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Contents of the Volume

i. Copyright Notice
ii. Editorial Board Members
iii. Chief Author and Dean
iv. Table of Contents
v. From the Chief Editor's Desk
vi. Research and Review Papers

1. Functional Properties based Statistical Optimization of Foam Mat Drying Parameters for Potato (Kufri Chandramukhi). *1-10*
2. Ordination and Classification of Vegetation in Semi Arid Area of Pakistan. *11-19*
3. Isolation and Phytochemical Characterization of Bioactive Compounds from the Rhizomes of Cyperus Rotundus. L. *21-27*
4. Evaluation of Ovicidal and Larvicidal Activities of Methylene Chloride-Methanol Extract of *Annona Senegalensis* (Annonaceae) Stem Bark on *Heligmosomoides Bakeri* (Nematoda, Heligmosomatidae). *29-39*
5. Strategies of Rodent Control Methods at Airports. *41-47*
6. "Plantain of Guinea". The Atlantic Adventure of Banana. *49-65*

vii. Auxiliary Memberships
viii. Process of Submission of Research Paper
ix. Preferred Author Guidelines
x. Index

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Functional Properties based Statistical Optimization of Foam Mat Drying Parameters for Potato (Kufri Chandramukhi)

By Sreemoyee Chakraborty, Soumitra Banerjee & Saikat Mazumder
University Techno, India

Abstract- This study is focussed on statistical optimization of foam mat drying conditions of potato (kufri chandramukhi) based on functional properties of dried powder. During the preparation of the mat maximum foam expansion of 25% was found with interactive effect of 10 minutes of magnetic stirring and 2% concentration of glycerol monostearate (GMS). The range of factors employed for optimization of drying was different concentrations of GMS, temperature, time. The optimum drying condition obtained was at 600C for 135 minutes with 2% GMS. The value of percentage of final moisture content, coefficient of reconstitution, browning index, and percentage gelatinized starch was 1.89 ± 0.551, 0.914 ± 0.025, 0.013 ± 0.00175 and 70.00 ± 1.645, respectively was found at optimum drying condition. Time and interaction of temperature with time have significant effect on moisture content at $p<0.05$ level.

Keywords: *kufri chandramukhi, foam mat drying, functional properties, regression equation.*

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Functional Properties based Statistical Optimization of Foam Mat Drying Parameters for Potato (Kufri Chandramukhi)

Sreemoyee Chakraborty [α], Soumitra Banerjee [σ] & Saikat Mazumder [ρ]

Abstract- This study is focussed on statistical optimization of foam mat drying conditions of potato (kufri chandramukhi) based on functional properties of dried powder. During the preparation of the mat maximum foam expansion of 25% was found with interactive effect of 10 minutes of magnetic stirring and 2% concentration of glycerol monostearate (GMS). The range of factors employed for optimization of drying was different concentrations of GMS, temperature, time. The optimum drying condition obtained was at 60^{0}C for 135 minutes with 2% GMS. The value of percentage of final moisture content, coefficient of reconstitution, browning index, and percentage gelatinized starch was 1.89 ± 0.551, 0.914 ± 0.025, 0.013 ± 0.00175 and 70.00 ± 1.645, respectively was found at optimum drying condition. Time and interaction of temperature with time have significant effect on moisture content at $p<0.05$ level.

Keywords: kufri chandramukhi, foam mat drying, functional properties, regression equation.

I. Introduction

Potato (Solanum tuberosum), a starchy, tuberous crop from the perennial Nightshade family, is a major food crop, grown in more than 100 countries in the world and also emerged as fourth most important food crop in India. In India different types of potatoes growing are Kufri Sindhuri, Kufri Chandramukhi, Kufri Jyoti, Kufri Muthu, Kufri Lauvkar, Kufri Dewa, Kufri Badshah, Kufri Bahar, Kufri Lalima, Kufri Swarna, Kufri Megha, Kufri Ashoka, Kufri Jawahar and Kufri Sutlej (Indian National Horticulture Database, 2011). Global potato production rate is about 324 million metric tonne per year out of which India contributes to about 36.5 million metric tonne per year as per a study undertaken in 2010. West Bengal contributes to about 32% of India's total harvest. Due to inadequate storage facilities and processing units about 17-20% is wasted and due to its perishability more than 50% of states surplus is wasted during transportation, finally only and due to its perishability more than 50% of states surplus is wasted during transportation, finally only about 5% of the world's potato crop is traded internationally (Food Processing Industries Survey, West Bengal).

Foam- mat drying (originally developed by Morgan et al. in 1959 at the Western Regional Research Laboratory of the U.S. Department of Agriculture) is a promising new development in the field of drying aqueous foods. It is a process in which the transformation of products from liquid to stable foam follows air drying at relatively low temperatures to form a thin porous honey-comb sheet or mat which is disintegrated to yield a free-flowing powder having better reconstitution properties than drum dried and spray dried products (Kadam et. al., 2010).

Kufri Chandramukhi is a perennial type of potato, grown in Hoogly district of West Bengal, India, is recorded to have higher carbohydrate content than most other varieties. Foam mat drying of potato of Chandramukhi variety for development of potato powder this investigation has been carried out with the specific objective of optimization of the concentration of foaming agent (glycerol monostearate), drying time and temperature for effective foam mat drying of cooked potato mash.

In this study, foam mat drying of potato is the function of percentage of GMS, temperature and time. The parameters were optimized in this study using factorial design of 3^2 and 3^3 levels at $p< 0.05$. The functional properties of dried powder such as final moisture content, coefficient of reconstitution, percentage gelatinized starch and browning index are used as a key players for deciding of optimum condition for foam mat drying.

The easiest and most widely used approach to study linear and interaction of different factors have been response surface methodology (RSM). RSM is a collection of mathematical and, statistical technique useful for analyzing and optimizing the response of multivariate system. In RSM, generally attempts are made to identify the responses of system as a function of explanatory variables. In this study, different linear and interactive effect of different factors has been studied.

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II. Material & Methods

a) Collection and Preparation of Raw material

Potatoes used in this study were of Chandramukhi variety freshly collected from local market of South Kolkata (cultivated in Tarakeswar, Hoogly district, West Bengal, India). The potatoes had initial moisture content of 82.34gm of moisture/100gm wet weight. The potato samples were washed with running tap water and distilled water respectively to make it free from dirt and soil and blotted with a tissue paper for removal of excess surface water. The potato samples were then peeled and cut into slices of equal thickness of 10±0.3 mm each. The sliced potato samples were blanched in hot water (Temperature-$90^0 \pm 2^0$ C) containing 2 gm NaCl/100 gm of water of sodium chloride (NaCl) and 2mg of potassium meta-bisulphate/1000gm of water for 10 minutes and followed by preparation of mash in a mixer grinder. The potato mash was gelatinized in an autoclave at 10 psig pressure for 15 minutes (Chakraborty et. al., 2013).

b) Optimization of Stirring Time for Effective Foaming of GMS (Foaming Agent)

At first glycerol monostearate (GMS) was weighed in different amounts (1%, 2%, and 3% respectively) then mixed with a refined vegetable oil and water in a ratio of 2:1:10 respectively and heated in boiling water bath (90^0-100^0C) and stirred till GMS gets evenly dispersed to form slurry. The potato mash and water was added (in a ratio of 10:1 respectively) to the three different slurries and stirred at 300 rpm for 5, 10 and 15 minutes in a magnetic stirrer (Eltek, Model – 2011) to form a thick foam slurry. Optimization of stirring time was done in terms of measurement of foaming properties i.e. maximum foam expansion (i.e., maximum foam volume) and maximum foam stability (i.e. minimum drainage volume). The water was added because due to high viscosity of the cooked potato mash proper foaming was not being possible. GMS also acted as a stabilizer of the foam for different cases.

The foam expansion was measured as described by Akiokato et. al., (1983).

$$FE = \{(V_1-V_0)/ V_0\}*100$$

Where FE is the foam expansion (%), V_1 is the final volume of foamed potato slurry (ml) and V_0 is the initial volume of potato slurry (ml).

A criterion for good foam stability was its uniformity and the lack of fluid drainage in 60 minutes after its preparation. (Kadam et. al., 2010). The foam obtained from the potato slurries were filled into a transparent graduated cylinder and kept at room temperature (i.e. 25^0C) for 3 hours. The reduction in foam volume was measured as an index for the foam stability. The foamed slurry prepared at optimized stirring time condition used further for drying parameters optimization.

c) Optimization of Drying Parameters

Drying of foamed potato mash was done on the basis of variation of drying time-temperature-percentage GMS profile. The experiments were carried out on the basis of experimental design at 3^3 and 3^2 full factorial design. Functional effect of interacting factors (i.e. time, temperature and percentage GMS) for 3^3 full factorial design and functional effect of interacting factors (i.e. time, temperature) for 3^2 was studied.

A batch type tray drier was run intermittently in order to stabilize the desired temperature (i.e. 50^0C, 55^0C, 60^0C respectively) inside the chamber. The homogeneous foamed potato slurry as well as the non foamed potato slurry was poured over muslin cloth and spread to an equal thickness of 10 mm. similar samples were poured to Petri plate to equal thickness and kept along with the sheets for drying. These sheets were kept in an aluminium tray size of 80X40 cm having 7 mm diameter holes. The trays were then placed on the tray stand in position for drying. The foamed and non-foamed potato slurries were dried at different time-temperatures profile. The Petri plates were taken out of the drying chamber at different time intervals for determination of weight loss. Moisture content was recorded using Dhona balance having least count of 0.1 mg on initial and final weight basis. Drying was stopped at the required times (5hrs, 6hrs, and 7hrs). Final moisture content of each of the sample was obtained by A.O.A.C method. A crispy flaky powder was obtained which was grounded to a fine powder and packed in LDPE zip pouches separately and functional properties of powders were determined.

Primary optimization of effective drying condition was done on the basis of superior functional properties of the powder. At primary optimization % of GMS and drying temperature was optimized, time was not optimized though time was included in the experimental design because the superior functional properties may develop before the pre-set time of primary optimization. The study was performed in this method to properly focus on the optimization condition. So, a set of foamed potato slurry prepared at optimum foaming condition was dried at primary optimized drying condition and samples were withdrawn at 15 minutes interval for determination of functional properties, on the basis of superior functional properties the final drying time required for effective foam mat drying was optimized.

i. *Design of Experiment for Drying*

The factorial design with three levels of treatment temperature, time and percentage of GMS respectively was assigned for 3^3 full factorial designs and three level of temperature and time respectively, for 3^2 full factorial designs in case of drying without GMS and the designs leads to 27 sets and 9 sets of experiments respectively. The factorial design was

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applied to estimate the relationship between variables of foam mat drying of potato mash. The three range level of factors are 50^0C, 55^0C, 60^0C for temperature (-1, 0, +1 level respectively), 5 hours, 6 hours, 7 hours for time (-1, 0, +1 level respectively), 1%, 2%, 3% for percentage of GMS (-1, 0, +1 level respectively).

ii. *Determination of Functional Properties*

The functional properties of dried powder such as moisture content, co-efficient of reconstitution, browning index, and gelatinized starch was determined. All the experiments were performed on triplicates basis.

iii. *Final Moisture Content*

The moisture content was determined according to A.O.A.C method, gravimetrically at 130^0C for 2 hours and then to constant weight.

iv. *Determination of Co-efficient of Reconstitution*

1gm of dried potato powder sample was taken in a previously weighed and dried centrifuge tube mixed thoroughly with 25ml of distilled water for 5 minutes by a vortex mixer and finally centrifuged at 3000rpm for 15 minutes. The supernatant was drained off and the final weight was taken. The co-efficient of reconstitution can be calculated by the following formulas:

Co-efficient of reconstitution = [Rehydration Ratio / Dehydration Ratio]
Rehydration Ration = [Weight of Rehydrated Material / Weight of Dehydrated Material]
Dehydration Ratio = [Weight of prepared material before drying / Weight of dried material]

v. *Determination Browning Index*

The browning index was determined using the procedure described by Hendel et. al., (1955). 2gm of grinded sample was extracted with 20 ml of 2% acetic acid solution and then filtered through a filter paper (Whatman No. 3). An aliquot of the filtrate was mixed with an equal volume of acetone and filtered again. The absorbance of the extracted colour solution was measured at 420 nm using a double beam UV-VIS spectrophotometer (Chemito Spectrascan UV 2700, Thermo Fisher Scientific) using a 1 cm cell. The results are expressed in terms of the optical density (Leeratanarak et. al., 2005).

vi. *Determination of Gelatinized Starch*

Gelatinized starch was determined by modified Chiang and Johnson (1977) method followed by Dextrose Equivalent titration method.

0.2gm of powdered sample was measured accurately and transferred to a conical flask and 5ml distilled water was added to it. Then 1ml of α-amylase solution (4unit/ml) and 8ml of glucoamylase solution (4unit/ml) was added to the conical flask. After proper mixing of the contents, the test tube was incubated in a hot water bath for 30 minutes at 40^0C with occasional shaking. After that, the enzymes were inactivated by holding the conical flask in boiling water for about 1-2 minutes. The final volume is measured. The enzymes break the starch to reducing sugar which was then estimated by Lane- Eynon method i.e. by Dextrose Equivalent titration method.

d) Proximate Analysis of Potato Powder

The potato powder prepared at optimized drying condition was used for proximate composition analysis. Moisture content, ash content, fat content, total protein, crude fibre content, and total starch content were done according to A. O. A. C method.

e) Localization study

In localization study contour plots were obtained by using Statistica (version 7) (Stat Soft, Inc., USA). The Contour plot describing combined effect between pair of time-temperature, GMS content-temperature, and GMS content-time on various functional properties was studied.

f) Determination of Regression Equation

RSM Modelling

Functional relationships between the independent variables (percentage GMS, temperature, time) and dependent variables (final moisture content, coefficient of reconstitution, and browning index) were studied. The regression equation were determined using multiple regression technique by fitting second order regression equation (Khuri and Cornell, 1987) of the following type

$$Y=\beta_o+\sum_{i=1}^{n}\beta_i X_i+\sum_{i=1}^{n}\beta_{ii}X_i^2+\sum_{i=1}^{n}\sum_{j=i+1}^{n-1}\beta_{ij}X_iX_j+e$$

where β_0, β_i, β_{ii}, β_{ij} are regression coefficients of variables for intercept, linear, quadratic and interaction terms, respectively, X_i, X_j are the independent variables, Y is the dependent variables n is number of independent variables.

The relationships between the responses were judged by correlation coefficients of determination (R^2). The significance or P-value was decided at a probability level of 0.05.

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III. Results & Discussions

a) Optimization of Optimum concentration of GMS and Stirring Time

Table 1 : Effect of different percentage of GMS and Stirring Time on Foam Expansion & Stability

Percentage of Glycerol Monostearate (%)	Stirring Time (minutes)	Foam Expansion (%)	Reduction of foam volume w. r. t initial foam volume (in ml) after a time interval of					
			30 min	60 min	90 min	120 min	150 min	180 min
1	5	15	0	0	1	3	7	10
2	5	20	0	0	0	0	1	1
3	5	20	0	0	0	0	2	5
1	10	20	0	0	0	1	3	5
2	10	25	0	0	0	0	0	0
3	10	25	0	0	0	0	0	0
1	15	18	0	1	2	5	9	13
2	15	23	0	0	0	0	0	0
3	15	23	0	1	1	2	2	3

Highest foam expansion is observed when the stirring time was increased from 5 to 10 minutes. Similarly the foam stability increases when stirring time is increased from 5 minutes to 10 minutes but decreases thereafter when the stirring time is increased to 15 minutes.

Foaming capacity as well as foam stability increased with increase in GMS concentration excepting in the case of addition of 3gm of GMS / 100gm of potato mash along with 15 minutes stirring in which a steady drainage of foam was noted. So, 10 minutes is determined as the optimum condition for effective foaming.

b) Optimization of Drying Parameters

Drying experiments were carried out on the basis of experimental design at 3^3 and 3^2 full factorial design. The factors were % GMS, temperature, and time. All the experiments conducted were repeated three times.

Table 2 : Functional Properties of Dried Powder at Different Drying Conditions

3**(2-0) full factorial design, 1 block , 9 runs						
Drying Condition Parameters			Functional Properties			
% of GMS	Temperature (°C)	Time (hrs)	Final Moisture Content (%)	Co-efficient of Reconstitution	Browning Index (Optical Density)	% Gelatinized Starch
-	50	5	7.37 ± 0.282	0.626 ± 0.061	0.019 ± 0.00115	60.57 ± 1.687
-	50	6	6.41 ± 0.285	0.644 ± 0.055	0.074 ± 0.00057	60.57 ±1.658
-	50	7	5.98 ± 0.531	0.645 ± 0.064	0.091 ± 0.00152	61.76 ± 1.715
-	55	5	6.67 ± 0.504	0.747 ± 0.043	0.021 ± 0.00173	61.76 ± 2.345
-	55	6	5.77 ± 0.319	0.769 ± 0.056	0.077 ± 0.00115	63.00 ± 1.725
-	55	7	5.33 ± 0.526	0.765 ± 0.065	0.095 ± 0.00117	63.00 ± 1.739
-	60	5	4.94 ± 0.496	0.773 ± 0.076	0.023 ± 0.00151	63.00 ± 2.080
-	60	6	4.88 ± 0.227	0.691 ± 0.058	0.080 ± 0.00157	61.76 ± 1.612
-	60	7	4.85 ± 0.230	0.677 ± 0.077	0.099 ± 0.00155	59.43 ± 1.635
3**(3-0) full factorial design, 1 block , 27 runs						
% of GMS	Temperature (°C)	Time (hrs)	Final Moisture Content (%)	Co-efficient of Reconstitution	Browning Index (Optical Density)	% Gelatinized Starch
1	50	5	2.53 ± 0.195	0.757 ± 0.043	0.012 ± 0.00115	58.33 ± 1.773
1	50	6	2.42 ± 0.431	0.760 ± 0.065	0.038 ± 0.00119	57.27 ± 2.645
1	50	7	2.35 ± 0.291	0.761 ± 0.081	0.067 ± 0.00100	59.43 ± 3.139
1	55	5	2.23 ± 0.325	0.770 ± 0.092	0.015 ± 0.00152	64.28 ± 2.086
1	55	6	2.20 ± 0.449	0.793 ± 0.034	0.039 ± 0.00157	68.47 ± 0.983
1	55	7	2.17 ± 0.247	0.791 ± 0.011	0.069 ± 0.00100	65.62 ± 2.732
1	60	5	2.01 ± 0.345	0.811 ± 0.075	0.013 ± 0.00155	70.00 ± 1.556
1	60	6	2.00 ± 0.111	0.789 ± 0.065	0.040 ± 0.00143	65.62 ± 2.385
1	60	7	2.00 ± 0.135	0.785 ± 0.039	0.075 ± 0.00123	58.33 ± 3.335
2	50	5	2.49 ± 0.333	0.868 ± 0.047	0.012 ± 0.00165	58.33 ± 1.347

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2	50	6	2.40 ± 0.346	0.871 ± 0.021	0.040 ± 0.00117	59.43 ± 2.775
2	50	7	2.33 ± 0.224	0.873 ± 0.033	0.072 ± 0.00173	61.76 ± 3.644
2	55	5	2.21 ± 0.292	0.888 ± 0.055	0.012 ± 0.00157	67.02 ± 3.414
2	55	6	2.17 ± 0.211	0.907 ± 0.078	0.042 ± 0.00145	68.47 ± 1.715
2	55	7	2.11 ± 0.398	0.899 ± 0.083	0.075 ± 0.00111	67.02 ± 1.670
2	60	5	1.89 ± 0.551	0.914 ± 0.025	0.013 ± 0.00175	70.00 ± 1.645
2	60	6	1.89 ± 0.526	0.895 ± 0.037	0.046 ± 0.00125	65.62 ± 3.188
2	60	7	1.89 ± 0.778	0.866 ± 0.056	0.076 ± 0.00100	57.27 ± 2.414
3	50	5	2.47 ± 0.299	0.827 ± 0.096	0.013 ± 0.00167	58.33 ± 1.371
3	50	6	2.39 ± 0.270	0.837 ± 0.075	0.039 ± 0.00178	59.43 ± 1.612
3	50	7	2.31 ± 0.234	0.841 ± 0.083	0.076 ± 0.00154	50.57 ± 2.713
3	55	5	2.19 ± 0.259	0.831 ± 0.043	0.013 ± 0.00185	64.28 ± 2.152
3	55	6	2.15 ± 0.398	0.835 ± 0.065	0.044 ± 0.00152	68.47 ± 1.713
3	55	7	2.15 ± 0.253	0.829 ± 0.034	0.075 ± 0.00188	63.00 ± 2.515
3	60	5	1.91 ± 0.324	0.813 ± 0.061	0.014 ± 0.00193	68.47 ± 3.162
3	60	6	1.91 ± 0.268	0.811 ± 0.045	0.048 ± 0.00102	64.28 ± 1.174
3	60	7	1.91 ± 0.481	0.801 ± 0.065	0.078 ± 0.00187	57.27 ± 0.987

From table: 2 it is observed that there is a decrease in percentage of final moisture content of potato foam with the increase in temperature-time and GMS profile of drying with the least moisture content of 1.89gm/100gms of potato powder belonging to the slurry subjected to drying for 5hrs at 60^0C and treated with 2gm GMS/100gm of potato mash. So, it can be concluded that effective foaming and foam stability leads to proper moisture removal from the sample.

From Table 2 it is observed that with increase in temperature and time of drying there is an initial increase in co-efficient of reconstitution but when the temperature- time profile of drying increases to 55^0C 7 hours from 55^0C 6 hours a slight decline in co-efficient of reconstitution is observed but the co-efficient further increases when temperature-time profile changes to 60^0C 5hours again after which there is a considerable drop in co-efficient of reconstitution. This phenomenon may be due to the combined effect of temperature and time of drying as higher heat absorption may affect the structure of the matrix and hence the reconstitutability. Sometimes in cases of heat sensitive products like potato high temperature and prolonged heating time negatively affects the product characteristics thereby decreasing its reconstitutable properties as well as it nutritive values thus decreasing its market acceptability.

From table: 2 it is observed that with increase in time of drying there is an increase in browning index at any particular temperature as well as at any particular time with increase in temperature there is an increase in browning index in some cases. The interactive effect of time-temperature on browning of the product shows that least browning occurs at the heat exposure of 5hrs and remains unaffected by change in combined effect of temperature and GMS concentration however samples not containing GMS i.e. non foamed samples show higher browning effect.

Table : 2 also indicate the effect of time temperature profile of drying on yield of gelatinized starch of the dried potato powder. Before drying the mash was initially pressure cooked which leads to gelatinization of the starch present in potato. A slight decrease in gelatinized starch content was also noted with excessive heat absorption this can be explained by the fact that with the change in enthalpy of the product there is a mild retrogradation of the starch.

From table: 2 primary optimization of drying condition is 2% GMS concentration, 60^0C, 5 hours where the functional properties are superior than other.

c) Study of Changes in Functional Properties during drying under Optimized Condition

From the table: 2, drying at 60^0C for 5 hrs with 2% GMS is primarily optimized (table: 2) to be the ideal condition for foam mat drying of potato mash. A set of foamed potato slurry prepared at optimum foaming condition was dried at primary optimized drying condition and samples were withdrawn at 15 minutes interval for determination of functional properties. The changes in the functional properties as a function of the interactive effects of temperature of drying and concentration of GMS at different time intervals were studied further under the optimized condition (table: 3). All experiments were done on a triplicate basis.

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Table 3 : Changes in Functional Properties at 15 Minutes Interval

Time (min)	Moisture Content (%)	Rehydration Ratio	Dehydration Ratio	Co-efficient of Reconstitution	Browning Index (Optical Density)	Gelatinized Starch (%)
15	66.722 ± 3.41	1.069 ± 0.117	1.339 ± 0.099	0.798 ± 0.013	0.013 ± 0.00180	67.90 ± 1.721
30	37.115 ± 2.97	1.275 ± 0.124	1.816 ± 0.143	0.702 ± 0.019	0.013 ± 0.00170	68.47 ± 1.552
45	26.391 ± 2.33	1.650 ± 0.098	2.256 ± 0.092	0.731 ± 0.015	0.014 ± 0.00210	68.81 ± 1.151
60	17.069 ± 2.71	2.185 ± 0.081	2.858 ± 0.088	0.764 ± 0.023	0.014 ± 0.00200	69.01 ± 1.691
75	6.981 ± 1.95	3.207 ± 0.133	4.014 ± 0.093	0.798 ± 0.011	0.014 ± 0.00140	69.19 ± 1.334
90	4.391 ± 1.31	3.737 ± 0.109	4.480 ± 0.089	0.834 ± 0.014	0.015 ± 0.0018	69.35 ± 1.362
105	3.473 ± 0.88	4.068 ± 0.087	4.672 ± 0.095	0.871 ± 0.021	0.015 ± 0.0016	69.78 ± 1.717
120	2.435 ± 0.76	4.386 ± 0.132	4.906 ± 0.121	0.894 ± 0.022	0.015 ± 0.0014	69.90 ± 1.258
135	2.135 ± 0.82	4.461 ± 0.091	4.983 ± 0.137	0.895 ± 0.017	0.015 ± 0.0011	69.90 ± 1.258
180	2.108 ± 0.37	4.471 ± 0.094	4.990 ± 0.112	0.896 ± 0.019	0.015 ± 0.0013	69.91 ± 1.324
240	2.077 ± 0.29	4.482 ± 0.099	4.998 ± 0.131	0.896 ± 0.016	0.015 ± 0.0017	69.91 ± 1.324
300	1.998 ± 0.53	4.501 ± 0.111	5.018 ± 0.105	0.896 ± 0.021	0.015 ± 0.00110	69.91 ± 1.324

From the observations made in table 3 it can be concluded that the changes in the functional properties and the moisture content becomes somewhat constant after 2hrs 15 minutes (135 minutes) of drying at 60^{0}C and hence the drying time of 5hrs becomes uneconomical. So the final optimization for foam mat drying of potato is 2% GMS, 60^{0}C, 2hrs 15 minutes (135 minutes).

d) Proximate Analysis of Potato Powder Prepared under Optimized Condition

The potato powder prepared at optimized drying condition was used for proximate composition analysis. Moisture content, ash content, fat content, total protein, crude fibre content, and total starch content were done according to A.O.A.C method. All the experiments were performed on triplicate basis

Table 4 : Proximate Composition of Dried Potato Powder

Parameters	Potato Powder (gm wt/100gm of potato powder)	
	Wet Basis	Dry Basis
Moisture Content	1.932±0.248	1.968±0.257
Ash Content	4.961±0.308	5.059±0.325
Fat Content	1.932±0.201	1.970±0.205
Total Protein	2.854±1.134	2.912±1.156
Crude Fibre	2.873±0.937	2.929±1.231
Total Starch	82.113±1.342	83.731±1.576

e) Localization study

Fig- 1 shows that final moisture content of 3^3 full factorial design of experiments (table: 2) as a function of time and temperature. It indicates that the reduction of percentage moisture content is more dependable on increment on temperature rather than increment of time.

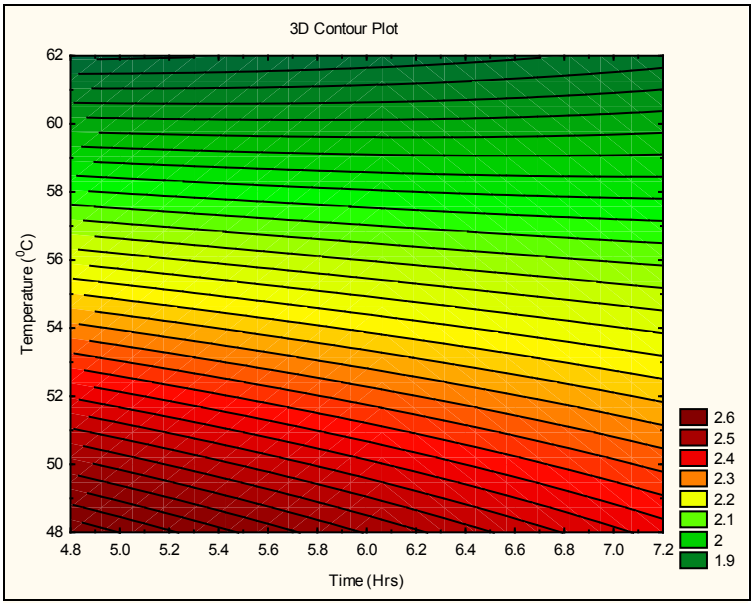


Figure 1 : Contour plot of the combined effect of time and temperature on Percentage of Final Moisture Content

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Fig- 2 shows that co-efficient of reconstitution of 3^3 full factorial design of experiments (table: 2) as a combined function of percentage of GMS and temperature. It is clearly indicates that value co-efficient of reconstitution is superior at mid level of percentage of GMS and higher level of temperature.

