortality (53.3% of all dead saplings), all with a mean height of less than 50 cm. Mortality of *B. boehmii* was second (16.7% of all dead saplings) followed by *B. spiciformis* (10%), with respective mean heights below 60 cm and above 120 cm. Other species which died were *C. spinosa, P. maprouneifolia*, *O. pulchra*, *T. sericea* (each 3.3%) and *E. africanum* (6.8%).

Changes in heights and numbers of stems of selected species

Changes in heights of five common species (*B. boehmii, B. africana*, *J. globiflora*, *M. glaber* and *P. maprouneifolia*) were not significantly different among sites or between thinning treatments but were significantly different among burning treatments ($F = 11.97$, $df = 2$, $p < 0.001$) with non-burnt saplings increasing in height while burnt saplings registered little growth, particularly in late burnt plots (Figure 5a). There were no significant differences in changes in height among species. The interactive effects of site and burn were significant ($F = 2.73$, $df = 4$, $p < 0.05$) with reductions in heights at Samapakwa in early burnt plots (Figure 5b). Numbers of stems of five selected species significantly increased with late burning ($F = 20.29$, $df = 2$, $p < 0.001$) (Figure 6a) but they were not

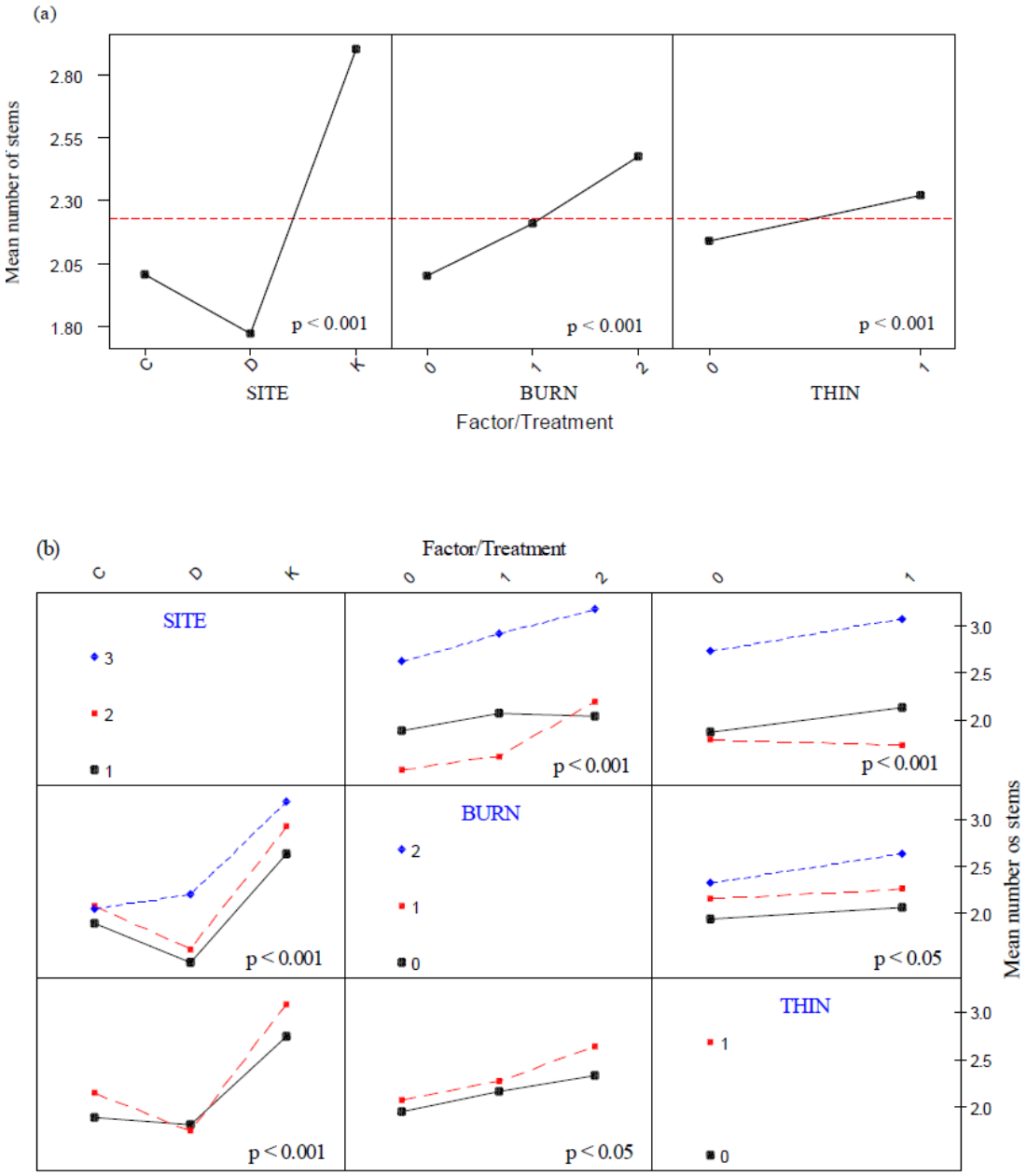

Figure 2. Main effects plots (a) and interactions plots (b) indicating the effects of site (C = Airstrip, D = Rongaronga, K = Samapakwa), burning (0 = no burn, 1 = early burnt, 2 = late burnt) and thinning (0 = no thinning, 1 = thinned) on the mean number of stems per plant (significance levels are shown in the respective graphs; NS = not significant)

significantly influenced by interactive effects between any two factors (Figure 6b). Site and thinning did not significantly influence numbers of stems. There were significant differences in changes in numbers of stems among species ($F = 4.40$, $df = 4$, $p < 0.01$), with above average increases in *M. glaber* and *P. maprouneifolia*. *B. boehmii*, *B. africana* and *J. globiflora* registered below-average increases in number of stems.

DISCUSSION

There were significant increases in numbers of stems with late burning and/or thinning, a result consistent with hypothesized trends. Woody plants in arid and semi-arid areas may respond to burning in various ways including resprouting or death followed by mass germination if there is adequate post-fire rain (Griffin and Friedel, 1984).

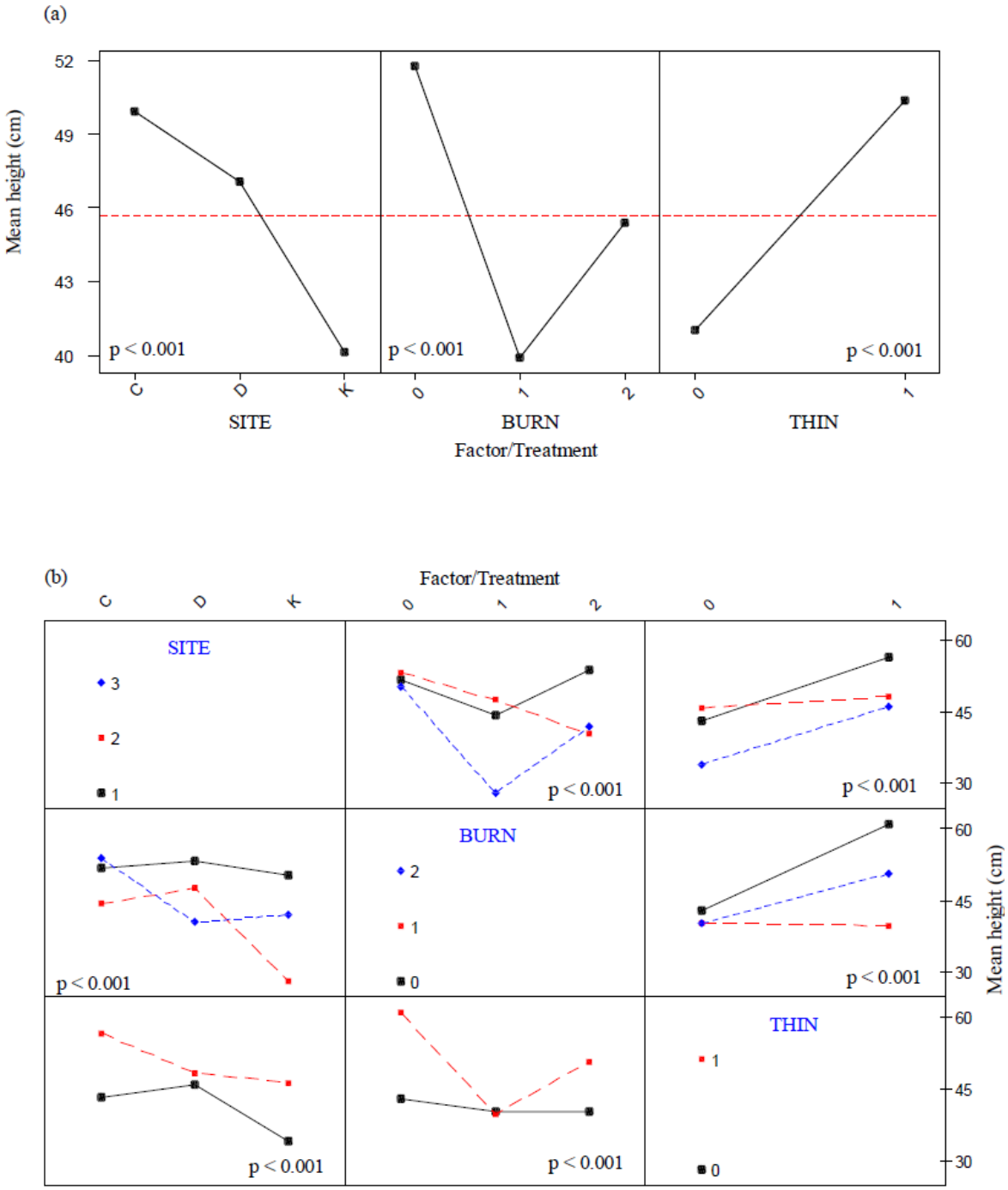

Figure 3. Main effects plots (a) and interactions plots (b) indicating the effects of site (C = Airstrip, D = Rongaronga, K = Samapakwa), burning (0 = no burn, 1 = early burnt, 2 = late burnt) and thinning (0 = no thinning, 1 = thinned) on the mean heights of shrubs (significance levels are shown in the respective graphs; NS = not significant)

Plants may also be induced to produce resprouts when the terminal meristem is damaged, irrespective of the damage agent. However, significant differences in numbers of stems among sites may be indicative of site-specific responses of plants. This may be a consequence of differences in species composition, site conditions and burn severity among sites. After-burn inspections indicated that some plants in burnt plots were unaffected by fire since they 'escaped' in islands where grass biomass was either low or absent. The responses of such

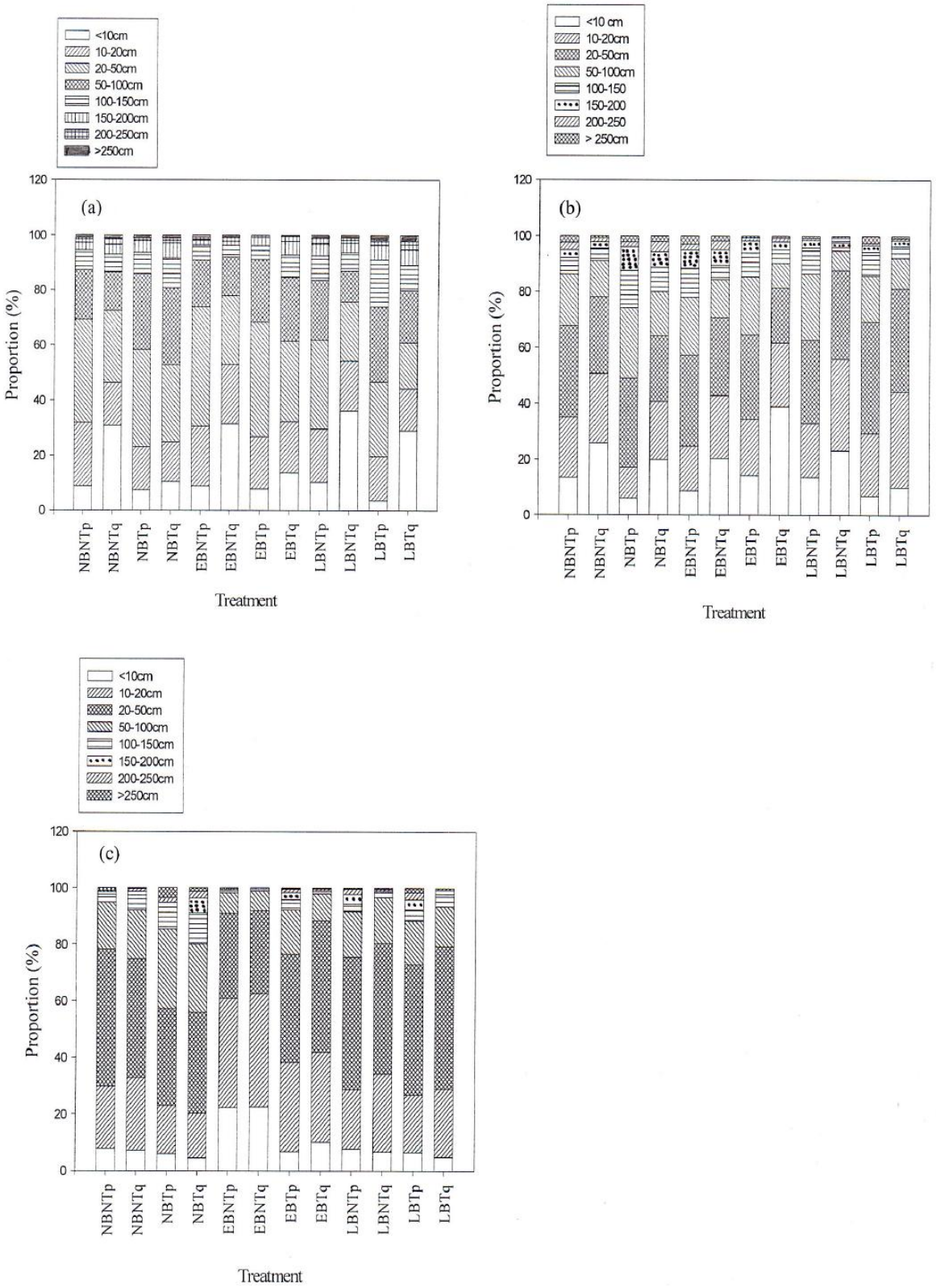

Figure 4. Changes in height class distribution patterns of shrubs and saplings at Airstrip (a), Rongaronga (b) and Samapakwa (c) at the start of the experiment (p) and 3 years later (q). NB = not burnt, EB = early burnt, LB = late burnt, NT = not thinned. T = thinned.

