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Highlights

- Amaranthus Hypochondriacus
- Characterization of Biofilm
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Biochemical Analysis of 25 genotypes of Grain Amaranths (*Amaranthus hypochondriacus* L.)

By R.M. Pandey , Mithlesh Dwivedi & Rekha Singh
Dr. Ram Manohar Lohia Avadh University, Faizabad

Abstract - Biochemicals such as Cholrophylls (a & b), Carotenoids, Protein, Vitamin C, Phenol, Niacin, Vitamin B1, Vitamin B2, Moisture analysis for 25 genotypes of Amaranthus hypochondriacus.L. has been worked out at Division of Genetics and Plant Breeding and Agrotechnology, National Botanical Research Institute, Lucknow in 2011. The analysis results with some genotypes with highly nutritious value. Out of the 25 genotypes studied, different genotypes having highest values for different biochemicals. AG 828, SKNA 211 and SKNA 21 are the best genotypes which will help in formation of products with superior nutritional quality. Thus the data emanating from the present study indicated the scope for utilizing best nutritional yielding lines for healthcare edible products and as the base material for developing nutraceuticals. The present investigation will also fill the gap regarding the processing of amaranth seeds for the development of superior quality edible food products for infants and also used in fast days. The highly nutritious lines of this crop are so high promising for supplemented nutritive food amelioration of nutritional deficiency.

Keywords : *Amaranthus hypochondriacus, Biochemicals, Chlorophyll, Vitamins.*

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R.M. Pandey [α], Mithlesh Dwivedi [σ] & Rekha Singh [ρ]

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Keywords : Amaranthus hypochondriacus, Biochemicals, Chlorophyll, Vitamins.

I. Introduction

Grain amaranths of the genus *Amaranthus* (Family – Amaranthaceae) embraces about 60 species wild (weedy) and cultivated types as an important subsidiary food crop for the people inhabiting to tropical and sub-tropical highlands of South and Central Americas and Asia (Sauer, 1967). There are mainly four species of grain amaranths viz. *Amaranthus caudatus* L., *A. cruentus* L., *A. edulis* Speg., *A. hypochondriacus* L. among which the later is extensively cultivated in Mexico and in the Himalayas of India from Kashmir to Arunachal Pradesh. It is an outbred-inbred crop upto 40% crossing varying in the different genotypes and geographical regions (Hauptli and Jain 1985; Jain *et al.* 1982; Pal and Khoshoo 1974;

Vietmeyer 1980; Walton 1968). The seed of grain amaranth is small but rich in protein (14-19 %), nutritionally more balanced because of better amino acid profile than other improved cereals, contains more lysine, 5- 8 % (Downton 1973; Misra *et al.* 1985; National Academy of Sciences 1975; Vietmeyer 1980; Pisarikova *et al.* 2005). *Amaranth* is a highly nutritious food. The leaves, shoots and tender stems are eaten as a potherb in sauces or soups, cooked with other vegetables. Biochemicals such as Carotenoids, ascorbic acid, phenols, Vitamin B1, B2 and niacin are important for human nutrition since some of Carotenoids serve as precursors of vitamin A while others have been shown to function as antioxidant (De Pee *et. al.*, 1995; Krinsky, 1989; Palozza and Krinsky, 1992). Ascorbic acid functions as antioxidant and anti cancer agent (Shibata *et al*, 1992). In stomach, ascorbic acid act as a scavenger of nitrites and free radicals formed during metabolic process (Cameron and Pauling, 1979). Phenolic compounds, exhibited the best antioxidant activity. Phenols are the organic acids which defend the plants from pest and diseases. Riboflavin (vitamin B2) is manufactured in the body by the intestinal flora and is easily absorbed, although very small quantities are stored, so there is a constant need for this vitamin. Niacin is needed for energy metabolism, proper digestion, and healthy nervous system. All B vitamins help the body convert food (carbohydrates) into fuel (glucose), which is used to produce energy. These B vitamins, often referred to as B complex vitamins, also help the body metabolize fats and protein. B complex vitamins are needed for healthy skin, hair, eyes, and liver. They also help the nervous system function properly, and are needed for good brain function. The aim of the present study was evaluation of the intra-species variation of grain *amaranth* by comparing the leaf biochemical components such as chlorophyll a and b, total chlorophyll, carotenoids, phenol content, leaf moisture, leaf protein content and Vitamins (C, B_1, B_2, B_3)

II. Methods and Materials

In the present investigation the material were seeds of 25 accessions (i.e. AG-303, AG141/1, SANA-20, AG828, AG198, AG114, AG198/2 , AG301, AG21BB, AG306 , GA-2, SKNA211 , APOLO LOKI , SKNA21 , SKNA-21-1 , GA-1, BGA-2, AG-114 (NBPGR), RMA-2, RMA-3 ,IC-120588, SKNA-23, RSUNA4, BGA-3, GA-2) of grain *amaranth*, which are being maintained at experimental field of Cytogenetics Laboratory of CSIR-National Botanical Research Institute, Lucknow.The leaf samples were cleaned and properly dried as required before analysis.

Chlorophyll (a, b and total) Caratenoid were estimated following Arnon (1949) methods.

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a) Reagent preparation

80% acetone - 80 ml acetone in 100 ml (80 ml acetone + 20 ml distilled water)

Sample – Fresh leaves sample weight 250 mg or 0.25g.

b) Procedure

The fresh leaves of all the given twenty genotype were weighted 0.25gram using Digital Analytical Balance C.165 citizen which was transferred to make homogenize with mortar and pestle with addition 10ml of 80% acetone, paste was made Chlorophyll extract was poured in a funnel having wattmaan filter paper and collect in volumetric Flask, collect all extract from the mortar, the green extract is gradually attained by adding 2-3 ml of acetone every time. Washing 3-4 time are giving and extraction continued until become colourless make volume to extract 25ml with 80% of acetone. Since the extract is subject to photo-oxidation. It is kept away from direct sunlight and store in the dark place. The O.D. of the chlorophyll extract is recorded on spectrophotometer using wavelength on 480, 510, 645, 652 and 663 nm continuously. The O.D. show the absorbance of solution.

c) Calculation

Following formula are used for the calculation

$$\text{Chlorophyll a (mg/g)} = \frac{12.7(O.D.663) - 2.69(O.D.645)\ xV}{W\ x1000}$$

$$\text{Chlorophyll b (mg/g)} = \frac{22.9(O.D.645) - 4.68(O.D.663)xV}{W\ x\ 1000}$$

$$\text{Total Chlorophyll (mg/g)} = \frac{O.D.652x1000xV}{34.5\ x\ Wx1000}$$

$$\text{Total carotinoids (mg/g)} = \frac{7.6(abs480) - 1.49(abs510)\ x\ \text{final volume}}{1000\ x\ \text{fresh weight}}$$

Determination of Crude Protein has been done by following Kjeldahl methods

d) Material Required

Alkali-40%NaoH

Receiver solution- Make 4% Boric acid solution with distilled water (warm) cool the solution at room temperature and add indicators-100ml of Bromocresol green and 70ml methyl red.

Kjeltabs- K_2SO_4 (3.5g) and $CuSo_4$.(0.4gm)

Digestor-2006 Digestor foss tector

Distillation unit -2200 Kjeltec Distillation unit.

Titration- 0.1N HCL burette etc.

Volumetric flask –according to sample.

e) Digestion

The fresh leaves sample of all given genotypes were weighed 0.25 grams using electronic digital analytical balance which was quantitatively transferred to the 250 ml Kjeldahl tube containing 12 ml of concentrated H_2SO_4 in which one kjeltab was added to each sample and was kept on FOSS Tecator Digestion unit at 380°c temperature for about 40-45 minutes. To evacuate the fumes coming from the digest and also prevent excessive acid losses, fume exhaust manifold was used. Placed the samples on the digester with exhaust manifold on top with water aspirator at full flow for the first five minutes of the digestion to evacuate moisture etc. and after five minutes the aspirating effect was essentially decreased with the help of flow regulator. A clear solution of ammonium sulphate was obtained as an indicative of complete digestion of samples.

f) Distillation

All digested samples were distilled using Auto-Kjeltec Distillation Unit. All samples after the digestion formed ammonium sulphate $(NH_4)_2SO_4$which was used as a standard to check the recovery of the distilling units. The distillation principles converted ammonium (NH_4) into ammonia (NH_3) by using an alkali (NaOH) and there after steam distill it into a receiver flask containing boric acid. A light green color solution is obtained after distillation.

g) Titration

The solutes were titrated against N/10 HCL for the detection of colorimetric end point until the color of the solution turns from light green to light pink. The observations were taken for each sample as the amount of N/10 HCL is consumed to end point. Similarly, the

blank was also run and titrated with N/10 HCl for the detection of end-point to avoid any error occurring while conducting the experiment.

h) Calculation

Lastly the nitrogen content in each sample were calculated as under;-

$$N_2 = \frac{(Tml\text{-}Bml) \times N \times 100 \times 14.01}{W}$$

Where, T =Titration volume for sample

B= Titration volume for blank in (ml)

N= Normality of acid

W= weight of sample

Determination of Protein: - % Protein=% Nitrogen x F (6.25)

Where,

F =Conversion factor (6.25)

Finally the protein content is obtained in each accession.

Estimation of Vitamin C

i) Reagent preparation

0.1% starch- 1g starch + 100ml distilled water and heat at 100°C for 2min and cool then use.

0.01M Iodine and KI - Dissolved 0.5076g of I_2 and 0.332KI in 120 ml water, Dilute with 200ml water.

j) Procedure

All fresh leaves sample of all given genotypes were weighed 0.25 grams using electronic digital analytical balance accurately and transfer for homogenized solution with mortar and pestle with 25ml distilled water ,filter that solution with Funnel and wattmaan filter paper then collected in conical flask and after few minute added 10, 10 drops of 0.1% starch solution ,as indicator. The solution obtain light green colour ,then titrated with 0.01M solution of Iodine and KI standard solution and count drop of standard solution which was titrated the leaves solution until change the light green colour solution in dark blue colour solution. It was measured the ascorbic acid in mg/g leaves solution with standard solution.

k) Calculation

Final vitamin C obtain in mg/g by the formula=Drop of standard solution x average of standard solution.

Estimation of total Phenol

l) Reagent preparation

Folin Ciocalteu reagent- 5ml (1:10 diluted with distilled water).

Galic acid or methanolic (methnol:distilled water -50:50) compound- 0.5 ml of 1:10 g/m for standard phenolic compound.

Aqueous sodium carbonate- 4ml 1M– solution.

Fresh leaves sample- .25g.

m) Procedure

All fresh leaves sample of all given genotypes were weighed 0.25 grams using electronic digital analytical balance accurately and transfer for making homogenized solution with mortar and pestle with 5ml Folin Ciocalteu reagent and add 0.5 ml Galic acid or methanolic compound then after 15 min maintain volume 4ml Aqueous sodium carbonate of 1M solution add measure at 765nm wavelength with spectrophotometer in the against of standard Phenolic compound.We use the wavelength for check the absorbance of solution, measured the phenol volume in the leaves sample. Calculate Phenol,

Final Phenol content was obtain in mg/g by the formula= O.D.at 765nm standard solution x average of standard solution x 100.

Estimation of Moisture content in the plant leaves

n) Procedure

All fresh leaves sample of all given genotypes were weighed by using electronic digital analytical balance accurately and write on then write pad, and then put in oven at 60°c for dry to loss the moisture for 24 hours and took weight and calculated the percent of moisture contain with these formula.

$$\% \text{ moisture} = \frac{\text{Freshweight} - \text{dryweight} \times 100}{\text{Freshweight}}$$

Estimation of Vitamin B_1

o) Reagent preparation

0.1M Perchloric acid.