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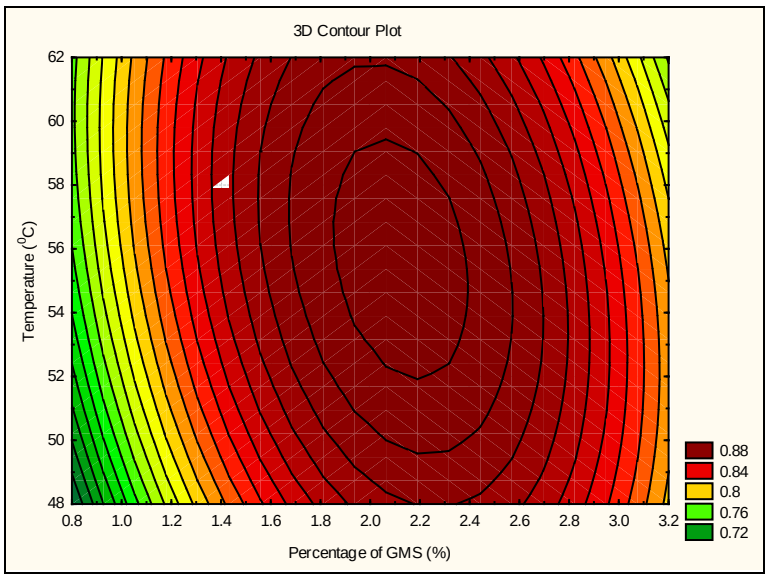


Figure 2 : Contour plot of the combined effect of percentage of GMS and temperature on co-efficient of reconstitution

Fig- 3 & 4 shows that browning index of 3^3 full factorial design of experiments (table: 2) as a combined function of time and temperature, time and percentage GMS respectively. Both the figure 3 & 4 clearly indicates that value browning index is mainly dependent on the period of heating time.

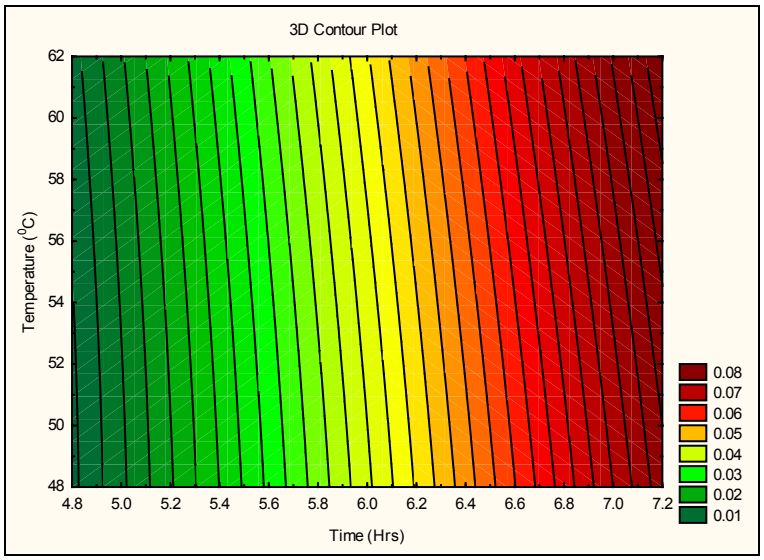


Figure 3 : Contour plot of the combined effect of time and temperature on browning index

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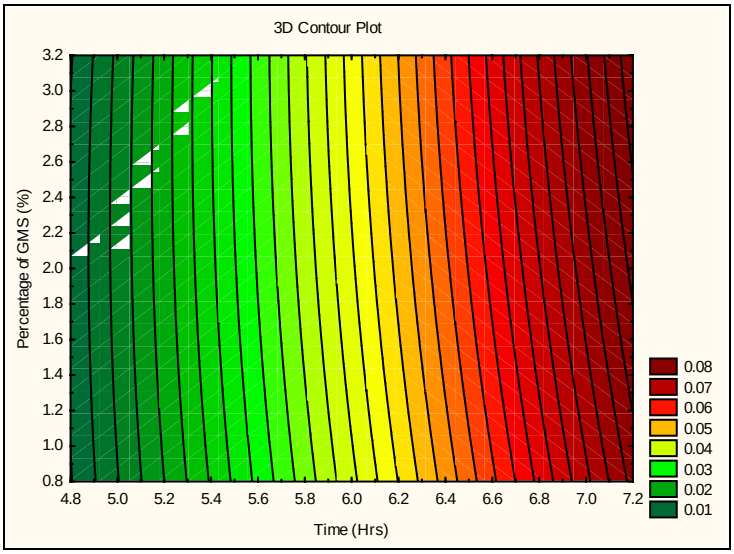


Figure 4 : Contour plot of the combined effect of time and percentage GMS on browning index

Fig- 5 shows that percentage of gelatinized starch of 3^3 full factorial design of experiments (table: 2) as a combined function of temperature and percentage of GMS. It is clearly indicates that value percentage of gelatinized starch is superior at mid level of percentage of GMS and slightly higher level than mid level of temperature.

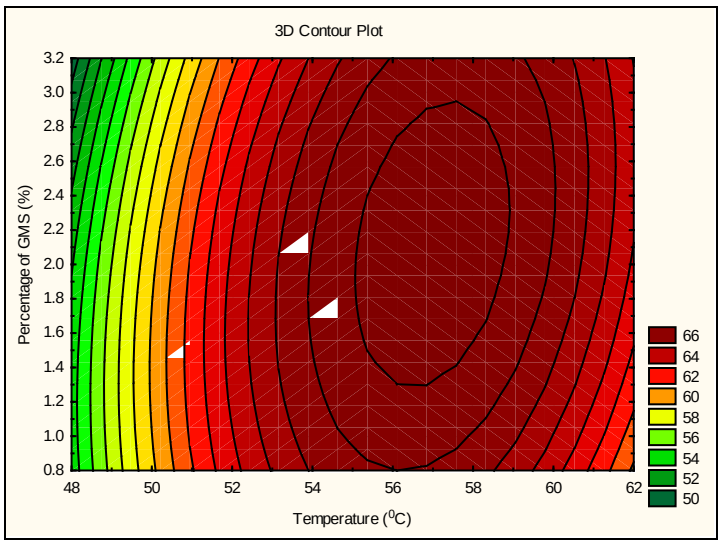


Figure 5 : Contour plot of the combined effect of temperature and percentage GMS on percentage of gelatinized starch

f) Determination of Regression Equation (RSM)

The second order model was fitted to response data of percentage of final moisture content, Co-efficient of Reconstitution, and browning index respectively of 3^3 full factorial design of experiments (table: 2) at a significance level of 0.05. Where the drying parameters percentage GMS, temperature, and time are independent variable and percentage of final moisture content, Co-efficient of Reconstitution, and browning index are dependent variable respectively.

It has been found that square term of GMS, time, interactive effect percentage GMS with temperature and interactive effect of temperature and time have significant effect at $p<0.05$ level on percentage of final moisture content (table: 5).

It was also found that except square term of time and interactive effect of temperature with time all other linear, quadratic, and interaction of different drying parameters have significant effect at $p< 0.05$ for Coefficient of Reconstitution (table: 5).

For browning index square term of time and interaction of percentage GMS with time have significant effect at $p<0.05$ level.

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Table 5 : Estimation of Regression Parameters, p value, and t value

Percentage of Final Moisture Content			
Effect	**Parameter**	**p**	**t**
Intercept	7.428889	0.000004[b]	6.64351[b]
% GMS	-0.036389	0.657073[a]	-0.45187[a]
% GMS*% GMS	0.030000	0.002079[b]	3.62785[b]
Temperature (^{0}C)	-0.076889	0.053982[a]	-2.07015[a]
Temperature*Temperature (^{0}C)	-0.000133	0.691903[a]	-0.40309[a]
Time (Hrs)	-0.556944	0.000214[b]	-4.68306[b]
Time*Time (Hrs)	0.005000	0.553403[a]	0.60464[a]
% GMS* Temperature (^{0}C)	-0.002500	0.047360[b]	-2.13773[b]
% GMS* Time (Hrs)	0.004167	0.485774[a]	0.71258[a]
Temperature (^{0}C)*Time (Hrs)	0.008167	0.000002[b]	6.98325[b]
Co-efficient of Reconstitution			
Effect	**Parameter**	**p**	**t**
Intercept	-1.99819	0.001331[b]	-3.8334[b]
% GMS	0.53136	0.00000[b]	14.1552[b]
% GMS*% GMS	-0.08444	0.00000[b]	-21.9068[b]
Temperature (^{0}C)	0.06849	0.001020[b]	3.9558[b]
Temperature*Temperature (^{0}C)	-0.00046	0.008606[b]	-2.9689[b]
Time (Hrs)	0.14525	0.017920[b]	2.6201[b]
Time*Time (Hrs)	-0.00394	0.320518[a]	-1.0233[a]
% GMS* Temperature (^{0}C)	-0.00312	0.000025[b]	-5.7172[b]
% GMS* Time (Hrs)	0.00008	0.975966[a]	0.0306[a]
Temperature (^{0}C)*Time (Hrs)	-0.00182	0.003944[b]	-3.3325[b]
Browning Index			
Effect	**Parameter**	**p**	**t**
Intercept	-0.002963	0.975348[a]	-0.031360[a]
% GMS	-0.006361	0.362943[a]	-0.934876[a]
% GMS*% GMS	-0.000444	0.533188[a]	-0.636091[a]
Temperature (^{0}C)	-0.001733	0.587515[a]	-0.552325[a]
Temperature*Temperature (^{0}C)	0.000009	0.754324[a]	0.318045[a]
Time (Hrs)	-0.001417	0.889542[a]	-0.140980[a]
Time*Time (Hrs)	0.001556	0.039805[b]	2.226317[b]
% GMS* Temperature (^{0}C)	0.000017	0.868048[a]	0.168669[a]
% GMS* Time (Hrs)	0.001500	0.007458[b]	3.036042[b]
Temperature (^{0}C)*Time (Hrs)	0.000183	0.080969[a]	1.855359[a]

a- not significant at $p<0.05$ level, b- significant at $p<0.05$ level

The student's t-test was performed to determine the significance of the regression co-efficient. The results of statistical analysis including the regression co-efficient, t and p values for linear, quadratic and combined effects of the variables were given in the table 5. The larger the magnitude of the t-value and the smaller the p-value, indicate more significant of the corresponding coefficient and its effect on functional properties as well as foam mat drying of potato mash. The p-values are used as a tool to check the significance of each of the coefficients and to understand the interactions between the best variables.

Table 6 : Multiple R, Multiple R^2, and Adjusted R^2 of Different Regression Equation

Regression Model Equation of	Multiple R	Multiple R^2	Adjusted R^2
Percentage of Final Moisture Content	0.996811	0.993632	0.990261
Co-efficient of Reconstitution	0.987038	0.974243	0.960607
Browning Index	0.998516	0.997033	0.995463

Joglekar and May (1987) have suggested for a good fit of a model, regression coefficient (R^2) should be at least 80%. All the R^2 values are the proportion of variation in the response attributed to the model was > 0.80 (table: 6), this means that this model fitted well with the experimental data.

The regression model equations from the parameters of table 5 for different responses of percentage of final moisture content, coefficient of reconstitution, and browning index have used to predict the data for different responses. Correlation of experimental and predicted data provided an

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observation that a good relationship exit between experimental and calculated data means high values of correlation coefficient (r) and coefficient of determination (R^2) (table: 7).

Table 7 : Correlation of Experimental vs. Predicted values

Responses	Correlation Co-efficient (r)	Coefficient of Determination (R^2)
Percentage of Final Moisture Content	0.9931	0.9966
Co-efficient of Reconstitution	0.9870	0.9743
Browning Index	0.9987	0.9975

IV. Conclusion

The interactive effect of concentration of foaming agent, time and temperature of drying on the reconstitutability, browning index, final moisture content and percentage of gelatinized starch present in the product was conducted. The process which yielded the superior functional properties is drying at 60^0C for 2hrs 15 minutes and foamed with 2% glycerol monostearate. It can also be concluded that GMS as a foaming agent has properly good effect on functional properties as well as drying.

Joglekar and May (1987), have suggested for good fit of a model, regression coefficient (R^2) should be at least 80%, all the R^2 value of different modelling equation in this study are more 80% (i.e. $R^2 > 0.80$) and also a high similarity was observed between the predicted and experimental results of different cases, which reflected the accuracy and applicability of regression equations or regression model equation of the study.

V. Abbreviations

^{0}C - Degree centigrade
Psig- Pounds per square inch gauge
KMS -Potassium metabisulphite
GMS- Glycerol monostearate
NaCl – Sodium Chloride
p - Significance level
R^2 – Coefficient of determination
r- Correlation coefficient

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Ordination and Classification of Vegetation in Semi Arid Area of Pakistan

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Abstract- A survey of natural vegetation of Tehsil Takht-e- Nasrati, District Karak was undertaken in spring 2010-2011. Total 66 species were recorded during spring. Hierarchical Cluster Analysis (HCA), and Detrended Correspondence Analysis (DCA) were used for the plant community analysis. Plant species of each community type are presented together with the information on dominance and sub-dominance species. Four plant co- mmunities association i.e. Prosopis- Fagonia-Saccharum association, Zizyphus-Saccharum-Acacia association, Fagonia-Zizyphus-Eragrostis association and Aerua-Acacia-Cymbopogon association were recognized. Classification and ordination techniques provided very similar results based on the floristic composition and communities similarity. The results produced the source for the mapping division of plant life communities.

Keywords: *HCA, DCA, spring, community association, tehsil takht-e-nasrati.*

GJSFR-C Classification : *FOR Code: 961306*



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Ordination and Classification of Vegetation in Semi Arid Area of Pakistan

Musharaf Khan [α], Farrukh Hussain [σ], Faridullah [ρ] & Shahana Musharaf [ω]

Abstract- A survey of natural vegetation of Tehsil Takht-e-Nasrati, District Karak was undertaken in spring 2010-2011. Total 66 species were recorded during spring. Hierarchical Cluster Analysis (HCA), and Detrended Correspondence Analysis (DCA) were used for the plant community analysis. Plant species of each community type are presented together with the information on dominance and sub-dominance species. Four plant co- mmunities association i.e. Prosopis-Fagonia-Saccharum association, Zizyphus-Saccharum-Acacia association, Fagonia-Zizyphus-Eragrostis association and Aerua-Acacia-Cymbopogon association were recognized. Classification and ordination techniques provided very similar results based on the floristic composition and communities similarity. The results produced the source for the mapping division of plant life communities.

Keywords: HCA, DCA, spring, community association, tehsil takht-e-nasrati.

I. Introduction

Ordination techniques are commonly used in phytosociology. This may be done either by arranging the points along the axis or by forming the scatter diagram with two or more axis. Detrended Correspondence Analysis (DCA), an indirect gradient analysis technique in which the distribution of species is not controlled by environmental variables rather, it focuses to analyze the pattern of species distribution. Environmental data for DCA is not required and species data is used to assume the gradients (Sagers & Lyon, 1997). Ordination techniques are widely used by the ecologists to study the relationship between vegetation and environment. Khaznadar et al., (2009) conducted a study in Chott El Beida wetland, a RAMSAR site in Setif, Algeria to study distribution of plants community and environmental factors. The collection was done from sixty vegetation plots. TWINSPAN and Detrended Correspondence Analysis (DCA) were used as the analysis techniques. A similar study was conducted by Ahmad et al., (2010) along motorway (M-2), Pakistan using multivariate techniques i.e., DECORANA. Results showed two major and sixteen sub-communities from 397 quadrats. The study was helpful for implementation and conservation planning and for the improvement of road sides. To study the relationship between vegetation and environment, a study was conducted by He et al., (2007) in the Alxa Plateau of Inner Mongolia, China which resulted in the detection of six characteristics vegetation groups by using the Detrended corr-espondence analysis (DCA). Ahmad (2009) studied the herbaceous vegetation in Margalla Hills National Park, Islamabad, Pakistan. Four vegetation groups were recognized by TWINSPAN. El-Bana et al., (2009) studied *Juniperus phoenicea* L. and associated vegetation at three mountains in Egypt, resulted in the recognition of four vegetation types along with juniper by TWINSPAN and DCA analysis techniques. Jabeen and Ahmad (2009) conducted a study to analyze the vegetation and environment data of Ayub National Park, Rawalpindi. PCOrd 5 and CANOCO 4.5 were used and data was recorded by quadrat method. 44 plants species from 30 quadrats were recorded. Many researchers (Dasti & Malik, 1998; Malik & Hussain, 2008; Saima et al., 2009; Ahmad, 2009; Ali & Malik, 2010; Ahmad et al., 2010; Khan & Hussain, 2012) have studied different aspects of vegetation structure and classification and ordination distribution patterns in different parts of Pakistan. Classification and ordination is an invaluable method for vegetation survey and assessment involving inv-estigation of characteristics of plant communities using simple and rapidly employing field techniques (El-Ghanim et al., 2010). In the present study, an effort has been made to investigate and analyse correlation of communities with key environmental factors. The Tehsil Takht-e-Nasrati comprises one of the richest and most interested ecosystems on earth. The community structure and distribution patterns of research area have not been given due attention till the date by the plant ecologists, and hence poorly understood (Khan, 2012). The particular objectives of present study include quantifying the vegetation in spring season of Tehsil Takht-e-Nasrati, District Karak using ordination techniques for upcoming conservation and providing base line data of ecological important area.

II. Research Area

The Tehsil Takhti Nasratti is situated at 32.47o to 33.28o North and 70.30 o to 71.30o East. The Tehsil is bounded by Tehsil Banda Dawood Shah on the North West, Tehsil Karak on the North East, District Mianwali and District Lakki Marwat on the South East, and Tribal

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area Adjoining District Bannu on the South West (Fig. 1). The total area of Tehsil is about 613.66 Sq. kilometer. Majority of the area consists of rigged dry hills and rough fields areas i.e. 323.97 Sq. kilometers and agriculture land is about 289.7 Sq. kilometer. The major income source of the people is Agriculture, which is rain depended. The area is situated at 340 m above the sea level. In the year 2010, 62.5 mm. y-1 of rainfall recorded. The area is very hot in summer and very cold in winter. June and July are the hottest months, where as December and January are the coldest months. In the year 2010 the mean maximum temperature was 39.5 Co, in the month of the May, where as the mean minimum temperature was as low as 4 C o, in the month of January. The wind speed was different in different years. In the year 2009 the wind speed was high 6 Km per hour (h) in the month of July whereas in the year 2010 it was high in the month of April 7.2 Km. h-1 (Table 1).

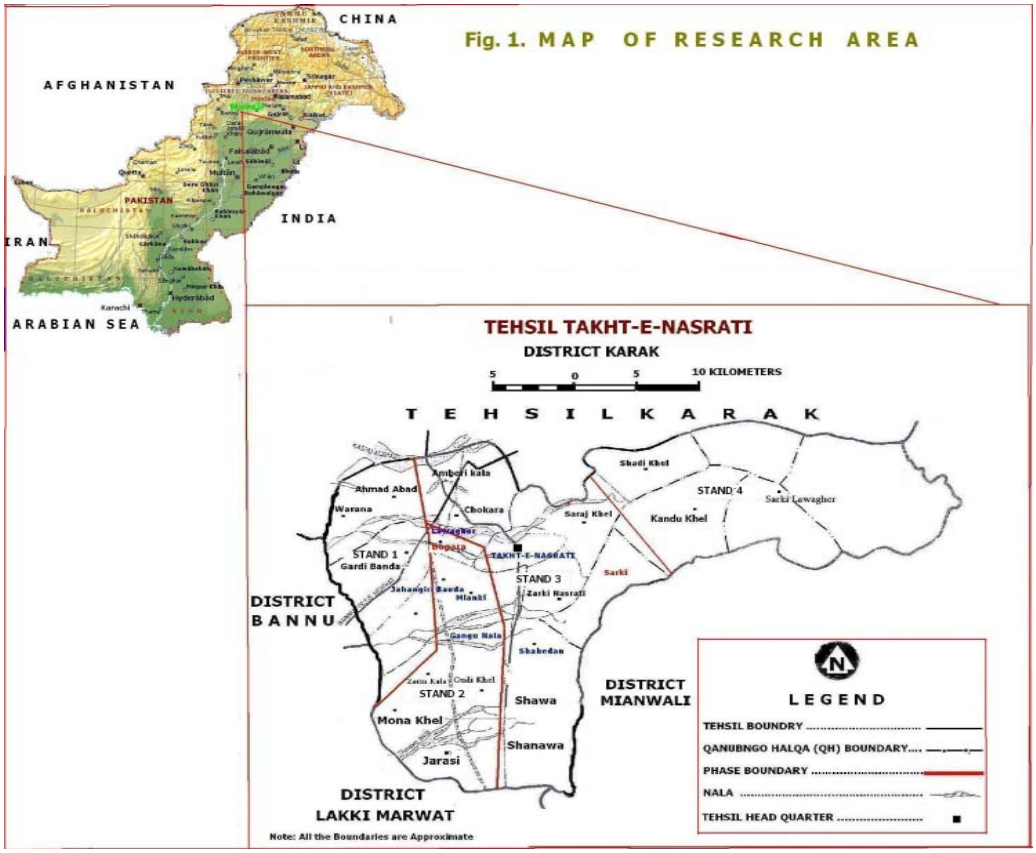


Figure 1 : Map of Tehsil Takht-e- Nasrati showing research spots.

Table 1 : Meteorological data of Tehsil Takht -e –Nasrati, District Karak for the year 2001-2010.

Months	Temperature (C°)		Humidity (%)		Rainfall (mm)	Soil temperature (C°) Average	Wind speed (Km Per Hour)
	Max	Min	Max	Min			
January	19.18	4.26	75.80	35.24	27.43	7.03	2.9
February	21.69	7.29	77.39	42.23	37.72	9.14	3.2
March	28.20	12.06	75.38	35.23	37.17	13.89	3.5
April	34.74	17.94	66.12	29.42	36.54	19.02	5.2
May	38.32	22.33	59.66	30.73	31.6	21.87	5.4
June	39.50	25.9	59.96	32.89	74.24	25.78	5.5
July	38.44	25.76	73.33	38.76	121.6	26.77	5.2
August	36.66	25.29	75.68	42.61	108.3	26.37	4.1
September	35.47	21.95	77.21	39.29	61.58	23.49	3.7
October	32.33	16.79	71.55	35.51	15.13	20.09	3.5
November	26.71	10.01	71.56	36.66	5.80	14.10	3.2
December	21.93	5.67	75.20	35.90	15.38	8.96	3.1
Mean	31.1	16.27	71.57	36.21	47.71	18.04	4.04

Source : Agricultural Research Farm Ahmad Wala Karak.

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III. Materials and Methods

a) Field data collection

Floristic data were collected from 22 randomly selected sites from 4 stand selected on the basis of altitude. Quadrat method was used for the collection of vegetation data. Each field site comprised of 10 Quadrats for each plant layer i.e. tree (10X10m), shrubs (5X5m) and herbs (1X1m). The latitude and longitudes were recorded for each site using a Global Positioning System (GPS). Sampling was completed in spring season. The spring season starts in March -April, when most of the plants are in flowering stages. Collected samples were pressed, dried and transported to herbarium of University of Peshawar Khyber Pakhtunkhawa, Pakistan, where they were identified and classified following Stewart (1972) and Nasir and Ali (1972) and a fraction of angiosperms of Tehsil Banda Daud Shah by Khan, (2004).

b) Data analysis

Vegetation attributes including frequency, density and cover were recorded along with environmental coordinates like latitude, longitude, altitude and slope using GPS. The importance value of each species was compiled adding RD, RF and RC following Hussain (1989). On the basis of the highest importance values of the first three dominant species from each layer, the communities were established and named. All the species data, as well as the field sites communities, were used for the analysis. The data was classified using standard methods Hierarchical Cluster Analysis (HCA) and Detrended Correspondence Analysis (DCA) (Hill, 1979) to summarize biological records and position of communities in groups during spring. The plant life associations were named after the highest value of three dominant species. DCA ordination offered two significant ordination axes on the basis of weight for communities. Detrended Correspondence Analysis (DCA) were performed to describe compositional gradients in the vegetation. All analysis was performed using the software PCORD ver. 4.16 (McCune & Mefford, 1999).

IV. Results

The arrangement of plant life record is commonly vegetation orientation and main query disquiets the classification and explanation of the vegetation in addition to inconsistency of ecological arrangement. Distinctive multivariate techniques are generally fruitful and commonly used for plant life arrangement position. Though, distinctive multivariate analyses do not directly take into explanation relations in their computation and are not particularly designed to vegetation structures rationalization. The ordination may be defined as the position of communities designed to set apart group types, location, relative position, standing of communities in a season of particular area. In other words, the ordination is the sound or clear arrangement of split communities or species in a season of a particular area. In present work the ordination of communities in spring is given as follows:

a) Hierarchical Cluster Analysis

In spring season, 66 species were present in 22 communities in 4 different stands on the basis of altitude. The Hierarchical Cluster Analysis shows that the relationship among 22 communities during spring were inclusion iv into 21 cluster cycling where in cycle 1 it shows the relation of 2 communities at 1.0928E+03 and last i.e. cycle 21, 22 communities were connected with one another at 1.0144E+05 with 3.29 % chaining. Further more, on the basis of relationship it marked out distinct 4 groups associations by different level, cycling and similarity of communities. The depiction of each one group association is as below:

i. *Prosopis-Fagonia-Saccharum association*

In group 1, 32 species comprising 6 trees, 6 shrubs and 20 herbs and grasses were present. The dominant species of association with highest mean important value were *Prosopis farcta* (IV = 28.4), *Fagonia cretica* (IV = 22.6) and *Saccharum bengalense* (IV = 21.9). Furthermore, it comprises 4 communities i.e. *Prosopis-Saussurea-Saccharum* community (PSS), *Prosopis-Periploca-Aerua* community (PPA), *Fagonia-Prosopis-Saccharum* community (FPS) and *Phoenix-Saussurea-Saccharum* community (PSS) which raised at 4.2195E+04 in cycles 15 (Table 2; Fig. 2).

ii. *Zizyphus-Saccharum-Acacia association*

The group 2 becomes visible at 4.8435E+04 in cycle 16 that contains *Acacia-Saccharum-Citrullus* community (ASC), *Calligonum-Zizyphus-Saussurea* community (CZS), *Zizyphus-Cenchrus-Saccharum* community (ZCS), *Zizyphus-Aerua-Calligonum* community (ZAC), *Zizyphus-Saccharum-Cynodon* community (ZSC) and *Zizyphus-Calligonum-Fagonia* community (ZCF). Moreover, 42 species in which 4 trees, 7 shrubs and 31 herbs were present. Where mean highest important value was represented by *Zizyphus maurtiana* (IV = 54.08), *Saccharum bengalense* (IV = 19.84) and *Acacia nilotica* (IV = 17.26) (Table 2; Fig. 2).

iii. *Fagonia-Zizyphus-Eragrostis association*

In cycle 17 at 5.5773E+04, the *Fagonia-Zizyphus-Eragrostis* association was structured that composed of 47 species of 7 trees, 14 shrubs and 26 herbs in which the mean highest important value 34.5, 28.1 and 19.9 presented *by Fagonia cretica*, *Zizyphus maurtiana and Aerua persica* respectively. It consists of 6 communities i.e. *Fagonia-Zizyphus-Saccharum* community (FZS), *Fagonia-Phoenix-Capparis* community (FPC), *Fagonia-Withania- Zizyphus* community (FWZ), *Dichanthium-Withania-Zizyphus* community (DWZ),

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Eragrostis-Zizyphus-Capparis community (EZC) and *Salvia-Zizyphus-Rhazya* community (SZR) (Table 2; Fig. 2).

iv. *Aerua-Acacia-Cymbopogon association*

In *Aerua-Acacia-Cymbopogon* association, 34 plant species comprising 5 trees, 11 shrubs and 18 herbs and grasses were present. The dominant species on the basis of important value were *Aerua persica* (IV = 49.4), *Acacia modesta* (IV = 21.2) and *Cymbopogon jwarancusa* (IV=20.9). It was structured at 6.3334E+04 in cycle 18 covering *Cymbopogon-Rhazya-Zizyphus* community (CRZ), *Aerua-Saccharum-Zizyphus* community (ASZ), *Aerua-Rhazya-Acacia* community (ARA), *Aerua-Punica-Acacia* community (APA), *Aerua-Acacia-Capparis* community (AAC) and *Zizyphus-Aerua-Capparis* community (ZAC) (Table 2; Fig. 2).

b) Detrended Correspondence Analysis (DCA)

Ordination of the communities by DCA explains that the communities with high weight and structured 4 groups. On Axis 1, the groups 4, 3, 2 & 1 were structured with mean DCA weight 241, 138, 34.6 and 23.8 at Eigen values of axes (0.495) respectively. While on Axis 2, the 1, 2, 3 and 4 groups were produced with mean DCA weight as 145, 74, 135 and 69 at EIG (0.206) respectively. Furthermore, the group 1, 2, 3 and 4 were composed of 4, 5, 3 and 5 communities respectively. Other communities that were not present in groups were ASC, CZS, EZC, FPC and FPS with DCA weight 231, 221, 67, 84, 207 at Axis 1 and 220, 190, 116, 88 and zero at Axis 2 respectively. These groups show different vegetation types during spring seasons (Fig. 3).