Table 1. Summary of sapling mortality indicating numbers of saplings and their original mean heights subjected to different burning and thinning treatments (NBNT = non-burnt, not thinned; NBT = non-burnt, thinned; EBNT = early burnt, not thinned; EBT = early burnt, thinned; LBNT = late burnt, not thinned, LBT = late burnt, thinned). All 5 species are combined.

Treatment	Number of experimental saplings (n)	Number of dead saplings	Mortality after 3 years (%)	Original height of dead plants (cm) (mean ±SE)
NBNT	177	1	0.6	68.0 (±0.0)
NBT	165	0	0.0	N/A
EBNT	159	7	4.4	58.1 (±13.4)
EBT	149	7	4.7	73.9 (±23.5)
LBNT	170	7	4.1	65.6 (±19.4)
LBT	157	8	5.1	42.0 (±4.9)
Total/overall	977	30	3.1	59.6 (±7.8)

plants would be similar to those under no burn (control) conditions. Increases in numbers of stems due to burning significantly differed among species. *M. glaber* and *P. maprouneifolia* registered above-average increases in numbers of stems. These results are indicative of individualistic, species-specific responses to fire, which was dependent on site. This underlines the importance of local interactions among abiotic and biotic factors in influencing small-scale patterns of individual plant responses to fire in semi-arid environments. This could be brought about by the fire history of each site prior to the experiment. Samapakwa (site K) experienced relatively low fire frequencies compared to Airstrip (site C) and Rongaronga (site D) (Mapaure et al., 2009). Frost (1999a) indicated that in *B. africana* and *T. sericea*, numbers of basal sprouts decreased (after an initial increase) with successive fire events, and attributed the initial increase to a mechanism of maximising carbon gain by plants.

Under conditions of severe fire, resprouting provides a selective advantage to plants, enabling them to quickly re-establish themselves and have competitive advantage over plants which regenerate from seed only (Keeley et al., 1999; Moreira et al., 2012). Increases in the numbers of stems with fire ensures that, through mobilisation of resources from rootstocks or lignotubers, plants regain their above-ground biomass in relatively short time to ensure their survival. In the absence of fire, species that survive by vegetative regeneration from the rootstocks are capable of continuously regenerating their canopy with basal sprouts (Keeley, 1992).

Burning significantly reduced plant heights while thinning led to significant increases in their heights. Interactions among treatments also significantly influenced height responses of plants. Whereas reductions in heights in burnt plots were largely due to top-kill by fire, height decreases in non-burnt plots were due to large mammal browsing. Most *Brachystegia boehmii* and *Burkea africana* saplings were heavily browsed resulting in significant height reductions. Large mammalian herbivores tend to prefer tender, nutritious leaves and shoots such as resprouts due to their high palatability (Holt and Coventry, 1990; Dörgeloh, 1999; Moe et al., 1990). *Brachystegia boehmii* was most affected by elephant browsing, an observation which could further explain its local disappearance from some sites in the area observed by Mapaure and Moe (2009). Frost (1999b) indicated that in drier environments, it was not important whether a fire was early or late but its presence or lack of it made an important difference. This assertion is supported by results of this study where the differences in effects of early and late burns were minimal but only significant between burnt and non-burnt saplings. Belsky (1984) reported similar results where mean heights of tree saplings were significantly reduced by browsing in Serengeti National Park, Tanzania. Height reductions through browsing could outweigh height increases in non-browsed shrubs and saplings resulting in net height reductions recorded during the study.

Observations soon after fire indicated that some plants particularly lower height classes were burnt to ground level but their basal resprouts had reached considerable heights at the time of re-assessments. This was particularly true for *C. spinosa*. In Serengeti National Park, 92 and 68% shrubs less than 1 m and 1-2 m in height, respectively, were burnt back to ground level during fires (Norton-Griffiths, 1979), an observation supporting current findings. Increases in heights after tree thinning can be explained by the reduction in above-ground competition between shrubs/saplings and small trees.

The mean plant height was higher in late burnt than early burnt plots, a finding contrary to expectation. Elsewhere, it was however shown that shrubs resprouting after high intensity fires (often characteristic of late dry season burns) had substantially higher rates of shoot elongation than after low intensity fires (Hodgkinson, 1992). This was influenced by above-average precipitation received after the October burn during the experimental period, resulting in improved shoot regrowth.

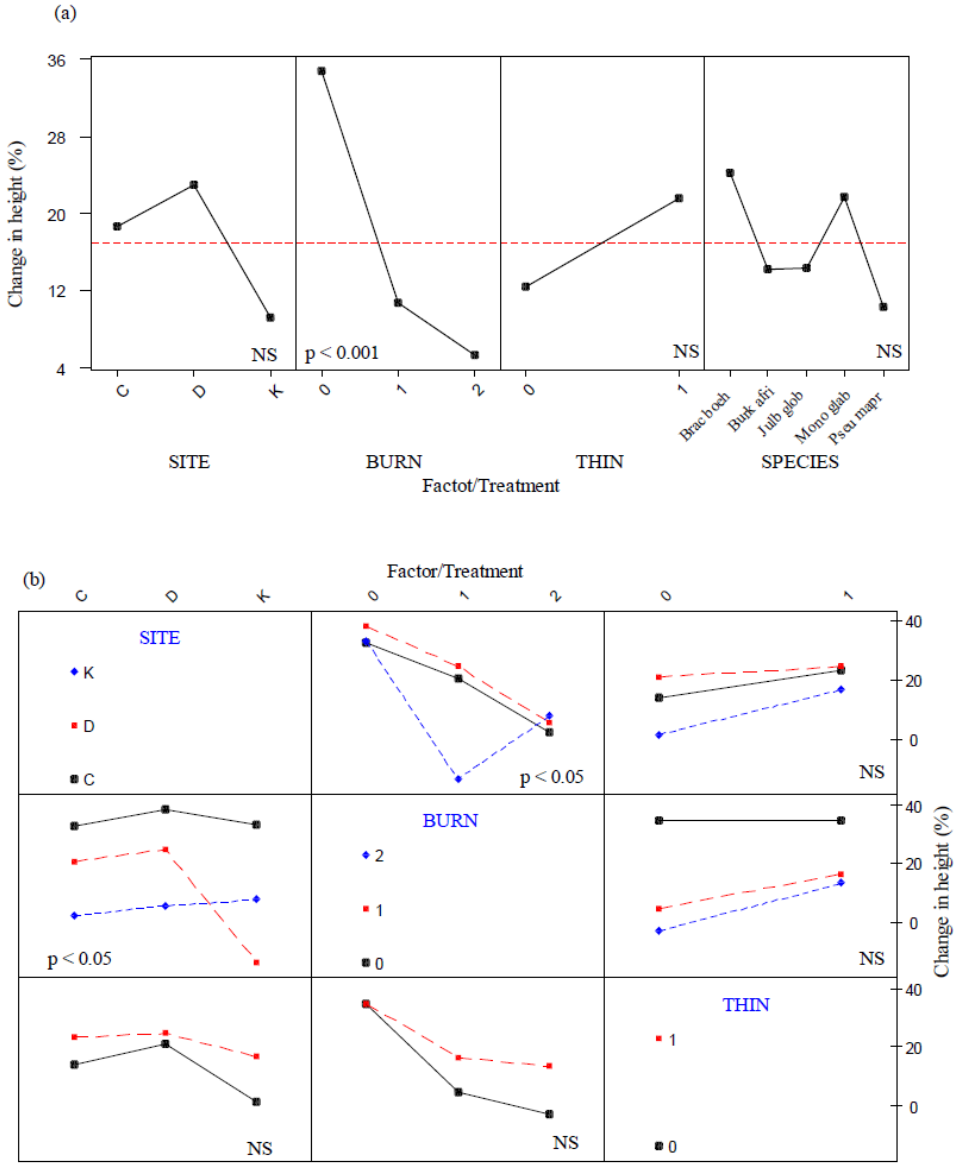

Figure 5. Main effects plots (a) and interactions plots (b) indicating the effects of site (C = Airstrip, D = Rongaronga, K = Samapakwa), burning (0 = no burn, 1 = early burnt, 2 = late burnt) and thinning (0 = no thinning, 1 = thinned) on mean heights of saplings of five common species (Brac boeh = *Brachystegia boehmii*, Burk afri = *Burkea africana*, Julb glob = *Julbernardia globiflora*, Mono glab = *Monotes glaber* and Pseu mapr = *Pseudolachnostylis maprouneifolia*) (Significance levels are shown in the respective graphs; NS = not significant)

Height class distributions indicate a clearer picture of changes in vertical structure of shrub/sapling communities. Increases in relative proportions of plants in lower height classes (<10, 10 to 20 cm) were due to a combination of two processes: First, a net recruitment of new seedlings, particularly *E. africanun* and *J. globiflora*, which led to large increases in numbers of small plants (irrespective of treatment) during the rainy season. Second, plants that were burnt back or top-killed were reduced to lower height classes leading to increases in proportions of plants in the lower height classes. This is consistent with the observation by Griffin and Friedel (1984) who reported significant differences in structure of shrub layers burnt in either season compared to that of non-burnt controls.

There was a consistent height reduction in the range 50-100 cm in non-burnt plots, which can be attributed to browsing. Browsing within this height range was probably

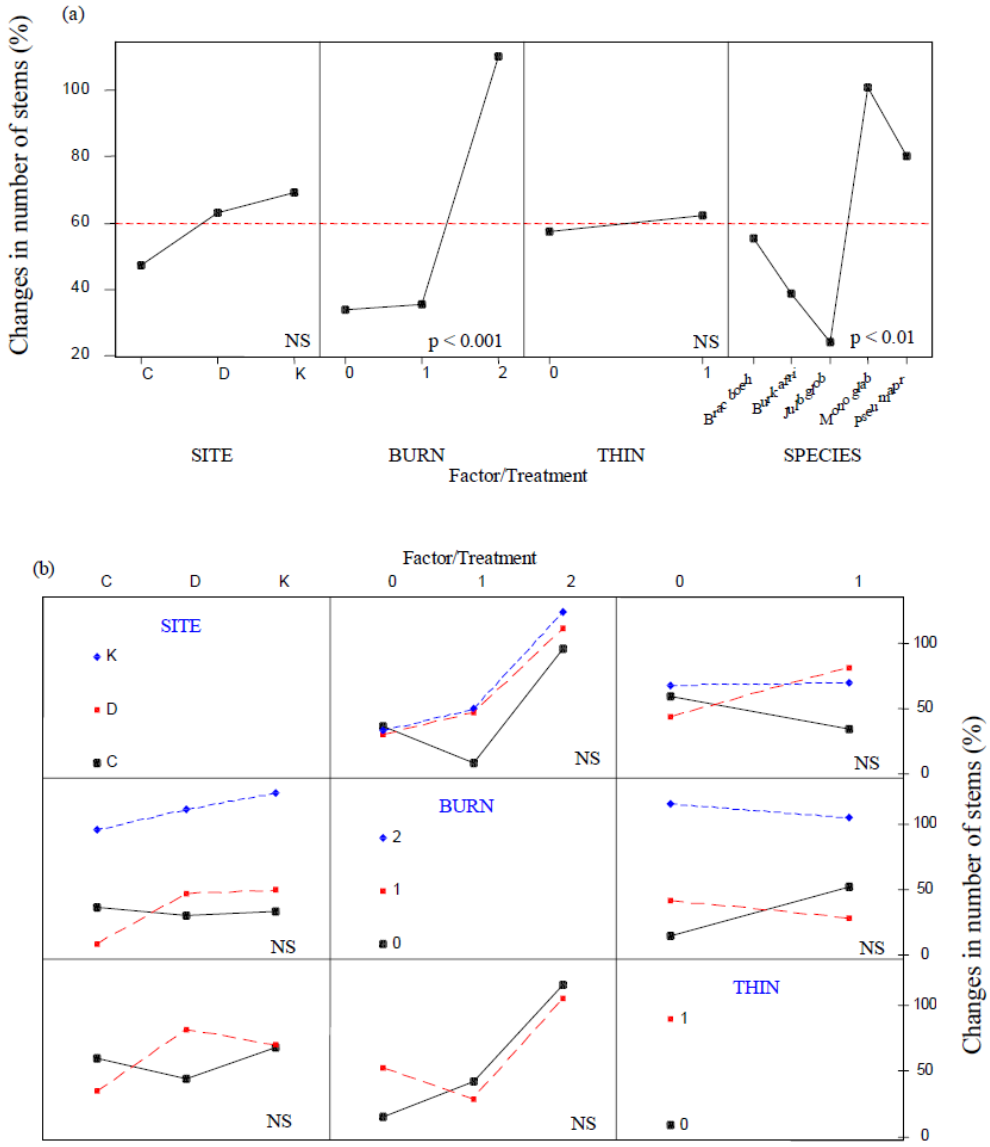

Figure 6. Main effects plots (a) and interactions plots (b) indicating the effects of site (C = Airstrip, D = Rongaronga, K = Samapakwa), burning (0 = no burn, 1 = early burnt, 2 = late burnt) and thinning (0 = no thinning, 1 = thinned) on number of stems of saplings of five common species (Brach boeh = *Brachystegia boehmii*, Burk afri = *Burkea africana*, Julb glob = *Julbernardia globiflora*, Mono glab = *Monotes glaber* and Pseu mapr = *Pseudolachnostylis maprouneifolia*) (Significance levels are shown in the respective graphs; NS = not significant)

by smaller mammals (such as impala) rather than elephants as the latter browse mainly between 1 and 2m (Guy, 1976; Belsky, 1984).