Acetic anhydride.

Anhydrous Formic acid.

Fresh leaves sample.

p) Procedure

All fresh leaves sample of all given genotypes were weighed 0.14 grams using electronic digital analytical balance accurately and transferd for made homogenized solution in mortar and pestle with 5ml of anhydrous formic acid R for total thiamin part extraction filter the sample's all extract from mortar with waatmaan filter paper and funnel then collect in volumetric flask sample colour obtained light colour and added 50ml of acetic anhydride our solution colour obtained violet blue colour ,then titrated whole solution with 0.1M perchloric acid until the colour change in light pink colour. Note the titrated end point from the burette compared with blank and then find out the vitamin b_1 content from the leaves sample solution. 1ml of 0.1M Perchloric acid is equivalent to 16.37 mg of $C_{12}H_{17}N_5O_4S$.

Estimation of Niacin

q) Reagent preparation

0.1M Sodium Hydroxide-

Phenolphthalein

Fresh leaves sample

r) Procedure

All fresh leaves sample of all given genotypes were weighed 0.25grams using electronic digital analytical balance accurately and transferred for homogenized solution with 5ml distilled water in mortar and pestle, filtered the sample's all extract from mortar with filter paper and funnel then collected in volumetric flask then add 10ml of Phenolphthalein as indicator and then titrated whole solution with 0.1M Sodium Hydroxide until the sample solution colour change in light pink colour. Note the titrated end point from the burette compare with blank and then find out niacin the from the sample solution.Calculate niacin 1ml of 0.1M Sodium Hydroxide is equivalent to 12.31 mg of $C_6H_5NO_2$.

Estimation of VitaminB_2

s) Reagent preparation

Sample- fresh leaves = 0.2g

Glacial acetic acid R = 0.2ml diluted with 50ml distilled water.

Sodium acetate R = 0.10g diluted with 10 ml distilled water

t) Procedure

All fresh leaves sample of all given genotypes were weighed 0.2 grams using electronic digital analytical balance accurately and transferd for homogenized solution in mortar and pestle with 3ml distilled water, then filtered the sample's all extract from mortar with filter paper and funnel then collected in volumetric flask, add 0.2ml Glacial acetic acid; it was change sample colour and added 7ml sodium acetate solution sample colour obtained light colour. And measure the absorbance at 444nm.and calculates the specific absorbance at 328nm.

III. Result

Chlorophyll a, chlorophyll b, total chlorophyll and carotenoids content were estimated and presented in Table 1 and figures-1&2 for all 25 accessions showed AG-141/1 had maximum chlorophyll a content was 24.47 mg g^{-1} followed by RSUNA4 3(21.79 mg g^{-1}) , SKNA23 (19.75 mg g^{-1}). The maximum chlorophyll b content was SKNA211 (15.55mg g^{-1}) followed by AG-303 (12.21 mg g^{-1}) and BG1(8.78 mg g^{-1}). The maximum total Carotenoid content was GA-1(12.49 mg g^{-1}) followed by AG306 (12.37mgg^{-1}) and AG-198 (12.35 mg g^{-1}). The highest protein content (%) of grain *amaranth* leaves are 3.50, 3.15, 3.15 and 2.45 respectively for genotypes AG-198, AG-301, AG-306, AG-21BB and AG-114 NBPGR ,SKNA-21(figure-3). Vitamin C (Ascorbic acid) showed maximum values for RSUNA4 (8.40 mg/g) followed by BGA-2(6.63 mg/g) and SKNA -21-1(6.19 mg/g) and minimum for AG-301(1.83mg/g)(figure-4). In the present case accessions such as GA-2, RMA-2, AG-141/1, AG-306, AG-301, phenol content was 6.83, 5.97, 5.96, 5.65 mg g^{-1} respectively in high content. The lower phenol content SKNA 21, SKNA 21-1, APOLOA LOKI 1.66, 2.36 2.74, mg g^{-1}(figure-5(a)& (b)). The highest moisture content was noted in % Genotype AG-141/1(82.9) followed by Genotype AG-303,(82.6) ,AG-306 (81.9) and the lowest moisture content was RMA-3(65.10), GA-2(68.38), GA-1(68.89), APOLOALOKI (70.07)(figure-6). The maximum vitamin B_1 content was observed 116.33 mg/g (SKNA-211), 64.07 mg/g (AG-198), 53.95 mg/g (IC-120588) 52.7 mg/g (AG-141/1) and minimum content 8.43 mg/g (AG-114 (NBPGR),18.55 mg/g (RMA-2), 28.66 mg/g (GA-2) (figure-7) .Higher Vitamin B_3 (niacin) content for AG-828 (73.86mg/g) followed by genotype AG198/2 (52.93 mg/g) and SKNA-211(49.29 mg/g) and the lower content was genotype IC120588 (12.31 mg/g) followed by genotype RMA-2(14.77 mg/g) BGA-3(16.00 mg/g) (figure-8) . Vitamin B_2(Riboflavin) content was maximum for genotype SKNA-20 (4.72mg/g) followed by genotype AG198 (4.35 mg/g) and AG198/2(3.00 mg/g) and the minimum content was for genotype of AG-114 NBPGR (1.20mg/g) followed by genotype BGA-3 (1.71 mg/g) SKNA-21(1.79 mg/g) (figure-9).

Cluster analysis was also performed with the help of WARD Clustering Method with 0.05 level of significance in order to find out the variation level. From the dendogram, three main clusters were observed, out of which one cluster comprising of genotypes shows maximum variation. These genotypes are very distant from the other genotypes which again indicate that there is a possibility of getting good recombinants if these genotypes are crossed with other genotypes.

The hybridization process can improved.The protein and other traits can also be thus improved. This information can be utilized for successful breeding strategies. *A. hypochondriacus* L. using Euclidean clustering analysis, to identify promising genotypes, which can be used in different for genetic improvement program of this crop. There are 23 cluster show in the chart and Accessions with more leaf protein have potential to increase nutritional value and. However, knowledge about amaranth leaf composition is still marginal. Using Euclidean cluster analysis 25 accessions were distributed in 3 clusters (at 9.0 euclidean distance) of which cluster I contained maximum (16) accessions, cluster II (6) and cluster III (2) accessions. The I genotypes cluster euclidean distance with 25 genotypes cluster at 9.0, 24 genotypes cluster at 8.0,23 genotypes cluster at 7.0, respectively. The

determination of chemical composition of leaf is necessary for variety evaluation, on the basis of high nutritive value for human diet. Cluster I and III were found more diverse than others and therefore can be used for developing recombinants.(figure-10).

IV. Discussion

Amaranth is useful in preventing retarded growth and improving health and strongness in children. Nutrition" (also called nourishment or aliment) is the provision, to cells and organisms, of the materials necessary (in the form of food) to support life. Many common health problems can be prevented or alleviated with a healthy diet. The human body contains chemical compounds, such as water, carbohydrates (sugar, starch, and fiber), amino acids (in proteins), Chlophylls, Carotenoid, Phenol, Vitamins (DNA and RNA).These compounds in turn consist of elements such as carbon, hydrogen, oxygen, nitrogen, phosphorus, calcium, iron, zinc, magnesium, manganese, and so on. All of these chemical compounds and elements occur in various forms and combinations both in the human body and in the plant and animal organisms that humans eat. Cholrophylls and Carotenoids, Protein, VitaminC, Phenol, Neacin, VitaminB1, VitaminB2, Moisture analysis in the present study has shown some genotypes with highly nutritious value. Out of the 25 genotypes studied, AG 828, SKNA 211 and SKNA 21 are the best genotypes on which further work can be done in order to improve the quality of these genotypes. This will help in formation of products with superior nutritional quality. Thus the data emanating from the present study indicated the scope for utilizing best nutritional yielding lines for healthcare edible products and as the base material for developing nutraceuticals which will certainly help in stylizing cottage or small-scale industries and will create more jobs to villagers for improving economic condition of the rural people highly dependent on agriculture.

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Table 1 : Biochemical Analysis for 25 genotypesof grain amaranths.

Genotypes	Chlorophyll a mg g^{-1}	Chlorophyll b mg g^{-1}	Total Chlorophyll mg g^{-1}	Total Carotenoid mg g^{-1}	Phenol (mg/g)	Niacin (mg/g)	Vitamin C (mg/g)	Vitamin b1 (mg/g)	Vitamin b2 (mg/g)	Protein	Moisture %
AG-303	18.16	12.21	30.38	7.32	5.29	24.62	1.77	38.78	2.9	0.7	82.6
AG-141/1	24.47	1.28	25.75	9.33	5.97	24.62	2.21	52.27	2.93	3.15	82.9
SANA-20	17.82	7.61	25.43	4.59	4.41	24.62	3.1	33.72	4.72	1.75	75.07
AG-828	19.59	8.31	27.91	5.74	4.96	73.86	3.1	64.07	2.31	2.8	76.32
AG-198	13.91	4.95	18.86	12.35	5.92	14.77	2.65	37.09	4.35	3.5	79.03
AG-114	13.56	2.87	16.43	12.17	5.54	36.93	2.65	35.41	2.7	2.1	72.9
AG-198/2	11.34	6.07	17.41	11.81	5.32	52.93	1.77	35.41	3	1.75	72.8
AG-301	10.3	8.23	18.53	11.85	5.65	39.39	1.33	47.21	2.59	3.5	77.9
AG-21BB	9.69	2.83	12.52	10.97	3.9	22.16	3.54	65.75	2.04	3.15	77.6
AG-306	13.33	3.3	16.63	12.37	5.96	34.47	3.98	52.27	2.83	3.15	81.9
GA-2	19.27	4.44	23.72	5.15	6.83	27.08	2.21	37.09	2	0.7	68.38
SKNA-211	8.06	6.52	14.57	2.78	5.42	49.24	1.77	116.33	2.39	0.35	75.98
APOLOA-LOKI	8.07	2.57	10.64	3.18	2.74	33.24	2.65	32.03	2.41	1.4	70.07
SKNA-21	9.48	2.33	11.82	3.22	1.66	27.08	4.42	52.27	1.79	2.1	72.61
SKNA-21-1	12.61	15.55	28.16	4.23	2.36	24.62	6.19	23.6	2.43	2.1	73.5
GA-1	17.32	8.78	26.1	12.49	5.34	23.39	3.1	37.09	2.72	2.1	68.89
BGA-2	7.78	3.88	11.66	3.09	4.6	27.08	6.63	42.15	2.02	1.4	71.7
AG-114 (NBPGR)	9.64	4.34	13.97	12.16	4.87	23.39	3.98	8.43	1.2	2.45	71.3
RMA-2	13.72	2.68	16.4	3.38	6.1	14.77	3.98	18.55	2.47	2.45	77.86
RMA-3	17.68	5.67	23.35	6.1	4.81	23.39	3.54	37.09	2.01	1.4	65.1
IC-120588	15.46	6.37	21.83	5.45	4.27	12.31	4.87	53.95	3.65	1.05	75.7
SKNA-23	19.75	7.99	27.74	6.85	4.6	27.08	3.98	40.46	1.99	1.75	71.23
RSUNA-4	21.78	0.04	21.82	2.83	4.34	36.93	8.4	35.41	2.14	0.35	73.4
BGA-3	11.78	6.37	18.15	4.44	5.32	16	4.87	38.78	1.71	2.1	76.67
GA-2	14.24	3.23	17.47	3.7	5.85	28.31	3.54	28.66	1.79	1.4	73.56
Average	**14.35**	**5.54**	**19.89**	**7.1**	**1.66**	**12.31**	**1.33**	**8.43**	**1.2**	**0.35**	**65.1**
Maximum	**24.47**	**15.55**	**30.38**	**12.49**	**6.83**	**73.86**	**8.4**	**116.33**	**4.72**	**3.5**	**82.9**
Minimum	**7.78**	**0.04**	**10.64**	**2.78**	**4.88**	**29.69**	**3.61**	**42.55**	**2.52**	**1.95**	**74.6**