V. Discussion

Cluster analysis segregates the communities of similar character into major groups of plant life. In spring, 4 groups were structured. The chaining percentage between communities association was high 3.29 in spring. From this it was noticed that the chaining percentage would be high with high quantity and presence of species in an area. In spring, species were mostly found in all sites in less or high quantity while species presence is restricted to specific area due to diverse factors. Most factors that occur during spring in under investigated area were high grazing, cutting, non availability of water, soil erosion and uprooting of plant species. Ahmed & Yasmin (2011) analyzed natural vegetation of two zones along Hanna Lake, Baluchistan using DECORANA and classify the vegetation into plant communities. Major group is the objective to give structure to plant life. However, cluster analysis is a helpful preliminary position for competent judgment and adjoining neighbors of vegetation. Greater the homogeneity within communities and greater will be the similarity in the clustering. The cluster analysis was used to give clear picture of the plant life in an area in the form of tree - shape. In hierarchical clustering the principle is to structure a hierarchical chain of communities' groups sorting from groups of community position at the bottom to a comprehensive group at the top. The graphically diagram which represents the hierarchy in the structure of upturned tree expresses a dendogram that clarifies the arrangement in which position were united (bottom-up outlook) or group were divide (top-down outlook).

Detrended Correspondence Analysis (DCA) was used to give the shape to the communities on the basis of weight. This method is also used to give cleared picture of plant life in specific area in spring season. The present results conclude that the plant species composition was different in spring season in the same area. On axis 1, communities DCA weight was high in plains and low in hilly area. However, DCA has limitations, making it best to remove extreme outliers and discontinuities prior to analysis. DCA consistently gives the most interpretable ordination results, but as always the interpretation of results remains a matter of ecological insight and is improved by field experience and by integration of supplementary environmental data for the plant life sample sites. Ali & Malik (2010) applied the Detrended Correspondence Analysis (DCA) to identify environmental gradients to define vegetation distribution in green belts, gardens and parks of Islamabad city and classified the flora into 4 major association groups. El-Ghanim et al. (2010) studied the vegetation at Hail region north of central Saudi Arabia where multivariate techniques results showed 7 vegetation groups. Ahmad et al. (2010b) analyzed the vegetation along motorway (M-2), Pakistan by using multivariate techniques. In the investigated area, the fore mentioned facts noticeably indicate that slope, edaphic factor, harsh erosion, crushing of herbs and supply of rain water were the key source of plant life discrepancy. These geomorphologic aspects restrict the limitations and composition of plant communities. Distant from the reality that the site changeable are definitely significant for explaining the major plant life nature the association between the results of cluster analysis and DCA planes allow a direct analysis of scores of position data in DCA plane in relation to area up-and-down. The DCA technique provided interpretable and dynamic results than other ordination techniques and the length of first axis was greater than 3.0 and in terms of communities or species turnover. Jongman et al. (1995) recommended that if plants species or communities turnover is larger than 1.9 standard deviation then DCA techniqe is advanced option of ordination. Detrended Correspondence Analysis (DCA) was carried out to express compositional ascents in the plant life. DCA was presented using a default value for rescaling and detrending. Rare species and divergent communities were downweighted in DCA ordination.

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The different association produced by cluster analyses are designed a first two axes as a sprinkled diagram. The DCA ordination axes may signify in same way the main substrate weight that affect the community in these records and have been used by the community and area characteristic of the relationship to argue the dominant characteristics of the location and plant life association. Cluster and DCA analysis are very helpful in communities' and species classification in addition to give structure to plant life. Such type of study was also carried out by Saima et al. (2009) who stated that tree density, pH and soil texture were the major determinant of vegetation pattern. There was thin vegetation in the investigated area and species was present in patches. The ecologists have tried to quantify the division of species beside the ecological gradients. There is an association between plant life sample and resources available (Ahmad et al., 2009b and Jabeen & Ahmad, 2009). The ordination by means of cluster analysis and DCA help us skillfully in evaluating the classification of plants and structure of entire habitat of plant life. Malik & Hussain (2008) conducted a study to work out the relationship between remote sensing data and vegetation communities of ecological importance using multivariate techniques and stated that the ordination methods proved effective in summarizing basic, general structure of the plant community types and to some extent indicated correspondence with their spectral signatures. This study pointed out that the climatic environment of region has restricted enlistment of area and the plant life was changed with the change of seasons and altitude. Our result agrees with Dasti & Malik (1998) who stated that altitude is an environmental factor which affecting plants association. Plant ecologists have commonly been aware that plant life shows an inconsistency over a wide range of particular scales and area that have built up methods for studying the classification of vegetation. The area show less rainfall than 200 mm and consist of thorny trees like *Zizyphus spp*, A. *nilotica,* A. *modesta*. Trees are sprinkled, roots longs, leaves thick and small in most plant species therefore, the investigated area fall into tropical thorn forests. The value of altitude as an ecological factor affecting plant species association is not considering, surprising its close correlation with precipitation and interruption of rain (Danin et al., 1975; Evenari et al., 1982).

VI. Conclusion

The study demonstrated the potential of different classification and ordination analysis such as HCA and DCA in detecting the main environmental gradients and one could isolate a subset of environmental factors that led to a reasonable (ecologically meaningful) interpretation for important gradients in a few dimensions. It is used as a perfect way to study and helps skillfully in evaluating the biodiversity and conservation of intact habitat and plant life in specific area. This study pointed out that climatic environment of region has privileged conscription of area and association of plant was changed with the change of altitude. Plant ecologists have commonly been conscious that vegetation shows a discrepancy over a broad variety of particular scales and area. Therefore, it is needed that we apply the multivariate techniques i.e. HEC and DCA methods for studying the degree of plant life differences.

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Table 2 : Mean relative importance value of species in different associations during spring distinguished through cluster analysis of Tehsil Takht-e-Nasrati, Karak.

S. No	Species Name	Groups			
		1	2	3	4
1	*Acacia modesta* Wall.	0	0	7.55	21.2
2	*Acacia nilotica* (L.) Delice.	4.6	17.3	5.56	4.07
3	*Dalbergia sissoo* Roxb.	2.9	12.4	0.79	2.97
4	*Gymnosporia royleana* Wall. ex M. A. Lawson.	0	0	0.78	0
5	*Monotheca buxifolia* (Falc.) A.D.	0	0	1.02	0
6	*Phoenix dactylifera* L.	16.6	0.87	10.7	1.32
7	*Prosopis farcta* (Banks & Sol.) J.F. Macbr.	28.4	0	0	0
8	*Prosopis juliflora* (Sw.) DC.	0	0	0	0.86
9	*Tamarix aphylla* (L.) Karst.	2.03	0	0	0
10	*Zizyphus maurtiana* Lam.	14.2	54.1	28.1	19.7
11	*Astragalus psilocentros* Fisch.	0	1.9	2.09	8.88
12	*Calligonum polygonoides* L.	0	15.3	2.3	0
13	*Calotropis procera* (Wild.) R. Br.	9.79	10.3	5.59	2.24
14	*Capparis decidua* (Forssk.) Edge worth.	0	0	7.49	11.9
15	*Capparis spinosa* L.	0	0	0	8.5
16	*Cassia angustifolia* Vahl.	0	0	3.19	0
17	*Datura metel* L.	13	7.99	1.1	0
18	*Periploca aphylla* Decne.	12.7	5.28	3.26	0.73
19	*Punica granatum* L	0	0	0	8.81
20	*Rhazya stricta* Decne.	0	0	15.7	20.2
21	*Ricinus communis* L.	6.5	2.83	0.83	0
22	*Saccharum bengalense* Retz.	21.9	19.8	6.34	9.02

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23	*Saccharum spontaneum* L.	1.38	0	3.03	0.75
24	*Withania coagulans* (Stocks) Dunal.	0	0	14.2	2.15
25	*Zizyphus nummularia* (Burm.f.) W. & A.	0	0	4.87	4.93
26	*Aerua persica* (Burm.f.) Merrill.	16.3	5.28	19.9	49.4
27	*Asparagus gracilis* Royle.	0	5.98	4.19	9.25
28	*Asphodelous tenuifolius* Cavan.	0	2.15	2.27	0
29	*Cenchrus biflorus* Hook. f.	4.17	0	0	0
30	*Cenchrus ciliaris* L.	0	12.3	15.9	12.8
31	*Centaurea iberica* Trev.Ex. Spreng	0	0	1.32	1.53
32	*Chenopodium album* L.	2.7	4.76	0	0
33	*Citrullus colocynthis* L. Schrad.	1.7	5.57	1.32	0
34	*Convolvulus arvensis* L.	6.9	2.3	0	0
35	*Convolvulus pluricaulis* Choisy.	0	0	2.82	7.64
36	*Crotalaria medicaginea* Lam.	0	1.22	0	0
37	*Cymbopogon jwarancusa* (Jones) Schult.	0	0	0	20.9
38	*Cynodon dactylon* (L.) Pers.	5.08	3.4	13.1	8
39	*Cyperus rotundus* L.	0	3.65	1.41	0
40	*Dichanthium annulatum* (Forssk) Staph.	0	0	11.5	8.44
41	*Echinops echinatus* D.C.	0	2.18	9.08	7.16
42	*Eragrostis poaoides* Beauv.	12.9	11.9	21.7	19
43	*Erodium malacoides* (L.)L. Her. Ex Ait.	0	0.81	0	0
44	*Euphorbia helioscopia* L.	5.89	6.44	0	0
45	*Euphorbia prostrata* Ait.	2.47	4.37	0	0
46	*Fagonia cretica* L.	22.6	6.68	34.5	8.2
47	*Hypecoum pendulum* L.	0	3.53	0	0
48	*Ifloga fontanesii* Cass.	12.1	10.7	0	0
49	*Ipomoea hederacea* (L.)Jacq.	0	0	1.32	0
50	*Kickxia ramosissima* (Wall) Janchen.	0	1.63	0	3.3
51	*Launaea nudicaulis* (L.) Hook. f.	0	7.7	8.1	4.25
52	*Malcolmia africana* (L.) R.Br.	1.8	2.04	0.66	0.44
53	*Malva parviflora* L.	0	2.75	0.98	0
54	*Malva neglecta* Wallr.	0	4.58	0.98	1.87
55	*Medicago laciniata* (L.) Mill.	0	0	0.49	1.66
56	*Melilotus indicus* (L.) All.	0	3.87	0	0
57	*Peganum hermala* L.	7.6	0	0	0
58	*Plantago ciliata* Desf.	9.9	4.34	0	0
59	*Plantago ovata* Forssk.	11.1	4.3	0	0
60	*Solanum nigrum L.*	4.99	3.3	0.75	0
61	*Salvia moorcroftiana* Wallich ex Benth.	0	0	12.4	0
62	*Saussurea heteromalla* (D.Don) Hand.	20.5	7.61	1.97	0
63	*Silene conoidea* L.	3.01	2.93	0.66	0
64	*Solanum incanum* L.	0	0	2.41	3.63
65	*Solanum surattense* Burm .f.	9.64	4.63	4.17	4.3
66	*Vicia sativa* L.	4.65	9.03	1.61	0

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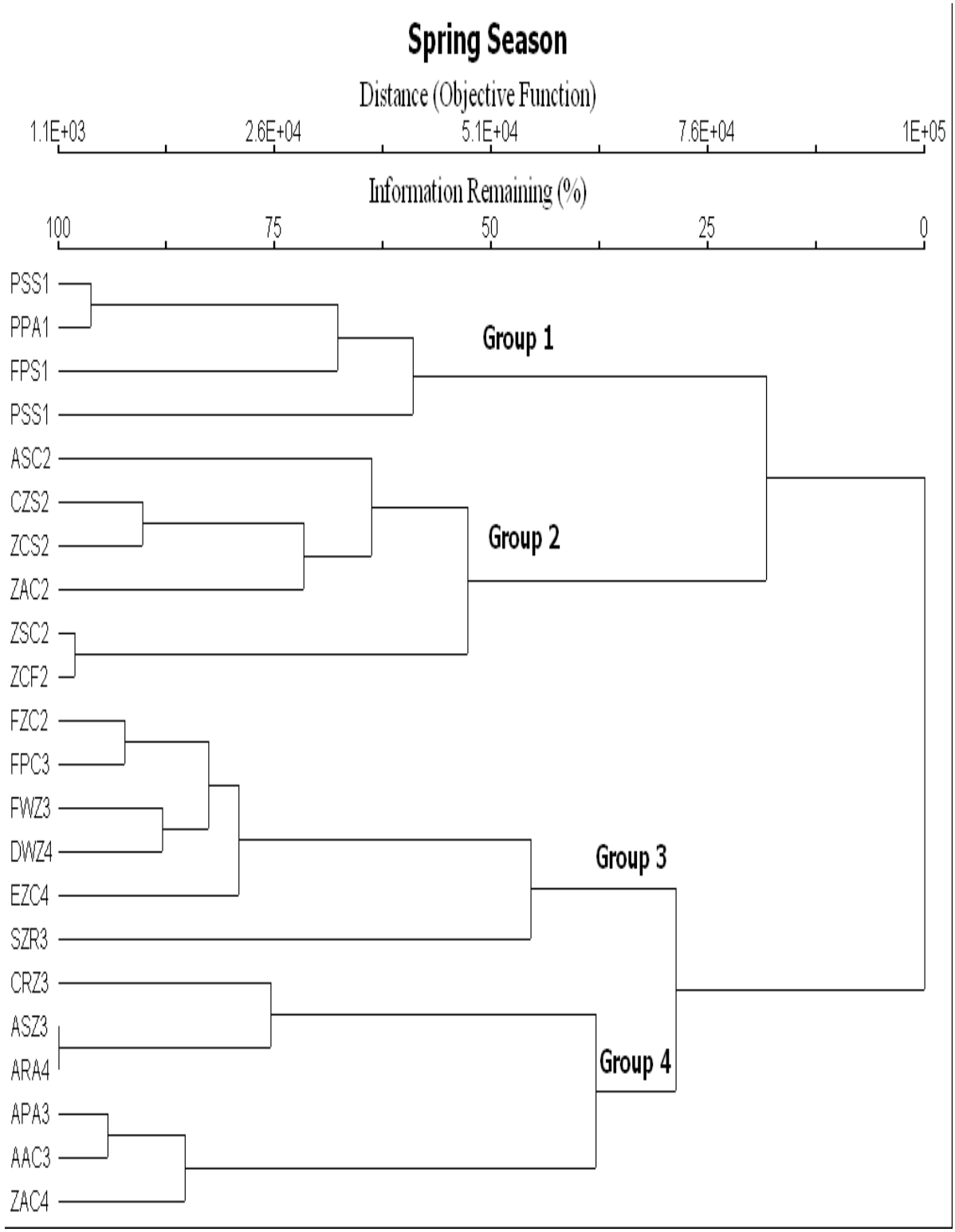


Figure 2 : Two way cluster dendrogram showing grouping of different communities into association during spring, Tehsil Takht-e-Nasrati, District Karak.

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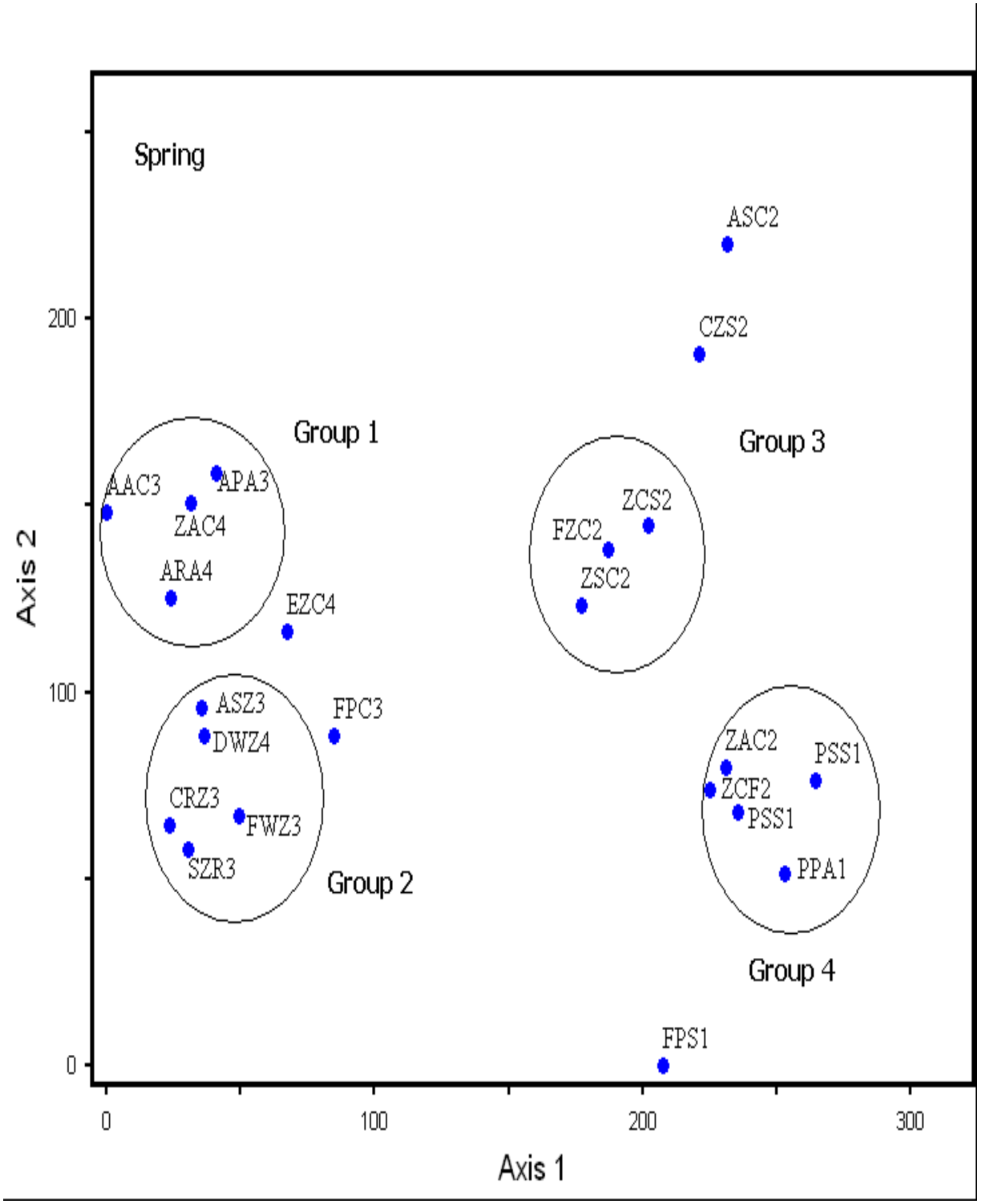


Figure 3 : Detrended Correspondence Analysis (DCA) of communities during spring, Tehsil Takht-e-Nasrati, District Karak.

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Isolation and Phytochemical Characterization of Bioactive Compounds from the Rhizomes of Cyperus Rotundus. L

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Abstract- Phytochemical constituents of plants with varied phytochemical, physiological and biochemical activity has received attention to use them as food, medicine, cosmetics etc. In fact many plants extracts have been shown to exert biological activity invitro and invivo which justified the healing potential of ethnomedicine. Using phytochemical analysis, UVvisible spectrum and GC-MS studies, the active compound that are rich in rhizomes were tested. From the investigation it was found that phenolic compounds and tannin are rich in rhizomes. An important compound that formed a dominant peak was identified to be 3, 3, 3, trifluoro -N-(-4-fluorophenyl)- bicyclo (4.1.0) heptanes. Hence it is essential to study the traditional health care systems.

Keywords: *cyperus rotundus, phytochemical analysis, uvanalysis, GC-MS analysis.*

GJSFR-C Classification : *FOR Code: 270199p*



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Isolation and Phytochemical Characterization of Bioactive Compounds from the Rhizomes of Cyperus Rotundus. L

Jeyasheela. R [α], K.Chairman [σ], C. Padmalatha [ρ] & A. J. A. Ranjit Singh [ω]

Abstract- **Phytochemical constituents of plants with varied phytochemical, physiological and biochemical activity has received attention to use them as food, medicine, cosmetics etc. In fact many plants extracts have been shown to exert biological activity invitro and invivo which justified the healing potential of ethnomedicine. Using phytochemical analysis, UV-visible spectrum and GC-MS studies, the active compound that are rich in rhizomes were tested. From the investigation it was found that phenolic compounds and tannin are rich in rhizomes. An important compound that formed a dominant peak was identified to be 3, 3, 3, trifluoro -N-(-4-fluorophenyl)-bicyclo (4.1.0) heptanes. Hence it is essential to study the traditional health care systems.**

Keywords: cyperus rotundus, phytochemical analysis, uv-analysis, GC-MS analysis.

I. Introduction

Cyperus rotundus Linn., (Family -Cyperaceae),is a medicinal plant, also known as purple nut edge or nut grass, is a common perennial weed with slender, scaly creeping rhizomes, bulbous at the base and arising from the tubers which are about 1-3 cm long. The tubers are externally blackish in colour and reddish white inside, with a characteristic odour. The stems grow to about 25 cm tall and the leaves are linear, dark green and grooved on the upper surface. Inflorescences are small, with 2-4 bracts, consisting of tiny flowers with a red-brown husk. The nut is three angled, oblong-ovate, yellow in colour and black when ripe. Cyperus rotundus is indigenous to India, but are now found in tropical, subtropical and temperate regions (Pooley et al, 1998). The genus Cyperus includes common weeds found in upland and paddy fields in temperature to tropical regions. C. rotundus, which are used as traditional folk medicines for treatment of stomach and inflammatory diseases (Gupta et al 1971; Singh et al., 1970). The Cyperus rotundus L have been reported to contain oils, alkaloids, glycosides, saponins, flavonoids, tannins. The rhizomes of C. rotundus have been used in ancient medicine in India for fever, dysentery, purities, pain, vomiting and various blood disorders (Kirtikar et al., 1944). In particular, plant extracts offer a rich potential source of novel anti – platelet agents (Ballabeni et al., 2007).

C. rotundus has been reported to contain sesquiterpenes, hydrocarbons, epoxides and ketones and also used as anti-inflammatory estrogenic, ant-ipyretic, antiemetic, diuretic, hypotensive agent (Aslam, 2002). There are some reports on the phytochemical analysis of species belonging to Cyperus rotundus found in the literature. These scientific studies on the species of this genus showed the presence of constituents belonging mainly to the groups of sesquiterpenes, flavonoids, tannins sterols, alkaloids, benzoquinones and essential oils (Zargari, 1990 and Lawal and Oyedeji, 2009).

A novel norsesquiterpene, named norcyperone , and three known compounds:(-)-clovane-2,9-diol , rosenonolactone , and 5α,8α-epidioxy-(20S,22E,24R)-ergosta-6,22-dien-3β-ol were isolated from the rhizomes of Cyperus rotundus L.(Yan Xu et al.,2008).The rhizome oils of this plant from different countries also showed compositional differences, suggesting the existence of phytochemical varieties. Cyperene (19.2-30.9%) and α-cyperone (4.5-25.2%) were the most abundant constituents of the oils of Nigerian and Tunisian species, but the concentrations of other main components varied (Ekundayo et al., 1991 and Kilani et al., 2005). Root extracts of C. rotundus (CR) showed the presence of β-sitosterol, cyperene, cyperol, flavonoids, ses-quiterpenoids, ascorbic acid and polyphenols (Sonwa and Konig, 2001).

II. Materials and Methods

a) Preparation of the plant extract

Ethyl acetate and methanol extracts were obtained by using soxhlet apparatus. The two extracts, with different polarities were concentrated by evaporating it to dryness under reduced pressure by rotary vaccum evaporator (New Lab, Company) to obtain the respective extracts and each residue was stored at 4 0C. These two extracts were resuspended in Dimethyl sulfoxide. The extracts were then stored at -180c until further analysis.

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b) Column chromatography – Methanol extract

The column was packed with silica gel G for column chromatography (60 – 120) and built the column with n-hexane mesh. The slurry of methanol root extract (3g) was introduced in to the column.

The 50ml of fractions were collected by eluting hexane: ethyl acetate (H: EA) and also followed by ethyl acetate: methanol (EA: M). The order of elution is H:EA(70: 30),H:EA (60: 40), EA100% , EA:M(98:2) ,EA : M (95:5) ,EA : M (90 : 10) ,EA: M (80:20) EA : M (60 ; 40), EA : M (40 : 60), EA : M (20 : 80), 100% Methanol. The collected fractions were then concentrated using rotary vacuum evaporator under reduced pressure was used for further pharmacological and spectral analysis.

c) Column chromatography – Ethyl acetate extract

The column was packed with silica gel G (60 - 120mesh) for column chromatography and built the column with dichloromethane. The slurry of ethyl acetate root extract (6g) was introduced into the column.