These observations therefore imply that height reductions in burnt plots were not due to fire alone but browsing had a further negative effect. Belsky (1984) showed that a combination of browsing and burning in the Serengeti National Park maintained the height of tree saplings below 30 cm for three years compared to height increases of up to more than 75 cm in non-burnt, non-browsed plots.

Plants taller than 200 cm were less affected by fire, an indication that a critical height for plants to escape fires at current fire intensities in the study area was around this value. Most shrubs and saplings were burnt back to below 50 cm, a height within which they were maintained by frequent fires. Fire-suppressed multi-stemmed plants (often referred to as `gullivers') have been shown to persist in plant communities for long periods (Bond and van Wilgen, 1996; Skowno et al., 1999; Smit et al., 2010).

If the fire-free interval increases, most 'gullivers' grow taller than 200cm and escape the fire trap, a scenario that may explain the increases in woody cover and densities of small trees recorded in the study area over a period of time (Mapaure and Moe, 2009). Hodgkinson (1998) noted that the proportion of plants surviving the passage of fire is determined by the height structure of each population of plant species present.

Monitoring of individual plants through time gives a relatively good indication of mortality as revealed by tagged plants in this study. Thinning of the woody component usually results in an increase in grass biomass (Knoop and Walker, 1985). This in turn leads to more intense fires, especially in the late dry season. It is therefore, expected that there would be higher reductions in heights of saplings in thinned plots, more so in thinned, late burnt plots. However, not all mortality can be attributed to fire as other factors could also have interactively caused plant death. Mortality figures presented in this paper were based only on plants whose tags could be found at the end of the experimental period, and excluded missing tags. It is therefore possible that mortality rates were either under- or over-estimated, depending on whether missed saplings were dead or alive (but not located), respectively. Griffin and Friedel (1984) reported considerable deaths of plants in the absence of fire, particularly in the smallest size-classes. Moisture stress is one of those factors that can cause significant mortality in young plants, even in the absence of fire. This probably happened at Airstrip since fire tended to be patchy at that site. Herbivore foraging on post-fire green flush may have a double effect; trampling of young plants, and heavy browsing on others, rendering them weak and sometimes resulting in death. Since all dead saplings (except one) in this study were in burnt plots, it is however; highly likely that fire was largely responsible for sapling deaths recorded in this study.

Fire-related mortality was recorded only in saplings less than a metre high, and mostly single-stemmed. If height and initial numbers of stems are cautiously taken as surrogates for age, this could indicate that dead saplings were relatively young and thus, of low fire-tolerance due to their limited below-ground biomass. *J. globiflora* and *B. boehmii* appeared particularly susceptible at this young stage compared to other species such as *M. glaber* and *P. maprouneifolia*. Despite being somewhat susceptible to fire at an early stage, *J. globiflora* and *B. boehmii* seemed tolerant once the height was above 50cm but the former species was less browsed by large mammals compared to the latter. This observation could further explain long-term increases in relative importance of *Julbernardia globiflora* reported by Mapaure and Moe (2009) in the area. Current results are consistent with observations from an Australian savanna where a high percentage of established seedlings of a number of species were killed by fire but survival increased with height reaching (depending on species) a maximum of up to 60 cm (Norton-Griffiths, 1979). Most species in savannas are easily killed by fire as seedlings and saplings. However, their resistance and tolerance increases with age up to the point where they become senescent, and again become susceptible (Kruger, 1984). Therefore, it is important for range managers of protected areas to have a holistic approach in managing the woodland-fire-herbivore system and to treat different patches of miombo woodland differently due to their varied responses to similar treatments.

Conclusions

This study has shown that the lower stratum of woody layer in semi-arid miombo woodland responds differently to fire treatments, depending on local site conditions and post-fire herbivore browsing. Thinning of the trees led to increases in heights of saplings and shrubs due to reduced above ground completion with trees. Burning, especially late burning, led to significant increases in numbers of stems through basal resprouting.

Resprouting provides a selective advantage to plants by enabling them to quickly re-establish themselves and ensures that the plants regain their above-ground biomass in a relatively short period of time to ensure their survival. Burning significantly reduced heights of plants and resulted in significant changes in the height structure of the lower woody stratum of shrubs and saplings. This is mainly due to the fact that frequent burning keeps shorter plants in a fire trap, and with natural recruitment during the wet season, there tends to be increases in proportions of shorter plants.

This study has also shown that different species may respond differently to fire, largely due to their individual life-history characteristics. Mortality of saplings due to fire was generally lower than anticipated, showing that these species are tolerant to fire and have adaptive life history characteristics to survive fires. Hence, fire management practices in semi-arid miombo woodlands must take cognisant of the differential responses of individual plant species to fire as well as differences in site characteristics the lead to differences in plant responses.

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Anti-emetic activity of some members of the family, Euphorbiaceae of Lahore region

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The present investigation aimed to evaluate the anti-emetic activity of some members of the family Euphorbiaceae of Lahore region. The selected plants were *Euphorbia prostrata, Euphorbia splendens, Euphorbia hirta, Euphorbia helioscopia, Ricinus communis, Jatropha integerrima, Putranjiva roxburghii* and *Phyllanthus emblica*. Anti-emetic activity was carried out by the preparation of aqueous extract of these plants. Anti-emetic investigations showed that aqueous extracts of *E. prostrata*, *P. roxburghii* and *P. emblica* have high potential to reduce the frequency of retching in $CuSO_4$ induced emesis in four days old chicks. Frequency of retching after treatment of aqueous extracts of *E. prostrata*, *P.a roxburghii* and *P. emblica* were 24.8 ± 0.89, 17.4 ± 0.89, 15.2 ± 0.58, respectively, which statistically proved significant at $p<0.01$ from the control (87.2 ± 1.46, 82.6 ± 1.46, 82.6 ± 1.45, respectively) values.

Key words: Antiemetic, Euphorbiaceae, $CuSO_4$.

INTRODUCTION

The use of plants by traditional people has started long time ago, which has laid basis for the discovery of modern medicines. Some years earlier, mankind was well aware of the medicinal characteristics of few plants growing around him (Sofowora, 1982; Hill, 1989). Drug discovery programs have started from the earliest times of mankind's history (Cotton, 1996).

A chemical substance that induces motion sickness, nausea and vomiting is called emetic. Emesis occurs in such cases when a person ingests certain chemical substances such as salt water, mustard water and copper sulphate, zinc sulphate, yellow mercuric subsulphate, alumen and apomorphine. When a toxic substance has been ingested immediately, an emetic is used for their removal from the body (Decker, 1971; Moder and Hurley, 1991).

An anti-emetic is a drug that is widely used to control motion sickness, severe nausea and vomiting. Various types of anti-emetic drugs are used to control nausea and vomiting. Some of these works on the brain by preventing the activation of the medullar chemoreceptor trigger zone or vomiting centre and other drugs work on the gut by speeding up the rate at which the stomach empties and so help to move food through the intestines more quickly (Zachary et al., 2004).

The family Euphorbiaceae has been selected because many of the members of family Euphorbiaceae has been used as folk medicine in the treatment of different diseases like *Croton tiglium* used as a pain reliever and dry cough (Koche et al., 2010) and *Eclipta alba* (Saxene et al., 1993), *Euphorbia fusiformis* used for Hepatitis (Anusuya et al., 2010), etc.

METHODOLOGY

Selection of plants

Eight plants were selected (*Euphorbia prostrata, Euphorbia*

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splendens, Euphorbia hirta, Euphorbia helioscopia, Ricinus communis, Jatropha integerrima, Putranjiva roxburghii and *Phyllanthus emblica*) of the family Euphorbiaceae for the study of anti-emetic activity. These plants were selected on the basis of easy approach, easy growth and easy availability and mainly on the basis of ethnomedicinal importance.

Collection of plants

These plants were collected from different areas of Lahore (Lahore College for Women University, Lahore, Lawrence Garden Lahore and Harbanspura, Lahore). These plants were authenticated by Dr. Tahira Aziz Mughal Assistant Professor in Botany Department of Lahore College for Women University, Lahore. Plant specimens were deposited as voucher specimen in Prem Madam Herbarium of Lahore College for Women University, Lahore, for the record and further reference.

Preparation of plant material

Drying and grinding of plants

The plant material was washed with water and dried in shade, then cut into small pieces and ground in an electrical grinder to be obtained in a powder form.

Extraction

Aqueous extract preparation

The crude aqueous extract of the powdered plant material was prepared by using the standard methods (Onyeyili et al., 2001). Twenty grams of the powdered plant material was mixed with 100 ml of distilled water in a 1 L beaker and boiled for 1.5 h. After boiling, it was allowed to cool at room temperature and then filtered by using Whatman 1 filter paper.

Evaporation of solvent

The solvent was removed by rotary evaporator to obtain crude extracts of plant materials. These extracts were stored at 4°C.

Preparation of stock solution

Stock solution was prepared from crude extracts of plants and stock solution was further diluted into different dilutions according to the requirement of the activity either in milligram or microgram.

Anti-emetic activity

Anti-emetic activity of the plant extracts was examined by the methodology of Yang et al. (1999).

Selection of animals

Four days old chicks (male) weighing 35 to 45 g were selected for anti-emetic activity. They are purchased from Tollinton Market, Lahore.

Preparation of animal house for chicks

Chicks were kept in a wire cage. The wire cage was 17 inches in length and 15 inches in width. The chick housing facility was maintained at standard conditions. Water pot was kept in chick house. Chicks need at least two inches of bedding material beneath them, so straw or dry leaves were spread beneath them.

Feeding routine

Chicks were fed at regular intervals with poultry feeding purchased from Tollinton Market Lahore. The feed was left with them all the time because they will stop eating when they have had enough.

Record of chicks

The information about each chick's group and its age, weight (grams) was recorded at the start and at end of the experiment.

Chemical used to induce the emesis

$CuSO_4$ was used to induce the emesis. For this purpose 1% $CuSO_4$ was prepared by dissolving 1 g $CuSO_4$ in 100 ml distilled water.

Chemical used as diluter

0.9 % saline water drip (Medisol Pvt Ltd, Pakistan) of 1 liter was used.

Preparation of dilutions

Four different concentrations (25, 50, 75 and 100 mg/kg) from crude aqueous plant extract were prepared in saline water.

Induction of emesis

Emesis was induced by 1% $CuSO_4$ in the chicks orally by 1 ml/20 g body weight.

Experimental plan

The antiemetic activity was determined by calculating the mean decrease in number of retching in contrast with those of the control. All the young male chicks were divided into 12 groups (A, B, C, D, E, F, G, H, I, J, K and L) and groups B, C, D, E, F, G, H, and I were further divided into subgroups. Each group or subgroups had 3 chicks.

Group A

The group A treated as control, was not given any treatment.

Groups B, C, D, E, F, G, H and I

These groups of chicks were administrated with 1% $CuSO_4$ to induce emesis and after 10 min the number of retching reflexes or emetic action was recorded, and then plant extracts were given in the form of dilution of 25, 50, 75, and 100 mg/kg with the help of a dropper.

Group B

Chicks of group B were administered aqueous extract of *E. prostrata*. This group was was further divided into four subgroups.

B_1: Chicks administered with aqueous extract of *E. prostrata* at the dose 25 mg/kg.
B_2: Chicks administered with aqueous extract of *E. prostrata* at the dose 50 mg/kg.
B_3: Chicks administered with aqueous extract of *E. prostrata* at the dose 75 mg/kg.
B_4: Chicks administered with aqueous extract of *E. prostrata* at the dose 100 mg/kg.