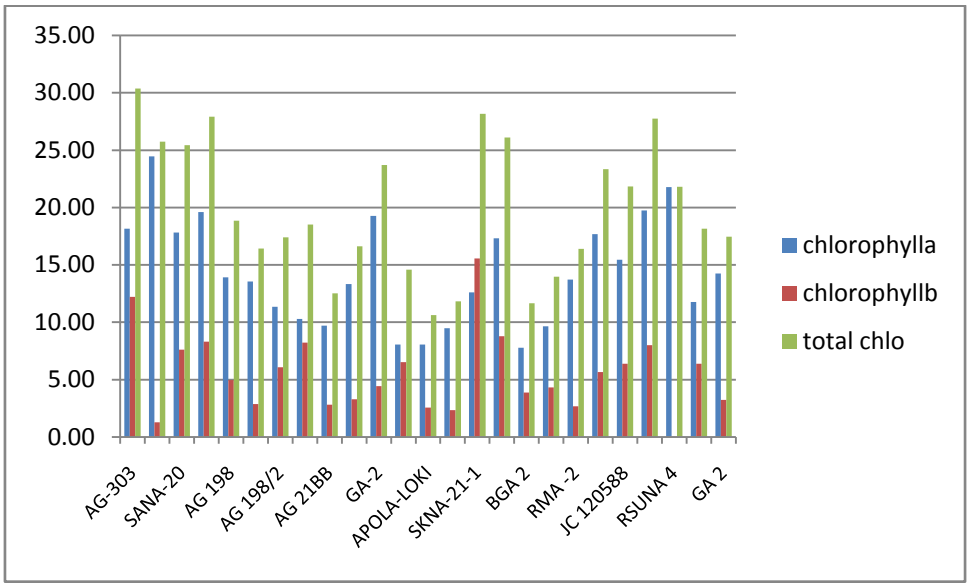

Figure 1 : Chlorophyll (mg/g) for twenty five accessions of grain amaranth (*Amaranthus hypochondriacus* L.).

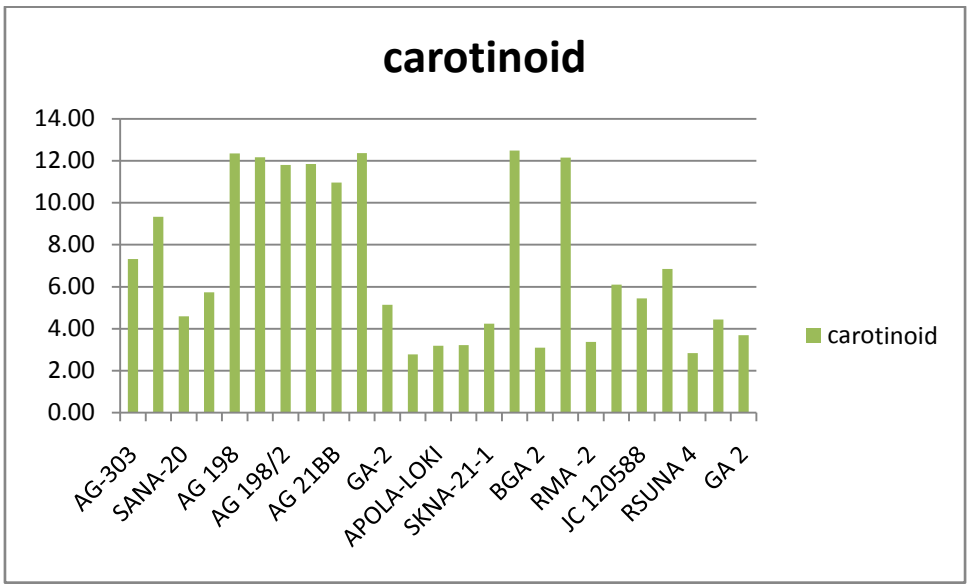

Figure 2 : Carotenoids (mg/g) for twenty five accessions of grain amaranth (*Amaranthus hypochondriacus* L.).

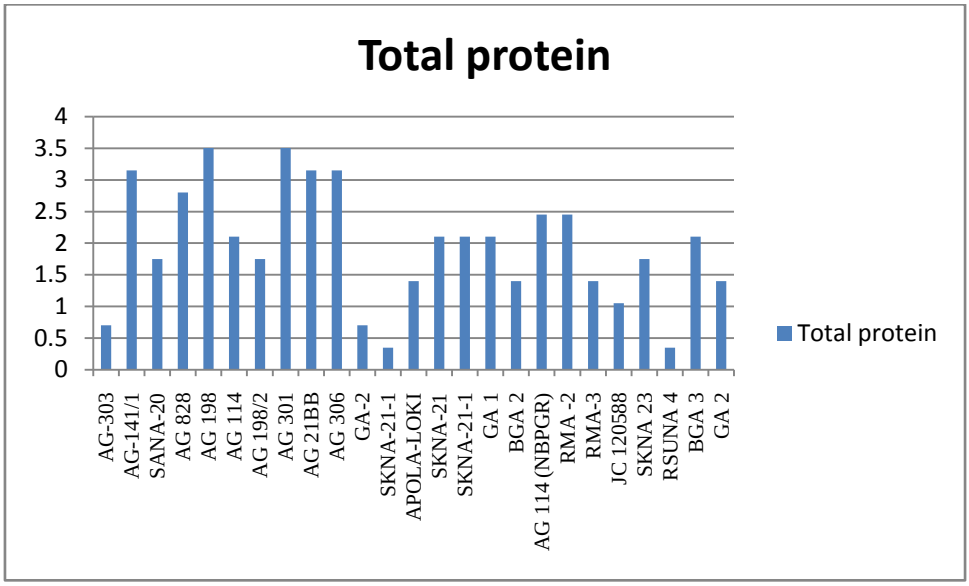

Figure 3 : Protein content (%) for twenty five accessions of grain amaranth (*Amaranthus hypochondriacus* L.).

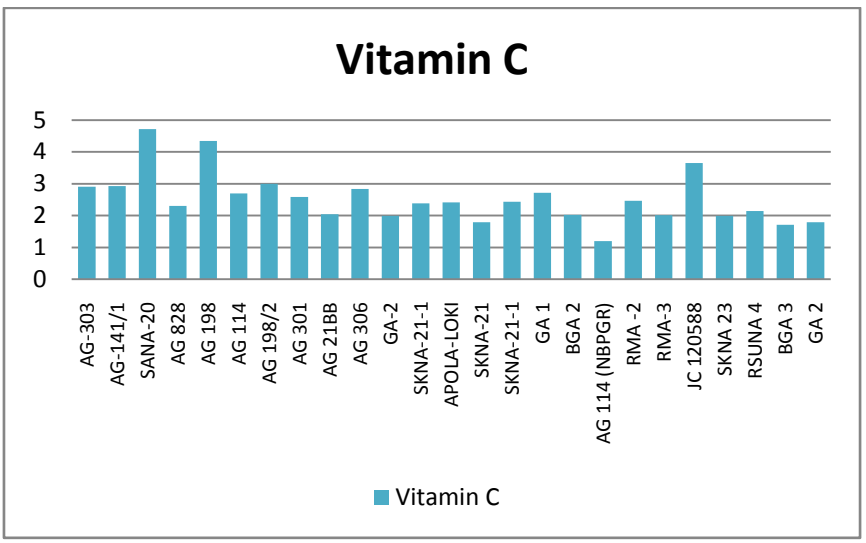

Figure 4 : Vitamin C (mg/g) for twenty five accessions of grain amaranth (*Amaranthus hypochondriacus* L.)

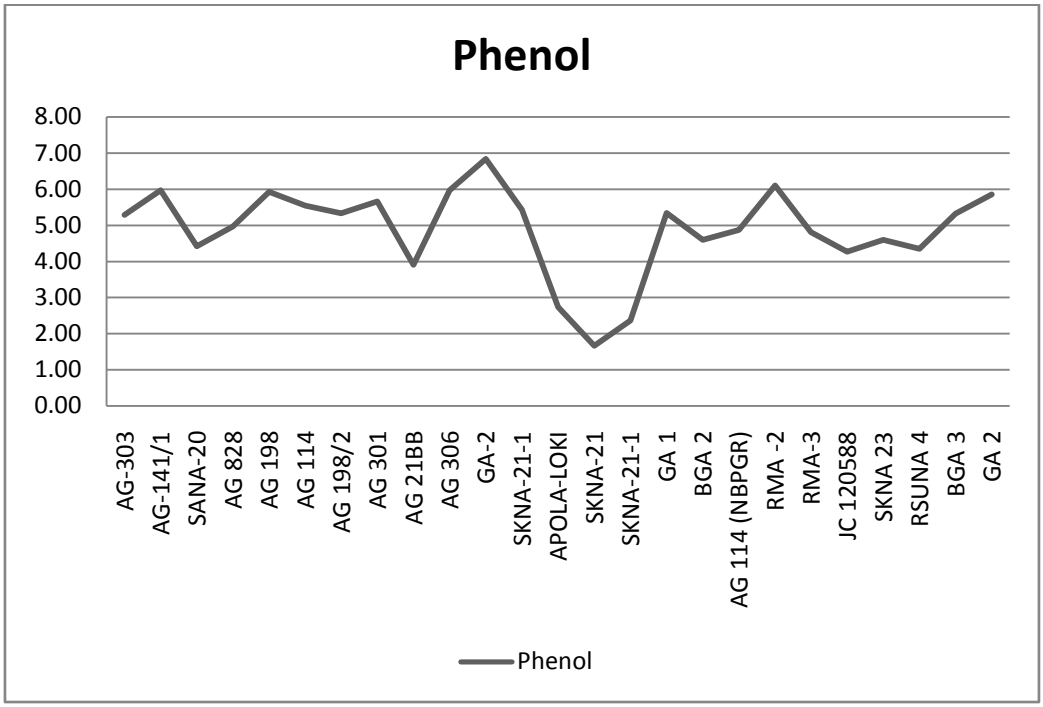

Figure 5 : (a)Phenol content (mg/g) for twenty five accessions of grain amaranth (*Amaranthus hypochondriacus* L.).

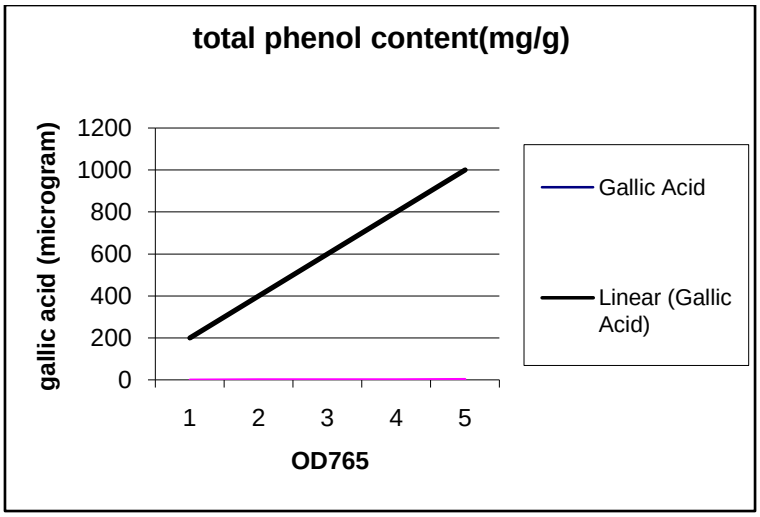

Figure 5(b) : Phenol content (mg/g) for standard.