The 50ml of fractions were collected by eluting dichloromethane: methanol (DCM: M). The order of elution is DCM : M (80 : 20) ,DCM : M (70 : 30) ,DCM : M (60 : 40) ,DCM : M (50: 50) ,DCM : M (40 : 60) ,DCM : M (30 : 70) ,DCM : M (20 : 80), DCM : M (10 : 90) , 100% Methanol. Finally the collected fractions were evaporated and concentrated using rotary vacuum evaporator under reduced pressure and were used for further pharmacological and spectral analysis.

d) Phytochemical Screening

Phytochemical screening of eluted fractions was tested for the presence of various phytochemical constituents .The analyses were carried out by following techniques.

e) Preliminary screening

The various fractions of methanolic and ethyl acetate root extracts of Cyperus rotundus L.were used to screen for the following the phytochemicals like Triterpenoids, reducing sugars, alkaloids, phenolic compounds, Saponin, xanthoprotein, tannins, aromatic acids, flavonoids, phytosterols small quantities of all the fractions were dissolved separated in distilled water and filtered. The filtrate was subjected to further analysis.

f) Detection of Triterpenoids

To the filtrate with one or two pieces of tin and three drops of thionyl chloride was added slowly, a violet or purple colour solution indicates the presence of triterpenoids.

g) Detection of Phenolic Compounds

i. *Ferric chloride Test*

To the filtrate add few drops of neutral ferric chloride was added and the appearance of an intense blue or violet colour were recorded. This indicated the presence of phenolic compounds.

ii. *Detection of saponins*

Small portion of the various filtrates were diluted with 20 ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. Formation of foamy layer was record, it indicated the presence of saponin.

iii. *Detection of Xanthoproteins*

To the filtrates add few drops of concentrated nitric acid was added with excess amount of ammonia .No red orange precipitate indicates the absence of xantho proteins in all fractions.

iv. *Detection of Tannins*

To the filtrate, 2ml of solution of gelatin was added white precipitate was seen which indicates the presence of tannins.

v. *Detection of Aromatic acids*

To small quantity of the various filtrates add saturated sodium bicarbonate, no brisk effervescence indicates the absence of Aromatic acids.

vi. *Detection of Flavanoids*

Small portion of the various filtrates were dissolved in alcohol and treated with magnesium metal followed by concentrated hydrochloric acid. Formation of Magenta colour indicates the presence of flavonoids.

vii. *Detection of phytosterols*

Small quantity of various filtrates were dissolved in 5ml of chloroform solutions were subjected to salkowski's and Liebermann-Burchard's test for the detection of phytosterols.

viii. *Salkowski's Test*

To the 1ml of above prepared chloroform solution, few drops of concentrated sulfuric acid was added. It gave red colour which indicates the presence of phytosterols.

ix. *Liebermann-Burchard's Test*

The above prepared chloroform solution was treated with few drops of concentrated sulfuric acid followed by 1ml of acetic anhydride solution. It gave green colour, which shows the presence of phytosterol.

h) Determination of total phenolics and tannins

The total phenolic content was determined according to the method described by Siddhuraju and Becker (2003).

i) Determination of total flavanoid content

The flavonoid content was determined by the use of a slightly modified colorimetry methoddescribed previously by Zhishen et al. (1999).

j) Phytochemical Separation by using TLC Method

Each fractions of the column eluted sample was subjected to TLC to find out the separation of single compound and confirmation from the fraction. Thin Layer Chromatography was performed on prepared plates with Silica gel F254 grade (Merck, Darmstadt,

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Germany) as stationary phase. A one-dimensional ascending development technique was used to detect the constituents of an extract on TLC plate. Visual detection was done in daylight and under UV light at a wave length of 254 and 344 nm depending on the nature of compounds separated.

k) UV-Spectrophotometry analysis

The absorbance spectra of the extracted samples were measured using UV visible spectrophotometer 2203 at wavelength range from 200-1000 nm. The detector used was diode array detector with deionized water.

l) GC - MS Analysis

The Clarus 500 GC-MS used in the analysis employed a fused silica column packed with Elite-1 (100% dimethyl poly siloxane, 30nm X 0.25nm ID X 1 m df) and the components were separated using Helium as carrier gas at a constant flow rate of 1 ml/min. The 2 sample extract injected into the instrument was detected by the Turbo gold mass detector (Perkin Elmer) with the aid of the Turbo mass 5.1 software. During the 36th minute GC-MS extraction process, the oven was maintained at a temperature of 1100C with 2 minutes holding. The injector temperature was set at 2500C (mass analyzer).

The different parameters involved in the operation of the clarus 500 GC-MS were also standardized (Inlet temperature: 2000C; Source temperature: 2000C). Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da. The MS detection was completed in 36 minutes. The detection employed the NIST Ver. 2.0 - year 2012 library.

III. Results and Discussion

Phytochemical screening of various fractions of methanol extract of Cyprus rotundus rhizomes reveals the presence of aromatic acids, triterpenoids, reducing sugars, alkaloids, phenolic compounds, saponins, tannins, flavonoids, phytosterols and absence of xanthoproteins (Table 1).

Table 1 : Phytochemical screening of Methanol extract fractions of Cyperus rotundus L. rhizomes.

S. NO	Phytoconstituents	Methanol extract fractions of *Cyperus rotundus* L. rhizomes										
		Hexane(H) :Ethyl acetate (EA) ; Ethyl acetate (EA) : Methanol (M)										
		H:EA 70:30	H:EA 60:40	EA 100%	EA: M 98:02	EA: M 95:5	EA: M 90:10	EA: M 80:20	EA: M 60:40	EA: M 40:60	EA: M 20:80	M 100%
1	Triterpenoids	+	+	+	-	+	-	+	-	-	-	-
2	Reducing sugar	+	+	+	+	-	+	-	-	-	-	+
3	Alkaloids	+	+	-	+	+	+	+	+	+	+	+
4	Phenolic compounds	+	-	+	+	+	-	+	-	-	-	-
5	Saponins	-	+	-	-	+	+	-	+	+	+	+
6	Xanthoprotein	-	-	-	-	-	-	-	-	-	-	-
7	Tannins	+	-	-	+	-	-	-	+	-	-	-
8	Aromatic acid	-	-	-	-	-	+	+	-	-	-	-
9	Flavonoids	+	+	+	-	-	+	-	+	+	-	-
10	Phytosterols	+	+	+	+	-	-	-	-	+	-	+

(+) Presence (-) Absence

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Table 2 : Phytochemical screening of ethyl acetate extract fractions of *Cyperus rotundus L*. rhizomes.

S.NO	Phyto constituents	Ethyl acetate extract fractions of *Cyperus rotundus* L. rhizomes								
		Dichloro Methane (DCM) : Methanol (M)								
		DCM:M 80:20	DCM:M 70:30	DCM:M 60:40	DCM:M 50:50	DCM:M 40:60	DCM:M 30:70	DCM:M 20:80	DCM:M 10:90	M 100%
1	Triterpenoids	-	+	-	-	-	+	-	-	-
2	Reducing sugar	+	+	+	+	+	-	+	+	+
3	Alkaloids	+	+	+	+	-	-	+	+	+
4	Phenolic compounds	+	-	+	-	-	+	-	-	-
5	Saponins	+	-	+	+	+	-	+	+	+
6	Xanthoprotein	-	-	-	-	-	-	-	-	-
7	Tannins	-	-	+	-	-	+	-	+	-
8	Aromatic acid	-	-	-	-	-	-	-	-	-
9	Flavanoids	+	-	-	-	-	+	+	-	-
10	Phytosterols	+	+	+	+	+	-	-	+	+

(+) Presence (-) Absence

Phytochemical screening of various fractions of methanol extract of Cyprus rotundus rhizomes reveals the presence of Triterpenoids, reducing sugars, alkaloids, phenolic compounds, Saponins, tannins, flavanoids, phytosterols and absence of xanthoprotein and aromatic acids. (Table -2)

The total phenolics in methanol extract, ethyl acetate extract and fraction DCM: Methanol (30:70) of ethyl acetate extracts are 57.46±0.84, 31.50 ± 0.51 and 22.63 ± 0.88 respectively. The total flavanoid in methanol extract, ethyl acetate extract and fraction DCM: Methanol (30:70) of ethyl acetate extracts are 1.10 ± 0.07, 0.29 ± 0.030 and 22 ± 0.05 respectively. The total tannin in methanol extract, ethyl acetate extract and fraction DCM: Methanol (30:70) of ethyl acetate extracts are 12.76 ± 0.83, 12.87 ± 0.19 and 5.32 ± 1.00 respectively (Table 3)

Table 3 : Estimation of Total phenolics, Total Flavanoids and total Tannins present in the Methanolic extract, Ethyl acetate extract and Ethyl acetate extract fraction DCM: Methanol (30:70) of Cyperus rotundus L. rhizomes.

S.No	Sample	Total Phenolics mg TAE/g extract	Total Flavanoid Mg RE/g extract	Total Tannin mg TAE/g extract
1.	Methanol crude	57.46 ± 0.84	1.10 ± 0.07	12.76 ± 0.83
2.	Ethyl acetate crude	31.50 ± 0.51	0.29 ± 0.03	12.87 ± 0.19
3.	Fraction DCM:M (30:70)	22.63 ± 0.88	0.22 ± 0.05	5.32 ± 1.00

Values are means of three independent analyses of the extract + standard deviation (n=3)
TAE – Tannic acid equivalent RE – Rutin equivalent

a) UV-SPECTRAL ANALYSIS

UV- spectrum of the test fraction EA: Methanol (80:20) of Ethyl acetate extract gives prominent peak of 241.6, 208.0, 248.0, 257.6, 979. 2 with 3. 025 ,3.064, 3.020, 3.013, 0.053absorbence which supports the bioactive compound in the root extract. (Fig-1)

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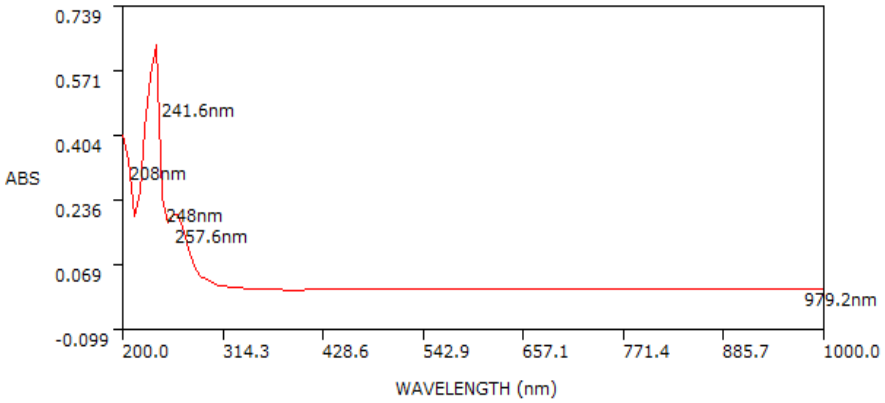


Fig. 1 : EA: M (80:20)

UV spectrum of the test fraction DCM: Methanol (30:70) of ethyl acetate extract gives prominent peak of 646.4 with 0.034 absorbance which supports the bioactive compound in the root extract. (Fig-2)

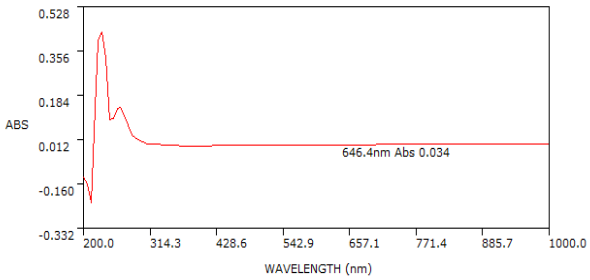


Fig. 2 : DCM: M (30:70)

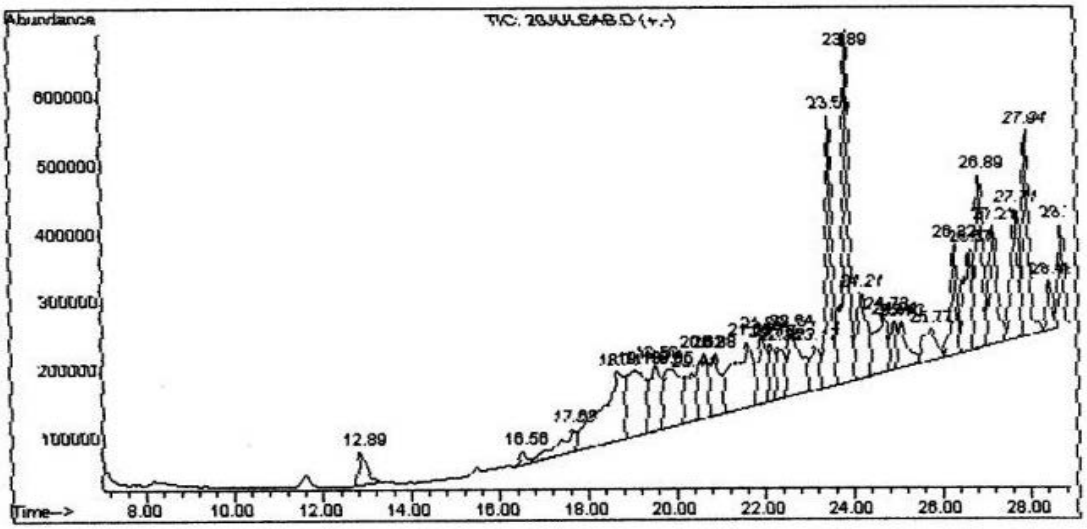


Fig. 6 : In GC analysis of the test fraction DCM: Methanol (30:70) of ethyl acetate extract of C.rotundus

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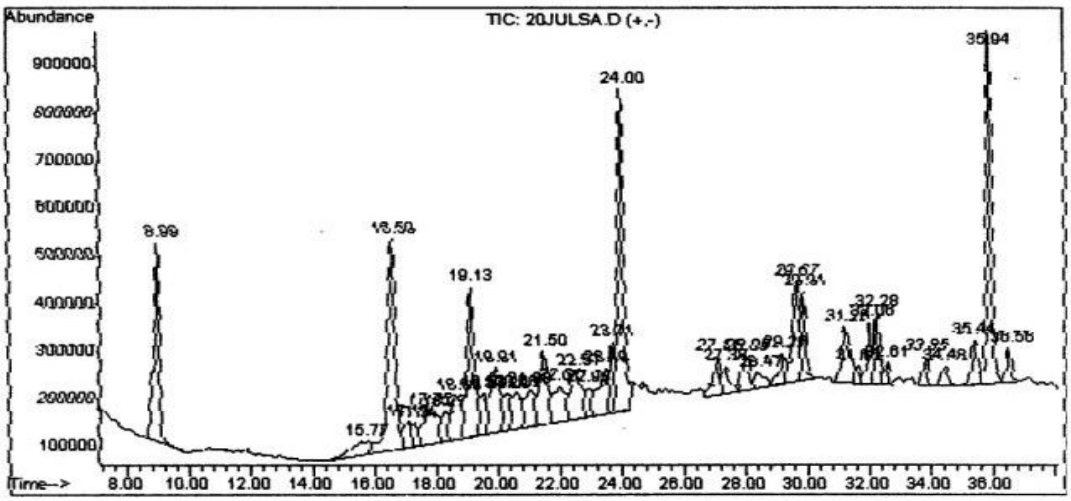
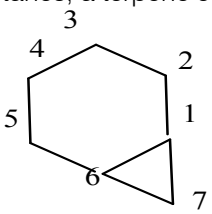


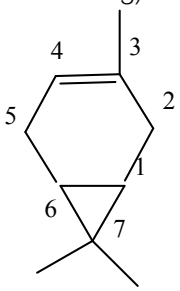
Fig.7 : In GC analysis of the test fraction ethyl acetate: Methanol (80:20) of methanol extract of C.rotundus

In GC analysis of the test fraction EA: Methanol (80:20) of methanol extract of C.rotundus gives prominent peak. The most abundant peaks with retention time 8.98, 16.59, 19.13, 24.00, 29. 68 and 35.94 were observed along with other peaks with smaller abundant out of the six more abundant peaks, the peak at 24.00 and 35.94 show the response for aromatic carboxylic acid and esters. Others are showing the response for aliphatic esters, acids and amides (Fig 6,7).

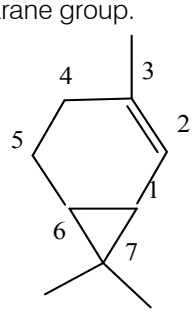
In GC analysis of the test fraction DCM: Methanol (30:70) of Ethyl acetate extract of C.rotundus gives prominent peak. The most abundant peaks with retention time 12.89, 23.50, 23.90, 26.90 and 27.94 were observed along with other with little abundant. Out of five peaks the most abundant peaks with RT 23.5 and 23.90 were quite interesting. The peak at 23.50 is due to Bicyclo (4.1.0)heptanes, a terpene compound



It belongs to bicyclic monoterpenoid class I (6+3 membered ring). It belongs to carane group.



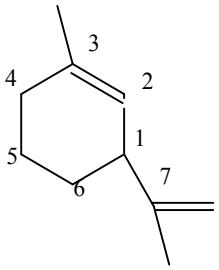
Car-3-ene



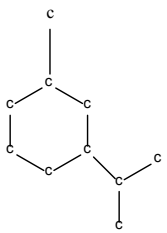
car-2-ene

The car-3- ene and car-2-ene are found to be present in natural source is essential oils. Finar, (1973). p-cymene, p-cymenene has been reported earlier in Cyperus rotuntus (El-Gohary 2004).

The carenes are obtained from m-cymene skeleton is.



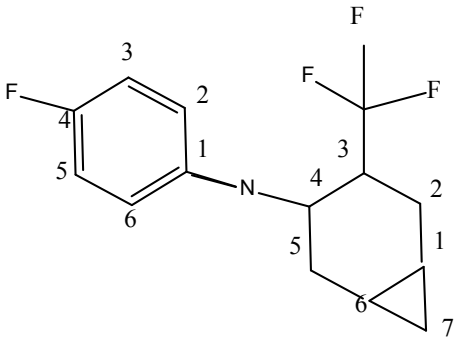
p-cymene



m-cymene

Sylvesterene are also reported in natural source as essential oil. The most probable compound belonging to the peak at 23.50 is justified by the above evidences.

The DCM: Methanol (30:70) fraction of ethyl acetate extract of Cyperus rotuntus rhizomes is found to contain.



3,3,3, trifluoro –N-(-4-fluorophenyl)- bicyclo (4.1.0)heptane

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This fraction shows excellent antimicrobial activities and antioxidant activities. Further phy - tochemical studies may lead to the exact bioactive compound principle. This most probable compound seems to be the first report in the rhizome extracts of Cyperus rotuntus

Recent literature survey in this plant indicates the bioactive potential of this plant Cyperus rotundus L. The plant root extract is found to contain various phytochemicals. The selected phytochemicals such as phenolic compound, flavanoid and tannin, have been estimated. The GC-MS pattern of ethyl acetate extract fraction dichloromethane: methanol (30:70) indicates the presence of 3,3,3, trifluoro –N-(-4-fluorophenyl)- bicyclo (4.1.0) heptane.

IV. Acknowledgement

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Evaluation of Ovicidal and Larvicidal Activities of Methylene Chloride-Methanol Extract of *Annona Senegalensis* (Annonaceae) Stem Bark on *Heligmosomoides Bakeri* (Nematoda, Heligmosomatidae)

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Abstract- Infections of animals with gastrointestinal nematodes constitute a world wide health problem. The aim of this study was to assess the effectiveness in vitro anthelmintic of Methylene Chloride/Methanol (1:1 volume mixture) extract of Annona senegalensis (Annonaceae) the barks of the stem on Heligmosomoides bakeri eggs and larvae (for ovicidal and larvicidal activities, respectively). Annona senegalensis is included in the list of the plants that have anthelminthic activity in the traditional medicine, just as with other plants like Albizia anthelmintica (Mimosaceae), Canthium mannii (Rubiaceae), Nauclea latifolia (Rubiaceae) and Carica papaya (Caricaceae). The plant material was collected from the peripheral savanas of Foumban, Noun Division, West Region of Cameroon. The final concentrations of plant extract tested were: 5 000, 3 750, 2 500, 1 250 and 625 **µ**g/mL; 4 % Tween 80 aqueous solution was used as negative control. Ovicidal and larvicidal activities were assessed thru egg embryonation and hatching rates and thru mortality rate of L1 and L2 larvae, respectively.

Keywords: *heligmosomoides bakeri; annona senegalensis; ovicidal; larvicidal; anthelminthic.*

GJSFR-C Classification : *FOR Code: 820303*



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Evaluation of Ovicidal and Larvicidal Activities of Methylene Chloride-Methanol Extract of *Annona Senegalensis* (Annonaceae) Stem Bark on *Heligmosomoides Bakeri* (Nematoda, Heligmosomatidae)

Francesco D'Angelo [α], Wabo Poné J [σ], Yondo Jeannette [ρ], Komtangi Marie Claire [ω], Sauro Vittori [¥] & Mpoame Mbida[§]

Abstract- Infections of animals with gastrointestinal nematodes constitute a world wide health problem. The aim of this study was to assess the effectiveness in vitro anthelminthic of Methylene Chloride/Methanol (1:1 volume mixture) extract of *Annona senegalensis* (Annonaceae) the barks of the stem on *Heligmosomoides bakeri* eggs and larvae (for ovicidal and larvicidal activities, respectively). *Annona senegalensis* is included in the list of the plants that have anthelminthic activity in the traditional medicine, just as with other plants like Albizia anthelmintica (Mimosaceae), Canthium mannii (Rubiaceae), Nauclea latifolia (Rubiaceae) and Carica papaya (Caricaceae). The plant material was collected from the peripheral savanas of Foumban, Noun Division, West Region of Cameroon. The final concentrations of plant extract tested were: 5 000, 3 750, 2 500, 1 250 and 625 µg/mL; 4 % Tween 80 aqueous solution was used as negative control. Ovicidal and larvicidal activities were assessed through egg embryonation and hatching rates and through mortality rate of L1 and L2 larvae, respectively. The extract produced low but dependant concentration on egg embryonation and hatching rates. With the highest extract concentration (5 000 µg/mL) embryonnation and hatching rates of 20.8 % and 16.1 % were obtained respectively. On the contrary, a strong larvicidal activity was observed. L1 mortality rates of 100 % and 96.7 % were recorded respectively, in the two most concentrated extract (5 000 and 3 750 µg/mL) just after six hours of exposition. L2 larvae appeared more resistant as the two most concentrated extracts (5000 and 3 750 µg/mL) produced larvicidal mortality rates of 96.1 % and 90.0 % respectively, just twenty four hours after the administration of the treatment. These results suggest that the extract of *A. Senegalensis* bark stem used, possess high larvicidal properties. Further more in vivo, studies assess the effects on adult worms and toxicity on mice hosts are still needed.

Keywords: heligmosomoides bakeri; Annona senegalensis; ovicidal; larvicidal; anthelminthic.

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I. Introduction

Infections of animals with gastrointestinal nematodes constitute a world wide health problem. These parasites frequently cause death in heavily infected host, resulting in important economic losses. Chronic infections are dangerous and can cause a reduction of milk and meat production, fertility and growth in animals. The anthelminthics discovered in the '60s, and which were largely used in the last 50 years, limited the problem until resistance, and multi-resistance, appeared in nematode populations (Holmes, 1985; Zajac et Gipson, 2000). Actually, in certain regions of the world, mostly among the developing countries, the situation is very serious (Van wyk et al. 1999). In fact, in tropical and sub-tropical zones, the spread of gastro-intestinal parasitic infections is strongly increased by climatic factors, such as temperature and humidity, and poor hygiene (Satrija et al. 1995). In Cameroon, the Permanent Secretary of the National Programme for the control of Schistosomiasis and Gastrointestinal Helminthiases reported in January 2006 that, over 10 millions natives are infected with various intestinal parasites and that 2 milions suffer from schistosomiasis. These infections affect children of school age especially, influencing stronly their growth, intellectual development and vulnerability to other diseases (Ngangout et al., 2012). The control measures used today depend on vaccination (Newton & Mun, 1999), breeding of sheep resistance races (Gray, 1997), improving food quality (Wallace et al. 1998) and breeding dairy farming methods (Bargers, 1999; Niezen et al. 1999). In addition, some biological measures are being developed such as the optimization for the use of anthelminthics already available on the market (Larsen, 1999; Van Wyk & Bath, 2002).

In this context, the search for new nematocidal agents is still a priority for humans and for livestock (Witty, 1999). Synthetic anthelminthics still dominate the

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animal pharmaceutical industry and they are widely sold at high prices (see International Federation for Animal Health website: http://www.ifahsec.org). The massive and strategic use of modern anthelminthic drugs is widely spread to control worm infections. Unfortunately, the high cost of these medicines, the increasing development of resistant strains of gastro-intestinal parasites and the shortage of veterinarians, especially in poor countries, push livestock breeders and herdsmen towards traditional medicine using therapheutic plants (Kaboré et al., 2005).

For a long time, humans have developed throughout the world a traditional pharmacopea based on the knowledge of medicinal plants. This knowledge became highly enriched over time through the observation of animal behaviour and through experimental tests. In many cases, the information thus acquired is only orally transmitted and it is therefore dangerously being put out in favour of modern medicine. In fact, traditional medicine still represents the cheap possible and readily available treatment for the natives. it is also an important potential source for the development of new pharmacological agents. Thus it is clear that modern ethnobotanical research is both important and urgent (Schillhorn Van Veen, 1997; Hammond et al., 1997). various studies, and some of these studies have shown that many plants contain several molecules with antiparasitic activity, such as quinine and artemisin, that are presently widely used (Kayser et al., 2003), while some other plants show promising anthelminthic activity (Al-qarawi et al., 2001; Onyeyilli et al. 2001). However, studies that scientifically evaluate the effectiveness of traditional medicine are very few (Lans & Brown, 1998; Veira et al., 1999), and publications related to Africa are even fewer (Aké Assi & Guinko, 1991; Nfi et al., 2001). Besides, the need for stringent regulation mechanisms and secrecy over the use of traditional medicine remains the major stumbling block (Gqaleni et al., 2007). Furthermore, the inadequate toxicological evidences about the safety of these medicinal plants is still an unsolved problem (Fennel et al., 2004).

The resistance against medically active substances is particularly pronounced in the case of gastrointestinal nematodes of sheep and goats (Sangster, 1999), and especially in the cases of Haemonchus contortus and Heligmosomoides polygyrus (now known as *H. bakeri*) (Diehl et al., 2004; Githiori et al. 2003).

Annona senegalensis (also known as African custard apple), is one of the medicinal plants that are commonly used in Central Africa against gastrointestinal parasite infections. This is a flowering plant of the custard apple family that takes the form of either a shrub or a small tree, growing up to, between two and six meters tall. Its bark has a smooth or a coarse texture, and may be gray-silver or gray-brown. It is leaf-scared, with nearly round flaking, showing lighter-hued spaces under the bark. Branches have thick, gray, brown or yellow tomentum when they are still new. Leaves are from green to blue-green and flowering white creamy. The fruits are made up of numerous fused, fleshy, bumpy, ovoidal or globular carpels. *Annona senegalensis* is origin to east and northeast, west and central, and southern tropical Africa, and to islands in the western Indian Ocean. In South Africa, it is found in KwaZulu-Natal, Limpopo and Mpumalanga (Anonymous, 2010). In vitro studies on aqueous and ethanolic extracts of the bark of stem, of this plant, have already confirmed their ovicidal and larvicidal activity on nematodes (Ngangout et al., 2012). In fact, the bark of this plant was used in early history to prepare tisanes and tea herbs for treating a wide array of ailments such as: intestinal and tissue parasitic worms, diarrhea, gastroenteritis, lung infections, toothaches and even snakebites (Anonymous, 2010). The aim of this study was to assess the in vitro ovicidal and larvicidal activity of Methylene Chloride/Methanol 1:1 volume mixture of extract from bark of the stem of *A. senegalensis* on *H. bakeri* eggs and larvae obtained from the faeces of experimentally infected mice.

II. Materials and Methods

a) Plant materials

The bark of stem of *A. senegalensis* used in this experiment was collected from the peripheral savanas of Foumban, Noun Division, West Region of Cameroon. It was then air dried while protected from sunlight and dust. Finally, the bark was well cut in small pieces (3 cm x 4 cm), ground and kept in the laboratory for further use.

b) Preparation of plant extract

The procedure used was according to Wabo Poné et al. (2006, 2010). Briefly, 200 grams of stored powder were macerated in 1.5 L of methanol (purity > 95%) and 1.5 L of Methylene Chloride (purity > 95%). The Methylene Chloride-methanol mixture was used for its greater capacity to extract some specific compound materials of the plant. The mixture was placed in an airtight glass jar and kept in a dark place. It was stirred daily to accelerate extraction. Seventy two (72) hours later, it was filtered through two metallic sieves (mesh sizes: 500 µm and 150 µm), then through a cotton layer and finally through filter paper of pore size of 2.5 µm. The solution obtained was then concentrated in a rotavapor at ~80 °C for 2 hours (rotation rate: ~150 rounds/min). The extract obtained, was poured in two large glass containers, covered with paper, and kept in the oven at 40-45 °C for 24 hours to allow it for a complete evaporation of the solvents. Two hundred (200) mg of the obtained extract were dissolved in 0,8 mL of Tween 80 (to facilitate the mixture with water). Warm water was finally added to bring the solution to a

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volume and prepare 20 mL of stock solution at 10 000 µg/mL. Through a series of dilutions were made to obtain the following concentrations: 7 500, 5 000, 2 500 and 1 250µg/mL.