Group C

Chicks of group C were treated with aqueous extract of *E. splendens*. This group was further divided into four subgroups.

C_1: Chicks treated with aqueous extract of *E. splendens* at the dose 25 mg/kg.
C_2: Chicks treated with aqueous extract of *E. splendens* at the dose 50 mg/kg.
C_3: Chicks treated with aqueous extract of *E. splendens* at the dose 75 mg/kg.
C_4: Chicks treated with aqueous extract of *E. splendens* at the dose 100 mg/kg.

Group D

Aqueous extracts of *E. hirta* was given to chicks of group D. This group was further divided into four subgroups.

D_1: 25mg/kg aqueous extract of *E. hirta* was given to chicks.
D_2: 50mg/kg aqueous extract of *E. hirta* was given to chicks.
D_3: 75mg/kg aqueous extract of *E. hirta* was given to chicks.
D_4: 100mg/kg aqueous extract of *E. hirta* was given to chicks.

Group E

Therapeutically, aqueous extract of *E. helioscopia* was given to the chicks of group E. This group is also divided into four subgroups (E_1, E_2, E_3, and E4) on the basis of dosage concentration (25, 50, 75and 100 mg/kg).

Group F

Chicks of group F received the treatment of aqueous extract of *R. communis*. Group F chicks were further divided into four subgroups (F_1, F_2, F_3, and F_4) on the basis of different dosage concentration.
F_1 received the treatment of 25 mg/kg, while F_2, F_3, and F_4 received the dosage of 50, 75 and 100 mg/kg, respectively.

Group G

Chicks of group G received an application of aqueous extract of *J. integerrima*. This group was further divided into four subgroups.

G_1: Chicks received aqueous extract of *J. integerrima* at the dose 25 mg/kg.
G_2: Chicks received aqueous extract of *J. integerrima at* the dose 50 mg/kg.
G_3: Chicks received aqueous extract of *J. integerrima* at the dose 75 mg/kg.
G_4: Chicks received aqueous extract of *J. integerrima* at the dose 100 mg/kg.

Group H

Aqueous extract of *P. roxburghii* was given to chicks of group H. This group was further divided into four subgroups.

H_1: Aqueous extract of *P. roxburghii* at the dose of 25 mg/kg was given to chicks.
H_2: Aqueous extract of *P. roxburghii* at the dose of 50 mg/kg was given to chicks.
H_3: Aqueous extract of *P. roxburghii* at the dose of 75 mg/kg was given to chicks.
H_4: Aqueous extract of *P. roxburghii* at the dose of 100 mg/kg was given to chicks.

Group I

Chicks of group I were treated with methanolic extract of *P. emblica*. This group was further divided into four subgroups.

I_1: Chicks therapeutically treated with 25 mg/kg aqueous extract of *P. emblica*.
I_2: Chicks therapeutically treated with 50 mg/kg aqueous extract of *P. emblica*.
I_3: Chicks therapeutically treated with 75 mg/kg aqueous extract of *P. emblica*.
I_4: Chicks therapeutically treated with 100 mg/kg aqueous extract of *P. emblica*.

Group J

Chicks of group J were administrated 50 mg/kg Motilium or Domperidone (Janssen and Janssen Pakistan (Pvt) Ltd.) which served as positive control.

Group K

125 mg/4 ml Gravinate or Dimenhydrinate syrup (Searle pharmaceutical Pakistan (Pvt) Ltd.) was given to chicks of group K which served as positive control.

Group L

Chicks of group L weretreated with 50 mg/kg Metodine or Diiodohydroxyquinoline which served as positive control.

Dosage administration

After dosage administration, the results were recorded by counting number of retching reflexes for the next 10 min.

Calculation of antiemetic activity

The percent inhibition was calculated by the following formula: Inhibition (%) =A-B/A × 100

Where A is frequency of retching before treatment and B is frequency of retching after plant treatment.

Statistical application

The standard error means (SEM) and statistical significance difference was determined by unpaired student t-test with the help of statistical software SPSS 19.

DISCUSSION

Phytochemical screening showed the presence of alkaloids, phytosterol, phenols, flavonoid, tannins and phlobatannins in the extracts of *E. prostrata*, *E. splendens*, *E. hirta*, *E. helioscopia*, *R. communis*, *J. integerrima*, *P. roxburghii* and *P.s emblica* (Tahira et al., 2010).

Table 1. Anti-emetic effect of aqueous extract of *E. prostrata* on copper sulfate induced emesis in young chicks.

Concentration of plant extract (mg/kg)	Average weight (g)	*Mean ± S.E.M. of no. of retching/10 min		Inhibition (%)	Remark
		Before plant treatment	After plant treatment		
25	39.5	66.7 ± 2.03	36.8 ± 2.03	44.8	Insignificant
50	37.4	72.4 ± 1.46	34.3 ± 0.89	52.6	Significant
75	35.8	67.3 ± 2.03	26.7 ± 0.67	60.3	Significant
100	40.7	87.2 ± 1.46	24.8 ± 0.89	71.5	Significant

*Significantly different from the control value p<0.01.

Table 2. Anti-emetic effect of aqueous extract of *E. splendens* on copper sulfate induced emesis in young chicks.

Concentration of plant extract (mg/kg)	Average weight (g)	*Mean ± S.E.M. of no. of retching/10 min		Inhibition (%)	Remark
		Before plant treatment	After plant treatment		
25	38.2	56.3 ± 1.77	33.6 ± 2.03.	40.3	Insignificant
50	42.8	60.4 ± 1.34	32.7 ± 1.46	45.8	Insignificant
75	44.5	65.7 ± 1.77	31.3 ± 1.77	52.3	Insignificant
100	36.7	66.7 ± 2.03	25.4 ± 0.89	61.9	Significant

*Significantly different from the control value p< 0.01.

Table 3. Anti-emetic effect of aqueous extract of *E. hirta* on copper sulfate induced emesis in young chicks.

Concentration of plant extract (mg/kg)	Average weight (g)	*Mean ± S.E.M. of no. of retching/10 min		Inhibition (%)	Remarks
		Before plant treatment	After plant treatment		
25	37.3	65.2 ± 1.21	43.7 ± 1.21	32.9	Insignificant
50	35.6	79.3 ± 1.46	46.4 ±1.46	41.4	Insignificant
75	39.8	59.4 ± 1.21	28.7 ± 0.89	51.6	Significant
100	42.4	78.2 ± 1.21	31.3 ± 0.89	59.9	Significant

*Significantly different from the control value p< 0.01.

Four days old chick is a useful animal model selected for anti-emetic effects because this animal possesses acute emesis that is observed in man. This animal also serves as a useful animal for evaluating the involvement of the brain in the observed anti-emetic effects of the extracts (Tijani et al., 2008).

Emesis (nausea and vomiting) is caused by interaction of gastrointestinal system, the vestibular system and the various areas of the brain like chemoreceptor trigger zone (CTZ) in the brain (Gan et al., 2003). Emesis occurred due to copper sulfate, because copper sulphate causes the excitation of visceral afferent nerve fibers of gastrointestinal tract. It has also been observed that the peripheral $5HT_4$ plays a major role in copper sulphate induced emesis (Andrews et al., 1990). An aqueous extract of *E. prostrata* exhibited significant inhibition (71.5%) of emesis at the dose of 100 mg/kg (Table 1).

Lower concentrations of aqueous extract of *E. splendens* showed insignificant results but at the dose of 100 mg/kg it showed significant inhibition (61.9%) of emesis (Table 2). 59.9% inhibition of emesis was observed by the therapeutic treatment of aqueous extract of *E. hirta* at the dose 100 mg/kg (Table 3). An aqueous extract of *E. helioscopia* at the dose of 25, 50, 75 and 100 mg/kg exhibit 23.5, 35.1, 49.8 and 55.2% inhibition of emesis respectively (Table 4). The frequency of retching in young chicks reduced (61%) significantly by the administration of aqueous extract of *R. communis* only at higher concentration (100 mg/kg) (Table 5). An aqueous extract of *J. integerrima* therapeutically reduced the copper sulfate induced emesis in young chicks by increasing the concentration of plant extract (Table 6). Significant inhibition of emesis observed by the application of aqueous extract of *P. roxburghii* even at lower concentration like 25 mg/kg showed 61.7% inhibition of emesis in young chicks (Table 7). An aqueous extract of *P. emblica* at the dose of 25, 50, 75 and 100 mg/kg showed 59.9, 63.7, 70.8 and 81.6 % inhibition of emesis, respectively (Table 8).

The most important findings of the present study are that, all the selected plants significantly reduced the frequency of copper sulphate induced retching in four days old chicks as compared to commercially available medicines: Gravinate, Metodine and Motilium (Table 9).

Table 4. Anti-emetic effect of aqueous extract of *E. helioscopia* on copper sulfate induced emesis in young chicks.

Concentration of plant extract (mg/kg)	Average weight (g)	*Mean ± S.E.M. of no. of retching/10 min		Inhibition (%)	Remark
		Before plant treatment	After plant treatment		
25	42.7	75.2 ± 1.46	57.5 ± 1.16	23.5	Insignificant
50	36.9	71.8 ± 1.77	46.6 ± 1.86	35.1	Insignificant
75	39.4	78.4 ± 1.46	39.3 ± 0.89	49.8	Significant
100	41.7	76.5 ± 1.21	34.2 ± 0.89	55.2	Significant

*Significantly different from the control value p< 0.01.

Table 5. Anti-emetic effect of aqueous extract of *R. communis* on copper sulfate induced emesis in young chicks.

Concentration of plant extract (mg/kg)	Average Weight (g)	*Mean ± S.E.M. of no. of retching/10 min		Inhibition (%)	Remark
		Before plant treatment	After plant treatment		
25	40.6	78 ± 1.53	55 ± 1.77	29.4	Insignificant
50	35.3	75 ± 1.74	46.4 ± 1.77	38.2	Insignificant
75	44.8	79.7 ± 1.23	41.7 ± 1.86	47.7	Insignificant
100	40.5	63.4 ± 1.21	24.7 ± 0.89	61	Significant

*Significantly different from the control value p< 0.01.

Table 6. Anti-emetic effect of aqueous extract of *J. integerrima* on copper sulfate induced emesis in young chicks.

Concentration of plant extract (mg/kg)	Average weight (g)	*Mean ± S.E.M. of no. of retching/10 min		Inhibition (%)	Remark
		Before plant treatment	After plant treatment		
25	36.8	77.5 ± 1.76	41.4 ± 1.21	46.5	Insignificant
50	37.4	70.7 ± 1.21	29.2 ± 0.77	58.6	Significant
75	43.2	77.8 ± 1.86	28.2 ± 0.89	63.7	Significant
100	40.5	87.6 ± 1.77	31.3 ± 0.89	64.2	Significant

*Significantly different from the control value p< 0.01.

Table 7. Anti-emetic effect of aqueous extract of *P. roxburghii* on copper sulfate induced emesis in young chicks.

Concentration of plant extract (mg/kg)	Average weight (g)	*Mean ± S.E.M. of no. of retching/10 min		Inhibition (%)	Remark
		Before plant treatment	After plant treatment		
25	36.5	67 ± 2.31	25.7 ± 0.89	61.7	Significant
50	40.8	78.4 ± 1.46	25.4 ± 0.89	67.6	Significant
75	43.4	88.3 ± 1.21	25.7 ± 0.89	70.8	Significant
100	44.3	82.6 ± 1.46	17.4 ±0.89	78.9	Significant

*Significantly different from the control value p< 0.01.

Table 8. Anti-emetic effect of aqueous extract of *P. emblica* on copper sulfate induced emesis in young chicks.

Concentration of plant extract (mg/kg)	Average weight (g)	*Mean ± S.E.M. of no. of Retching/10 min		Inhibition (%)	Remark
		Before plant treatment	After plant treatment		
25	37.8	78.4 ± 1.21	31.3 ± 0.89	59.9	Significant
50	38.5	77.8 ± 1.86	28.2 ± 0.89	63.7	Significant
75	39.8	88.3 ± 1.21	25.7 ± 0.89	70.8	Significant
100	41.4	82.6 ± 1.45	15.2 ± 0.58	81.6	Significant

*Significantly different from the control value p< 0.01.

Table 9. Anti-emetic effect of commercial medicines on copper sulfate induced emesis in young chicks.

Commercial Medicines	Average weight (g)	*Mean ± S.E.M. of no. of Retching/10 min		Inhibition (%)	Remark
		Before plant treatment	After plant treatment		
Motilium (50 mg/kg)	35.7	87.6 ± 1.76	17.4 ± 0.89	80.9	Significant
Gravinate (125 mg/4 ml)	44.6	65.7 ± 1.76	11.7 ± 0.89	82.2	Significant
Metodine (50 mg/kg)	37.9	66.7 ± 2.03	10.6 ± 0.89	84.6	Significant

*Significantly different from the control value $p < 0.01$.

These medicines showed anti-emetic activity by acceleration of gastrointestinal tract movement (Akita et al., 1998).