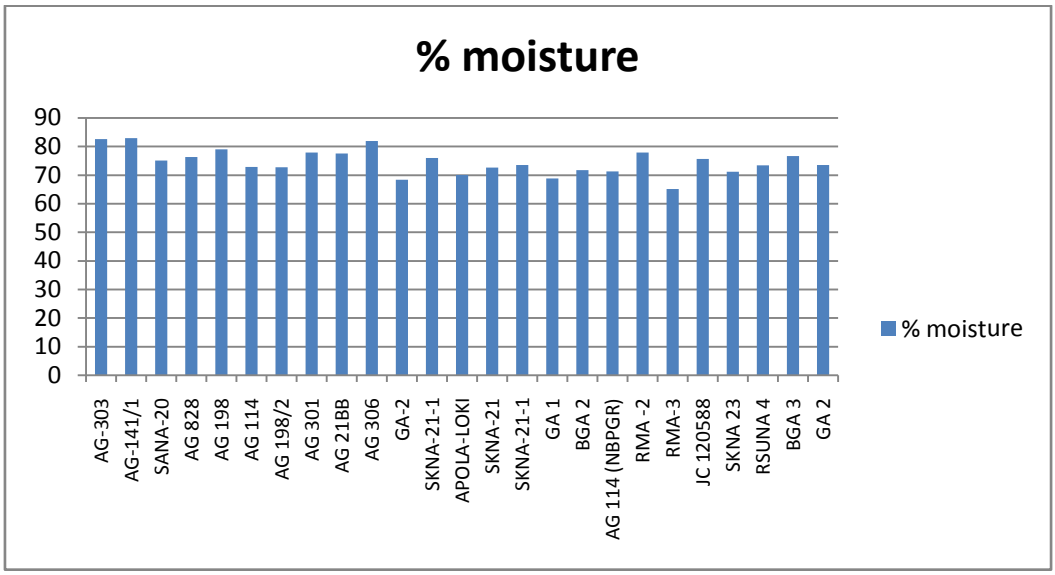

Figure 6 : Moisture (%) for twenty five accessions of grain *amaranth* (*Amaranthus hypochondriacus* L.)

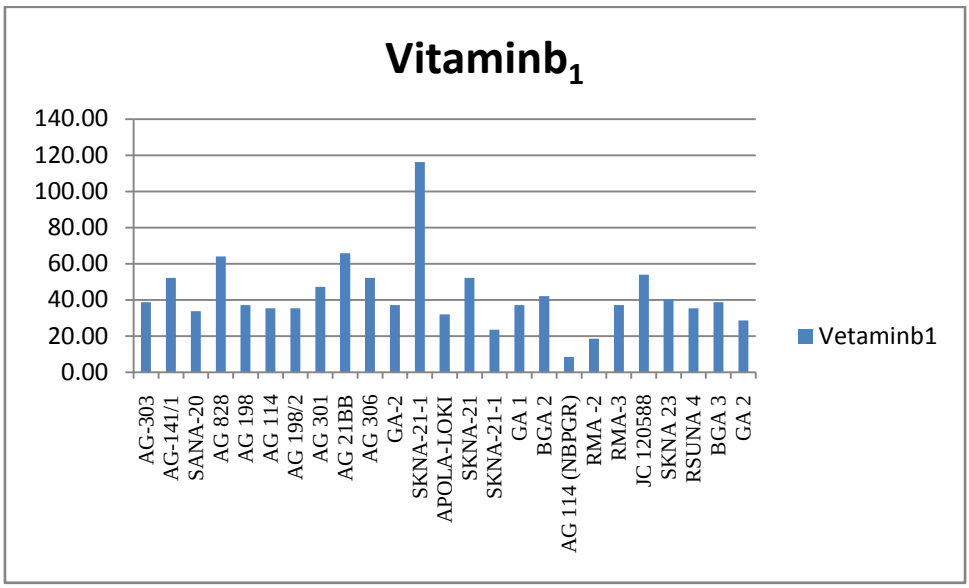

Figure- 7 : VitaminB_1 (mg/g) for twenty five accessions of grain amaranth (*Amaranthus hypochondriacus* L.).

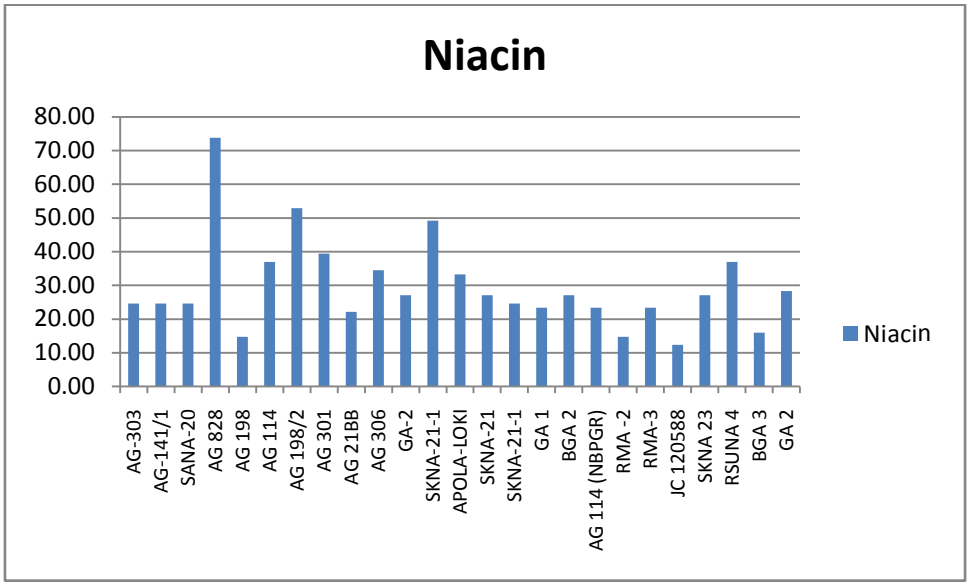

Figure 8 : Niacin (mg/g) for twenty five accessions of grain amaranth (*Amaranthus hypochondriacus* L.)

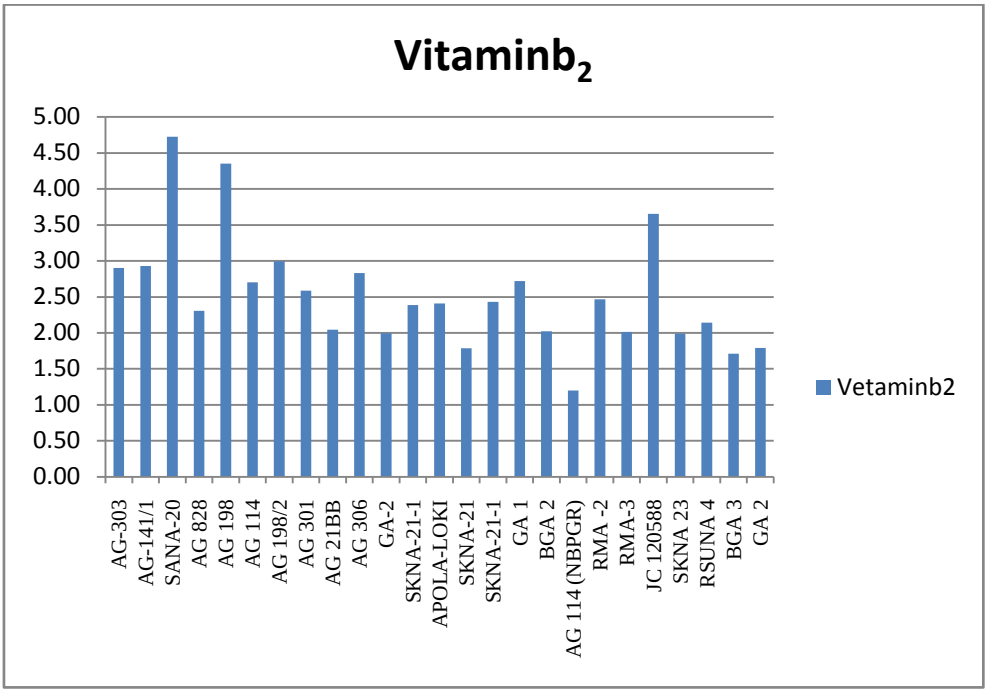

Figure 9 : Vitamin B_2 (mg/g) for twenty five accessions of grain amaranth (*Amaranthus hypochondriacus* L.)

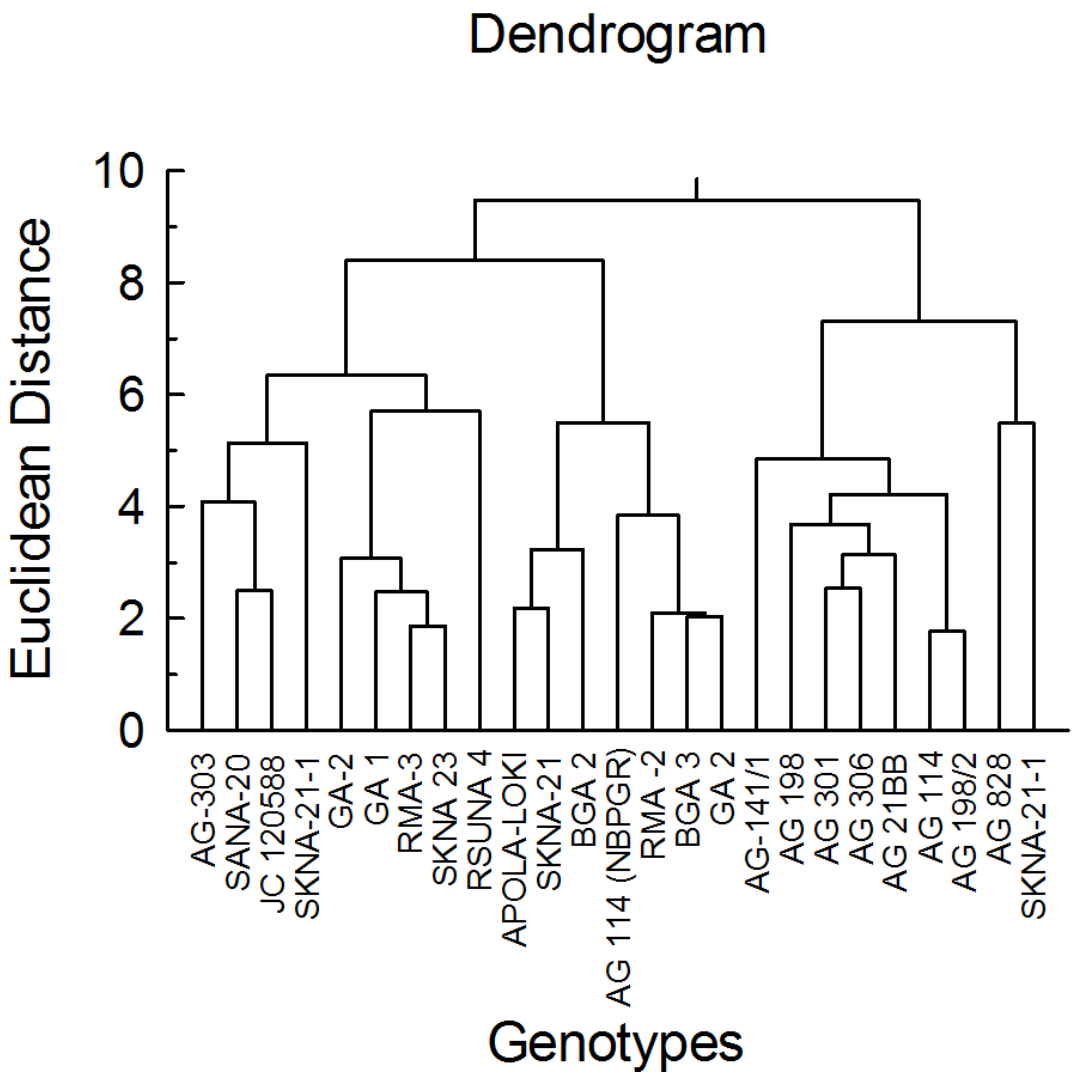

Figure 10 : Cluster analysis of twenty five genotypes of Grain amaranth (*Amaranthus hypocondriacus.L)*