0.8 mL of Tween 80 were added to 19.2 ml of distilled water to obtain a solution at 4 % V/V, used for negative control.

c) Recovery of nematode eggs

Mice (Mus musculus) were infected by oral gavage with their natural nematode, *Heligmosomoides bakeri* (previously known as Nematospiroides dubius, H. polygyrus and H. p. bakeri).The eggs of *H. bakeri* were obtained from the faeces of mice as follows. Each day, ~100 mg of faeces were collected from the animal cages, homogenized in a mortar adding a small volume of water, suspended in saturated salt solution (NaCl 40 % W/V). Then the solution was cleared of organic debri by filtration through a 250 µm mesh-size sieve into a beaker and finally poured into three glasses of U-vials until the formation of a meniscus at the top. A cover slide was thus deposited on each vial, in direct contact with the solution. Three (3) minutes later, the cover slides were removed and put on a slide and soon analysed under an optical microscope Olympus-CH under the 4x objective to look for eggs. Slides and cover slides containing the eggs were finally rinsed with tap water into a 50 mL vial. The vial was allowed to stand for 30 minutes for the sedimentation of the eggs at the bottom. Then, to completely remove the salt solution, 40 mL of solution were accurately siphoned out using a syringe and replaced with the same amount of tap water. This operation was repeated each 30 minutes three times. Finally the supernatant was removed and the remaining solution was distributed into 24 Petri dishes (Ø = 35 mm) each with 1 mL.

Figs 1 and 2 show *H. bakeri* fresh and embryonated eggs seen through the optical microscope, respectively. Here it is easy to recognize the L1 stage larvae still within the shell of the eggs.

d) Evaluation of ovicidal activity

The ovicidal activity was determined using two different parameters: the effect on the embryonation rate and the hatching rate. To evaluate the effect on the embryonation rate, a percentage of embryonation inhibition (EI) was calculated using the following formula:

$$EI\ \% = 100 - [\left(\frac{Number\ of\ L_1\ larvae}{Number\ of\ fresh\ eggs\ in\ culture}\right) x100]$$

The eggs in 6 Petri dishes were counted immediately after their introduction (20-40 eggs/Petri dish) and soon each, Petri dish received 1 mL from each one five different extract concentrations or 1 mL of Tween 80 solution (4 % V/V) for the negative control. After 48 hours, the larvae in each Petri dish were counted. It is assumed that the embryonated eggs contain the 1st larval stage.

To evaluate the hatching rate, a percentage of hatching inhibition (HI) was calculated by the formula below:

$$HI\ \% = 100 - [\left(\frac{umber\ of\ L_1\ larvae}{Number\ of\ embryonated\ eggs\ in\ culture}\right) x\ 100]$$

In this case, 1 mL of each one of five different concentration extracts or Tween 80 solution (4 % V/V) was introduced into 6 different Petri dishes 24 hours after the collection of eggs, just when these start to hatch. The counting of the larvae was done 6 hours later, when 90 % of eggs are hatched in the negative control.

In all Petri dishes, the final tested concentrations were behalfed from the initial ones due to the addition of egg suspension (1mL) resulting as follows: 5 000, 3 750, 2 500, 1 250 and 625 µg/mL for the plant extract, and 2 % V/V Tween 80 aqueous solution for the negative control. Each treatment was repeated 4 times.

e) Recovery of nematode L1 and L2 larvae

To obtain larvae of the parasite, some eggs, kept inside the vials, were maintained in culture at room temperature (24-25 °C) for fixed periods of time.

L1 larvae were soon identified after hatching from eggs. The larvae were incubated in the extract and tween around 30 hours after the collection of the eggs from faeces. This is when the number of larvae in the negative control reaches 90 % of the number of eggs present in a Petri dish.

To obtain L2 larvae, a solution of faeces collected from the mice free of parasite was added to the egg suspension and kept in a temperature room (24-25 °C) for about 48 hours. The addition of the extract was made only after the identification of L2 larvae. In fact, there are some morphological and specific characteristics that can be exploited, during the qualitative analysis under the microscope, to distinguish between the first and second stage of larvae (L1 larvae are smaller than L2 larvae and do not present the internal esoskeleton that is characteristic of adult larvae. Further more, L1 larvae movements are limited while L2 larvae agitate so fast in "S-shaped" movements like a snake. Figs 3 and 4 show these evident differences between L1 (Fig. 3) and L2 (Fig. 4) larvae.

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f) Evaluation of larvicidal activity

To assess the effect of larval extract on the survival, 1 mL of solution containing each 15-25 larvae, previously poured into 6 Petri dishes, was mixed with the same volume of extract at the following final concentration (5 000, 3 750, 2 500, 1 250 and 625 µg/mL). The negative control thus reached at the ratio of

$$MR\ \% = 100x(\frac{Number\ of\ dead\ larvae}{Number\ of\ larvae\ in\ culture})$$

Immobile and straight shaped larvae were stimulated with a light-heating ray for about 20-30 seconds to confirm death (Fig. 5). Furthermore, their external layer is not uniform and presents holes and irregularities. In all the cases, the number of dead or immobile larvae was counted 2, 4, 6, and 24 hours after incubation in the extract.

g) Statistical analysis

The inhibitory 50 % concentrations (IC50) for embryionation and hatching rates were calculated using the regressive line of the data obtained from the tests, using the high value of the coefficient of determination (R2). The lethal 50 % concentrations (LC50) for L1 and L2 larvae mortality rates were determined using the regressive line of the probit as a fonction of the natural logarithm of the concentrations (also in this case the coefficient of determination R2 was taken into account). The mean percentages of inhibition and larval mortality were compared using Chi-square test and the differences were considered significant at $P < 0.05$.

III. Results

The yield of the extraction process with the mixture of Methanol and Methylene Chloride (1:1 V/V) was of 8.95 %, thus resulting in 17.9 g of dried extract (starting from 200 grams of dried powder).

The effect of various concentrations of plant extracts on embryonnation rate are shown in Figure 6a. The negative control (Tween 80 aqueous solution at 2 % V/V) hardly affected embryonation rate (4.50% inhibition rate). Embryonation inhibition rate was concentration dependant. The highest concentration of extract (5000 µg/mL), yielding the highest embryonation inhibition rate (20.80%). The calculated IC50 value for the embryionation rate is 15 930 µg/mL (R2 = 0,92).

The percent inhibition of *H. bakeri* eggs hatching, in various concentration of Methylene Chloride-Methanol exctract of *A. senegalensis* barks of the stem, is shown in Figure 6b. In the negative control the mean hatching inhibition rate of the eggs is very low (only the 3 % of eggs affected). Forty eight (48) h post-treatments hatching inhibition rate was clearly concentration dependant (9.00%. at 625 µg/mL to 16.10% at 5 000 µg/mL).The calculated IC50 value for the hatching rate is 23 910 µg/mL (R2 = 0, 97).

2 % V/V of Tween 80 in water. All the treatments were repeated 4 times.

To evaluate larval mortality, the number of dead or immobile larvae was accurately counted under the microscope with the 4x objective. Then, the percentage of mortality rate (MR) was calculated using the following formula:

The effect of *A. senegalensis* extract on the survival of *H. bakeri* L1 larvae is reported in Figure 7a which presents the mean mortality rate obtained two, four, six and twenty four hours post-treatment. The negative control didn't cause any larval mortality. Larval mortality was concentration and time dependant. The highest value (100 %) being recorded in 5 000 µg/mL extract concentration in only 6 hrs. The calculated LC50 values for the L1 larvae mortality rate are as follows: 5 000 µg/mL (R2 = 0.97), 426 µg/mL (R2 = 0.76), 167 µg/mL (R2 = 0.92), 25 µg/mL (R2 = 0.93) for 2, 4, 6 and 24 h, respectively. Figure 7b shows the activity of *A. senegalensis* barks stem extract on *H. bakeri* L2 larvae survival. The absence of activity in the negative control demonstrates the safety of 2 % Tween 80 aqueous solution for *H. bakeri* larvae. The concentration and time dependence recorded for L1 larvae was also observed in this case. The calculated LC50 values for the mortality rate are as follows: 21 320 µg/mL (R2 = 0.92); 2 407 µg/mL (R2 = 0.96); 1 849 µg/mL (R2 = 0.98); 236 µg/mL (R2 = 0.84) after 2, 4, 6 and 24 h respectively.

IV. Discussion

Taking into account the data presented, it is thus easy to understand that the Methylene Chloride-Methanol extract of *A. senegalensis* barks stem, possesses some anthelminthic properties against *H. bakeri* eggs. These findings could be due to the polarity of the solvents used for the extraction and consequentely to the secondary metabolites extracted from the plant material. Thus methanol (a highly polar solvent; dielectric constant 32.6) will extract tannins, catechins, terpenoids, polyphenols and alkaloids while Chloride (apolar solvent; dielectric constant 9.1) will extract semi-polar and apolar compounds, such as heterosides, terpenes, sterols, coumarins and carotenoids (Marie-Magdeleine et al. 2010a). Many studies have revealed the saponins among the most active compounds in terms of nematotoxic activity because of their specific interactions with the cell membranes and with the collagen proteins present on the cuticles of the parasite larvae, causing changes in cell wall permeability and cellular death (Heng et al.,2004; Argentieri et al.. 2008; Eguale & Giday, 2011). In this work, saponins were not present in the *A. senegalensis* Methylene Chloride and Methanol extracts. Our hypothesis is that, the compounds extracted by the two solvents are not capable of passing through the

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parasite fresh egg shell, and therefore cannot affect mean embryonation and hatching rates of *H. bakeri* eggs.

On the other hand, the effect of the extracts against L1 and L2 *H. bakeri* larvae is very high. Mortality rate, reaching 100 % in some cases, as described in the previous session. In each assay, the data obtained in the negative controls demonstrates that the used excipient (Tween 80 2 % V/V) can be considered totally ineffective and thus any activity observed in assays can be completely ascribed to plant extracts. The results show that *A. senegalensis* Methylene Chloride/Methanol extracts are much more active on L1 larvae than on L2 ones. These findings could be related to the presence of flavonoids and alkaloids, known to possess strong larvicidal properties. In fact, the activity of flavonoids is attributed to their anti-oxidative and free-radical scavenging capacity (Middleton et al., 2000). As for alkaloids, they creat alkaline conditions which are deleterious to the survival of parasitic larvae that normally prefer acidic conditions (Wabo Poné et al. 2011). Thus the larvicidal activity observed, may be related to two different mechanisms of action, the first, creating unsuitable environmental conditions for the survival of larvae and the second, affecting the internal organs of the larvae either through. interference with the neuromuscular physiology or blockage of energy metabolism (Nchu et al., 2011). To this purpose, it is necessary that the active compounds are found within the larval body and spread in the intestinal cells (Marie-Magdeleine et al. 2010b). The active molecules may either enter the larvae through the cuticle or through ingestion, during feeding. It is possible that the Methylene Chloride-Methanol extract of *A. senegalensis* could contain a large spectrum of active compounds, characterized by different modes of actions. In fact, the bark of plant stems represents the site of the biosynthesis and storage of secondary metabolites that probably are responsible for the biological properties of medicinal plant extracts.

The Methylene Chloride/Methanol extract of *A. senegalensis*, compared to the aqueous and ethanolic extracts of the same plant,[6] show a lower effect on the eggs of *H. bakeri* but, at the same time, a surprisingly higher activity on the parasite larvae.

In conclusion, the observations brought out in this work, reveal that the Methylene Chloride/Methanol extract of *A. senegalensis* shows an ovicidal and larvicidal activity. The activity of the plant, very high against *H. bakeri* L1 and L2 larvae, is probably related to the combined interaction of the extracted compounds as described above. However, in vivo further studies are needed to confirm its efficacy and mostly, to investigate the potential presence of toxic effects.

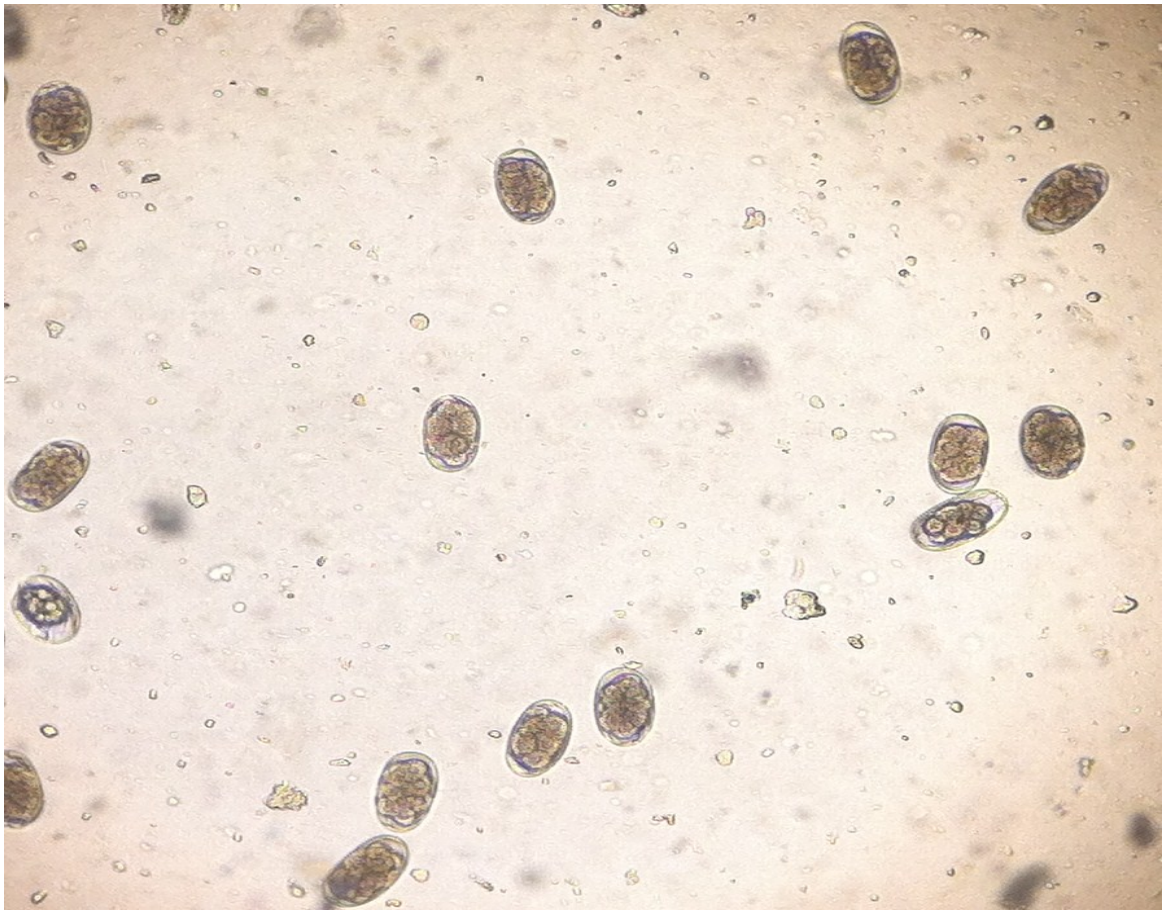


Figure 1 : Fresh eggs of *Heligmosomoides bakeri* (x100)

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Figure 2 : Embryonated eggs of *Heligmosomoides bakeri* (24 hrs-old). X 100



Figure 3 : L1 larvae of *Heligmosomoides bakeri* (x100)

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Figure 4 : L2 larvae of *Heligmosomoides bakeri* (x 100)

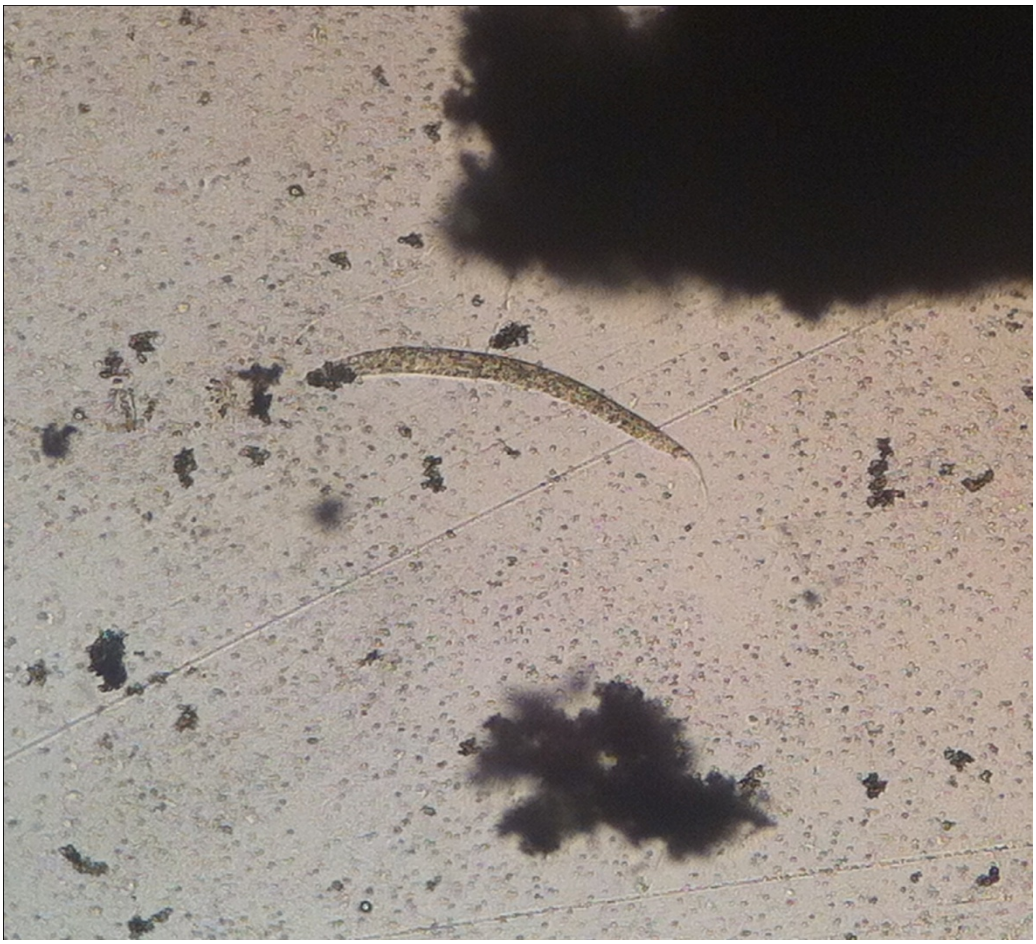


Figure 5 : *Heligmosomoides bakeri* dead larva (x100)

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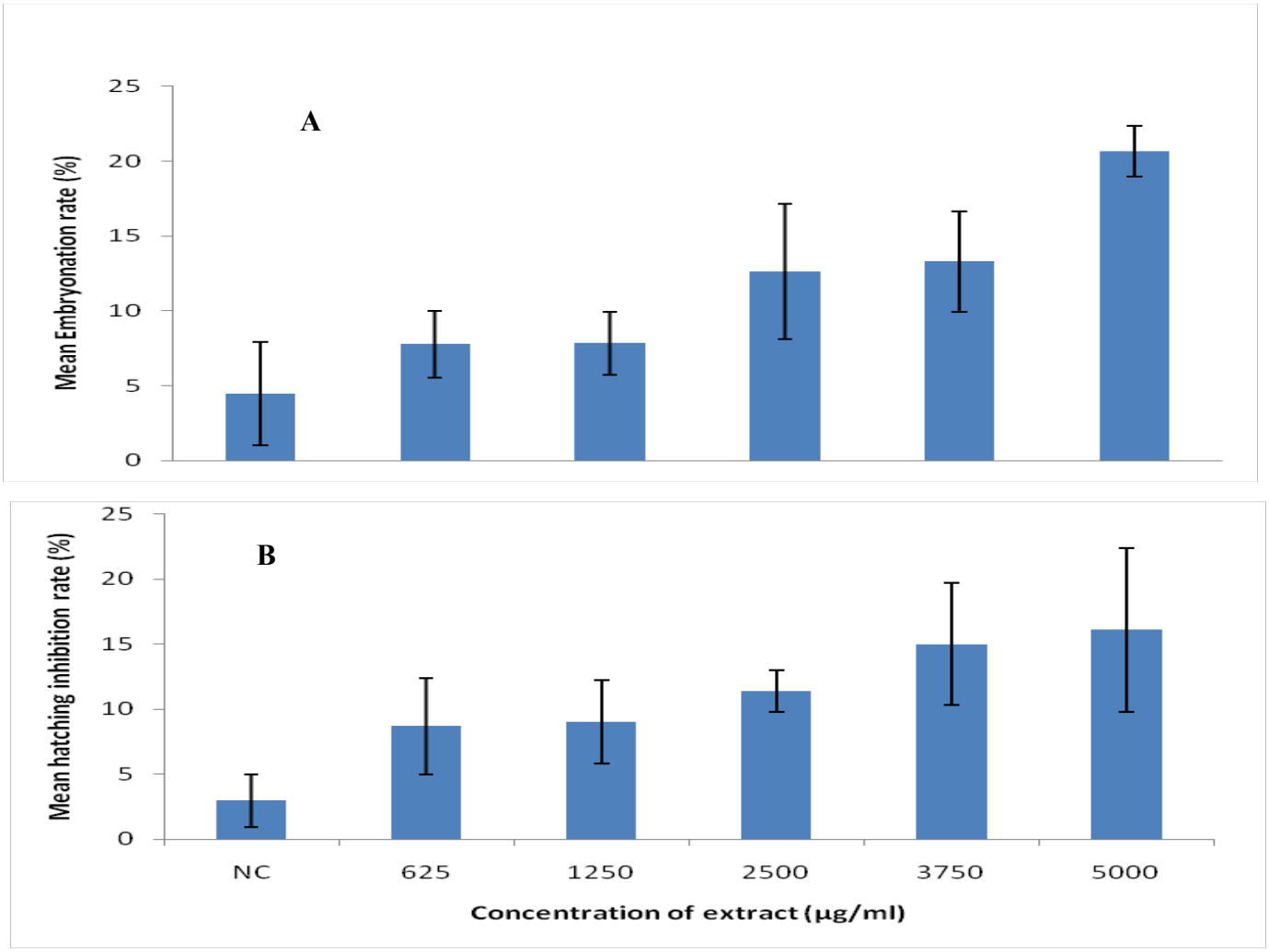


Figure 6 : Effect of *Annona senegalensis* extract on mean embryonation inhibition rate (A) and on mean hatching inhibition rate (B) of *Heligmosomoides bakeri* eggs. Legend: NC= Negative control

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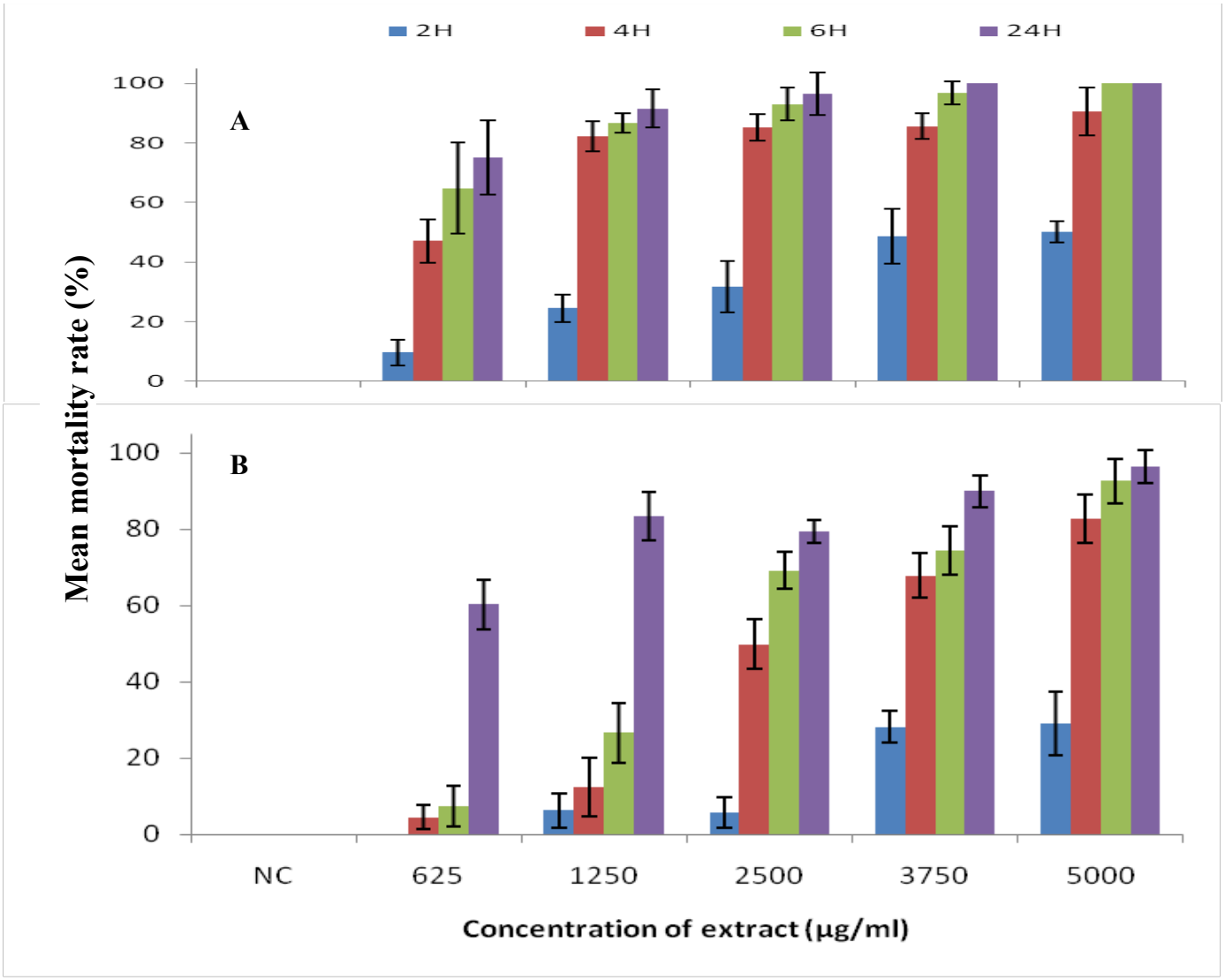


Figure 7 : Effect of *Annona senegalensis* extract on mean mortality rate of L1 larvae (A) and L2 larvae (B) of Heligmosomoides bakeri. Legend: NC= Negative control

V. Aknowledgements

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Strategies of Rodent Control Methods at Airports

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Abstract- Rodent populations at airports can cause human safety issues by attracting raptors ... be, expended to reduce of rodent populations at an airport may decrease birds population in the area and therefore, reduce the risk that raptors pose to aircraft. Rodent populations can be reduced by population management (i.e., use of rodenticides) or by habitat management (i.e., vegetation management, barriers, and land uses) that reduces the area's carrying capacity for rodents. We discuss potential approaches to reduce rodent populations at airports within the context of an integrated pest management strategy.

Keywords: aircraft, habitat management, integrated pest, management strategy, raptors, rodent, rodenticides, vole, wildlife damage.