The observed anti-emetic activity of plant extracts in the present study may be attributed to its phytochemical (alkaloids, phytosterol, phenols, flavonoid, tannins and phlobatannins) constituents, like in previous studies, shogaol and gingerols isolated from *Zingiber officinale* showed the anti-emetic activity (Akita et al., 1998) and diarylheptanoids isolated from *Alpinia officinarum* (Itokawa et al., 1981a, 1985b; Uehara et al., 1987), *Alpinia conchigera*, *Alpinia oxyphylla* (Itokawa et al., 1982c; Shoji et al., 1984), *Alpinia bleparocalyx* (Kadota et al., 1994), and *Curcuma comosa* and *Curcuma xanthorrhiza* (Uehara et al., 1987) showed highest anti-emetic activity. These plants have been used in Chinese traditional medicine as the anti-emetics.

Therefore, it is concluded that these plants *E. prostrata, E. splendens*, *E. hirta*, *E. helioscopia*, *R. communis*, *J. integerrima*, *P. roxburghii* and *P. emblica* showed significant anti-emetic activity and thus provides the basis for their use in traditional or folk medicines for the treatment of emesis.

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Testing of striga resistant composite maize varieties for response to two levels of nitrogen fertilizer up-take

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Trials were carried out using five composite maize varieties in *a Striga lutea* (Lour) endemic soil of Temidire-Eruwa, Oyo State, Nigeria, in 2004 and 2005. The composite maize varieties were tested under varied fertilizer types, nitrogen (N) concentrations and artificial striga infestation. The objective was to test these striga resistant maize varieties for yield and agronomic performance under the above conditions. The result showed that, variety and year of evaluation differed significantly (P<0.01) for almost all agronomic characters such as plant stand, days to anthesis (silking and tasselling), plant and ear heights as well as root and stalk lodging resistance at both 100 and 200 kgN/ha. Variety x year interaction were also significantly different (P<0.01) for all agronomic characters except root lodging and husk tip cover. Fertilizer type differed only for days to silking, plant height and plant aspect, while year x nitrogen source and variety x nitrogen source was highly significant (P<0.01) for field weight. Plant stands were generally better in 2004 than 2005 for all varieties except Acr 97syn-W and DMR-LSR-W. The composite maize varieties were able to tolerate high N-concentration except DMR-LSR-Y which do not utilize excess nitrogen above 100 kgN/ha. Use of striga resistant maize varieties concomitantly with nitrogen fertilizer is recommended for farmers in *S. lutea* endemic ecology, for higher grain yield.

Key words: Composite maize, fertilizer type, nitrogen (N) concentrations, striga resistance, yield.

INTRODUCTION

Maize is an important cereal crop in the farming system of tropical and subtropical Africa. It constitutes the bulk of the diet of people and livestock as a source of energy for survival and growth. Maize is adaptable to almost all agro-ecologies of Nigeria where moisture is adequate for its growth (Olakojo and Olaoye, 2006). Among the major problems of maize production especially in the southern and Northern Guinea savanna is striga parasitic weed, a pest capable of reducing yields by between 30 and 70% and sometimes results in total crop failure especially in the endemic area of maize producing belts of Nigeria. Many workers had suggested various control measures for striga. These include: the use of nitrogen fertilizer (Igbinnosa et al, 1996; Kim and Tanimonure, 1993; Kim and Adetimirin, 1997; Olakojo et al., 2001), breeding for striga resistance crop genotypes (Kim, 1994; Ogunbodede and Olakojo, 2001; Olakojo and Olaoye, 2005, 2006a, b) and the use of striga resistant genotypes in combination with adequate moisture supply (Olakojo and Olaoye, 2006).

Other suggested control measures include: cultural control method using manual hoeing, pulling of striga seedlings from the field before flowering and crop rotation using leguminous crops such as cowpea and soybean on maize fields in alternating years (Parkinson and Boxque-Perez, 1988). Use of suicidal striga germination or trap and catch cropping, whereby the striga seeds are stimulated to growth by planting susceptible host plant

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like sorghum and maize, striga seedlings, are then ploughed off before flowering with the susceptible host plant. This method is very effective at reducing striga seed density in the soil if practice for at least three consecutive years (Bashir, 1987).

Our experience at Moor Plantation, however, shows that integrated control method seems to be the best. The present study therefore made use of both resistant host plants and nitrogen fertilizers to assess yield performance of maize under artificial striga infestation and varied N-concentrations. The objective of the study therefore was, to test some striga resistant composite maize varieties under varied fertilizer types and N concentrations for yield and agronomic performance, with a view to making valid recommendation for control of *Striga lutea* in Southern Guinea savanna of Nigeria.

MATERIALS AND METHODS

Inoculation and planting of maize

Five composite maize varieties (Acr 97 syn-Y, Acr 97 sym-W, DMR-ESR-Y, DMR-LSRY and DMR-ESR-W) were selected and tested in Temidire, Eruwa, *S. lutea* endemic area of Oyo State, Nigeria. Land preparation was done mechanically by ploughing, harrowing and ridging, using tractor. Seeds were planted in four-row plots of 3 x 5 m at 75 x 50 cm at 2 seeds per hill to obtain a plant population of 53, 333 stand/ha. Planting was done under natural infestation in a highly endemic soil. The 200 kg N treated plots were directly opposite the 100 kg N treated maize plots.

Design

The design was a factorial plot design of 5 x 4 where maize varieties was the main plot, having five varieties and four fertilizer types as sub plots. The evaluations were carried out under the two separate nitrogen concentrations, 100 (low) and 200 kg N (high) per hectare. The nitrogen concentration was not taken as a factor, but as a treatment. `The trial was carried out for 2 years (2004 and 2005) and was a randomized complete block design (RCBD) with 3 replications. NPK 15-15-15, urea, urea+NPK, fertilizers were used while none fertilizer treated plot served as control. They were applied at 100 and 200kg N/ha.

Data taken

The following parameters were taken from the two middle rows of each plot: Plant stand, days to 50% silking and pollen shed, plant and ear heights (cm), root and stalk lodging, husk tip cover, plant aspect, (using a rating of 1-5, where 1 = excellent and 5 = poor), plant harvest and field weight (kg/plot) according to Kim (1994).

Data analysis

Data were analyzed using Mstat, to compute analysis of variance (ANOVA) and significant differences were determined at probability levels of 5 and 1%. Significant interactive means were separated using standard error at $P<0.05$, while differences in character means were determined at $P<0.05$.

RESULTS

Table 1 presents mean squares (MS) for maize agronomic characters under varied fertilizer types (N) and concentrations. Years of evaluation differed significantly for all agronomic characters such as plant stand, days to silking, days to pollen shed, plant and ear heights as well as root and stalk lodgings for both 100 and 200 kg N/ha at $P<0.05$ and/or 0.01. Variety (V) also differed significantly for maize agronomic characters at both levels of N- concentration except for plant aspect, root and stalk lodging, as well as husk tip cover which were significantly different from one variety to another. The magnitude of MS for plant and ear heights and plant stand were generally larger than the other traits. For example, year of evaluation recorded MS of 18302.70 and 9381.08, respectively for plant height under low and high N concentrations as against 18.4 each for stalk lodging at low and high nitrogen concentrations.

Response of maize varieties to nitrogen sources were significantly different for days to silking, plant heights and plant aspect at $P<0.05$ and 0.01 for low and high N concentrations. Year x variety interaction was significant for plant stand, days to silking and pollen shed under 100 kgN/ha, as well as ear height under 200 kgN/ha. First order interactions of year x nitrogen were not significant for any of the agronomic maize characters at both levels of N-concentrations (Table 1).

Mean square (MS) for the interaction of variety x year for yield related characters are presented in Table 2. Years of evaluation were significantly different for plant harvest and field weight at both nitrogen levels with means of 896.53 and 1613.33 for plant harvest and, 11.49 and 2.38 for field weight at low and high N-concentrations, respectively. Variety was significantly different for plant harvest and field weight only at 100 kg/ha N concentration. Variety x year interactive means for these two characters were also significant at low (100 kg/ha N) concentration, significant field weight was obtained at high level of N. Similarly, year x nitrogen source and variety x nitrogen source were significant for field weight at low N-concentration (Table 2). The MS magnitudes of plant harvest were fairly larger than that of field weight in all sources of variation assessed. Second order interaction of Y x V x N were not significant for either of these two yield characters.

Table 3 presents the interactive means of variety x year interaction for maize agronomic characters. Varieties differed significantly from one year of evaluation to another for plant stand, days to silking, days to pollen shed and ear height at $P<0.05$. Plant establishment count (plant stand) was generally better in year 2004 as compared to 2005, in all varieties except for Acr 97 Syn-W and DMR-ESR-W with higher plant stand in year 2005 than 2004. In year 2004, Acr 97 Syn-W recorded plant stand of 15.00 as compared to 18.0 in 2005, similarly, DMR-ESR-W recorded 17.6 in 2004 as compared to

Table 1. Mean square (MS) for maize agronomic characters of *S. lutea* tolerant maize varieties under artificial striga infestation, fertilizer types and N concentrations.

Source of variation	DF	Plant stand	Days to silking	Days to pollen shed	Plant height	Ear height	Plant aspect	Root lodging	Sta1k lodging	Husk tip
Replicate (R)	2	448.93 (505.08)	44.12 (17.43)	16.24 (23.25)	3421.9 (4711)	2513.26 (1351.27)	1.25	3.80	0.11 (5.16)	0.83
Year (Y)	1	112.13* (40.83)	16.13** (13.33**)	31.00** (1.41)	18302.70** (9381.08**)	(2548.40** (816.40*)	1.00	7.0*	18.40** (18.4**)	0.01
Variety (V)	4	514.84** (414.49**)	1782.80** (244.12**)	230.36** (172.97**)	2017.11** (2568.05**	399.28* (981.59**)	0.22	2.67	1.03 (0.65)	0.17
Y x V	4	290.78** (261.62**)	269.20** (43.12**)	94.69** (54.80**)	168.28 (790.82)	297.78 (664.05**)	0.22	2.69	1.03 (1.38)	0.36
Nitrogen source (N)	3	0.13 (4.67)	9.20** (1.26)	0.18 (0.67)	1211.94* (218.74)	398.40 (92.23)	0.83*	0.94	0.18 (0.16)	0.07
Y x N	3	0.13 (4.69)	9.20 (1.26)	0.18 (0.67)	343.50 (141.07)	10.54 (285.76)	0.83	0.34	0.18 (0.16)	0.18
V x N	12	1.44 (6.80)	29.46 (2.55)	3.98 (2.84)	454.00 (314.49)	130.31 (188.79)	0.17	0.96	0.17 (0.39)	0.17
Y x V x N	12	1.45 (6.80)	29.46 (2.55)	3.98 (2.84)	120.11 (281.24)	74.33 (171.88)	0.15	1.05	0.17 (0.3A)	0.26
Error	78	26.89 (43.88)	251.78 (2.90)	2.85 (3.95)	430.4 (378.23)	160.61 (188.32)	0.29	1.49	0.62 (0.48)	0.28
Total	119									

*, ** Significant at P<0.05 and 0.01 respectively; + Figures in parenthesis are for 200 kgN while those outs of parenthesis are for 100 KgN. DF = Degree of freedom.

22.8 in 2005. Days to silking was generally longer in year 2004 as compared to 2005 in almost all varieties ranging from 50.0 to 59.6 in 2004 and 49.5 to 56.5 in 2005. DMR-LSR-Y was however, found to be responsible for the significant differences in its ANOVA, with 59.6 days to silking in 2004 as against 54.7 in 2005. In the same vein, days to pollen shed varied for DMR-LSR-Y in the two years. While it took 61 days for pollen to shed in 2004, this was reduced to 53.7 days in 2005 (a difference of 7.6 days). Acr97 syn-W was found to be responsible for significant differences observed in these maize varieties for ear height with a mean of 53.0 cm in 2004 and 69.2 cm in 2005. All other varieties tested recorded significantly higher ear heights in 2005 as compared to 2004 (Table 3) except DMR-LSR-Y with a slight decrease in ear height in 2005. Across variety means were generally higher in 2004 for all agronomic characters with 22.4, 54.0 and 54.3 for plant height, days to silking and days to pollen shed, respectively.

Interactive means of nitrogen x year for ear aspect are presented in Table 4. Mean ear aspects for all varieties were generally favored by NPK in year 2005 by nitrogen from all sources with reduced ear aspects of 2.5, 2.2 and 2.3 for NPK, urea and NPK + urea in 2004 as against 2.24, 2.3

Table 2. Interactive means of variety x year interaction for maize yield characters.

Source of variation	DF	Plant harvest	Field weight
Replicate (R)	2	893.02 (751.40)	0.15 (0.23**)
Year (Y)	2	896.53**(1613.33**)	11.49**(2.38**)
Variety (V)	1	147.51** (64.83)	0.50** (0.03)
V x Y	4	120.26**(103.16)	0.44**(0.55**)
Nitrogen source (N)	3	3.08 (7.87)	0.12(0.04)
Y x N	3	9.22(3.22)	0.24**(0.06)
V x N	3	6.29(11.62)	0.09**(0.09)
Y x V x N	12	8.39(8.63)	0.08(0.03)
Error	12	36.30(51.21)	0.08 (0.23)
	78		
Total	119		

*, ** Significant at P<0.05 and 0.01 respectively; + figures in parenthesis are for 200 KgN, while those outs of parenthesis are for 100 kg N.
DF = degree of freedom.