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RSM for Accelerated Biofilm Formation that Facilitates Bioremediation and Characterization of Biofilm

By R. Vidhyalakshmi & C. Vallinachiyar
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Abstract - Pseudomonas stutzeuri a sewage isolate has been reported to produce extracellular polymeric substances(EPS) invitro and forms high quality biofilm in natural environment .An attempt is made to increase the extracellular polymeric substances and its Biofilm forming ability of the isolate using Design Expert-8 a statistical software .Various factors like concentration of carbon source, Nitrogen source , pH, Temperature are known to influence the process of EPS synthesis. FTIR reports strongly infer the presence of anionic glycoprotein polymer with stretching- NH vibrations around 3100 - 3500nm and –CH vibration around 1637.99 indicating the presence of aldehyde group. HPLC further ensured the presence of glucose ,galactose ,rhamnose and verbacose at a concentration of 30%,22%,26%,25%and 5% respectively. Phenol sulphuric acid assay and Barfoards assay showed the presence of 60% and 30% of carbohydrate and protein components respectively. The polymer showed metal binding ability of 26% and 42% of lead and copper respectively complimented by its anionic nature. Extracted EPS showed noticeable emulsifying effect on diesel, engine oil, petrol and grease that can route this to successful tool for bioremediation.

Keywords : *Extracellular polymeric substances, RSM, Biofilms, Anionic polymer.*

GJSFR-G Classification: *FOR Code: 030305*

Strictly as per the compliance and regulations of:

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R. Vidhyalakshmi [α] & C. Vallinachiyar [σ]

Abstract - Pseudomonas *stutzeuri* a sewage isolate has been reported to produce extracellular polymeric substances(EPS) invitro and forms high quality biofilm in natural environment .An attempt is made to increase the extracellular polymeric substances and its Biofilm forming ability of the isolate using Design Expert-8 a statistical software .Various factors like concentration of carbon source, Nitrogen source , pH, Temperature are known to influence the process of EPS synthesis. FTIR reports strongly infer the presence of anionic glycoprotein polymer with stretching- NH vibrations around 3100 - 3500nm and –CH vibration around 1637.99 indicating the presence of aldehyde group. HPLC further ensured the presence of glucose ,galactose ,rhamnose and verbacose at a concentration of 30%,22%,26%,25%and 5% respectively. Phenol sulphuric acid assay and Barfoards assay showed the presence of 60% and 30% of carbohydrate and protein components respectively. The polymer showed metal binding ability of 26% and 42% of lead and copper respectively complimented by its anionic nature. Extracted EPS showed noticeable emulsifying effect on diesel, engine oil, petrol and grease that can route this to successful tool for bioremediation.

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I. INTRODUCTION

A Biofilm is an aggregate of microorganisms in which cells adhere to each other on a surface. These adherent cells are frequently embedded with in a self produced matrix of extra cellular polymeric substances. (EPS) Biofilms have some major roles in Bioremediation .In brief bioremediation process uses microorganisms to remove detoxify or immobilize pollutants and does not require addition of harmful chemicals. Bioremediation is suitable for large area where contaminant concentration are relatively low and the hydrology of the soil does not support an aggressive chemical remediation statergy. In the last few years researchers have described the mechanisms of Bioremediation for numerous priority pollutants, including chlorinated hydrocarbons, polyaromatic hydrocarbons and heavy metals.

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II. BIOFILM FORMATION

Biofilm community are known to mobilize these accumulated heavy metals and involve in degrading the compound. Bacterial attachment is mediated by fimbriae and exo polysaccharide that act to form a bridge between bacteria and the conditioning film. These biofilms are used for the treatment of waste water and sewage .If the contaminated water pass through biofilm the microorganisms in the biofilm would eat and thus remove the harmful organic material from the water. They can be used for remediation of contaminated soil and ground water for cleaning up oil and gasoline spills. P.*aeruginosa* forms surface – associated communities called Biofilms. Compared with free swimming culture, Biofilms resist clearance by the host immune system and display increased resistance to antimicrobial agents[1] Psedomonas *species* forms bio emulsifier of peptide glycolipid type where the sugars are hydrophilic and create a good micelle that are semi soluble and involve them in pseudo solubilization [2].Microbial biofilm formed on abiotic surfaces is an important area of research because of the wide range of possible aspects and the disinfected resistance of the cells. The comparative and comprehensive analysis of all documented data concerning EPS production can enable the development and effective control strategies for Biofilms. The potential role of EPS has been documented by [3].Generally membrane biofilm have been believed to be minimized during the operation of membrane Biofilm Reactor for waste water treatment and reuse. The biofilm on the membrane surface was responsible for the removal of low molecular weight Organic matter by use of easily degradable organic matters. [4]The extracellular matrix produced by Bacillus *subtilis* B-1 an environmental strain forming robust floating biofilms was purified and investigated. [5]The structure of Biofilm was dramatically influenced by EPS production or capsule formation .This work is a study on the effect of various factors like pH, Temp and oraganic chemical components like carbon source and protein source using statistical software Design Expert-8 on EPS synthesis. The isolate being obtained from natural environment, forms EPS of high application value like

metal Binding ability and emulsifying property suitable for Bioremediation process.

III. Materials & Methods

a) Screening and Isolation

Number of sewage samples with high Biofilm were screened for EPS former. A Basal nutrient Broth was used for primary isolation. Nutrient Agar and Mac conkey Agar medium were used for isolation process using streak plate technique. Mucoid colonies were selected for further Biofilm forming ability. Selected colonies were then analyzed by Biochemical tests.

b) Invitro Biofilm Formation

Prepare Brain heart infusion broth and inoculate the isolates. Add 100ml of sterile BHI broth to micro titer plate in all the wells and 100μl of standardized inoculum 1x10^{8}CFU/ml (app) of various isolate into the well. Incubate for 24 - 48 hours time at preferred temp and remove the supernatant. Discard the non-adherent cells by washing with 100 μl of sterile phosphate buffer saline. Fix the biofilms by incubating them for 1 hour at 60°C and then stain with 100μl of Huckel crystal violet for 15 min was with water to remove the excess stain. Dry the plates for 30 min at 37 and resolublize the dye in 150ul of 95% v/v ethanol in the well, Measure the absorbance (A620) of the resolublized dye with the micro titer plate reader.

RSM To Enhance EPS Forming Ability. The method involves number of empirical techniques to evaluate the correlation of experimental factors and predict the critical concentration of dependent and independent variables. Based on prior experiments the factors like pH, Temp carbon source (Sugar),protein (yeast extract) were found to be major influencing factor for EPS production.

Thus these variables were selected to find the optimized condition for higher polymer production using CCD and RSM. The range & level of experimental variables investigated in this study are presented in Table – 1.

The central value zero chosen for experiment were glucose – 3gm/100ml, yeast extract – 0.3gm/100ml, pH -7 and Tem -33°C.For statistical calculations the variables X_i were coded to x_i according to equation 1.

$$X_i = x_i\,(x_i - x_i^2)/\Delta x \qquad (1)$$

After considering several experimental designs, a four variable experimental design proposed by Box Behnken was used to optimize the critical composition required for high yield of Exo polysaccharide. TABLE -2. A quadratic model was used to estimate the response of dependent variable .Where Y is predicted response and A, B, C, D are independent variables, b_0 is constant and b_1 ,b_2 ,b_3 ,b_4 are coefficients.

$$Y = b_o + b_1A + b_2B + b_3C + b_4D \qquad (2)$$

The production was optimized by using Box Behnken design when EPS production is related to independent variables by a response equation.3

$$Y = f(x_1 , x2_2 , x_3 , x_4 \ldots\ldots\ldots x_n)$$

The true relation between Y and X may be compli2cated; in most cases it is not known. A quadratic polynomial can be used to represent the function in the range interest (Annadurai & Sheeja)

$$Y = R_0 + \sum R_iX_i + \sum 2R_{ii}X_i2 + \sum\sum R_{ij}X_iX_j + E\text{--}3$$

Where X_1, X_2, are independent variables which affect the response Y. R_o , R_i , R_{ii} , R_{ij} (i=1-k and j=1-k) are known parameters, E is the random error.

IV. Characterization of Eps

a) FTIR analysis by KBR technique

The major functional groups of the EPS were identified using FTIR spectrum. 0.5 mg of dried sample was ground with 150mg of KBr crystals. The mixture was pressed using hydraulic press. The discs were subjected to FTIR analysis using Perkin Elmer IR spec.

b) NMR analysis

Bruker Advance 600 MHz sample was exchanged twice with D_2o with intermediate lyophilisation and then dissolved into 500μl of D_2O to a final concentration of 50 mg/ml, chemical shifts are reported in ppm, relative to sodium-d4-trimethylsilyl propionate for H- and $CPCl_3$ for ^{13}C – NMR spectra.

c) Emulsifying activity

EPS dissolved in 5ml distilled water (0.5 w/w) was mixed with 5 ml each of hydrophobic substances in test tube. The tubes were vortexed to homogeneity and left to stand for 24 hours at 4° C. Emulsifying activity was expressed as the percentage of total height occupied by the emulsion after 24 hours,petrol, kerosene, diesel, lubricant oil and grease were the hydrophobic substances used.

d) Heavy Metal Binding Ability

Metals salts like cobalt chloride and lead acetate were used 0.5% w/v of anionic polysaccharide were put into dialysis tubing in flask with 20ml of each metal salt solution and shaken at 1000 rpm for 24 hours at 30 ° C. The quantity of metal bound to polymer was calculated by measuring the ions in solutions at 0 hours and remaining after 24 hr by atomic absorption spectrometry. Controls were made with 5ml distilled water in dialysis tubing with various metal salt solutions.

V. Results

a) Identification

The isolate that formed high amount of Extra cellular polymeric substance was identified as P.stutzeuri. The organism formed large mucoid colonies on nutrient Agar and no colonies on Mac-conkey medium. To our surprise, the organism could also grow on high salt medium – Mannitol salt Agar proven as selective medium for staphylococcus aureus. P.Stutzeuri formed pink colonies on Mannitol salt Agar. The organism was germ negative and non-motile, non capsulated. FITR – Reports revealed the presence of amino groups and aldehyde groups specific for protein – polysacharide nature of extracellular polymeric substance. The stretching C-H vibrations around 1637.99 clearly indicates the presence of anionic carbohydrate. Bonds around 3100 to 3500 clearly ensures the presence of N-H groups which indicates presence of carbohydrates with proteins in the Exoploymeric substance.

HPLC – Reports further ensures presence of glucose, fructose, galactose, Rhamanose, verbose at a concentration of 30%,22%,26%,25% and 5% respectively. The extracted Extracellular polymeric substance could bind 26% of lead and 42% of copper as detected by Atomic Absorbtion spectrophotometer, as they were identified as anionic carbohydrates. The extracted Exopolymeric substance showed good Emulsification index on diesel, Engine oil, petrol and kerosene. (Table-3)

Statistical approach towards increased production of extracellular substances is summarized here in form of tables and equations. (Data not given fully)

Statistical testing of the model was done by partial sum squares – Type III ANOVA and the results are tabulated (Table – 4). The calculation of regression analysis gives the value of the determination coefficient (R^2 = 0.9469) which indicates that only .06%of the total variations are not explained by the model and the P value Prob F less than 0.0500 indicate model terms are significant. This proves all the factors are significant EPS forming ability. The statistical analysis of the design shows a high precision of the quadratic model that reflects the high degree of fitting between the predicted and the experimental data.(Table-1). This great similarity between the predicted and the observed results reflects the accuracy and the applicability of the Box Behnken model in the optimization of this Biofilm forming process.