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Abstract- Rodent populations at airports can cause human safety issues by attracting raptors ... be, expended to reduce of rodent populations at an airport may decrease birds population in the area and therefore, reduce the risk that raptors pose to aircraft. Rodent populations can be reduced by population management (i.e., use of rodenticides) or by habitat management (i.e., vegetation management, barriers, and land uses) that reduces the area's carrying capacity for rodents. We discuss potential approaches to reduce rodent populations at airports within the context of an integrated pest management strategy.

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I. Introduction

Worldwide, rodents have been, and continue to be, the major vertebrate pest group. Much effort has been, and continues to be, expended to reduce their numbers and damage (Witmer *et al.,* 1995). Rodents are implicated in many types of damage, including crop and tree damage, structural property and cable damage, disease transmission, and significant predation on native species of animals and plants on islands to which rodents have been accidentally introduced (Witmer *et al.,* 1998). Numerous books have appeared in the last decade from all continents or regions of the world, addressing rodent damage and its management (e.g., Corrigan 2001, Singleton *et al.,* 1999).At the same time, rodents have many important ecological roles and most species are not major pests. Some of the roles include soil mixing and aeration, seed and spore dispersal, influences on plant species composition and abundance, and a prey base for many predatory vertebrates.

Birds and other wildlife are a serious problem at U.S. airports. Certain species are more hazardous to aviation safety than others, most often due to the size and behavior of the species. Raptors, including hawks, vultures, and eagles, were the fourth most common bird group reported in bird strikes to the Federal Aviation Administration from 1991 - 1997, and hawks specifically were the fifth most common bird species group reported in bird strikes in Canada during the same time period.

Red-tailed hawks were the fifth most common bird spe-cies reported in U.S. Air Force bird strikes from 1985 - 1999, resulting in over $12 million in damage costs. Since raptors are protected under the Migratory Bird Treaty Act, the ability to directly manage raptor populations is limited. Management of their habitat, however, is often more easily accomplished. Raptors are attracted to airport habitats that provide their basic necessities: food, water, and cover. Small mammals, such as mice and voles, are attractive prey for raptors.

Reduction of small mammal populations at an airport may decrease raptor populations in the area and therefore, reduce the risk that raptors pose to aircraft. Reduction of small rodent populations can be achieved through a variety of methods, including habitat manipulation and the use of rodenticides. Habitat management can be accomplished through a grass height management regime or through the introduction of an endophyte-infected grass which may support fewer herbivores, both of which are currently being studied. Zinc phosphide, a rodenticide, was tested for efficacy at Kansas City International Airport. This presentation will discuss these options and the implications of the studies (Witmer and Fantinato ,2003).

Airports often provide good year round habitat for rodent populations. Rodents at airports can cause damage directly by gnawing and burrowing activities. Larger rodents (e.g., beaver, porcupine, woodchucks) can pose a direct collision hazard to aircraft moving on the ground. It should be noted, however, that larger mammals such as deer and coyotes are considered a much more serious direct strike hazard than are rodents or other mammals (e.g., Dolbeer *et al.,* 2000). Perhaps the most serious hazard posed by a sizeable rodent population at airports, however, is the indirect hazard of attracting foraging raptors with an associated raptor aircraft strike hazard (e.g., Barras and Seamans 2002). Raptors pose one of the most hazardous groups of birds at the airport setting (Cleary *et al.,* 2002). Unfortunately, many of our activities at airports result in good habitat for rodents (e.g., allowing tall grass in an effort to reduce loafing habitat for flocking birds) or reduced predation of rodents (e.g., perch removal, bird hazing, carnivore-proof perimeter fencing, and raptor and carnivore capture and relocation; see discussion by Barras and Seamans [2002]).

In this paper, we provide background information on the biology and ecology of rodents and the habitats available to rodents at airports. We also discuss human activities and land uses at or near

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airports that can benefit or adversely affect rodents and, hence, influence the potential for raptor aircraft collisions. The recommendations are not meant to contravene, in any way, the existing authorities, rules, and regulations of federal, state, and local governmental agencies regarding wildlife, land management activities, and airport management.

II. The Nature of Rodents

Over a third of all mammalian species in the world are rodents. They occur on most, if not all, continents. Species have adapted to all life-styles: terrestrial, aquatic, arboreal, and fossorial. Most rodent species are small, secretive, nocturnal, adaptable, and have keen senses of touch, taste, and smell. For most species, the incisors grow throughout the animal=s life, requiring them to be constantly gnawing to keep the incisors at an appropriate length and position. Rodents are known for their high reproductive potential; however, there is much variability among species as to the age at first reproduction, size of litters, and the number of litters per year. Under favorable conditions, populations of some species such as the microtines (e.g., voles) can irrupt, going from less than 100 per ha to several thousand per ha in the period of a few months (e.g., O'Brien 1994). As part of this life strategy, individuals of most rodent species have short life-spans and the annual mortality rates in a population may be as high as 70%. Although rodents are good dispersers, unless conditions are very favorable, mortality rates during dispersal are quite high.

There are many interesting dynamics to various rodent populations that should be understood to better facilitate their management and to reduce damage. The population goes through an annual cycle that may include high and low densities, active and inactive periods, reproductive and non-reproductive periods, and dispersal periods. To avoid inclement periods, some species exhibit a winter dormancy (hibernation), and some species have a summer dormancy (estivation) during hot, dry periods. Some species exhibit multi-year cycles; for example, the microtines often reach population peaks (irruptions) every 3-5 years. Raptors may be attracted to areas such as airports during the "highs" of these population cycles (Baker and Brooks 1981). Even when vole populations "crash", those that survive in grassy "refugia" are able to quickly reproduce and re-invade formerly occupied areas (e.g., Edge *et al.,* 1995, Wolff *et al.,* 1997).

Clearly, it is important to know which rodent species occur at the airport and to have a good understanding of their biology, population dynamics, and ecology along with their relationships to damage, land uses, and human activities.

III. Monitoring Rodent Populations

It is important to monitor rodent populations at airports. Monitoring allows you to identify the problem species and to conduct pro-active actions, not just retroactive actions. Several to numerous rodent species may occur in any given area, but in many situations only one (or a few) species is causing damage or a problem situation (e.g., high numbers of foraging raptors). Knowing what species are present allows the development of control strategies which account for non-target species and minimize non-target losses. Monitoring rodent populations is also very important because densities can fluctuate dramatically within a year and between years. Monitoring also provides additional information on the rodent population: do they breed throughout the year, how rapid is reinvasion, and how far and quickly are animals dispersing.

Obtaining accurate estimates of population density is difficult and costly, in terms of labor, time, and resource requirements. Often, an index that efficiently tracks the population is adequate. A wide array of methods exist for monitoring rodent populations, including trap grids or transects, plot occupancy, open and closed hole indices for burrowing species, bait station or chew card activity and food removal, and runway or burrow opening counts (Engeman and Witmer 2000, Witmer and VerCauteren 2001).

Airport personnel or a contractor should develop and implement a rodent monitoring protocol. This may require some trials with trap placement and potential, palatable baits. Once an effective protocol is developed, it should be implemented in certain areas both inside and outside the perimeter fence. Care must be taken to insure that traps, wire flags, and other materials used in the field for rodent management do not contribute to foreign object damage.

IV. Developing an Integrated Pest Management Strategy

While vertebrate Integrated Pest Management (IPM) has not been as fully explored and implemented as has IPM for invertebrate, weed, and plant disease pests, there has been considerable progress in recent decades. Rodenticide application, causing rapid and large-scale population reduction, continues to be an important tool in rodent damage management. These reductions, however, are short-term and there is a growing concern with the environmental hazards and safety issues associated with rodenticide use. Great strides have been made to better understand the nature of rodent populations, why damage occurs, how damage can be predicted and reduced by non-lethal approaches (physical, chemical, behavioral, and cultural), and how to apply ecologically based rodent management strategies (e.g., Singleton *et al.,* 1999).

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The general equipment, methods, and strategies used to manage rodents, including rodenticides, have been presented in detail by **Buckle and Smith (1994) and Hygnstrom *et al.,* (1994).** Many new approaches (use of disease agents and fertility control) have proven ineffective or ill-conceived for vertebrates in the preliminary testing phases.

The strategies adopted for Managing Rodent Pests (MRP) varies from agro ecosystems to the other such as desert and semi-desert ecosystems. However, the present work was initiated to through a beam of light on the Management Strategies of Rodents (MSR) within different Ecosystems. The conclusion that has been achieved from the conducted experiments could be summarized in the following points: (1)survey and population density of rodent species in the area (2) The differences in species composition of rodents depending on locality, habitat type and preferred food.(3-)The rodent species preferred the vegetable baits in the traps. This can be useful to prepare rodent baits to capture rodents.(4) The control of rodents depends upon the locality, neighboring and available food.(5)-Mechanical, biological and chemical control methods can be used effectively in an Integrated Pest Management Approach (IPMA) for the regulation of the rodents population density (**Desokey, 2007).**

We can develop an effective IPM strategy for rodent population and damage management that involves rodent population management, habitat management, and people management (Table 1). Although we seek a relatively easy and long-term solution to the problem, these often do not exist. Therefore, continual, diligent efforts using multiple methods are required. Once an IPM strategy is applied, it is important to monitor the results and to adjust activities as necessary (i.e., incorporate a feedback loop and practice adaptive management).

Table 1: Potential approaches to the management of lower populations of rodents at airports

Habitat Management	Population Management
Burrowing destroying	Trapping
Sanitation (food and debris removal)	
Remove wetlands, riparian habitats, standing water	Rodenticides use
Manage substrates, soil Compaction	Enhance natural predation (counter-productive; attracts predators) Manage substrates, soil
The differences in species composition of rodents depending on locality, habitat type and preferred food.	
Plant monoculture of endophytic grasses or unpalatable plants	Fertility control (future?)
Manage vegetation height and amount with mowing, herbicides, burning, or plowing; remove plant residues	Introduce rodent disease or parasite (future?)
Use artificial turf or other surface cover which prevents burrowing (not practical?)	Mechanical, biological and chemical control methods can be used effectively in an Integrated Pest Management Approach (IPMA) for the regulation of the rodents population density.
The control of rodents depends upon the locality, neighboring and available food	
Establish rodent-proof barriers (at the perimeter fence), extending above and below the ground surface (needs testing)	
Use crops (soybeans, corn) or livestock grazing outside perimeter fence that do not support high populations of rodents	
Remove animal travel and dispersal corridors leading into airport property	

Several manuals have been developed for guidance on managing wildlife populations and habitats at airports **(e.g., Cleary and Dolbeer 1999, Transport Canada 2002).** These manuals stress the need to reduce the attractiveness of airports to wildlife through habitat manipulation.

V. Habitat Management

All rodents require food, shelter, and water. The shelter provides protection from predators, inclement weather, and a favorable place to bear and rear their young. Although rodents require water, those water requirements vary greatly by species. Because rodent food and cover (i.e., vegetation) can be influenced by

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human activities, there has been considerable development of strategies to reduce populations and damage by manipulating vegetation (Table 1). We will discuss some of these habitat management approaches, but caution that many of them have not been thoroughly investigated or tested on a large scale (e.g., Barras and Seamans 2002).

Good sanitation should be practiced on all areas of the airport. It is especially true around food processing facilities, dumpsters, and employee outdoor eating areas (Barras and Seamans 2002). Commensal rodents, in particular, are prone to exploit these areas. Debris piles (rocks, metal, boards, branches and plant clippings) should not be created as they provide protective cover that most rodents will utilize as burrows, dens, and nest sites. Additionally, airport personnel should anticipate a potential influx of rodents when major airport construction or demolition occurs. Wet lands, surface water, and riparian areas all provide very good habitat for rodents and other wildlife because of the close proximity of food, cover, and water .These habitats should be removed, or minimized in area, within the perimeter fence and out to 5,000-10,000 feet of aircraft movement areas (Cleary and Dolbeer 1999).

Vegetation height and plant residues can be managed by a number of physical and chemical means---burning, plowing, herbicide application (e.g., Tracy 1999), and mowing (Cornely *et al.,* 1983, Witmer and VerCauteren 2001). It has been well documented that rodent population densities are generally lower when vegetation height is maintained at 20 cm (8 inches) or less (Allen 1998 and Barras *et al.,* 2000). Mowing is the most commonly used practice to achieve this goal, but it should be recognized that plant residues (i.e., cuttings or thatch) should not be allowed to build up as these provide good overhead cover as well as insulating nest materials for rodents (e.g., Peles and Barrett 1996). Tall grass may dampen the cycles observed with microtines (Getz and Hoffman 1999), with relatively high numbers being maintained year-round. Tall grass can also allow small, resident populations to build up rapidly (Birney *et al.,* 1976). In some situations, even with mowing, vole populations have quickly increased to pre-mowing levels (Edge *et al.,* 1995). Another consideration is that mowing outside the perimeter may result in an influx of rodents to airport property if better cover exists there.

Grass or vegetation type is also an important consideration. Certain types of grass (bluegrass, creeping fescue) appear to be less supportive of rodents than other types such as tall fescue (Sullivan and Vandenbergh 2000). Some varieties of grass, called endophytic grasses, contain an alkaloid-producing fungus that can improve the hardiness of the grass and reduce herbivory. Some preliminary studies suggest that endophytic grass fields support lower rodent densities (Pelton *et al.,* 1991).

Other species of plants may be unpalatable to rodents. Trials are currently underway with a plant called meadowfoam to assess its natural repellency of wildlife (Sharon Gordon, personal communication). With any of these approaches, it would be important to maintain essentially a monoculture of the plant type to prevent the availability of an alternative food source. Grasslands at airports are typically neglected, except for mowing, so extra effort and expense would be required to maintain monocultures. Artificial turf has even been suggested as a way to restrict rodent habitat, but in most situations, the approach may be prohibitively expensive.

Barriers to rodent movement or burrowing should be considered. The ability of rodents to construct and maintain burrow systems could be reduced by heavy compaction of the site's soil where vegetation occurs over it. Alternatively, a substrate (e.g., gravel, very fine sand) less supportive of intact burrows could be used. Another possibility would be a layer of mesh or woven material placed over the surface that would allow grass to grow through, but would not allow rodents to move between the surface and the subsurface. Finally, a barrier (e.g., cement or metal flashing) could be established at the perimeter fence, extending at least 25 cm (10 inches) above and below the soil surface to restrict rodent dispersal on to the airport proper. An alternative to this type of barrier would be a shallow, horizontal trench extending out from the perimeter fence about 5 meters (16.4 feet) filled with gravel or other material that would make above and below ground movement difficult for rodents. Of course, these barriers would only be effective if the existing rodent population within the perimeter could be successfully eliminated, or greatly reduced, by the use of rodenticides within the perimeter fence. Also, tall vegetation or deep snow cover, may allow rodents to gain access over vertical barriers. While repellents may have some potential to exclude voles from areas, more research and field trials are needed before effective, commercial products become available (Witmer *et al.,* 2000).

Land uses outside the perimeter fence should not be supportive of rodent populations, especially if a rodent-proof barrier cannot be established. Of course, any of the above vegetation management approaches could be implemented on lands managed by the airport outside the perimeter fence. Additionally, cereal grains should not be grown as these crops support rodents as well as grain-eating birds (Barras and Seamans 2002). Certain crops, such as soybeans and corn, are much less supportive of rodent populations. On the other hand, corn fields may attract other mammals and birds. Also, intensive livestock grazing is less supportive of rodent populations (Moser and Witmer 2000). Travel ways or dispersal corridors that could be used by wildlife (tree and shrub cover along streams flowing to or from the airport) should also be eliminated (e.g., Barras and Seamans 2002).

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VI. Rodent Population Management

Populations of rodents can be reduced by a variety of means. Although methods such as trapping, burning, flooding, and drives have been---and are still being---used in developing countries, many parts of the world have come to rely on rodenticide baits for rodent control (Singleton *et al.,* 1999; Witmer *et al.,* 1995). Considerable development has gone into making rodenticides effective, efficient, and relatively safe for use in buildings or the environment. The use of rodenticides is closely regulated by federal and/or state and provincial governments. In many cases, they can only be applied by a certified pesticide applicator.

Trapping is not very practical for rodent population management, except with some of the larger rodents such as beaver, woodchucks, and porcupines. Trapping can also be used to help control commensal rodents within buildings. Perhaps the most important use of traps in rodent management, however, is as a tool for monitoring rodent populations as discussed earlier.

Rodenticides, in many situations, are the most practical and effective way to reduce a large, widespread rodent population. There are two general classes of oral rodenticides. Acute rodenticides (including zinc phosphide and strychnine) usually kill with a single feeding. In contrast, chronic or multiple-feeding rodenticides (including warfarin, diphacinone, and chlorophacinone) usually require a period (days) of feeding before killing. The distinction has become somewhat blurred because the anticoagulant group includes first generation (examples given) and second generation (bromadiolone, brodifacoum, difethialone) anticoagulants. Second generation anticoagulants are very toxic and can usually kill within several days of a single feeding. These materials are generally not available for field application. Use patterns generally allow rodents to feed continuously at bait stations or on bait blocks, however, so that second generation materials offer no practical advantage in many situations. An additional group of rodent toxicants includes the fumigants (e.g., gas cartridges, aluminum phosphide, methyl bromide) which are used in building fumigation or in burrow systems that are closed after application.

Broadcast baiting with zinc phosphide (ZP; 2% active ingredient) on oats or wheat has worked well for vole (and other small rodent) control at some airports (e.g., Witmer 1999). The bait should be applied early in the year, during a dry period, and pre-baiting with "clean" oats (or wheat) should be done to get good bait acceptance and to avoid the development of "bait shyness" (whereby rodents don't consume a lethal dose, become sick, and won't touch the bait again). ZP does pose a primary hazard to any animal that consumes it so it should be used carefully. On the other hand, ZP is considered to pose very low secondary hazards (to scavengers or predators) because it disperses quickly as phosphide gas and does not bio-accumulate (Johnson and Fagerstone 1994). Rodents do not become bait shy when anticoagulants (chlorophacinone, diphacinone) are used, but there may be greater secondary hazards because the compounds do bio-accumulate. In some situations, the use of bait stations is required for anticoagulant use. If one rodenticide is not working, it is often recommended that a different one be tried. It is preferable to apply rodenticides during more vulnerable times in the rodent's life cycle---often early or late in the year when succulent vegetation for foraging is less abundant.

On the other hand,found that bait consumption acceptance of rodents with the addition of natural Materials, seeds powder of coriander (*Coriander sativum*); anise (*Pimpinella anisum*) and yeast, vanillia unnatural Materials as attractants. Also, The active burrows of rodent were decreased when used Aluminum phosphate fumigation (Dsokey,2011)

Airport personnel or contractors should establish an effective rodenticide program to control rodent populations. An effective program would provide a ready tool for a pro-active response to an irrupting rodent population, as determined by the population monitoring protocol.

Other methods of rodent population reduction are not practical or may be counter-productive in an airport setting (e.g., enhancing natural predation) or are not yet registered for field application (introduction of rodent disease agents or parasites, use of fertility control materials).

VII. Conclusions

Dealing with rodent problems, especially in complex settings with many constraints such as airports, may be difficult. Multiple approaches are available and possible, however, and should be woven into a rodent IPM strategy (Table 1). In some cases, it will be necessary to experiment with approaches on a small scale to see which will be most effective and practical in a specific setting. In general, vegetation, overall setting, and land uses of the airport and adjacent properties should be managed so as to be less supportive of rodents, hence attracting less activity by raptors. The rodent population should be carefully monitored with a standardized protocol so that direct population control can be quickly implemented, if necessary. Hopefully, research will continue to provide a better understanding of rodent populations and access to new or improved methods of population and damage reduction.

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"Plantain of Guinea". The Atlantic Adventure of Banana

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Abstract- "Plantain of Guinea". The Atlantic Adventure of Banana. It is studied the historical process of the first move of Musaceae to the West Indies, along with sugarcane, in the context of a transatlantic network. Historical texts are critically analysed; evidence about cultivations and species in Europe, Africa and the archipelagos in Macaronesia is shown; longterm historical mistakes are discussed and some hypotheses about the Atlantic transport of biotypes or hybrids during the sixteenth century are suggested.

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“Plantain of Guinea”. The Atlantic Adventure of Banana

Manuel De Paz-Sánchez

Abstract- ***“Plantain of Guinea”. The Atlantic Adventure of Banana.*** **It is studied the historical process of the first move of Musaceae to the West Indies, along with sugarcane, in the context of a transatlantic network. Historical texts are critically analysed; evidence about cultivations and species in Europe, Africa and the archipelagos in Macaronesia is shown; long-term historical mistakes are discussed and some hypotheses about the Atlantic transport of biotypes or hybrids during the sixteenth century are suggested.**

Keywords: musa spp., bananas and plantains, history of the musaceae, history of culture.

Resumen- ***“Plátano de Guinea”. La aventura atlántica del plátano.*** **Se estudia el proceso histórico del primer traslado de musáceas [*Musaceae*] a las Antillas, junto con la caña de azúcar, en el contexto de una red trasatlántica. Se analizan críticamente textos históricos; se muestran evidencias sobre cultivos y especies en Europa, África y archipiélagos de la Macaronesia; se comentan errores históricos de larga duración y se plantean algunas hipótesis sobre el transporte atlántico de biotipos o híbridos durante el siglo XVI.**

Palabras clave: musa spp., bananas y plátanos, historia de las musáceas, historia cultural.

I. Introuction

In 1736 Linnaeus dedicated a monograph to the *Musa cliffortiana* and, in the epilogue, in order to praise its beauty and eulogise the global diffusion of its cultivation, he included an elegant composition (Linnaeus, 1736: 50) by the neo-Latin poet Hendrik Snakenburg:

> Salve; hospitali sede beatior,
> Quam si vel Indus vel Tropicus tuo
> Pinguescat e fructu, et saporis,
> Musa, satur nihil optet ultra.

This monograph, as Mark Griffiths has highlighted, allowed Linnaeus to refine his method and taxonomic system (Griffiths, 2007: 23, 25). Half a century ago, the Colombian botanist and historian Víctor Manuel Patiño (1912-2001) held that the division which is usually made between plantains (*Musa paradisiaca*, *Musa* spp.) as vegetable or starchy vegetable substitute for bread, and bananas (*Musa sapientum*, *M. Cavendishi*, etc.) as fruit of consumption like a dessert or sweet, was a rbitrary regarding a large part of equin octial America, since both plantains and bananas were used green, as a vegetable or because of its starch, and when mature both were consumed as fruit (Patiño, 1969: 297-298).

With few exceptions, the familiar eating bananas are naturally occurring hybrids among the various subspecies of *Musa acuminata* and interspecific hybrids between *M. acuminata* and *M. balbisiana* (Ploetz *et al*., 2007). These same authors highlight that

> *Musa* taxonomy is confused by several factors including the sterility, ancient domestication, and hybrid origins of the cultivated varieties (cultivars), and the unwillingness of many to adopt newer, correct names. For example, Linnaean binomials such as *M. paradisiaca* (French plantain) and *M. sapientum* (Silk) are still used decades after the cultivars to which these names refer were recognized as *M. acuminata* x *M. balbisiana* hybrids (Ploetz *et al*., 2007).

The relevance of the *Musaceae* could be appraised according to four essential concepts, as Nelson, Ploetz and Kepler emphasise. Firstly, its “extraordinary significance to human societies, produces the fourth most important food in the world today (after rice, wheat, and maize), bananas and plantains”. Secondly, the fact that “*Musa* species grow in a wide range of environments and have varied human uses, ranging from the edible bananas and plantains of the tropics to cold-hardy fibber and ornamental plants”. Thirdly, its evolution and genetic diversity: “These large, perennial herbs, 2-9 m (6.6-30 ft) in height, evolved in Southeast Asia, New Guinea, and the Indian subcontinent, developing in modern times secondary loci of genetic diversity in Africa, Latin America, and the Pacific”. And, in short, despite it is not regarded as “invasive, *Musa* nonetheless is a persistent plant that competes relatively well with other species with-in managed agro-forestry settings” (Nelson *et al*., 2006).

II. Tempus Adventus

When did the *Musaceae* arrive in the Canary Islands before moving to the New World? “Thomas Nichols, who enumerated several introduced crops he saw on his visit to Madeira and the Canary Islands in 1526 [sic], used the Spanish word *plátano* to describe his first encounter with the plantain”, and he stated about the fruit of the *plántano* [sic] that “when it is ripe it is blacke, and in eating more delicate then any conserve” (Carney & Rosomoff, 2009: 41-42). However, this must have occurred around 1556, when Nichols

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visited the Canary Islands for the first time, since "according to his own words, it seems that he was born in the city of Gloucester by the year 1532" (Castillo, 1992: 66).

The first edition of his book, *A Pleasant Description of the Fortunate Ilandes, called the Ilands of Canaria, with their straunge fruits and commodities*, was published in London (1583), and later on the work was spread by Richard Hakluyt in *The Principal Navigations, Voyages and Discoveries of the English Nation* (London, 1589-1600). This contributed to forge the image of the Canary Islands in England, at least until late in the eighteenth century (Castillo, 2000: 75-76; Castillo, 2009: 31).

The text by Nichols about bananas, as reproduced by Hakluyt, reads:

> but especially the PLANTANO which groweth neere brooke sides, it is a tree that hath no timber in it, but groweth directly upward with the body, having marvelous thicke leaves, and every leafe at the toppe of two yards long and almost halfe a yard broad. The tree never yeeldeth fruit but once, and then is cut downe; in whose place springeth another, and so still continueth. The fruit groweth on a branch, and every tree yeeldeth two or three of those branches, which beare some more and some lesse, as some forty and some thirty, the fruit is like a Cucumber, and when it is ripe it is blacke, and in eating more delicate then any conserve (Hakluyt, 1599, II (2): 316).

The error which sets Nichols' visit to the Canaries in 1526 is very old since it stems from the travels' compilation by Hakluyt, who placed a brief "note concerning an ancient trade of the English Marchants to the *Canarie-ilands*", in which it is alluded to the year of Lord 1526 (Hakluyt, 1599, II (2): 315). Some years after this edition, in 1629, the French Pierre Bergeron (c.1580-c.1637) collected it in his *Traité de la Navigation* (Bergeron, 1629: 220; Bergeron, 1735, I: col.116), from whom must have took it, in turn, José Viera y Clavijo (Viera y Clavijo, 1772, I: 47; Viera y Clavijo, 1950, I: 55). Bergeron (1629: 225-226) also reproduced the description about bananas made by Nichols.

Much later authors followed the trail of the anachronism. Bonnet translated the text and published it in Castilian in 1933, based on the cited edition (1599) of the collection by Hakluyt. This earned him the applause of the Canary Islanders historians of the period, who indicated that "Mr. Bonnet has rendered a great service to the history of the Archipelago by translating into Spanish such an interesting account". The article was entitled "Description of the Canaries in the Year 1526, Made by Thomas Nicols [sic], English Factor". He included, annotated, the description of the *plántano*: "estando muy maduro, la cáscara se ennegrece; es por demás delicioso al gusto que la más regalada conserva que se pueda hacer" (Bonnet, 1933: 209; Benítez-Padilla, 1959: 144).

Nichols had also written about sugar production in Tenerife, where he said "there are 12 sugar houses called *Ingenios*", and drew attention to the extraordinary fertility of the Valle de La Orotava, "there is also one league of ground which […], it is thought that the like plot of ground is not in all the World" (Hakluyt, 1599, II (2): 317; Bonnet, 1933: 210-211), centuries before Humboldt did it.

The cultivation of sugarcane in the Canaries was initiated, in the last quarter of the fifteenth century, with plants from Madeira. In 1502 the conqueror of La Palma and Tenerife, Alonso Fernández de Lugo, transferred to Catalan merchants some of his lands in Los Sauces (La Palma), a "very rich in sugarcane plantations" zone, where other settlers from Catalonia also invested (Pérez-Vidal, 1983, II: 309; Fabrellas, 1952: 457-459). Slavery was inserted in this plantation economy. The trade between the Canaries and Africa was already common at this time since the routes had been opened a long time ago by the Portuguese (Aznar *et al*., 2012: 55-63).