Table 3. Interactive means of variety x year interaction for maize agronomic characters.

Variety	Plant stand		Days to silking		Days to pollen shed		Ear height(cm)	
	2004	2005	2004	2005	2004	2005	2004	2005
Acr 97syn-Y	19.0	18.1	54.3	56.5	55.3	55.5	68.5	73.4
Acr 97syn-w	15.6	18.0	54.6	55.4	55.6	54.0	53.0	69.2
DMR-ESR-Y	30.3	27.1	51.3	50.3	50.0	52.5	59.5	73.0
DMR-LSR-Y	29.3	16.3	59.6	54.7	61.3	53.7	65.1	64.2
DMR-ESR-W	17.6	22.8	50.0	49.5	49.3	50.7	55.0	67.4
Mean	22.4	20.0	54.0	53.3	54.3	53.3	60.2	69.4
S.E (0.05)		0.66		0.2		0.2		1.63

Table 4. Interactive means of fertilizer type x Y interaction for ear aspects.

Variety	NPK		Urea		NPK + Urea		Control	
	2004	2005	2004	2005	2004	2005	2004	2005
Acr97syn-Y	3.0	2.3	2.0	2.3	2.6	2.3	2.3	2.0
Acr97syn-W	3.0	2.3	2.3	2.3	3.0	2.0	2.0	2.0
DMR-ESR-Y	3.0	2.3	2.0	2.6	2.3	2.6	2.3	2.3
DMR-LSR-Y	2.6	2.3	2.0	2.3	2.0	2.0	2.6	2.3
DMR-ESR-W	2.6	2.0	2.3	2.0	2.3	2.3	2.0	2.0
Mean	2.5	2.24	2.2	2.3	2.3	2.24	2.2	2.12
S.E (0.05)								0.06

and 2.24 in year 2005. It was also observed that DMR-LSR-Y Acr 97syn-W and DMR-ESR-W were better utilizers of nitrogen for improved ear aspect. They recorded generally low ear aspects when supplied with urea and the mixture of the two especially in 2005 as compared to other varieties. Character means of *S. lutea* tolerant maize varieties under varied fertilizer types and concentrations are presented in Table 5. Maize varieties treated with 200 kg N/ha generally established better than those treated with 100 kg N/ha with a mean plant stands of 22.87 as compared with 22.40 to 100 kg N. Days to 50% silking and pollen shed also differed significantly at P<0.05 for both N concentrations. Plant and ear heights differed significantly for the two levels of N. Values obtained from 200 kg N/ha were generally higher than those obtained from 100 kg N. The reverse was, however, the case in DMR-LSR-Y for these two traits. Stalk lodging resistance were generally better

Table 5. Character means for *S. lutea* tolerant maize varieties under varied nitrogen concentrations.

Variety	Plant stand		Days to silking		Days to pollen shed		Plant height (cm)		Ear height (cm)		Stalk lodging (1-5)	
	100 kgN	200 kgN	100 kgN	200 kgN	100 kgN	200 kgN	100 kgN	200 kgN	100 kgN	200 kgN	100 kgN	200 kgN
Acr 97syn-Y	18.54	22.12	55.58	55.45	55.41	54.95	163.37	168.91	70.95	76.75	0.83	0.66
Acr 97syn-W	16.83	22.08	55.50	55.04	54.87	54.20	144.83	152.50	61.12	68.12	0.58	0.87
DMR-ESR-Y	28.70	29.79	49.75	50.83	51.25	51.37	164.79	170.21	66.29	71.45	0.83	0.54
DMR-LSR-Y	22.83	21.45	58.25	57.20	57.54	56.91	156.62	152.71	64.71	62.42	1.16	0.45
DMR-ESR-W	20.25	18.62	48.08	49.79	50.04	50.33	146.95	147.81	61.21	61.37	0.87	0.75
Mean	22.40	22.87	53.43	53.67	53.82	53.55	153.32	158.44	64.85	68.02	0.85	0.66
S.E (0.05)	0.66	0.85	0.23	0.22	0.25	0.25	2.67	2.51	2.58	1.77	0.10	0.08

Table 6. Character means for yield related maize traits under varied N concentration.

Variety	Plant harvest		Field grain weight	
	100 kgN	200 kgN	100 kgN	200 kgN
Acr97syn-Y	13.75	14.42	0.75	0.82
Acr97syn-W	11.75	15.00	0.52	0.85
DMR-ESR-Y	18.45	17.66	0.91	0.82
DMR-LSR-Y	13.58	13.16	0.80	0.76
DMR-ESR-W	14.45	15.16	0.76	0.81
Mean	14.40	15.08	0.75	0.82
S. E. (0.05)	0.66	0.92	0.03	0.05

under 200 kgN than 100 kgN in all varieties of maize tested. Lodging resistance was reduced to 0.85 in 100 kgN and to 0.66 in 200 kgN across varieties at P<0.05 (Table 5).

Table 6 presents character means for *S. lutea* resistant maize varieties for plant harvest and field weight. Plant harvests were generally higher for 200 kgN in the three varieties than 100 kgN mean while in DMR-ESR-Y and DMR-LSR-Y, it was slightly lower as compared to 100 kgN, but not at significant threshold. Plant harvest ranges from 11.75 to 18.45 for 100 kgN and 13.16 to 17.66 for 200 kgN in the tested varieties. In the case of the field grain weight, the composite maize varieties responded differently to varied levels of N. While Acr 97-Syn-Y, Acr 97 Syn-W and DMR-ESR-W recorded increased field weight with increase in N from 100 to 200 kg/ha, DMR-ESR-Y and DMR-LSR-Y recorded relatively lower field weight as N concentration increases from 100 to 200kg (Table 6).

DISCUSSION

The results from the evaluation showed that year of evaluation as well as maize variety differed significantly for almost all agronomic characters at both levels of nitrogen (100 and 200 kgN/ha) concentrations. Similar result was reported by Olakojo and Olaoye (2006) where interaction between fertilizer type and maize variety was highly P<0.01 significant for grain yield under striga infestation. Although other workers such as Vogt et al. (1991) and Olakojo et al. (2011) have reported on similar findings, farmers in striga endemic ecologies should make use of nitrogen fertilizer to enhance better performance of maize agronomic characteristics and more importantly grain yield.

Maize varieties evaluated showed significant

differences to different fertilizer types, especially for days to silking, plant and ear heights as well as plant aspects. This suggests that, the fertilizer types can be selected to influence these agronomic maize characters for grain yields. Olakojo et al. (2001) reported that urea and sulphate of ammonia contributed significantly to maize field weight if striga resistant maize variety is used, while NPK and urea reduces significantly striga syndrome rating in susceptible maize varieties. Similarly, variety x year interaction was significant for days to silking and pollen shed under low and high N concentrations. Farmers and agronomist may therefore influence days to silking and pollen shed under *S. lutea* infestation to a reasonable extent using ideal N concentration. Interactive means of variety x year further revealed that varieties of maize use performed differently for plant harvest and varieties to utilize prevailing climatic weather conditions for varied yield performances. This was further confirmed when across varietal means for both yield and agronomic traits were relatively higher in year 2004 than 2005. Olakojo et al. (2004) had earlier reported that increase in moisture supply to maize plants under striga stress do significantly enhance higher yield. Meteorological data for this location shows that precipitation was relatively higher in 2004 than 2005.

Response of maize varieties to fertilizer types revealed that DMR-LSR-Y and DMR-LSR-W were better N users for enhanced ear aspect. The two varieties recorded significantly lower ear aspect which was good for an improved maize variety, the lower the ear aspect, the better the maize variety. It was discovered that higher (200 kgN/ha) N concentration on maize seedlings significantly enhances stalk lodging resistance. Stalk lodging resistance of 0.45 to 0.87 at 200 kgN/ha seems to be relatively better than that of 0.5 to 1.16 at 100vkgN under striga infestation. It therefore, implies that higher N concentration does not only enhance higher field weight, it also confers on maize better lodging resistance and ear aspect.

Conclusion

The composite maize varieties responded differently to vary N-concentration. Acr 97 Syn-Y and Acr 97 Syn-W as well as DMR-ESRY tolerated higher (200 kgN/ha) N-concentration, hence could be applied at reasonable quantity to enhance higher field weight. DMR-LSR-Y on the other hand did not utilize excess of N concentration above the optimum of 100 kg/ha, thus, should be discouraged. In fact, it reduces field weight by as much as 5%.

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Full Length Research Paper

Variation of alkaloids in the Kenyan *Zanthoxylum gilletii* (De Wild Waterman)

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Zanthoxylum gilletii **is an African indigenous deciduous tree which is important for its medicinal use in many communities to treat a wide range of ailments. This study was conducted to identify the alkaloids present in the bark, root and leaves of the Kenyan *Z. gilletii*. The plant materials were randomly sampled, dried at room temperature, powdered and subjected to thin layer chromatography (TLC), high performance liquid chromatography (HPLC) and liquid chromatography mass spectrometry (LC-MS) analyses for the alkaloid confirmatory tests. The bark, root and leaf extract contained peroxysimulenoline, sanguinarine, fagarine I, norchelerythrine (dihydroavicine or demethylnitidine), trans-fagaramide, 8-methylnorchelerythrine and dihydronitidine alkaloids. The distribution of the alkaloids appeared to be quite variable within different plant parts and different regions. The identified alkaloids have been documented to be useful for their medicinal value in humans and also protect the plants against predation. The medicinal value of *Z. gilletii* may be due to its contents of varied alkaloids. The information on alkaloidal variation in the species has potential value and practical applications in chemotaxonomy, toxicology and pharmacognosy. The present findings may be useful in optimizing the processing and wild-harvesting of these alkaloids.**

Key words: *Zanthoxylum gilletii,* chemotaxonomy, pharmacognosy, liquid chromatography-mass spectrometry.

INTRODUCTION

Zanthoxylum gilletii is an indigenous deciduous tree growing 10 to 35 m high, belonging to the family *Rutaceae*. The genus *Zanthoxylum* is distributed world-wide from the tropics to the temperate zones. There are over 200 species from small shrubs to large trees (USDA, 2003). In Africa, *Z. gilletii* is widely distributed in countries such as Sierra Leone to Kenya, Sudan, Angola, Malawi, Zambia and Zimbabwe. The bark is chewed and the juice swallowed for the treatment of stomachache (Kokwaro, 1993). The stem bark decoction is commonly used for back pain and externally for all urinogenital complaints including infections. The root bark or the fruit pulp is a liniment for rheumatism and all kinds of pain. The decoction of young leaves eases coughs and is said to be effective on gonorrhea and bilharzia. Several phyto-chemical studies have identified numerous compounds with medical and antioxidant potential including alkaloids, xanthophylls, phenolic acids, sapo-nins, coumarins and hydroxycinnamic acids (Islam and Ahsan, 1997). Phytochemical investigations carried out on a related species, *Zanthoxylum chalybeum* have yielded pure crystalline alkaloids (Olila and Opuda, 2001). In Nigeria, chemical investigations of *Z. gilletii* showed the presence of furoquinoline alkaloid, skim-mianine, the cinnamic acid amide, fagaramide and benzo phenanthridine alkaloids, nitidine, dihydrochelerythrine and chelerythrine alkaloids

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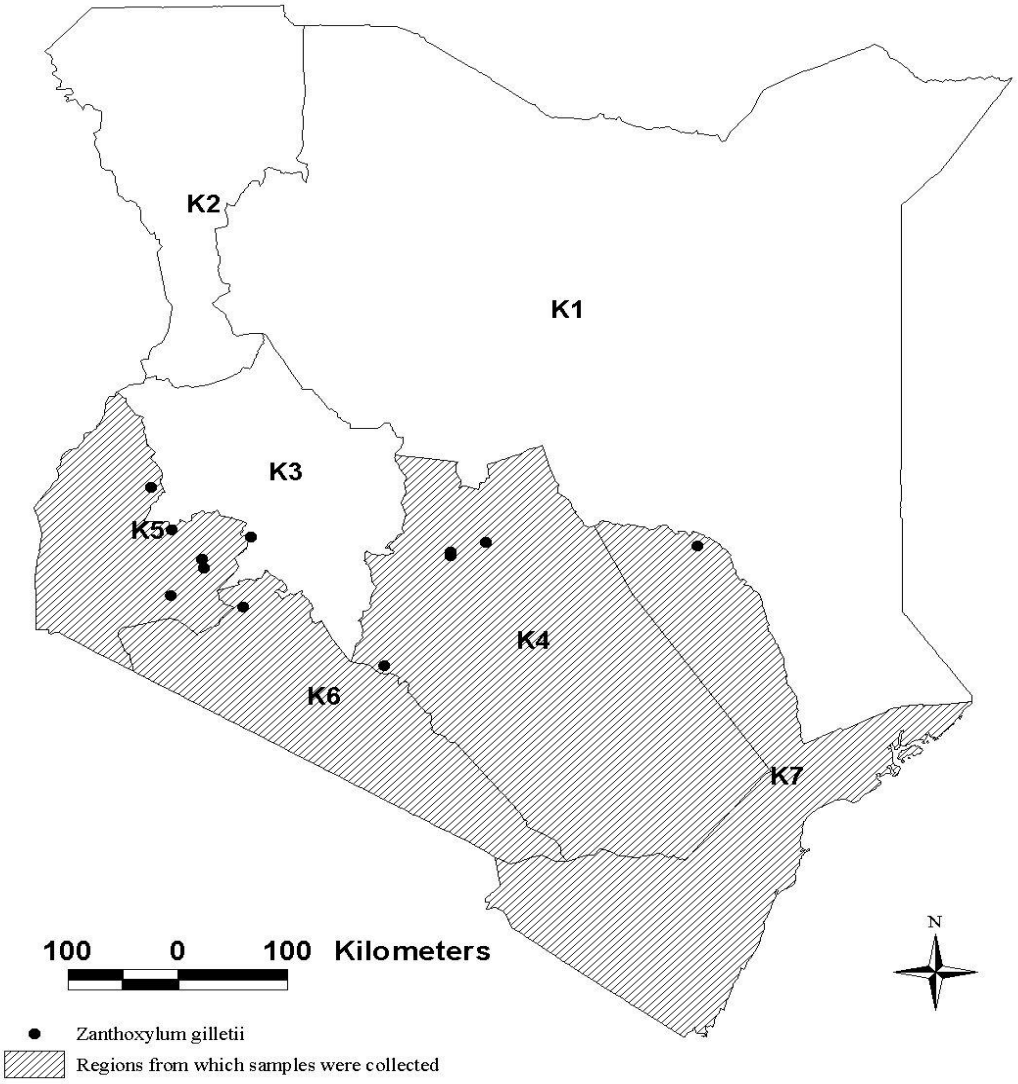

Figure 1. Geographical distribution of *Z. gilletii* in Kenya (Source: Seeds for Life Project, National Museum Kenya database).