VI. Discussion

Increasing population accumulates heavy pollution directly & indirectly. We indeed are in need of eco friendly chemicals and process that help us to make this planet a comfortable place for life. This thought evolved in Bio medication processes where living organisms and there products are used in removal of contaminants. In nature bacteria and fungi frequently inhabit distinct environmental riches at the interface between two phases such as air and water or water and a substratum. In these locations cells are anchored together by means of multivariate combination of biomolecules which form a barrier surrounding the cells and acts to protect against adverse conditions such as temperature or from chemical attack, such as chlorine in potable water. [6]Response of biofilms to toxic compounds has been modeled using mono-type inhibition kinetics [7] Bacterial biofilm formation is thought to enhance survival in natural environment and during interaction with hosts. [8]The Plc R mutant of B.*cereus* strain ATCC 14579 developed significantly more biofilm than the wild type and produced increased amounts of bio surfactant. Bio surfactant production is needed for Biofilm formation. [9] Bacterial extracellular polysaccharides are a key constituent of the extracellular matrix material of biofilms. [10] The extracellular polymeric substance extracted in this study is known to constitute 66% of extracellular polysaccharides and 10% of protein. Bio stabilizers are known to disperse one liquid to other. They are proteins with hydrophobic moiety and initially bind to hydrocarbon in a reversible manner. Polysaccharides then attaches to protein and stabilizes the oil in water emulsion. The EPS extracted in this study is more efficient in this process as they are reported to have high emulsifying index. The actual and potential applications of phototrophic biofilms in waste water treatment, bioremediation , fish feed production, bio hydrogen production and soil improvement. [11]

The role of bacteria P.Putida in regulating the mobility of heavy metals in the soil environment was reported .[12] P. *stutzeri* is closely related to P.*putida* species of phytogenetic tree and also it could accumulate 26% lead and 42% copper. The anionic character of biofilm enables them to interact with metal cations and to form minerals. Biofilm mediated mineral formation on plant leaves that can enhance the leaf fossilization process has also been documented .[13]

FTIR analysis of EPS in this study reported they are anionic and thus implicate proven effects on metal binding property. Studies on Bio emulsifiers from marine Streptomyces Sp S1 was documented [14]. The fact is that these EPS may have marked by different functions based on its chemistry. [16] EPS with glycoprotein nature could be applied in the field of Bio and nano-technology such as lipid films and silicon wafers[17] Thus the EPS obtained from P.*stutzeuri* may have a promising application in such fields.[18] Presence of sugar and protein may help to render the protein more soluble.It is common to examine samples from waste water to screen bacteria with unusual metabolic properties such as degradation of anthropogenic compounds for Bioremediation.

Though the conventional method is simple and easy to apply for arriving at an optimal situation for biofilm formation, using statistical method to design an optimal medium is economical and often accurate with fewer residues as experienced by the author[19] use of statistical method for optimization process was proved to be cost effective. The use of Design Expert-8 model proves to be less time consuming though it needs expertise and prior experience with the product.

Table 1 : Observed and predicted value

Test No	Observed Value	Predicted Value	Residual Value
1.	.21	.21	-3.667
2.	.33	.33	6.333
3.	.22	.23	-9.667
4.	.23	.23	3.333
5.	.21	.22	-8.16
6.	.20	.21	-9.333
7.	.24	.23	6.000
8.	.21	.20	4.833
9.	.20	.20	8.125
10.	.27	.27	8.125
11.	.21	.21	-5.70
12.	.26	.26	-5.70
13.	.27	.27	-7.083
14.	.22	.22	4.792
15.	.23	.24	-2.37
16.	.21	.21	3.125
17.	.20	.20	4.042
18.	.26	.26	-5.958
19.	.19	.18	6.875
20.	.23	.24	-3.125
21.	.24	.24	3.75
22.	.25	.24	8.75
23.	.29	.29	4.167
24.	.21	.21	5.417
25.	.23	.22	8.200
26.	.23	.22	0.011
27.	.21	.22	-0.015
28.	.21	.22	-0.015
29	.23	.22	0.010

Table 2 : ANOVA table

Sample mg/ml	Diesel	Engine oil	Petrol	Kerosene	Tween 80	Copper binding	Lead binding
.5	20%	5%	negligible	10%	20%	3%	2%
1	50%	10%	negligible	30%	28%	8%	5%
1.5	80%	4%	10%	50%	40%	17%	12%
2	100%	51%	30%	60%	78%	26%	18%
2.5	20%	5%	50%	30%	80%	42%	26%

Table 3 : Emulsifying property and Metal binding property

Source of Variation	Sum of Squares	Degree of Freedom	Mean Square	F value	P value
A	.65	1	.057	17.83	<.0001
B	0.013	1	.65	101.44	.0036
C	0.023	1	.013	44.33	.0005
D	0.018	1	.023	10.34	.0011
AB1.600E-003	1	1.600E-003	.018	0.99	-
AC2 250E-004	1	2.250E-004	1.47	<0.0001	-
AD0.010	1	0.010	0.21	0.5637	-
BC1 .006E-003	1	1.600E-003	9.16	0.3411	-
BD0.010	1	0.010	1.47	0.2761	-
CD6.250E-004	1	6.250E-.004	9.16	.0013	-
$A^2$0.012	1	0.012	.57	.2966	-
B^2.602E-004	1	2.602E-004	10.78	.1156	-
$C^2$0.043	1	0.043	0.24	.0003	-
$D^2$9.081E-003	1	9.081E-003	39.16	.0123	-
Pure error	7.348E-003	4	8.32	0.3553	-
Correlation total	.82	28	_	_	-

VII. Conclusion

To conclude we say that Biofilm forming bacteria on the whole help as more in bioremediation and using statistical software like Design Expert help us in achieving a highly efficient process with less labor and is more economical. The cell free EPS of *P.stutzeri* binds heavy metals and also shows good emulsifying activity towards problem causing hydrocarbons and serve as better tool in bioremediation .As the prevalence of *P.stutzeri* or its growth is supported by a wide range of temperature and pH this serve as a promising tool in waste management. Further studies include other factors that influence this biofilm formation and tests for other supplemented activities of this EPS.

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Combining Ability Estimates for Egg Production Traits from Line X Tester Analysis

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Abstract - The present investigation was undertaken to study the combining ability for egg production traits using the line x tester mating design. Four local male lines (Baheij, Bj; Matrouh, Mt; Silver Montazah, SM and Golden Montazah, GM) and two commercial female testers (Lohman Brown, LB and Lohman Selected Leghorn, LSL) were used in this study. Combining ability estimates are important genetic attributes to chicken breeders in predicting improvement via hybridization and selection programs. The magnitude of specific combining ability (SCA) variance was evident from mean squares, indicating that egg production traits had been controlled by non-additive genes. However, among the four male lines, Silver Montazah (SM) showed maximum general combining ability (GCA) effects for number of eggs at 90 d., of laying (EN1), number of eggs at 180 d., of laying (EN2), number of eggs at 240 d., of laying (EN3), number of eggs till 52 wks., of laying (EN4), average egg weight at 52 wks., of laying (EW4) and egg mass throughout 52 wks., of laying (EM) studied traits. The second high GCA scoring parent for EN1, EN2, EN3, EN4, EW4 and EM was GM parental line.

Keywords : *line x tester analysis, combining ability estimates, local strains of chicken.*

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Abstract - The present investigation was undertaken to study the combining ability for egg production traits using the line x tester mating design. Four local male lines (Baheij, Bj; Matrouh, Mt; Silver Montazah, SM and Golden Montazah, GM) and two commercial female testers (Lohman Brown, LB and Lohman Selected Leghorn, LSL) were used in this study. Combining ability estimates are important genetic attributes to chicken breeders in predicting improvement via hybridization and selection programs. The magnitude of specific combining ability (SCA) variance was evident from mean squares, indicating that egg production traits had been controlled by non-additive genes. However, among the four male lines, Silver Montazah (SM) showed maximum general combining ability (GCA) effects for number of eggs at 90 d., of laying (EN1), number of eggs at 180 d., of laying (EN2), number of eggs at 240 d., of laying (EN3), number of eggs till 52 wks., of laying (EN4), average egg weight at 52 wks., of laying (EW4) and egg mass throughout 52 wks., of laying (EM) studied traits. The second high GCA scoring parent for EN1, EN2, EN3, EN4, EW4 and EM was GM parental line. While the male lines Golden Montazah (GM) and Matrouh (Mt) were good general combiners for age at sexual maturity (ASM) -34.3 and -30.0, respectively. Moreover, GM parental line was the best general combiner for average egg weight through the 1st 90 d., of laying (EW1), average egg weight at 180 d., of laying (EW2) and average egg weight at 240 d., of laying (EW3) traits. Regarding the experimental testers, LSL represented higher estimates of GCA effects for ASM, EN2, EN3 and EN4, while LB tester represented higher estimates of GCA for EW1, EW2, EW3 and EW4 traits. The GCA and SCA mean squares estimates suggested that all the studied traits could be improved through hybridization. However, the hybrids SM x LB and Bj x LSL exhibited maximum SCA effect for annual egg production. Consequently, the priority should be given to parents SM and Bj lines and LB and LSL testers for improving egg production yield.

Keywords : line x tester analysis, combining ability estimates, local strains of chicken.

I. Introduction

The effectiveness of crossing for genetic improvement of quantitative characters in the fowl remains a controversial issue. Determining good characteristics of various lines is possibly exploit heterosis during hybridization, and may evolve more favorable genes to their progenies. The concept of combining ability helps to identity desirable combiners that may be utilized to exploit hybrid vigorous. It is especially useful in testing the ability of parents to attain high performance when crossed with different testers. Tester is common to be inbred or outbreed lines even single cross tester, which is considered a rabid method for developing the best 3-way and/or double cross combination. Oldemeyer *et al.*, (1968) stated that good tester varieties must be chosen with of different origin than the material being tested, relatively broad genetic base and inherently poor in performance. One of the methods used to estimate the variance components and effects due to general and specific combining ability is line x tester mating design (Kempthorne, 1957). Two types of combining ability, general and specific, have been recognized in quantitative genetics. General combining ability was found to be important for almost all traits (Fairfull *et al.*, 1983; Singh *et al.*, 1983 and Gupta *et al.*, 2000; Szydlowski and Szwaczkowski 2001 and Abou El-Ghar *et al.*, 2009). However, specific combining ability was more widely important than has been reported elsewhere (Fairfull and Gowe, 1990; Wei *et al.*, 1991 a, b; Wei and van der Werf, 1993; Abou El-Ghar *et al.*, 2003 and Abou El-Ghar and Abdou, 2004). The objectives of this study were to estimate the combining ability effects, to estimates the additive and dominance mean squares and to estimate the contribution of various genetic variance components to the total variance for egg production yield. These estimates would provide guidelines to the fowl breeders to launch effective breeding strategies.