Unfortunately there is no complete evidence, apart from what Gonzalo Fernández de Oviedo says, on early dates, about the presence of *Musaceae* in the Canaries. The poet Vasco Díaz Tanco could see bananas in the Islands around 1525, since he sang to them in his *Triumphos*, which were published in Valencia around 1530 approximately. In the stanzas by this poet from Extremadura local and foreign plants were mixed: "vi plátanos, cedros y linaloeles [aloe]", assuming, of course, that he refers to *Musaceae* and not to *Platanaceae*. He also praised the dragon trees (*Dracaena Draco* L.): "vi dragos perfectos muy medicinales" in the same stanza (Rodríguez-Moñino, 1934: 14, 21; García-Arranz, 1989: 30).

Another poet, Bartolomé Cairasco de Figueroa (1538-1610), born in Gran Canaria, mentions the voice *plátano* on several occasions. It seems clear that in the first part of its monumental *Templo Militante*, the reverent allusion is to the Virgin Mary, according to the biblical quote *et quasi Platanus exaltata sum iuxta aquas*; so there can be no doubt that he refers to *Platanus* × *hispanica* or London planetree. Probably, also comment on this tree the verses dedicated to Doramas, islander hero against conquest, born to freedom in the jungle of his name:

> Plátano, Fuente, amomo inusitado:
> En cuyo gremio estuvo nueve meses,
> El divino Doramas retirado
> (Cairasco, 1615, IV: 282).

It is of great interest the testimony of the nobleman-soldier-priest Juan Ceverio de Vera (Las Palmas [Gran Canaria], 1550-Lisbon, 1600), who travelled through America (Hispaniola, Panama,

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Colombia, Peru, Quito) between 1567 and, approximately, the late 1580s (Borges, 1980: 354, 358). In 1595 he went on a journey to the Holy Land, and in Tripoli (Lebanon) he not only saw bananas, but he also related them to the Canaries, to the Iberian Peninsula and to the New World. The first edition of his book, printed in Rome, is of 1596:

> I also saw bananas brought from Egypt, which is healthy and exquisite fruit; and although the trees have come from Canaria [Gran Canaria] to Spain, they are destroyed by the cold, because they want a temperate land and being in the streams of water; they give a single fruit, and being mature, the tree dries and leaves in place many sprouts. And in the Indies [West Indies] there are so many, that in the mountains they are raised without profit or owner (Ceverio de Vera, 1598: 93-94; Martínez-Figueroa & Serra-Rafols, 1964: 112).

In 1645, a group of Spanish Capuchins received bananas in Gran Canaria, during a layover of the missionary journey they went on to Congo. In Las Palmas, where they were feted, they were offered food in abundance, but they "only took a few lemons and fruits from India [sic], which in Canaria is called plátano; of which there are much abundance in the Congo, called *nicefos* in the language of that kingdom" (Pellicer de Tovar, 1649: 5).

These friars were very interested in the acclimatisation of trees in the Congo region. "There are not pears, apples or other similar to these, or born, although they sow them, as the religious have confirmed by experience"; but "three genus" of plants grew well there; in the first place the *niceffo* or *nicefo*, and then the ananas and the coconut palm. The *nicefo*, fairly extended, was grown throughout the year,

> Its trunk is not wood like the other trees, but made of the same leaves multiplied one upon another, which then spread on top and make a beautiful crown [...]. This plant or tree produces a very large cluster, full of *nicefos* (Pellicer de Tovar, 1649: 52-53).

Giovanni-Antonio Cavazzi da Montecuccolo (1621–1678) also referred to the *niceffo*, of which he reproduced some pictures, and he talked about its cultivation in Angola: "Il Niceffo, che gli Ambondi chiamano Maongio-à-Camburi, e pianta utilissima, [...] e produce una pigna capace di cento, sino a ducento frutti somigliantissimi a cetrioli". In addition, he recommended it for dysentery sufferers: "cibarli con frutti acerbi del Niceffo" (Cavazzi da Montecuccolo, 1687: 35-36, 143), so it could be a variety of cooking.

In 1678 Friar José de Sosa, talking about Las Palmas, described the "platanales which guard its shores and often serve as hedges". He also pointed out that the monastery of San Francisco had been founded in the most cheerful part of the town, and that in its magnificent vegetable gardens there were grown "sour fruit trees, plantanales [sic] and other fruits" (Sosa, 1849: 24-25; Sosa, 1941: 34 y 36).

Tomás Arias-Marín de Cubas (1643-1704) claimed in turn that in the late fifteenth century two vessels were chartered in Gran Canaria in order to defend the island from pirate attacks. The ships arrived in Guinea, where they took "blacks to service in the sugar mills", and brought "potatoes, maize, banana roots, yams, and other seeds" (Arias-Marín de Cubas, 1986: 220-221; Bonnet, 1933: 209-note 5). But this information raises doubts because of its inaccuracy and because it incorporates, at that time, maize and potatoes to the credible import of other plants and seeds.

The cultivation of vegetable gardens of platanales continued, however, during the seventeenth and eighteenth centuries (Rodríguez & Macías, 2012: 144). In La Gomera the banana was incorporated into the feast of proclamation of Luis I de Borbón (1725), in the town of San Sebastián, where "the church was seen the next day adorned with bananas and its clusters, and the procession accompanied by the confraternities", although in a certain way its uniqueness was stressed. But it is described in Hermigua where, "in olden days, there was *hazas* of sugar plantations and two sugar mills, today there are only vestiges", and in the *Valle de la Negra* [*Valley of the Black*], "covered by platanales and *iñames*" (*Dioscoreaceae*). In Los Sauces (La Palma) still existed the "sugar mill", the water flowed clear and there were "bananas, dates, lemons" (Viera y Clavijo, 1776, III: 66-67, 91, 93, 498; Viera y Clavijo, 1951, II-III: 450, 466, 467, 763).

The same could be said of Igueste de San Andrés (Santa Cruz de Tenerife), where throughout the eighteenth century small vegetable gardens of bananas and yams were sold. Not far away, the powerful *ingenio* of Taganana had operated with Portuguese technicians, free employees and slaves since the early sixteenth century, and the same could be said about the fertile lands of the Valle de La Orotava (Viña-Brito, 2006a: 363-364; Rivero-Suárez, 1990: 19-33).

There is something undeniable about all this. *Dioscoreaceae* and *Musaceae* subsisted in the Canaries, especially in areas which had stood out because of their sugar mills during the sixteenth century, since around the end of this century sugar production decreased. It was about irrigated regions where slaves had resided. In these strongholds, where it should have been cultivated at the same time as the sugarcane plantation, bananas survived as a mute witness of a special agrarian, food and, obviously, cultural past.

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III. Sugar, Bananas and Slaves

But when, exactly, did the first *Musaceae* arrive in the Canaries? At different times several authors from distinct scientific fields have echoed the "old tradition" of the arrival of the banana in the Canaries sometime in the fifteenth century, coming from Guinea and brought by Portuguese (López-Gómez, 1972: 16; Pérez-Marrero, 2000: 23; Ramírez *et al*., 2013: 53; Lassoudière, 2010: 117-118; Hall, 2009: 26; Crane & Balerdi, 2012, etc.).

On this subject, Langdon writes, "as far as Simmonds is concerned, the banana was carried by the Portuguese from Africa to the Canary Islands some time after 1402" (Langdon, 1993: 16; Simmonds, 1966: 313; Marin *et al*., 1998: 968). It has even been suggested that bananas "seem to have been brought from Guinea by two Franciscan friars" (Martínez-Figueroa & Serra-Rafols, 1964: 192), but the source is not indicated.

Viera y Clavijo also includes this ancient tradition in his *Dictionary of Natural History* when he states that the French gave to the banana the name of "*bananier*, taken from that of the *banano*, which is the one given to it by the natives of Guinea, from whose coast is tradition, the *plátano* was brought to our Islands" (Viera y Clavijo, 1869, II: 200-201). Nevertheless, a tradition is not evidence, and therefore this issue should be further specified.

The occupation of the eastern islands of Lanzarote and Fuerteventura by Jean de Béthencourt and Gadifer de La Salle, in 1402-1404, could not cause the cultivation of any type of *Musaceae*, as the plant does not occur on any of these islands. They have always produced cereals and, partially, grapevine, but the two islands are very poor in water resources, thus making it impossible to plant bananas or sugarcane. They were the granary (cereal producers) of the Canaries when it rained. With absolute certainty the amusing *Figure 9.7* (Camel transporting bananas with donkey in background, Canary Islands, n. d.), included by Carney & Rosomoff (2009: 165), and taken from Simmonds, does not refer to the Canaries: neither the child nor the clothing or the landscape is characteristic of the Archipelago. It is probably somewhere in the NW continental Africa.

The island of El Hierro, which joined as well the lordly conquest, also lacked sufficient water; in fact, the water used to drink was that distilled by the *Garoé*, a mythical tree, probably a variety of *Ocotea foetens*, by a phenomenon of horizontal rain. Nowadays, El Hierro has bananas and tropical fruits such as ananas, but these cultivations began in the last third of the twentieth century.

The great development of the banana in the Canaries came from the mid-nineteenth century, when the Englishman Alfred Diston introduced, from greenhouses in the UK (Paz-Sánchez, 2008: 69-70), the *Musa Cavendishi* that revolutionised the agricultural economy of the Archipelago thanks to the high European demand (Nuez-Yánez, 2005: 154; Macías-Hernández, 2011: 241).

The Crown of Castile dealt directly with the organisation of the conquest of the three islands which, in the last third of the fifteenth century, were still unoccupied. Thus, Gran Canaria was conquered between 1478 and 1483, La Palma between 1492 and 1493, and finally, Tenerife between 1494 and 1496. On these three islands (and to a lesser extent in La Gomera, which had a lordly jurisdiction) occurred, after the conquest, a remarkable development of the sugar plantation, whose works demanded importing slave labour: Blacks, Moors and baptised Moors. The first sugar mill began its work in Gran Canaria immediately after the conquest (Fernández-Armesto, 2002: 519).

Canary Islander planters acquired black slaves mainly in three ways: a) by means of exchanging captured Muslims in the Barbary area, which "was made in exchange for blacks from Guinea"; b) by purchasing to slave ships or Portuguese merchants who "travelled through the islands offering their human merchandise" (Rumeu de Armas, 1947: 84) to sugar mills and workplaces; and c) by expeditions led by the Canary Islander settlers themselves (against the international treaties) to the ports of Senegal, Guinea, Cape Verde or Magarabomba [Sierra Leone] (Lobo-Cabrera, 1983: 102-104; Aznar, 1983: 212-214; Fuentes-Rebollo, 2002: 238, 274; Rosa-Olivera, 1978: 184, 227, 236-237; Viña-Brito, 2006a: 376; Cortés-López, 1989: 44, 107-108; Green, 2012: 69, 81, 90, 133, 183).

Cape Verde became a usual market for the black slaves supply to the Canaries. In 1494, a Portuguese neighbour of Gran Canaria, along with two Spanish, armed caravels, went to Guinea and "caught, stole and captured many souls of blacks from Guinea and so brought them captives" (Lobo-Cabrera, 1983: 104; Pérez-Embid, 1948: 227). The monarchs were forced to ban Canary Islanders and Castilian settlers' raids into Guinea by several royal orders between 1477 and 1516 (Viña-Brito & Macías, 2012: 57, 226, 376).

In April 1503, the Catholic Monarchs ordered Juan de Silva, senior lieutenant of Seville, to arrest the guilty and to return to the king of Portugal

> certain blacks, melegueta, and other things that some neighbors of Lepe, Palos, Triana and Alcalá del Río took in Guinea where it was said Manicongo, in Santo Tomé, Príncipe island, Fernando Poo and Melegueta Coast, which was the land belonging to the king of Portugal and where they continually used to go to rescatar [swap] their vassals (Lobo-Cabrera, 1983: 104; Cortés-López, 1989: 156).

The gathering of melegueta pepper or Guinea grains (*Aframomum melegueta*), in Grain Coast [Liberia], came to demonstrate the ease with which slave trader could be involved in the introduction of spices and

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possibly of plants that they could market in their places of origin.

To the Canaries, essential link in the chain connecting the Old and the New World, some *Musaceae* should be moved in the late fifteenth century by Castilian, Catalan, Valencian, Portuguese or Genoese colonists who actually were going to look for slaves and spices. This move could occur between 1483 and early 1500, and would include taxa from Guinea-Congo and perhaps from the Senegambia region and Cape Verde.

IV. In Transitu Ad Novum orbem

When did the plantain or banana come to the New World? The testimony of Fernández de Oviedo, who, provisionally is the closest to the facts, clearly expresses that it happened in 1516, from Gran Canaria to Hispaniola, and that it was the Dominican Friar Tomás de Berlanga who carried it out. This is an accurate testimony which has been accepted by hundreds of scholars throughout time.

The first reference to banana can be read in the *Summary*, i.e., in the work *Oviedo de la natural hystoria de las Indias*, which was printed in Toledo on February 15, 1526:

> from Spain were brought the first, and they have multiplied so much that it is a thing of wonder to see the abundance that there is of them on the islands and in Mainland, where there are villages of Christians, and they are bigger than better and of better flavour in those parts than in these (Fernández de Oviedo, 1526: XLIIJ).

In *La historia general de las Indias*, published in Seville (Juan Cromberger, September 30, 1535), we read (book VIII, chap. I, § X):

These were not in these Indias, and were brought to them […] (Fernández de Oviedo, 1535: LXXIX).

> They were brought from the island of Gran Canaria in the year MDXVI [1516] by the Reverend Father Friar Tomás de Berlanga of the Order of Preachers to this city of Santo Domingo and from here they have extended [...]. The first were brought as I said from Gran Canaria, and I saw them there in the same city at the monastery of San Francisco in the year MDXX [1520], and so there are some of them in other Fortunate islands or Canary Islands. And I have also heard that there are some in the city of Almería in the kingdom of Granada: but to the true [...]: this fruit is from the Levant and the East India according to Genoese, and Italians and Greeks merchants [...] (Fernández de Oviedo, 1535: LXXIX *v*-LXXX).

And these came here owing to that Reverend Father Friar Tomás de Berlanga, to whom meritoriously the Imperial Majesty has made the mercy of the Bishopric of Castilla del Oro in Tierra Firme (Fernández de Oviedo, 1535: LXXX *v*).

The edition of Salamanca, in the office of Juan de Junta, was completed on May 2, 1547 and is entitled *Coronica de las Indias: la hystoria general de las Indias*, "corrected and amended". It does not offer significant differences on this point.

Now then, book VIII, chapter I, § 10 of the scholarly edition of 1851, by José Amador de los Ríos, shows striking changes in the paragraph referring to Almería:

> As to the truth they cannot be called plátanos (nor they are so); but that which is, *según he oído a muchos*, was brought this lineage plant from the island of Gran Canaria, the year one thousand five hundred and sixteen, by the Reverend Father Friar Tomás de Berlanga [...]. And I have also heard that there are some in the city of Almería in the kingdom of Granada, *y dícese que de allí pasó esta planta a las Indias, y que a Almería vino del Levante y de Alejandría, y de la India oriental*. I have heard Genoese and Italian and Greek merchants […] (Fernández de Oviedo, 1851, I: 291-292).

Does Fernández de Oviedo want to indicate that the plant could also be moved from Almería to the New World? So it seems, by judging the preceding text, which would be based on the manuscripts recovered in the nineteenth century and which, according to the "Warning" of the publisher, had suffered "great additions and amendments" by the hands of the author himself (Fernández de Oviedo, 1851, I: V-VI). The same text, with minor spelling changes, in the edition by Juan Pérez de Tudela (Fernández de Oviedo, 1959, I: 248).

V. Al-Andalus Gardens

Did bananas continue to be cultivated in Andalusia during the sixteenth century, as had happened in the Muslim period? Of course to a much lesser extent, but it seems that it was so. There is a testimony that even documents the eventual consumption of the fruit among certain courtiers. The doctor of Felipe II, Juan Fragoso, stated it; he was a man of a "balanced critical spirit" (Calbet, 1988: 7). Fragoso wrote *Discursos de las cosas aromáticas* (1572), and said about the "fruit called Musa" that

> This plant is known today in Almería, from where the fruit has been sent to some gentlemen of this Court, being a great gift and with good reason, being of such an exquisite and rare tree. These figs at first to be tasted, give discontent; but if one continues using them the person who eats them is not sick of them (Fragoso, 1572: 168).

Fragoso was an influential man. It was pointed out that he was one of the first Spanish doctors to quote Paracelsus; that he was fellow countryman, friend and classmate of Francisco Hernandez; and that his *Discourses* were not only about Asian plants, but also about many American plants, copying García da Orta

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and Nicolás Monardes (López-Piñero & Pardo-Tomás, 1996: 28, 52, 112; Fresquet-Febrer, 1995: 76-77).

Actually, Fragoso was inspired by the commented edition that Carolus Clusius had made about the celebrated *Coloquios dos simples, e drogas he cousas mediçinais da India* (1563) by the Jewish-Portuguese physician and botanist García da Orta (c. 1499-1568). Wisely, Clusius had decided to comment and publish this work in Latin, and his work is not a simple translation, but a scholarly study which preserved the authorship of Orta under the title *Aromatum et simplicium aliquot medicamentorum apud Indos nascentium historia* (1567).

Accompanied by Jakob Fugger, Clusius had visited Spain and Portugal in 1564. As a result of that journey he discovered the *Coloquios dos simples* by the Lusitanian and, impressed by his newness, he decided to translate the work immediately (Barona-Villar & Gómez-Font, 1998: 30-31). During his stay in Lisbon Clusius saw some bananas, which had a low fruition, but which were there, as if to prove they existed. The plant was known as Figuera Banana:

> Vlysipone, ubi aliquot plantas vidi, minime tamen fructiferas, nomen hoc retinet; vocant enim etiamnum Figuera Banana, id est, ficum Bananas ferentem (Orta & Clusius, 1567: 222; Clusius, 1605: 230).

José de Acosta, author of the influential *Natural and Moral History of the Indies* (1590), which Humboldt did not cease to praise, however had pointed out that the *plátano de Indias* did not grow in Spain or Italy, where grew the *Platanus* (Acosta, 1590: 248-249). But Juan de Guzmán, the first translator into Castilian (1586) of the *Georgics* by Virgil (Herreros, 1998: 192, 395, 419; Morreale, 2002: 597, 601, 615), was more prudent when he wrote the hemistich *et steriles platani malos gessere valentes* (*Georg*. 2, 70), saying that these *Platanus* were

> different from those of the Indies [West Indies], because in Seville there are those of the Indies, although they do not bear fruit here like they do there, and are small in comparison, it can thus be inferred that they were those brought from Asia (Guzmán, 1795, II: 189-190).

The cultivation of banana varieties among Spanish Muslims is a fact known and debated in the context of discussions on irrigation in Al-Andalus and its socio-political implications (Glick, Thomas F., 1994: 974; Retamero, 2009: 276; Essa & Ali, 2010: 61). Crops of *Musaceae* and other species, which required enough water and special care, were successfully introduced by means of specific hydraulic techniques. In the *Calendar of Cordoba* (attributed to Ibn Sa'id, tenth century), in the *Compendium of Medicine* (Abd al-Malik Ibn Habib, 790-c. 853) and in the *Anonymous Andalusian Calendar* (thirteenth century) there are mentioned the sugarcane (qasab al-sukkar), the cotton (al qutun), and the banana (al-mawz). According to the chronicle by Ibn Hayyan (eleventh century) during the first half of the ninth century, in the Cordoba court of Abd al-Rahman II it was discussed "the characteristics of the banana (al-mawz)" (Trillo-San José, 2004: 46-47, 50).

In the process of receiving and diffusing these vegetables in the West, it has to be taken into account, together with the commercial tradition of the Arabs, the "extensive kindred networks and the obligation of hospitality with friends and pilgrims". It has also been studied the introduction in Al-Andalus of the main varieties of *Musaceae* (Watson, 1983: 51; Watson, 1998: 117; Trillo-San José, 2004: 46-50).

The agronomist Abu Zacaria Iahia [Ibn al-Áwwam, Yahyà b. Muhammad], who flourished in Seville during the twelfth century, dedicated an interesting epigraph to the *Musaceae* in his *Book of Agriculture*, where he counseled a number of practical care for the cultivation and conservation of the fruits (Boutelou, 1878, I: 228-229).

VI. Fernández De Oviedo, Anghiera Y Varthema

Patiño (in several adaptations of his manual on *Cultivated Plants and Domestic Animals in Equinoctial America*, 1963-1969) considered that, if the banana had been moved from Andalusia to America and not from the Canaries, it would also be "clones adaptable to high latitudes". Moreover, he drew attention to the doubts raised by the text by Fernández de Oviedo (editions of 1851 and 1959) because he introduces certain inaccuracies ("as I have heard from many" or "it is said that from there moved"); because he indicates that he first saw bananas in 1520 in the Franciscan monastery of Las Palmas; and, as well, because he quotes the works by Anghiera and Varthema in order to describe the plant (Patiño, 1969: 300).

However, Fernández de Oviedo had ratified all the time the famous move and, besides, he had praised the merits of Berlanga when he was appointed Bishop of Castilla del Oro (Colombia-Isthmus), as already mentioned (Fernández de Oviedo, 1535: LXXX *v*; Fernández de Oviedo, 1851, I: 293; Fernández de Oviedo, 1959, I: 249-250).

On the other hand, it seems that he simply turned to the cited authors in order to get the necessary information and to provide a correct classification of a crop that he considered very useful. The surly though sharp Pietro Martire d'Anghiera had written about this unique biotype:

> De arbore, quam potius caulem appellauerim, quod sit vti carduus, medullosa non solida, quamuis ad lauri celsitudinem surgat, multa sunt repetenda, facta est mentio breuis de hac in primis decadibus (Anghiera, 1530: C).

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Text which Torres-Asensio had no difficulty in translating as follows:

> About the tree, which I better call cabbage because it is like a fluffy thistle, not solid but as high as the laurel, many things have to be repeated: in the first Decades it had been mentioned (Torres-Asensio, 1892, IV: 205).

Thus, what Fernández de Oviedo makes is to confirm that, apart from what he had heard from certain travellers, Anghiera had eaten this fruit, "called *musas*", in Alexandria, and it was clear that it was very different from *Platanus*. This author, besides, did not feel very attracted by the plant since, as he said, it "grows so much" that it renders the land useless for other crops (Torres-Asensio, 1892, IV: 207-208) more typical of Western European agriculture.

Fernández de Oviedo believed, on the contrary, that the *Musaceae*'s capacity to reproduce was one of its advantages. For this reason he confessed to own at least *cuatro mil pies de ellos* in his own farm,

> [...] and they have been moved to Tierra Firme and where they have been planted they have grown very well: *y no hay hombre de cuantos en esta tierra tenemos heredades en el campo que esté sin muchos de ellos. Bien creo que hay en mi hacienda iiij [cuatro] mil pies de ellos, y en otras muchas haciendas que hay, mayores que la mía, hay muchos más*, because they are very advantageous and the many there are of them are used by folk, and it is even a good income for their owners, for any expense is put into growing them (Fernández de Oviedo, 1535: LXXX; Fernández de Oviedo, 1547: id.).

The peculiar thing of this revealing passage, for it shows that our chronicler grew bananas on his farm and that he had direct knowledge of the plant, is that it does not appear in the editions of 1851 and 1959, to which scholars usually refer. The equivalent paragraph reads:

> [...] and they have been moved to Mainland and where they have been planted they have grown very well; and in the heredities which neighbours have on the island, there are countless numbers of these bananas, because they are very advantageous and they are used, the many there are of them, by folk, and it is even a very good income for their owners, for any expense is put into growing them (Fernández de Oviedo, 1851, I: 292; Fernández de Oviedo, 1959, I: 248).

It may be thought that, definitively, the author himself decided to delete that little paragraph; but other alternatives should not be dismissed, such as the loss of part of the material or the use (in 1851 and 1959) of defective copies of the original manuscript. Cuesta-Domingo had pointed out that Fernández de Oviedo "had lived for a quarter of a century" in America and, for that reason, his experiences and the information obtained allowed him to write a "great work in which the physical and human geography are combined" (Cuesta-Domingo, 2007: 118), despite its imperfections.

The second of the mentioned books is the *Itinerary* by the Bolognese Lodovico di Varthema (c. 1470-1517), who indeed had referred to the *plantain-tree* under the name of *malapolanda*, corruption, as it seems, of the Tamil *valei pullum*. Perhaps the most interesting thing is that this Italian traveller described three species: "The third sort are bitter. The two kinds above mentioned are good like our figs, but superior" (Badger, 1863: 162-163). Fernández de Oviedo, who mistakenly writes Ludovico de Vartenia [sic], an error that has endured throughout centuries (1851, I: 292, 1959, I: 249), points out that in Santo Domingo [Hispaniola], the plant also showed differences in the quality of the fruit, which could be an indication of the existence of species or perhaps different hybrids,

> And I also say that on this island this fruit is not all of a goodness, because there are some better fruits of these and tastier than others of the same fruit; but this may happen because of the soil or the disposition of the land as it happens with all other fruits in Spain and elsewhere (Fernández de Oviedo, 1535: LXXX).

It is possible, on the other hand, that he had first seen the plant in Las Palmas in 1520, for he was a long time linked to his destinations in Mainland, involved in political and administrative conflicts and travelling quite frequently between America and Spain. Simply, he knew that they had been moved to Santo Domingo in 1516 and, when he wrote his chronicle, he confessed he had seen them during a layover made in the Canaries. At the same time, he discovered that they held great attraction for ants,

> Ants are very keen on these bananas, and it is always seen on bananas great masses of them [...]; and in some parts there have been so many ants that [...] they have uprooted many of these bananas and thrown them out of the villages (Fernández de Oviedo, 1526: XLIIJ).

This is not a simple anecdote, which furthermore he repeats in all his works. Bartolomé de Las Casas, in Chapter CXXVIII of the Book III of his *History of the Indies*, talked, in almost marvellous terms, about the dreadful epidemic of ants that invaded, among other places, the city of Santo Domingo "for this time of year 18 and 19". This new biblical plague destroyed several vegetable gardens, and among them "one of the monastery of the Dominicans, a principal one with pomegranates and orange trees", and in the Vega, another famous vegetable garden of the Franciscans. At the end of the chapter Las Casas writes:

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> The cause that originated this anthill, some said and believed, was the brought and position of the bananas (Casas, 1876, V: 27; Casas, 1956, III: 472).

VII. Friar Tomás De Berlanga

The move by Friar Tomás de Berlanga, from Gran Canaria to Santo Domingo, not of a single and stunted specimen of *Musaceae*, but probably of several taxa or hybrids of the plant, around 1516, is credible, despite the fact that up to now no evidence has been found documenting his trip to America in this particular year.

The possibility that this move had taken place after 1526 is dismissed because of the publication, on the same date, of the said *Summary* [*Oviedo de la natural hystoria de las Indias*], where the writer already describes the importance of bananas in Hispaniola. There is, however, documentary evidence of Berlanga's first trip to the New World, as part of a group of Dominicans who set out from Seville "in mid-November 1510" for a voyage of "about a month and a half". New expeditions were chartered in 1515 (Figueras-Vallés, 2010: 48, 76, 85-89; Rubio y Moreno, c. 1930: 301, 330).

In 1517 Friar Tomás de Berlanga sent Friar Bartolomé de Las Casas "to the Peninsula in order to recruit peasants who wanted to go to the Indias [West Indies] to settle". Among the two hundred farmers who accepted the proposal there were seventy neighbours of Berlanga, since Friar Tomás had insisted that Las Casas had to go to his hometown "and not elsewhere" because, as Figueres-Vallés has pointed out,

> he knew his people would do an excellent job in the Indias, as did a fellow countryman of him, who was in Hispaniola, the bachelor surgeon Vellosa [or Velosa], who developed a system for whitening much better the sugar cane (Figueres-Vallés, 2010: 86-87).