(Adesina and Johannes, 1988). Other constituent compounds isolated included volatile oils, vanillic acid and hydroxyl benzoic acid (Adesina, 2005). Geographical variation may have some effects on the level of medicinal active ingredients of plants of the same species (Sheen et al., 1991).

Variation among plants of the same species may be due to age, climate, soil or season of the year. In Kenya, there is limited information on the active ingredients and variation of the secondary compounds in *Z. gilletii* across agro ecological zones and plant parts. Such knowledge on the chemical constituents of plants is desirable, not only for the search of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic materials such as tannins, oils, gums, precursors for the synthesis of complex chemical substances. The aim of this study was to identify the presence and variation of main alkaloids in *Z. gilletii* from Kenya.

MATERIALS AND METHODS

Study site

The plant samples (leaf, root and bark) were collected from trees growing in the Kakamega forest (altitude 1649 m, 00° 20' 195 N; 034° 52' 607 E), Southern Mau forest (altitude 2340 m, 00° 47' 357 S; 035° 34' 831 E) and Mount Kenya region (1768 m, 00° 14' 115 S; 037° 35' 031 E). The records for the distribution of the species were retrieved from voucher specimens at the National Museums of Kenya. Sampling points (K4, K5 and K6) correspond to the species range in Kenya (Figure 1).

The samples were cleaned then chopped into smaller pieces before being dried in the shade at 25 ± 2°C for ten days. The dried plant samples (leaves root and bark) were then ground into fine

Table 1. Solvent systems used in TLC (butan-1-ol, acetic acid and water; MAC = methanol, ammonia and chloroform).

Solvent system	BAW- 40 ml butan-1-ol: 10 ml acetic acid: 50 ml water	MAC*- 10 ml methanol: 1 ml ammonia: 89 ml chloroform
Plates	Silica 5553	Silica 5577
Standard(s)	Papaverive Chloride	Papaverine Chloride
Spray(s) used	Dragendorffs' reagent	Dragendorffs' reagent

powder in a grinder and sieved to give particle size of 50to 150 mm. The ground powder was then packed into 1 kg and stored in labeled zip locked polythene bags at room temperature before they were transported to the Jodrell Laboratory, UK for extraction and phytochemical analysis. In the laboratory, the samples were documented and given accession database numbers in the biological interactions' (BI) accession database.

Extraction of plant material

Nine bottles were labeled with BI accession database numbers. Into each of the labeled sample bottles, 0.5 g of dry powder sample was weighed, and this was repeated for all samples. To each vial, 5 ml of 100% methanol was added and shaken well and left for three days to extract. The extracts were filtered and analyzed by thin layer chromatography (TLC), high performance liquid chromatography (HPLC) and liquid chromatography mass spectrometry (LC-MS).

Thin layer chromatography (TLC) analysis

Solid Phase Sorbent was mounted on aluminum backed silica plates without florescent indicators F254 (sigma code 5334). Methanol extract (20 µl of each sample) was applied to the selected TLC plates (10 x 20 cm) and allowed to dry between each 5 µl application. Standard papaverine chloride (in 100% methanol) at a concentration of 2 mg/ml was then applied (5 µl), to the plate (Svendsen and Verpoorte, 1983). The solvent front was marked on the plate in pencil immediately on removal from tank and allowed to air dry in a fume cupboard. TLC-UV spectra were observed under the UV spectrophotometer (model UV- Desage) at wavelengths of 254 and 366 nm. The plates were further developed by spraying with the Dragendorffs' reagent and the retention factor (Rf) was recorded.

$$Rf = \frac{\text{Distance moved by compound}}{\text{Distance moved by solvent front}}$$

After viewing plates under the UV light, and before spraying with any reagents, the plates were scanned (a Camag TLC Scanner 3) under a range of wavelengths from 254 to 350 nm. The resulting data for different colour peaks, heights and bases of the peaks observed at 350 nm were analyzed and interpreted.

Alkaloid analysis

Each of the samples was filtered, dried and rehydrated in 10 ml of 0.5 M hydrochloric acid. Acidified extract was added to an equal volume (10 ml) of chloroform in a separating funnel, shaken and left to settle. The bottom layer (acidified chloroform) was drained into a labeled bottle and a second equal volume (10 ml) of chloroform was added to the aqueous extract, shaken and left to settle. The acidified chloroform layer was air dried in the fume cupboard while the 1 to 2 ml of aqueous layer was basified with concentrated ammonia and the pH confirmed using pH paper (Sigma). A volume of 10 ml chloroform was added to the basified sample in separating funnel, shaken and left to settle. The bottom layer was drained out into a 20 µl labeled vial and a second equal volume of chloroform added to the aqueous extract. The basified chloroform layers were allowed to dry in the fume cupboard while the aqueous layer was dried in a heat block at 40°C. The two chloroform layers (acid + basic) were rehydrated in 100% methanol. The dry aqueous layer was re-hydrated in 100% water, kept in the cold room prior to analysis. The alkaloid partition fractions were run in confirmatory alkaloid tests using TLC and LC-MS analyses (Table 1).

HPLC and LC-MS analysis

In order to remove all the particulates, the extracts were centrifuged at 10,000 rpm for 2 min and the clarified extracts were transferred into small clean labelled vials (BI 18826 - 18834 stock solutions) using a glass pipette. From the stock solution, 1 ml was placed into an HPLC vial (Chromacol) and 300 µl into a smaller LC-MS vial (Chromacol). HPLC analyses were carried out on a Waters 600 pump with a 600E controller, Waters 717plus autosampler coupled to a Waters 996 photodiode array detector controlled through a PC workstation running Empower software. Detection of alkaloids was achieved by scanning through 200 to 550 nm scans per second and data were collected for 30 min with a 10 min between injections to ensure column equilibrium between samples. Extracts were analyzed using gradient solvent programme with a flow rate of 1 ml per min with in-line degassing and an injection volume of 20 µl. Accurate mass LC-MS analyses were carried out on a ThermoScientific LTQ Orbitrap XL with an Electrospray source (ESI) operating on positive or negative mode with an Accela system (LC system). The data was analyzed using XCalibar software. Chromatography was achieved on a Phenomenex Luna C18 column 150 mm x 3 mm i.d. x 3 µm with a 0.4 ml/min flow rate and a 5µl injection volume. The samples were run in both positive and negative mode in full ms scan mode to allow data to be recorded as well as for accurate mass determination. Alkaloids present in the extracts were identified on their UV spectra and retention times (HPLC analysis) while LC-MS analyses was based on accurate mass, molecular formula and mass fragmentation pattern (µs/µs) were compared with known compounds from library of compounds based at the Jodrell Laboratory. The column used the HPLC analysis of a Phenomenex Luna C18 capillary column 250 mm x 4 mm i.d. x 5 µm. Extracts were analyzed using gradient solvent programmes (Table 2) with a flow rate of 1 ml/min with in-line degassing and an injection volume of 20 µl.

The LC-MC chromatography was achieved on a Phenomenex Luna C18 column 150 mm x 4.6 mm i.d. x 3 µm using a gradient (Table 3) with a 0.5 ml/min flow rate and injection volume of 10 µl.

RESULTS

The screening of alkaloids using papaverine chloride as

Table 2. Gradient conditions for HPLC analyses (A- methanol; B- water; C- 5% acetic acid in methanol)

Time	A	B	C
0	15	75	10
20	90	0	10
25	90	0	10
27	15	75	10
30	15	75	10

Table 3. Gradient conditions for LC-MS analyses (A- methanol; B- water; C- 1% formic acid in acetonitrile).

Time	A	B	C
0	0	90	10
20	90	0	10
25	90	0	10
27	0	90	10
37	0	90	10

the standard together with the sample extracts on TLC plate showed the best separation of the components of the extracts. On viewing the photographic illustration under UV-light at 366 nm, the colour display for leaf extracts were blue, purple and red, while the bark and root extracts showed yellow, purple, orange and blue coloration. Distinct orange coloration confirmed the presence of alkaloids in all the extracts after application of Dragendorff's reagent on the plates. Different colour bands showed the presence of different components in the root, leaf and bark extracts. The appearance of different colours in the TLC plate and formation of the various peaks viewed after the TLC scan represented the separation of the different compounds present in the extracts. As shown in the ESI - MS detector chromatograms of root bark and leaf extracts (Figures 2 to 4), there are varying peaks. The peaks represent the abundance and diversity of alkaloids in different plant parts as well as geographical locations. The detection of alkaloids in the root, leaf and bark extracts was based on retention times and peak pattern heights.

HPLC and LC-MS analysis

The HPLC and LC-MS analyses of the extracts confirmed the presence of peroxysimulenoline, sanguinarine, fagarine I, norchelerythrine (dihydroavicine or demethylnitidine), trans-fagaramide, 8-methylnorchelerythrine, dihydronitidine as alkaloids. Fagarine I and 8-methyl norchelerythrine were found to be present in all the extracts except in leaves from Kakamega and Mau forests. Norchelerythrine, dihydroavicine and demethylnitidine occurred in all plant parts but were absent in the leaves from all the regions. The retention time, molecular weights and chemical formula of the alkaloids found in *Z. gilletii* are shown in Table 4, while the molecular structures of the alkaloids are shown in Figure 5.

The occurrence of dihydronitidine and sanguinarine were restricted only to the roots and barks as opposed to trans-fagaramide that was evenly distributed in all the plant parts and across regions (Table 5).

DISCUSSION

Alkaloids are considered as the main bioactive constituents of many plant species including *Z. gilletii* (Pěnčíková et al., 2011). The extreme variation of alkaloids observed in the bark, roots and leaves should be taken into account if the plant is to be collected for medicinal purposes. Similar variation trends have also been reported in other plants in Northern Turkey (Çirak et al., 2008). The variations could be due to morphometric traits as well as other various environmental factors present at various locations along with altitudes (Kjaer et al., 2004). Other factors such as water availability, acidity or salinity could also contribute to variation in the concentrations of alkaloids in *Z. gilletii* across the studied regions in Kenya.