II. Materials and Methods

The current experiment had been carried out at El-Sabahiah Poultry Research Station, Animal Production Research Institute, Agriculture Research Center, Egypt.

a) Experiment Stock and Design

The progenies of eight F1 hybrids developed from line x tester mating design by crossing four local developed strains, used as male (*Lines*) in this study. They include: Baheij (Bj), Matrouh (Mt), Silver Montazah (SM) and Golden Montazah (GM) chickens together with females of two genetic stocks of commercial laying hens (*Testers*) i.e. Lohman Brown (LB) and Lohman Selected Leghorn (LSL), used in this experiment. Table 1 reflects the genetic stock designation of the four local strains as 295, 60, 180 and 60 dams for Baheij, Matrouh, Silver Montazah and Golden Montazah, and 100 and 100 dams of each of Lohman Brown and Lohman Selected Leghorn, respectively. The crossing plan of this

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experiment was that each line (10 males) was crossed to each of the two testers (20), thus we have 8 crosses. These crosses along with 6 parents, i.e., 4 lines and 2 testers, total entry being 14. The observations were recorded on 1195 hens, (595 lines, 200 testers and 400 lines x testers hybrids) were tested in a Randomized Complete Block Design with 5 replicates for providing information about the general combining ability of a line and expected to show good performance in specific combining ability in hybrid combinations.

Table 1 : Reflects the genetic stock designation and the crossing plan obtained

Lines	♀	♂	Testers	
			LB (♀)	LSL (♀)
Bj	295	10	20	20
Mt	60	10	20	20
SM	180	10	20	20
GM	60	10	20	20
Total	595	40	100	100

Bj = Baheij, Mt = Matrouh, SM = Silver Montazah, GM = Golden Montazah, LB = Lohman Brown and LSL = Lohman Selected Leghorn.

b) Management Conditions

All managerial practices were similar as possible as throughout the experiment for all replicates. Artificial insemination was applied by assigning 4 females per each male. Two hatches in each mating combination were used. For each hatch eggs were collected throughout 7 d and incubated in full-automatic draft machine. At hatch, all chicks were wing-banded and weighed to the nearest gram. The chicks were fed *ad libitum* commercial starter ration (19 % CP and 2800 KCal) up to 8 weeks of age, then the ration was changed by commercial grower ration (15 % CP and 2700 KCal) up to 20 weeks of age. During the production period the pullets were fed a commercial layer ration (16.5 % CP and 2750 KCal) and they were housed in individual cages and received 16 hr day light. At the onset of lay, eggs were recorded and weighed daily during the first 90 (d.) of production, then twice a week till the end of experiment.

The Studied Traits: 10 egg production traits were studied; i.e.

Age at sexual maturity (A.S.M)

Number of eggs at 1st 90 d. of laying (EN1),

Average egg weight through the 1st 90 d. of laying (EW1),

Number of eggs at 180 d. of laying (EN2),

Average egg weight at 180 d. of laying (EW2),

Number of eggs at 240 d. of laying (EN3),

Average egg weight at 240 d. of laying (EW3),

Number of eggs at 52 wks of laying (EN4),

Average egg weight at 52 wks of laying (EW4) and

Egg mass throughout 52 wks of laying (EM).

c) Statistical Analysis

The data derived from 8 crosses along with 6 parental lines were firstly analyzed in conventional analysis of variance for all characters prior to combine analysis to test the significance among the different genetic groups using the following linear model of SAS program (SAS Institute, 2000):

$$Y_{ijk} = M + G_{ij} + r_k + e_{ijk}$$

Where:

Y_{ijk} is the k[th] observation, M is the overall mean, G_{ij} is the effect of i[th] genotype, r_k is the effect of k^{th} replication and e_{ijk} is the random error. The procedures followed are possible to partition genotype source of variation into variations due to crosses, parents and parent vs. crosses. If these differences are found significant, line x tester analysis is done. Similarly, the line x tester analysis was partitioned into variations due to lines, testers and line x tester. Estimate of GCA of a tester (females) was obtained in terms of its performance in F1 hybrid combinations with all possible lines (male). Likewise, GCA of a line was determined in terms of its performance in F1 hybrid combinations with all possible testers. The lines and testers were considered as fixed effects. GCA and SCA effects were determined for each trait following (Kempthorne, 1957) as follows:

GCA lines (L) = X j – Y

GCA tester (T) = X i - Y

SCA (L x T) = X ij - X j - X i - Y,

Where:

X **j** = the mean of hybrid with a given line (male) averaged over all replications and testers (females),

X **i** = the mean of hybrid with a given tester (females) averaged over all replications and lines (males),

X **ij** = the mean of a given hybrid (L x T) averaged over replications,

Y = the experimental mean.

Standard errors (*SE*) for general and specific combining ability were calculated as follows:

SE for GCA of lines = $(Me/r*l)^{½}$, SE for GCA of tester = $(Me/r*t)^{½}$ and SE for Line x Tester = $(Me/r)^{½}$,

Where:

Me, is the respective mean square of line x tester error divided by number of

If the absolute effect of GCA or SCA is greater than the C.D, it is considered significantly different from zero. The critical difference (C.D) was calculated as follows: C.D = SE x t (tabulated).

III. Results and Discutions

a) Performance of genetic groups

Results of crossing Baheij, Matrouh, Silver Montazah and Golden Montazah local sire lines with two testers dames Lohman Brown and Lohman Selected Leghorn in line x tester mating design given in Table 2, reveals superiority of Silver Montazah (SM) parental line means in most of the studied traits i.e. number of eggs at 90 d., of laying (EN1), number of eggs at 180 d., of laying (EN2), number of eggs at 240 d., of laying (EN3), number of eggs till 52 wks., of laying (EN4), average egg weight at 52 wks., of laying (EW4) and egg mass throughout 52 wks., of laying (EM) traits 48±11 egg, 80±16 egg., 128±22 egg, 155±25 egg, 53.5±2 g., and 8±1.3 kg., respectively, while Matrouh (Mt) parental line was ranked second in EN1 42±5 egg, EN2 77±7 egg, EN3 100±9 egg and average egg weight at 240 d., of laying (EW3) 52.9±1 g., respectively. Contrarily, the parental line Golden Montazah (GM) had the earliest age at sexual maturity (A.S.M) 184±4 d., among all parental lines and it was superior in average egg weight at 90 d., of laying (EW1) 51.9±3 and average egg weight at 180 d., of laying (EW2) 52.4±3 g., traits. Regarding the experimental testers results showed that Lohman Brown (LB) was ranked first in most of traits studied 151±14 d. (A.S.M), 50±7 egg (EN1), 58.1±3 g. (EW1), 98±18 egg (EN2), 59.2±2 g. (EW2), 153±26 egg (EN3), 59.7±3 g. (EW3), 192±37 egg (EN4), 61.1±3 g. (EW4) and 12±2.7 Kg. (EM), while the other tester Lohman Selected Leghorn (LSL) was ranked second for the corresponding traits 153±8 d., 50±9 egg, 52.4±3 g., 91±10 egg, 55.7±3 g., 116±19 egg, 55.7±2 g., 168±25 egg, 56.4±2 g., and 11±2.7 Kg., respectively. Thus, the former results showed clearly that Silver Montazah and Golden Montazah local sire lines of chicken are considered to be fitting parental lines that play an important role in improving both egg number and egg weight traits, respectively. These findings agreed with those reported by (Kosba and Abd El-Halim, 2008 for egg number and egg mass at 90 d., of production, Abou El-Ghar *et al.*, 2009&2010 for egg weight and most of egg production traits and Iraqi *et al.*, 2012).

The results of tester's performance revealed that the testers Lohman Brown (LB) and Lohman Selected Leghorn (LSL) were gained either high or low egg production yield, respectively. It may conclude that they have either high or low frequency of favorable alleles for these traits. The same conclusion was reported by Lopez-Perez (1979). Furthermore, results of lines x testers analysis showed that the single cross Bj x LSL was the earliest hybrids in reaching sexual maturity (A.S.M) 184 d., while the single cross SM x LSL had better means of EN1 EW1, EN2, EN4 and EM (40, 119, 210 egg and 11 kg., respectively). Moreover, the single cross GM x LSL showed superiority in average egg weight at all laying periods studied (EW1 53.8 g., EW2 54.1 g., EW3 54.5 g., and EW4 54.2 g., as well as, the same hybrid (GM x LSL) had a higher means of annual egg number (EN3) and annual egg mass (EM) 153 egg and 11 kg., respectively. The same trend was found in lines x LB hybrids in Table 2, where the single cross SM x LB showed superiority means of EN1, EN2, EW2, EN3, EW3, EN4, EW4 and EM i.e. 41 egg, 117 egg, 53.7 g., 153 egg, 55.0 g., 209 egg, 55.8 g., and 12 kg., respectively. While, GM x LB single cross had the heaviest egg weight at the first 90 d. of laying (EW1 53.5 g.) and egg weight at 180 d. of laying (EW2 53.7 g.). Generally, the lines x tester (LSL) single crosses were achieved higher estimates for A.S.M 186 d., and 39, 114, 148, 201 eggs and 11 Kg., for EN1, EN2, EN3, EN4 and EM traits than the corresponding traits in lines x LB hybrids. Contrarily the lines x LB hybrids had the heaviest egg weight at different laying periods studied EW1, EW2, EW3 and EW4 52.7, 53.0, 53.4 and 53.7 g., respectively. It could be concluded that from the former results the lines x LSL single crosses were found to exhibit an outstanding higher egg production yield than the corresponding lines x LB hybrids, which has been associated with increased egg weight at different laying periods studied. The same finding was reported by Oldemeyer *et al.*, (1968) who stated that good tester varieties must be chosen with of different origin or relatively broad genetic base than the strains being tested. Also the former results showed clearly that there was a correlation between egg number and egg mass at the different periods of production, since egg mass could be affected mainly by the large proportion of variations in egg number trait. The same finding was reported by Abou El-Ghar *et al.*, (2010).

b) Phenotypic Variations

The differences among lines, testers and line x tester in Table 3 revealed that all egg production traits studied were statistically differ significantly ($P<0.01$) in between replicates except for egg number till 180 d., of laying (EN2), this finding indicating enough genetic variations for the genotypes and necessity of genetic analysis. In addition, the variations in between genotypes, between total parents and between hybrids were highly significant ($P<0.01$) for all egg production traits revealing the parents chosen were diverse and with a different genetic background. Moreover, the differences among lines and testers were insignificant with respect to all ten egg production traits. It also appears from Table, 3 that the interaction affects of hybrids vs. parents was highly significant differences ($P<0.01$) for all of the studied traits. On the other hand, the line x tester interaction was highly significant differences ($P<0.01$) except for all egg production traits, which indicating the presence of heterosis. These findings agreed with some Egyptian studies (Sheble *et al.*, 1990 and Iraqi, 2008) they reported that the

possibility of improving the most of native breeds through crossbreeding could be evidenced. Generally, the findings of variations for egg production traits were agreed the previous estimates of means of the different genetic groups; moreover, significant mean square of parents vs. hybrids noted that the non-additive genetic effects may control most of the studied traits. These findings were in agreement with those reported by (Fairfull and Gowe, 1990; Wei *et al*., 1991 a, b; Wei and van der Werf, 1993; Abou El-Ghar *et al*., 2003 and Abou El-Ghar and Abdou, 2004).

c) General and specific combining ability effects

Estimated general combining ability (GCA) effects determined in line x tester mating design were presented in Table 4. It was noticed that the negative values denote to desirable values for age at sexual maturity (ASM). Results presented in Table 4 showed that from the studied parental lines three showed significant negative GCA values -30.0, -12.4 and -34.3 for Matrouh (Mt), Silver Montazah (SM) and Golden Montazah (GM), respectively, while the parental line Baheij (Bj) gave insignificant positive GCA value 76.7 of ASM trait. These results indicate that the parental lines Mt, SM and GM had desirable genes for early sexual maturity and considered good combiners for breeding to age at sexual maturity. Among testers, the Lohman Selected Leghorn (LSL) tester gave a significant negative GCA -33.0 effects on ASM. Conversely, Lohman Brown (LB) tester gave insignificant positive effect of GCA 33.0 on the same trait. Fairfull *et al*. (1983); Singh *et al*. (1983) and Huang and Lee (1991) cited that GCA was significant for ASM.