Everyone had had "their eyes closed", wrote Fernández de Oviedo, until the "bachelor Gonzalo de Velosa, at his own expense of great and excessive spending", brought sugar masters to this island "and made a *trapiche* [rustic sugar mill] with horses and he was the first who succeeded in making sugar on this island". However, he clarified that, since some time ago, they had taken the first sugarcanes to Hispaniola, and also molasses had been produced; but it is obvious that Velosa had been characterised by a higher professional thoroughness. For this reason he had built a *trapiche* drawn by an animal on the banks of the Nigua River and, very soon, as we will immediately say, associated with Cristóbal and Francisco de Tapia brothers. "He brought the officers to do so from the islands of Canaria (Canary Islands), and ground and made sugar before anyone made it" (Fernández de Oviedo, 1535: XLII *v*; Fernández de Oviedo, 1851, I: 118).

Fernández de Oviedo was probably informed about the move of bananas to Santo Domingo, led by Friar Tomás de Berlanga, by the bachelor of Gonzalo de Velosa or, where appropriate, by someone from his old surrounding such as, for example, Cristóbal and Francisco de Tapia brothers. We know that the latter had been governor of the fortress of Santo Domingo, a position in which he was replaced in 1533 by Fernández de Oviedo himself (Pérez de Tudela, 1959, I: CXIX-CXX), so he put on recorded his new employment in the colophon of his *History* (1535): "governor of the fortress and castle of the city of Santo Domingo in Española island".

Thus, Fernández de Oviedo, given the quality and closeness of his informants, had not the slightest doubt when it comes to include the fact in his *History* as an indubitable truth, despite his corrector obsession of the advanced age. Velosa had invested heavily in the incipient sugar sector, and therefore he moved the technicians, tools, slaves and, naturally, some of the Canary Islands-African food traditions that, thereafter, would mark with an indelible stamp the cultural and food and agriculture history of the New World.

VIII. Investing in Hispaniola

In 1515-1516 occurred in Santo Domingo and indeed, in the Americas, a very special coincidence. The start-up of the first *ingenio* [sugar mill] worthy of such name came to coincide, technically, with the arrival of bananas in Hispaniola. The date of the inauguration of Velosa's *trapiche* is in Father Las Casas (chap. CXXXIX):

> Later, a neighbour of the city of Santo Domingo came to understand how to do it [sugar]; he was called bachelor Vellosa because he was surgeon, native of the town of Berlanga, about the year of 516; he was the first who made sugar in that city, after making some more suitable instruments, and thus it was better and whiter than the first of the Vega, and the first was that from which he made *alfeñique* [cooked sugar-paste] and I saw it [...] he managed to make one that is called *trapiche*, which is a mill or *ingenio* [sugar mill] that is drawn by horses (Casas, 1876, V: 28; Casas, 1956, III: 473).

Velosa was appointed surgeon of the city in 1511; but "between 1514 or 1515 the salary stopped to be paid to the physicist, apparently because he used the office negligently" (Mira-Caballos, 2010: 512), which, at first, would contradict his entrepreneurship; but the change in his financial stability may induced him to invest their savings in the *trapiche*. It was, in any case, an important investment with a high initial cost to make it profitable: sufficient sugarcane plantations, technology, workforce, commercial organisation to output products, etc. As Pérez-Vidal suggested "that development is not reached, apparently, in Hispaniola until 1515", since until that moment "sugar could only be obtained in small quantities and with inappropriate means" (Pérez-Vidal, 1983, II: 309; Phillips, 1985: 196).

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However, the overlap of the two dates, that of the arrival of the banana and the one of the start of the not handmade production of sugar, is surprising even in its imprecision. As Ortiz stressed in his brief chronological proposal: "1515 (or earlier): The first sugar harvest of the first rustic sugar mill. By *Gonzalo de Velosa*", and next, "1516: The introduction of the first *ingenio* (sugar mill). By *Gonzalo de Velosa* and *Francisco and Cristóbal Tapia* brothers" (Ortiz, 1987: 295).

Basically, it is a logical coincidence within the frame of reference of a temporal sequence that should be placed in the mid-1510s, when the enhancement of the Caribbean led to the New World resources that had already been experimented in the Mediterranean and, above all, in the Atlantic archipelagos of *Macaronesia*, where it is introduced the novelty of slave labour in the plantation (Viña-Brito, 2006b: 22-23). It is a phenomenon that is repeated: settlers, landowners, traders, slaves and industrial technicians, and also certain patterns of sugar technical and cultural tradition. The first initiative for the sugar exploitation, although using rudimentary methods, was that of the Catalan Miguel Ballester, though the first who cultivated the cane was Pedro de Atienza (Ortiz, 1987: 295-296; Pérez-Vidal, 1983, II: 305, 311; Cuevas, 1999: 7; Cantero *et al*., 2005: 112).

There was a transatlantic network between the archipelagos of *Macaronesia* and the Caribbean, which of course also includes Brazil in competition for new technological developments (Sánchez-Valerón & Martín-Santiago, 2003: 72, 88-89; Daniels & Daniels, 1988: 494, 496, 510). This historical reality was highlighted, already since 1773, by Viera y Clavijo:

> Our Islands were not secretive of their excellent *sweet canes*, or of the manner of making sugar in their *Ingenios*, since sugarcanes, ingenios, trapiches and officials, all came to America from the Canaries. What a colony so useful for that continent! (Viera y Clavijo, 1773, II: 476-477; Viera y Clavijo, 1951, II-III: 250).

The investment required to start an *ingenio* of those called "powerful" was not less than ten or twelve thousand gold ducats in order to have it "run-of-the-mill". The figure, undoubtedly raised, should not be surprising because it was needed to have,

> At least, continuously, eighty or one hundred blacks and even one hundred and twenty and some more, in order to have better all they need; and near a good herd or two of cows of one thousand or two thousand or three thousand of them, *que coma el ingenio* [which eat the *ingenio*]; apart from the great expense of officers and masters who make the sugar, and of carts to carry the cane to the mill and to bring wood, and people who continually cultivate the wheat and take care of and water the canes, and other necessary and of constant expense things (Fernández de Oviedo, 1535: XLIIJ; Fernández de Oviedo, 1547: id.; Fernández de Oviedo, 1851, I: 119).

The growing decrease in indigenous population, along with the decline in gold production, turned the sugarcane into the last hope of salvation; at the same time black slaves were the "indispensable means of production" in the change of the production model. Various subterfuges were resorted to, such as the demand that slaves who travelled to America had to be Christianized, which strategically could benefit the Canaries' intervention as a previous layover in the process of slave trade. But in the end, the interests of individuals triumphed and especially those of the Crown because, as the academic Joaquín Maldonado-Macanaz wrote,

> More certain is that the Catholic King Fernando had *situados* 500,000 gold escudos per year on the income of the Island, which came not only from the working of the mines, but also from the sugar industry transplanted there from the Canaries by Gonzalo Velosa, who founded the first *ingenio*, as well as from other sectors of agriculture to which Spanish population was dedicated (Maldonado-Macanaz, 1870: 9).

From 1518, by virtue of the *asiento* [slaving monopoly] or royal privilege that was granted by Charles V to Laurent de Gouvenot, Governor of Breza, it was intended to introduce in America some 4,000 blacks in the course of eight years (Ngou-Mve, 1994: 40; Friedman, 2000: 49; Phillips, 1985: 185) in order to guarantee the workforce in a plantation economy that was beginning to be efficient.

The situation had actually changed from 1516, with the arrival in Spain of Carlos V. The new governor of Santo Domingo, Rodrigo de Figueroa, had the support of the Crown for the promotion of sugar production, and as Phillips indicates, "the king also encouraged sending experts from the Canaries to the Caribbean, exempted technological equipment from taxation, and allowed on-the-spot production of the copper", necessary for manufacturing works of sugar. "Progress was rapid" (Phillips, 1985: 197).

In short, around 1516 all the factors were given for *Musaceae* to pass to America, as a traditional food of Africans that had also a great identity value. Benítez-Rojo and other authors have stressed that, along with various products of vegetal origin, banana "was an essential element of the African diet, so much so that in many places in the Caribbean it is still called *guineo*, that is, native to Guinea" (Benítez-Rojo, 1996: 96-97; Vesa-Figueras, 2003: 75). Yam constituted, in turn, a staple food in tropical areas of Africa such as, for example, Benin (Fernández-Armesto, 2002: 211).

It was also pointed out that, "as with sugar, the history of the banana plant in Spain's New World colonies begins at the gateway of the Canary Islands".

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The problem that arises is to know the type of *Musaceae* officially moved to the Americas, since "the European palate's attraction to the sweet banana would imply that Berlanga's clone was in fact the fruit type", which Fernández de Oviedo neither helps clarify (Carney & Rosomoff, 2009: 40, 113, 114). As Lassoudière suggests: "Des doutes subsistent sur les espèces : s'agit-il seulement de bananiers dessert? De bananiers plantains? Des deux?" (Lassoudière, 2010: 117).

IX. In the Footsteps of the Chosen Species

Was it a single biotype or were they several? It has been noted that, probably, it would be cultivars "Silk fig" or "French plantain", which are not "edible in raw, but after cooking" (Galán-Saúco & Cabrera, 1992: 4; Robinson & Galán-Saúco, 2011: 2-3). In the Caribbean, it was seen that the old term *Musa sapientum* would correspond to the hybrid "Silk fig" in Trinidad; to the "Figue pomme" in Haiti, Guadeloupe and Martinique; and to the "Cambur manzano" in Venezuela. The term *M. paradisiaca* would refer to the "French plantain" or *Dominico* of Venezuela (Cheesman, 1948: 293-296; Cheesman, 1950: 29-31; Haddad & Borges, 1971: 227-228).

García-Álvarez suggests that different varieties were introduced at different times and from different territories, which would give rise to names associated "with the geographical origin of the clones", and hence the "generic name *guineo* used in Eastern Cuba for banana" (García-Álvarez, 2001: 150). This is interesting, since bananas were grown in Santiago de Cuba around 1538, when it was described by "hum fidalgo Delvas" who accompanied Hernando de Soto, before moving to Florida. "Chamam se naquella terra *Plantanos* [sic], e sam de bom sabor e amaduram depois de colhidos", and he also highlighted the *batatas*, which "mantem muita gente, e principalmente os escrauos" (Burgos, 1557: XI *v*, XII; Costa de Macedo, 1844, I: 11-12).

The added *guineo* is therefore a form of cultural identification and, of course, a reference to the African origin of the plant, which had already been pointed out by Anghiera:

> *Ab ea Aethyopiae parte, quam vulgo Guinea dicitur, vbi est familiaris & sua sponte nascitur, primum aduentam ferunt* (Anghiera, 1530: C v).
>
> It is told that they first took it from that part of Ethiopia which is commonly said Guinea, where it is common and grows spontaneously (Torres-Asensio, 1892, IV: 208).

Acosta distinguished at least two types of bananas or *plantains*; some small, delicate and white, which in Hispaniola are called *dominicos*, and others "thicker and tough and red". He also stated that it was the most commonly used "fruit" in the Indies, "although it is said that its origin was Ethiopia and that from there came, and in fact blacks use it a lot" (Acosta, 1590: 247, 250).

The Inca Garcilaso de la Vega maintained that the *dominicos*, smaller but more rarer and tastier, received this name because the skin, when the fruit was ripe, "was black and white with patches" (Garcilaso de la Vega, 1609: 211; Garcilaso de la Vega, 1723: 282), imitating therefore the habit of the Dominican friars.

In the New Kingdom of Granada, Friar Pedro Simón takes the tradition that states that the *plantains* or bananas had been brought to America by "the Spanish from the Canaries", and maintained that they were of "good taste when raw if they are well seasoned; and they are also cooked". He also said that he had seen bunches of those that are "called of Guinea" with more than three hundred fruits, "though small" (Simón, 1626: 725).

The *Historical Relation* by Jorge Juan and Antonio de Ulloa talks about dominico and guineo bananas (Juan & Ulloa, 1748, I (2): 391); and in 1769 the botanist Gómez de Ortega distinguished, in a note to the voice "bananas" of the book by Commodore Byron *A Voyage Round the World*, the three species of *Musaceae* that were best known in his time. The big ones (bananas), the small ones (plantains guineos) and the medium ones (dominicos); "to the second species it is likely that its name come from Guinea, where it is produced a special abundance of them" (Byron, 1769: 11-12).

In his *Saggio di Storia Americana* the Jesuit Filippo Salvadore Gilij (1721-1789), who developed his missionary work in the Middle Orinoco, also drew attention to the varieties *guinèo* or *cambùre*, *artòni* and *dominico*, of which he says that "ed è un frutto di mezzo tra l'Artòne, e 'l Guinèo se non che il Dominico è di figura quasi triangolare" (Gilij, 1780, I: 212, 310-311).

Humboldt, who described fascinated the Valley of La Orotava (Tenerife), "the greatest sight, the richest of the universe", mentioned groups of bananas being part of such an idyllic landscape (Puig-Samper & Rebok, 2007: 289). In New Spain, when he defended his thesis on the existence of a type of pre-Columbian *Musa*, as had defended the Inca Garcilaso de la Vega, he suggested that Friar Tomás de Berlanga "could not transport from the Canary Islands to St. Domingo any other species but the one which is there cultivated, the *camburi*", and not "the *plátano [h]artón* or *zapalote* of the Mexicans". The first of these species, wrote, "only grows in temperate climates, in the Canary Islands, at Tunis, Algiers, and the coast of Malaga". He also stated that "the *guineo*, a variety of the camburi, as its name proves, came from the coast of Africa" (Humboldt, 1822a. II: 369, 371; Humboldt, 1822b, II: 233-234).

The fact is that while "in Peninsular Spain both plantains and bananas were called *plátano*", in Spanish America "sweet fruit banana was linguistically

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differentiated from the plantain (*plátano*) as *plátano guineo*" o *guineo* (Carney & Rosomoff, 2009: 103).

One possible explanation is that this linguistic phenomenon occurs for two principal reasons. In peninsular Spain crops of bananas, from the fall of Granada, were further reduced to marginal areas, and the fruit became, already from the sixteenth century, a courtly delicacy.

In the Canary Islands, with more suitable temperatures, *Musaceae* survived as I already said, but also marginally because of the change in the production model to vine and wine exports (seventeenth and eigthteenth centuries). Despite everything, lexical coincidences have been detected in the traditional glossary of the banana between the two shores of the Atlantic, and it has been suggested that, already since the sixteenth century, the plant was known in the Canaries as *plátano guineo*. "In this way the banana as well as sugarcane, yam and maybe the vine were known in America from the Canaries" (Leal-Cruz, 1996: 209-210). But surely it was called *plátano de Guinea* in the Canaries and it was in America where it was known later as *guineo*, because slavery, the culture of black Africa and also the biodiversity of the *Musaceae* carried much more weight there.

Thus, in the Americas, the lexical richness would be related to the process of introducing the plant, to the species and territories diversity (from Florida to Ecuador), and especially to the weight that in some of these countries had slavery, cultural resistance and miscegenation. The *guineo* is a culturally mestizo hybrid vegetable.

X. "Come Out the Guineo to Dance" [Calderón De La Barca]

Hermann Schacht (1814-1864), who visited Madeira and the Canary Islands in the mid-1850s, detected among others, species of *Musa sapientum*, *M. paradisiaca* and *M. Cavendishi*. He assured that they thrived in low areas; that they rarely grew to 600-700 feet; that in Tenerife they spread everywhere and formed "large plantations"; he offered some interesting data on the size of the *cepa* or trunk, on the type of cluster, on the bloom, etc. "The fruit of *M. sapientum* has the shape of a cylinder, elongated and flattened at both ends", when it is ripe it "has yellow colour with black spots that also appear on the trunk of the plant". The fruits of *M. paradisiaca* and *M. Cavendishi*, which are smaller, "are not cylindrical as the previous ones, but angular, and some people will prefer these ones". They could be eaten ripe or well roasted and cooked "as a vegetable". The *M. sapientum*, which was the one that had a higher stem (ten to twelve feet), opened its large leaves out on either side of a central rib, "and it soon splits due to the action of the wind" (Sarmiento-Pérez *et al*., 2012: 94-98).

It is striking that, in addition to food uses, Fernández de Oviedo also emphasised, from the *Summary* of 1526, morphological aspects that Schacht repeats accurately, for example, size and appearance of the leaves after the action of the wind; clusters with forty and fifty fruits more or less, which measure "a hand and a half", and are the bulk of the "wrist of an arm" (Fernández de Oviedo, 1526: XLIII *v*-XLIII). Without forgetting the differences in the quality of the fruits, which the chronicler attributed to the fertility of the soil.

In Cuba, the term *guineo*, alone or accompanied by the voice plátano, was clearly identified with a particular species of *Musaceae*, which according to Pichardo would be *M. sapientum*:

The GUINEO (*Musa sapientum*) is the smallest of all; its length does not exceed one-third of a foot, straight, cylindrical; when mature softest flesh (Pichardo, 1836: 212-213).

In later editions he insisted on that the "*plátano guineo* or *de Guinea* (*M. sapientum*)" was the smallest of them, although "near the bulk of the *macho*" [*M. paradisiaca*], and described it in similar terms. He added that its clusters were laden with excess of fruits, and that the vegetable could not be from the Caribbean region, since even the *dominico* or *hembrita*, or *Congo*, a variety, he states, of *M. regia* (small, delicately curved, soft and delicate when ripe), was from Nigricia [Negroland], as well as the *guineo*, while those of the *Otahití* and of *Orinoco* clearly expressed their origin. How could Columbus and the others talk about *ñame*, *yuca*, etc., and mute the interesting plátano?, he asks himself (Pichardo, 1849: 187; Pichardo, 1861: 213-214).

Thus, when a certain species of bananas left the Canaries in 1516 it is possible that it already bore the appellation *plátano* or *plántano de Guinea*, and that, with the passage time, it would be transformed, especially in the Caribbean islands (Santo Domingo and Cuba), into *plátano* or *plántano guineo*, since in both cases meant the same: bananas coming from the Gulf of Guinea, the banana of the black slaves. Indeed, from 1510 it becomes "legal the sending of black Guineans" to the Caribbean (Viña-Brito, 2006b: 21-22).

In the Canary Islands there has always been the *yerba* [grass] from Guinea [*Panicum maximum*], as well as in Cuba where, in addition, there is the "guinea fowl"; but Canary Islanders have also the "guinea pumpkin", long, curved and of very yellow flesh, perhaps like those grown in the nascent town of *Ingenio* (Gran Canaria), from the sixteenth century (Sánchez-Valerón & Martín-Santiago, 2003: 432; Morera, 2006: 642-643; Corrales & Corbella, 2009, I: 1074-1075).

Also, the *guineo* is a dance similar to the primitive *canario* (ancient dance that apparently came from the natives of the Canaries), and which came into fashion in Europe during the Modern Age. That is why the footman Rodrigo says, in act II of *The Ingratitude*

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Revenged by Lope de Vega: "and I can dance the canarie, / I'm a native of there, / and among sugar brought up", and therefore Sandoval writes, talking about black *guineos*,

> Among them there are many good musicians, to such a degree that from them people learned in Spain [...], the so celebrated dance, called the Canario, reformed Guineo by the Isleños Canarios (Sandoval, 1647: 45).

For this same reason, Mr. Pedro Calderón de la Barca (1600-1681) makes the king say, at the end of his burlesque comedy *Cephalus and Procris*, "The giant with the owners / come out the guineo to dance" (Calderón de la Barca, 1691: 416).

Fernando Ortiz already highlighted the need to give the corresponding gentilic name to certain nouns, with which they become dense signs of identity. Blacks and "then whites", he says, used in America the African names to "distinguish certain things from other similar things, according to their origins". Thus, it is said *fowl of Guinea*, *yerba of Guinea*, *plátano guineo*, *plátano burro* (which is *mature* in carabalí [Calabar]), *jutía arará*, etc." (Ortiz, 1987: 370).

In the general Spanish, the expression *guineo* soon became to identify slaves. Covarrubias defines *guineo*, in first meaning as a black of Guinea, and in second as "certain dance of prestos [rapid] and hasty movements". Guinea was "the land of the blacks" (Covarrubias, 1611: 457 *v*). Therefore, *guineo* meant black, and black was equivalent to slave. Sandoval says that such "Ethiopian guineos" were the most beloved of the Spanish. "These guineos are", he states, the most esteemed, sought-after and expensive blacks, "and those we commonly call honest, good-natured, with sharp wit, beautiful and willing, joyful of heart" (Sandoval, 1627: 41 *v*; Sandoval, 1647: 45).

Cervantes, in his comedy *La Entretenida* (*Jornada* I, 606-612), introduces Ocaña, a footman who complained about being treated like a *guineo*:

> ¿Yo no veo
> Que, cual si fuera guineo,
> Bezudo y bozal esclavo,
> Apenas entro en la sala
> Por alguna niñería,
> Cuando cualquiera me envía,
> Si no en buena, en hora mala?

It should not be very difficult to identify certain types of *Musaceae* with the name *guineo* or *plátano de Guinea* because, in fact, they were the favorite of the slaves, they were nutritious, versatile and intensely familiar and, therefore, with a powerful identity value. Viera y Clavijo, unlike other authors, equated the smaller bananas with those called *dominicos*,

> In our islands that species is preferred with good reason, which gives the smallest bananas, called *dominicos*, because of its delicate, soft and creamy of all its flesh, and which Linnaeus distinguished by the name of *Musa sapientum*, considered without doubt the daily bread of the philosophers of India (Viera y Clavijo, 1869, II: 203).

Although he tells bellow, once again, Berlanga's expedition in 1516 as well as the vision of Fernández de Oviedo in the Franciscan monastery of Las Palmas, four years later, he does not say that that was, specifically, the chosen species, perhaps because as a good naturalist he sensed that from the beginning of the history there had lived together in the island territory *Musaceae* taxa which, brought from Guinea and Andalusia, grew in monasteries' vegetable gardens, real spaces of culture of the oasis, before moving to the New World. After all, as Benítez-Padilla (1959: 147) wrote, "the function of the island is that of the oasis in the open field of the oceanic desert. It is nourished by caravans, at the same time it nourishes them".

In the fourteenth and fifteenth centuries when Portuguese navigators explored the most remote coasts of West Africa, "somatic mutations obviously occurred, resulting in a large number of clones and a secondary centre of diversity in Africa" (Robinson & Galán-Saúco, 2011: 2).

XI. Conclusion

In 1483, almost a decade before the discovery of America, the first efforts were being made to make the fertile virgin lands of Gran Canaria profitable. During the following decade of 1490, the islands of La Palma and Tenerife joined the Crown of Castile and were also incorporated in an intense production phase. The three islands were, along with Madeira and Santo Tomé, well equipped to start the first draft of Atlantic model of sugar plantation.

Why should we limit ourselves to think it was only one biotype which was moved, from the Gulf of Guinea to the Canary Islands, to feed the emerging endowments of slaves, and which then moved from the Canaries to Hispaniola? Perhaps it should be suggested the possibility of the reception, in the Canaries, of clones from Guinea, Cape Verde and other parts of Africa, as well as from Andalusia, the latter brought to the Canaries by Franciscan or Dominican missionaries, as a result of the conquest of Gran Canaria, La Palma and Tenerife, the richest in water and the most populated by indigenous (*canarios*, *benahoaritas* and *guanches*), who were also enslaved as, in fact, happened in America.

When the possibility of obtaining sufficient gold resources were exhausted in the Antilles, and the system diverted towards a model of sugar plantation, the islands of Macaronesia were put at the service of the new centers of demand for goods, equipment and services. Thus, technicians, slaves, and traditional food and resources were moved. The gentilic name served to

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differentiate plants, animals and humans. Under the pain of an extraordinary historical birth, these people were building new worlds, new spaces of effort, sacrifice and intense cultural diversity.

Nobody called the cane (*Saccharum officinarum*), cane of the East, of Sicily, of Spain, of Madeira or of the Canaries, though of course there are different varieties, since it was always known as sweet cane or sugarcane; white, unique and shining as a Roman goddess. The plantain and the banana needed a gentilic name to be recognised and differentiated, since, depending on the circumstances, they became viand (cooking banana) or smiling fruit, but its clusters are always bended towards the ground as if they recognise their humble origins.

XII. Acknowledgements

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(f) Results should be presented concisely, by well-designed tables and/or figures; the same data may not be used in both; suitable statistical data should be given. All data must be obtained with attention to numerical detail in the planning stage. As reproduced design has been recognized to be important to experiments for a considerable time, the Editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned un-refereed;

(g) Discussion should cover the implications and consequences, not just recapitulating the results; conclusions should be summarizing.

(h) Brief Acknowledgements.

(i) References in the proper form.

Authors should very cautiously consider the preparation of papers to ensure that they communicate efficiently. Papers are much more likely to be accepted, if they are cautiously designed and laid out, contain few or no errors, are summarizing, and be conventional to the approach and instructions. They will in addition, be published with much less delays than those that require much technical and editorial correction.

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Format

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Standard Usage, Abbreviations, and Units: Spelling and hyphenation should be conventional to The Concise Oxford English Dictionary. Statistics and measurements should at all times be given in figures, e.g. 16 min, except for when the number begins a sentence. When the number does not refer to a unit of measurement it should be spelt in full unless, it is 160 or greater.

Abbreviations supposed to be used carefully. The abbreviated name or expression is supposed to be cited in full at first usage, followed by the conventional abbreviation in parentheses.

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Acknowledgements: Please make these as concise as possible.

References

References follow the Harvard scheme of referencing. References in the text should cite the authors' names followed by the time of their publication, unless there are three or more authors when simply the first author's name is quoted followed by et al. unpublished work has to only be cited where necessary, and only in the text. Copies of references in press in other journals have to be supplied with submitted typescripts. It is necessary that all citations and references be carefully checked before submission, as mistakes or omissions will cause delays.

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28. Make colleagues: Always try to make colleagues. No matter how sharper or intelligent you are, if you make colleagues you can have several ideas, which will be helpful for your research.

29. Think technically: Always think technically. If anything happens, then search its reasons, its benefits, and demerits.

30. Think and then print: When you will go to print your paper, notice that tables are not be split, headings are not detached from their descriptions, and page sequence is maintained.

31. Adding unnecessary information: Do not add unnecessary information, like, I have used MS Excel to draw graph. Do not add irrelevant and inappropriate material. These all will create superfluous. Foreign terminology and phrases are not apropos. One should NEVER take a broad view. Analogy in script is like feathers on a snake. Not at all use a large word when a very small one would be sufficient. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Amplification is a billion times of inferior quality than sarcasm.

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33. Report concluded results: Use concluded results. From raw data, filter the results and then conclude your studies based on measurements and observations taken. Significant figures and appropriate number of decimal places should be used. Parenthetical remarks are prohibitive. Proofread carefully at final stage. In the end give outline to your arguments. Spot out perspectives of further study of this subject. Justify your conclusion by at the bottom of them with sufficient justifications and examples.

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- Significant conclusions or questions that track from the research(es)

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Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
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Approach

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- Give details all of your remarks as much as possible, focus on mechanisms.
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- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
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Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

INDEX

A

Annonaceae · 34, 50
Anthelminthic · 34, 36, 41, 47, 48, 49, 50
Argentieri · 41, 48

B

Benzoquinones · 25

C

Chlorophacinone · 57, 58
Cymbopogon · 14, 17

D

Detrending · 18

E

Embryonnation · 34, 40
Ethnomedicine · 25
Eynon · 4

G

Glucoamylase · 4

H

Heligmosomoides · 34, 36, 38, 43, 44, 45, 46, 47, 49, 50, 51
Hom- Ogeneity · 17

L

Larvicidal · 34

M

Monostearate · 1, 2, 3, 12
Monoterpenoid · 31

P

Perishability · 1
Pharmacopea · 36
Phenolics · 26, 29
Phytochemicals · 26, 32

Q

Quiterpenoids · 25

R

Reconstitutability · 7, 12
Retrogradation · 7
Rodenticides · 57
Rosenonolactone · 25

S

Saponins · 25, 26, 28, 41, 48
Senegalensis · 34
Sesquiterpenes · 25
Spectrascan · 4
Spectrophotometry · 28

T

Torrescasana · 62, 66

X

Xanthoproteins · 28



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