The restricted occurrence of dihydronitidine and sanguinarine alkaloids in the roots and bark concurs with the preference of the plant organs mostly harvested and used in traditional medicine. The presence of sanguinarine in the bark and roots confirmed in previous studies showed its usefulness in treating periodontal disease (Colombo and Bosisio, 1996). In countries like the United States derivatives such as sanguinarine chloride have even been included in commercial tooth-pastes and mouthwashes (Bruneton, 1999). These alkaloid activities validate the use of *Zanthoxylum* sp. in the traditional treatment of mouth ulcers and toothache. There is sufficient evidence that the Chinese use *Zanthoxylum armatum* (Hartley, 1966), Kenyans *Zanthoxylum chalybeum* (twigs) and Nigerians the roots of *Zanthoxylum zanthoxyloides* (Lam.) as teeth cleaning sticks. Even distribution of Fagarine I and 8-methylnorchelerythrine in the leaves, roots and bark could be due to their role as chemical defense against herbivores and other plant enemies. They could also serve as a plant natural source of insecticides and fungicides. The principal action of alkaloids is on the nervous system (Pěnčíková et al., 2011) and their high concentrations in plants protect them from grazing animals. Alkaloids such as trans-fagaramide, 8-methylnorchelerythrine and dihydronitidine have been

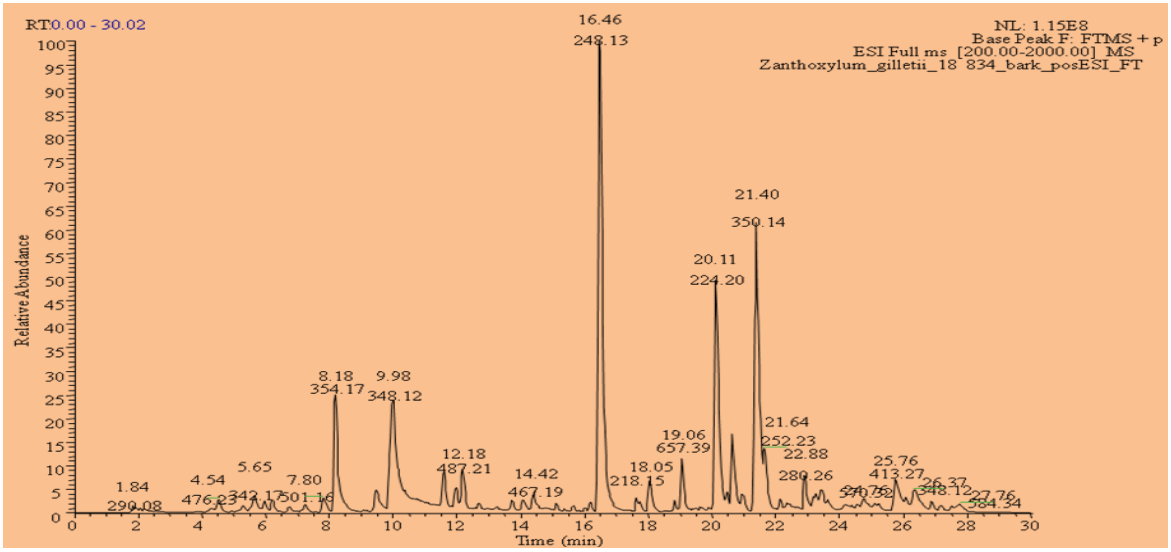

Figure 2. Liquid chromatography-mass spectrometry (LC-MS) profiles of methanolic bark extracts of *Z. gilletii*.

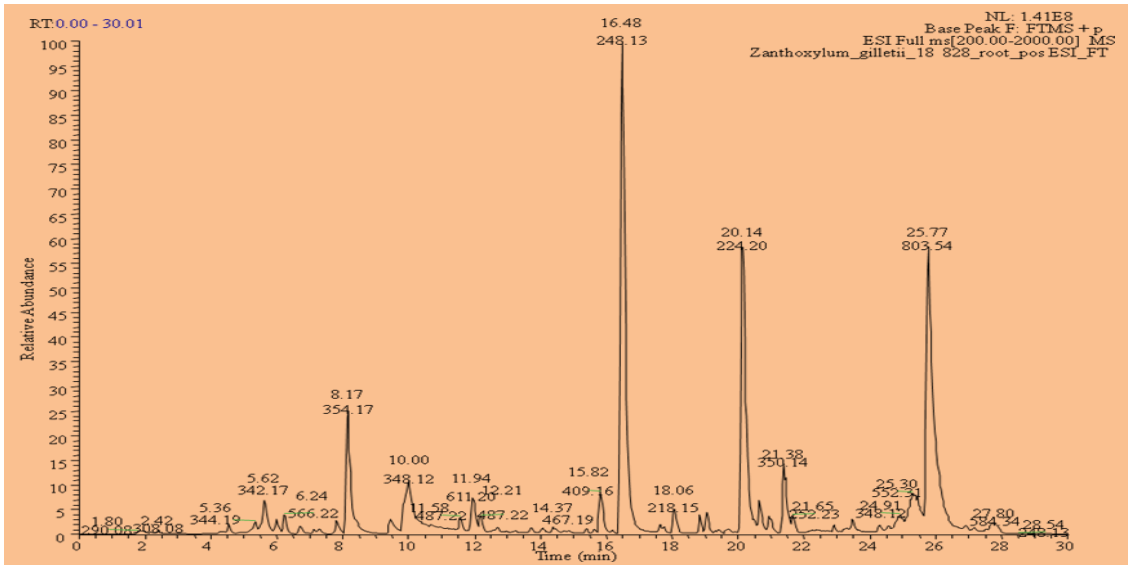

Figure 3. Liquid chromatography mass spectrometry (LC-MS) profiles of methanolic root extracts of *Z. gilletii*.

reported to help biologically in storage of waste nitrogen, cationic balancing and protection against parasites (Ting, 1982). Sanguinarine, which also shows specific toxic effects to herbivores and microbial pathogens, is proposed to function as an inducible defense compound (Schmeller et al., 1997).

In line with other African *Zanthoxylum* species such as *Zanthoxylum capense, Zanthoxylum chalybeum* and *Zanthoxylum davyi, Z. gilletii* is used for the treatment of snakebite (Mpondo in Transkei, Vhavenda in Limpopo) and severe coughs and colds (Kokwaro and Johns, 1998). The spines are used to manag e infected wounds, the leaves for chest pains, the stem bark to treat boils, pleurisy and toothache, and root preparations for mouth ulcers, sore throats and as an aphrodisiac (Mabogo, 1990). Root-bark decoctions are used by the Zulu as a tonic both for man and animals (Tarus et al., 2006) and to treat toothache.

Conclusions

Z. gilletii contains many alkaloids with antimicrobial and cytotoxic activities used to manage periodontal disease and caries in many African communities. The study has shown that presence of alkaloids should be taken

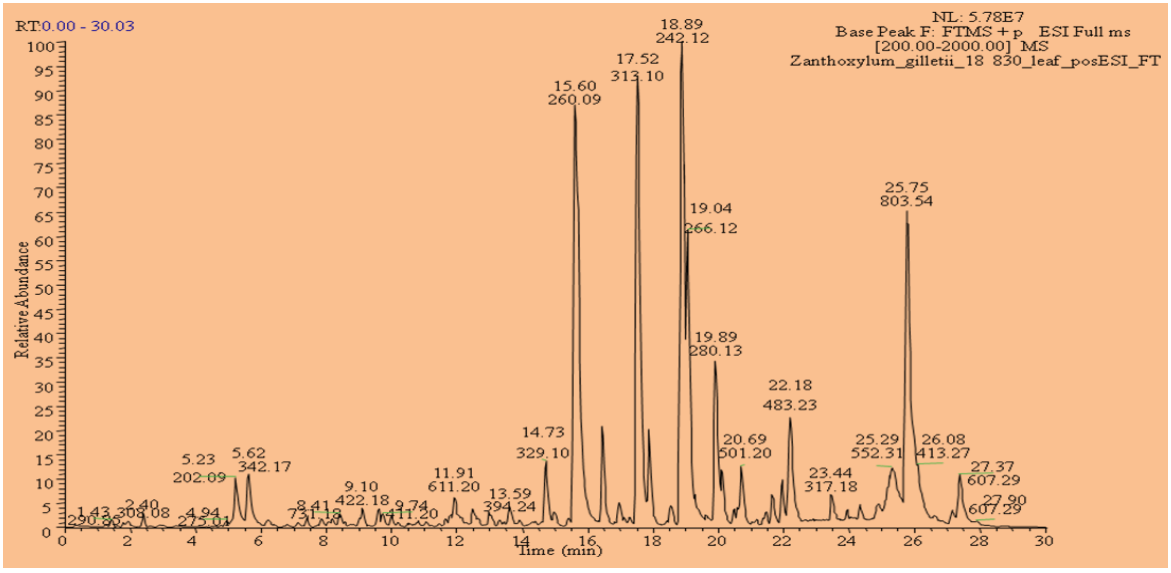

Figure 4. Liquid chromatography mass spectrometry (LC-MS) profiles of methanolic leaf extracts of *Z. gilletii*.

Table 4. Chemical formula, retention time and molecular weights of the alkaloids.

S/N	Alkaloid	Formula	Retention time	Molecular weight
1	Peroxysimulenoline	$C_{20}H_{23}NO_4$	5.60	341
2	Sanguinarine	$C_{20}H_{14}NO_4$	9.30	332
3	Fagarine I	$C_{21}H_{23}NO_5$	7.60	369
4	Norchelerythrine (dihydroavicine or demethylnitidine)	$C_{20}H_{15}NO_4$	9.48	333
5	Trans-fagaramide	$C_{14}H_{17}NO_3$	16.49	247
6	8-Methylnorchelerythrine	$C_{21}H_{17}NO_4$	10.00	347
7	Dihydronitidine	$C_{21}H_{19}NO_4$	21.4	349

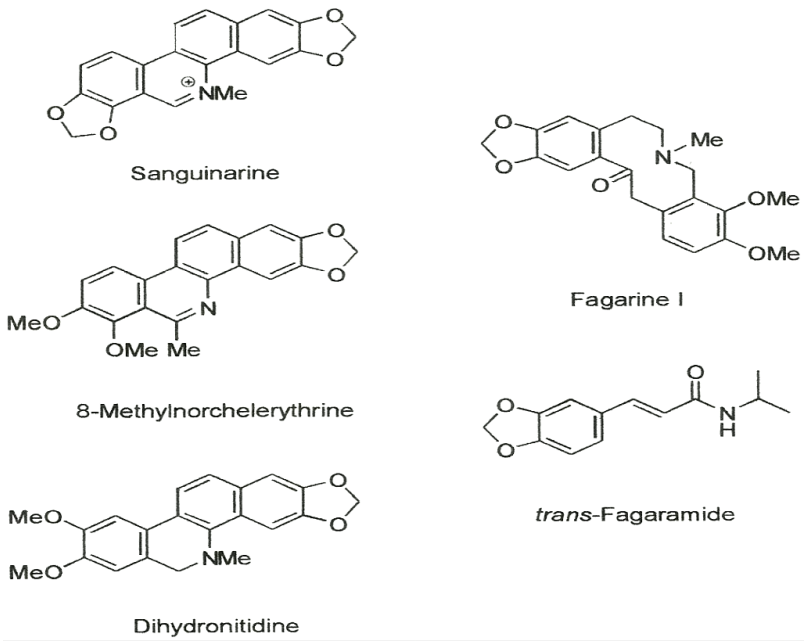

Figure 5. Structures of alkaloid isolates of *Z. gilletii* root, bark and leaf extracts.

Table 5. Alkaloid profiling in the extracts of *Z. gilletii* from the different regions and plant parts (R = root; B = bark; L = Leaf; √ = alkaloid present; X = alkaloid absent).

Alkaloid	Region								
	18826	18828	18832	18834	18829	18831	18830	18827	18833
	Kakamega Forest	Mount. Kenya	Mau Forest	Kakamega Forest	Mt. Kenya	Mau Forest	Kakamega Forest	Mount Kenya	Mau Forest
	R	R	R	B	B	B	L	L	L
Peroxysimulenoline	√	√	√	√	√	√	X	√	√
Sanguinarine	√	X	√	√	√	X	X	X	X
Fagarine I	√	√	√	√	√	√	X	√	X
Norchelerythrine	√	√	√	√	√	√	X	X	√
Trans-fagaramide	√	√	√	√	√	√	X	√	X
8-Methylnorchelerythrine	√	√	√	√	√	√	√	√	X
Dihydronitidine	√	√	√	√	√	√	X	X	X

into account if the plant is collected for medicinal purposes. It is also evidenced that the same plant species at different localities are chemically different and as a result, show variation in biological activity and potential toxicity. For use of alkaloids for chemotaxonomic purposes, it is important to take into account the plant part used, the different stages of development and genetic variation of plants and the geographical distribution. Finally, most of the medicinal uses of this species can probably be attributed to its rich diversity of alkaloids acting synergistically.

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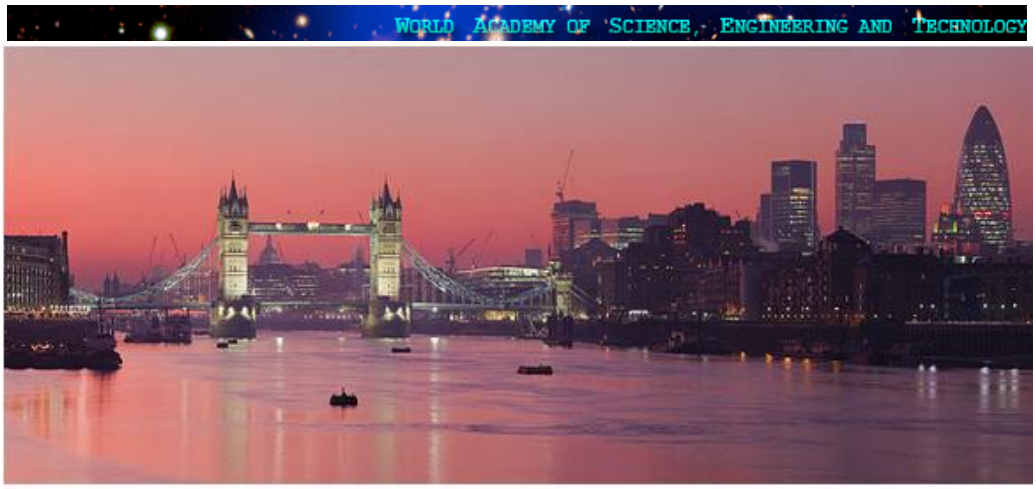

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