Concerning specific combining ability (SCA) effects, data obtained in Table 5 revealed that of the studied eight F1 crosses four showed significant negative SCA value -95.4, -29.9, -23.3 and -42.2 in the crosses Bj x LSL, Mt x LB, SM x LB and GM x LB, respectively. Although the insignificant positive SCA values were detected in four crosses, their values ranged from 95.4 in the cross Bj x LB to 23.3 in the cross SM x LSL. These results were in agreement with those reported by Fairfull *et al*., (1983) who found that general combining ability and specific combining ability effects were important for sexual maturity. Regarding egg number at the first 90 d., of laying (EN1) results obtained in table 4 revealed significant positive estimates of GCA effects 20.4 and 3.3 for SM and GM parental lines, respectively, these significant values indicated that the parental lines SM and GM were the best combiners for egg number during the first 90 d. of production. Unlikely, the insignificant GCA effects -6.2, -17.5, -2.0 and 2.0 were given by Bj, Mt parental lines and LB and LSL testers, respectively. These results agreed with those obtained by (Verma *et. al*., 1987 and Farghaly and Saleh, 1988). On the other hand, the estimated SCA effects in Table 5 showed that six crosses gave insignificant SCA values ranged from 4.2 to -8.6. On the other hand, a highly significant positive SCA value ($P<0.01$) was given by the cross Mt x LSL (8.6), while the significant SCA value ($P<0.05$) 6.1 was reflected by the cross SM x LB. These results agreed with those obtained by Verma *et al*., (1987). On the topic of GCA effects on egg production traits, the parental line SM showed the best significant positive GCA values of EN2, EN3 and EN4 traits 74.4, 59.8 and 142.1, respectively (Table 4). The parental line GM was ranked second among the parental lines, which reflects the significant positive values of GCA effects on EN2, EN3 and EN4 traits 17.2, 46.1 and 41.8, respectively. The same trend was found for LSL tester, which exhibits significant positive values of GCA effects on EN2, EN3 and EN4 i.e. 35.1, 22.7 and 51.0, respectively. The two parental lines Bj and Mt along with LB tester gives insignificant negative GCA values for the same traits, respectively. Therefore, the line SM as will as the tester LSL were considered the most superior genotypes for improving egg number at different laying periods. Moreover, estimates of specific combining ability effect values for egg number at different laying periods were presented in Table 5. Out of eight hybrid combinations, four had good estimates of positive specific combining ability (SCA) since they showed significant effects on EN2 i.e. 18.7, 23.3, 24.4 and 17.7 for Bj x LSL, Mt x LSL, SM x LB and GM x LB, respectively. For egg number at 240 d., of laying (EN3), three hybrids showed significant positive estimates of SCA were 19.0, 19.6 and 33.5 for Bj x LSL, Mt x LSL and SM x LB, respectively. Only two crosses reflected significant positive SCA effects on EN4 these values were 39.1 and 43.1 for Bj x LSL and SM x LB, respectively.

Concerning general combining ability effects on egg weight, Table 4 showed that the parental line GM had a significant higher GCA effect ($P<0.01$) of egg weight at different laying periods studied EW1, EW2 and EW3 12.2, 11.4 and 10.8, respectively. In addition, the parental line SM had the highest significant GCA effects ($P<0.01$) in egg weight at 52 wk., of laying EW4 (10.9). Therefore, the parental lines GM and SM were the most superior parental lines under this study. At the same time as, the LB tester was considered as good combiners for egg weight at different laying periods, it gains the significantly higher positive GCA values 2.4, 2.1, 3.1 and 5.7 for EW1, EW2, EW3 and EW4, respectively. Unlikely, the tester LSL showed the lowest negative insignificant GCA values -2.4, -2.1, -3.1 and -5.7 for EW1, EW2, EW3 and EW4, respectively. Therefore, it could be concluded that the parental lines Bj, Mt and LSL tester were not promising for egg weight traits. Generally, estimates of SCA effects listed in Table, 5 showed that the SCA effects in F1 crosses for EW1 being significant ($P<0.01$) 13.7, 4.1, 5.3 and 4.3 in Bj x LB, Mt x LSL, SM x LSL and GM x LSL F1 crosses, respectively. Moreover, the crosses Bj x LB, Mt x LSL,

SM x LB and GM x LSL showed significant positive SCA effect values 4.1, 3.2, 3.3 and 4.2 in EW2 trait, respectively. Additionally, EW3 showed significant positive SCA effect values 3.4, 9.8 and 7.0 for Bj x LSL, SM x LB and GM x LSL, respectively. The same significant and positive direction of SCA effects on EW4 were 4.2, 10.1 and 7.3 given by Bj x LSL, SM x LB and GM x LSL F1 crosses, respectively. Similar results for egg weights were obtained by Fairfull *et al.*, (1983); Verma *et al.*, (1987) and Farghaly and Saleh (1988).

Further discussion of the results of GCA effects on annual egg mass (EM) in table 4, that the parental line SM achieved the highest significant estimate of GCA effects on EM trait 9.8, while the corresponding estimate of GCA effects 3.8 was achieved by GM parental line. In addition, negative estimates of GCA for EM trait were -9.6 and -4.0 achieved by Bj and Mt parental lines, respectively (Table 4). The insignificant estimates of GCA on the bases of the testers were -1.4 and 1.4 for LB and LSL, respectively. Otherwise, the estimates SCA effects (Table 5), revealed that the single crosses SM x LB and Bj x LSL had significant ($P<0.05$) positive estimates of SCA effects for EM trait 4.3 and 2.8, respectively. And insignificant estimate of SCA effects on EM trait were given by the crosses Bj x LB, Mt x LB, Mt x LSL, SM x LSL, GM x LB and GM x LSL i.e. -2.8, -0.6, 0.6, -4.3, -0.9 and 0.9, respectively. From the previous results, it is concluded that the parental lines SM and Bj could be favored in GCA for EM trait. These results agreed with those reported by Fairfull *et al.*, (1983) and Gupta *et al.*, (2000) they found significant ($P<0.01$) effect of GCA for egg production traits, while, Hill and Nordskog (1958) cited that the SCA effects is more importance for egg production traits.

d) Components of Genetic Variance

The estimates of genetic variance components were presented in Table 6, results showed that the variances of GCA for lines (σ^2GCA_{Lines}) were higher than those for testers (σ^2GCA_{Tester}) for all characters studied. The SCA mean squares for egg production traits were about more than two times greater than the GCA mean square of lines and more than four times greater than GCA mean square of tester. Thus, the results of SCA (variances due to lines x testers) implied that non-additive type of variations was controlling all egg production traits, yet non-additive genes were more important than the additive genes because variance due to SCA was higher than that of GCA. These results agreed with those obtained by Hill and Nordskog (1958) who reported that the SCA is more importance for hen-day egg production. Also these observations were in agreement with (Wearden *et al.*, 1965; Amrit, 1980; Fairfull *et al.*, 1983; Huang and lee, 1991 and Shebl, 1991). Moreover, results regarding the magnitude of additive σ^2A and dominance σ^2d mean square components of genetic variance in Table 6 indicated that dominance mean square component (σ^2d) play an important role in the inheritance of all the characteristics measured. However, the dominant mean square σ^2d was larger than additive components σ^2A for ASM, EN1, EW1, EN2, EW2, EN3, EW3, EN4, EW4 and EM4 traits. Consequently, it could be concluded that the nature of gene effects were dominant for these traits. Similar to the findings of Fairfull and Gowe, 1990; Wei *et al.*, 1991 a, b; Wei and van der Werf, 1993; Abou El-Ghar *et al.*, 2003 and Abou El-Ghar and Abdou, 2004 obtained higher magnitude of σ^2d over σ^2A for number of egg production traits. On the other hand, Table 7 shows that the contribution of lines was greater than that of testers for all egg production traits studied, while the contribution of line x tester was greater than that of lines or testers for all characters of egg production traits.

IV. Conclusion

The results of SCA mean squares were about more than two to four times greater than the GCA mean squares of lines and testers, suggests the importance of non-additive variances for all egg production traits studied. The parental line Silver Montazah demonstrates the ability to distinguish the merit of the male lines. However, the higher GCA effects of male line SM and female tester LB for most of egg production traits indicate that both these parents may be preferred for hybridization programs. On the other hand, the SCA effects reveal that, for hybrid egg yield development, crosses SM x LB and Bj x LSL could be the better choice for improving egg production yield.

Table 2 : Means and S.E of some egg production traits from line x tester analysis in laying hens

Genotypes	Traits									
	A.S.M	EN1	EW1	EN2	EW2	EN3	EW3	EN4	EW4	EM
Baheij (Bj)	194±5	41±3	45.7±1	57±5	45.8±1	82±12	45.7±1	148±9	45.0±4	7±0.7
Matrouh (Mt)	187±12	42±5	47.2±1	77±7	51.4±1	100±9	52.9±1	110±12	53.4±1	6±0.6
Silver Montazah (SM)	190±9	48±11	47.4±3	80±16	49.9±2	128±22	52.3±2	155±25	53.5±2	8±1.3
Golden Montazah (GM)	184±4	35±5	51.9±3	67±9	52.4±3	89±13	52.1±3	135±19	52.2±3	7±1.0
Total lines (L)	191±8	43±8	47.0±3	67±15	48.2±3	99±25	49.1±4	145±21	49.2±5	7±1.2
Lohman Brown (LB)	151±14	50±7	58.1±3	98±18	59.2±2	153±26	59.7±3	192±37	61.1±3	12±2.7
Lohman Select. Leghorn (LSL)	153±8	50±9	52.4±3	91±10	55.7±3	116±19	55.7±2	168±25	56.4±2	11±2.6
Total Testers (T)	152±11	50±8	55.3±4	95±15	57.5±3	134±29	57.7±3	180±34	58.7±4	11±2.7
Total Parents	181±19	44±9	49.1±5	74±19	50.6±5	108±31	51.3±5	154±29	51.6±6	8±2.5
Bj x LB	209±22	38±4	52.4±2	101±5	52.3±2	136±12	52.1±2	172±16	52.3±2	9±1.0
Bj x LSL	184±2	38±6	49.2±3	112±13	51.0±8	145±18	52.2±6	190±18	52.0±6	10±1.5
Mt x LB	186±9	36±4	52.5±3	99±8	52.3±2	137±13	52.7±2	185±14	52.9±2	10±0.8
Mt x LSL	184±7	38±5	49.2±2	112±5	51.0±2	145±14	52.2±2	190±16	52.0±2	10±0.9
SM x LB	189±3	41±6	52.4±3	117±14	53.7±1	153±8	55.0±1	209±25	55.8±1	12±1.4
SM x LSL	187±13	40±5	52.9±3	119±10	52.6±3	151±15	52.4±3	210±21	52.7±3	11±1.3
GM x LB	185±4	39±4	53.5±2	110±10	53.7±2	149±9	53.8±2	196±11	53.9±2	11±0.8
GM x LSL	186±8	39±4	53.8±4	114±8	54.1±4	153±7	54.5±4	204±14	54.2±4	11±0.7
Total hybrids	189±13	39±5	52.4±3	110±12	52.8±4	146±14	53.1±3	195±21	53.2±3	10±1.3

A.S.M = age at sexual maturity, EN1= egg number at the first 90 d., of laying, EW1 = early egg weight at the first 90 d., of laying, EN2= egg number at 180 d., of laying, EW2 = egg weight at 180 d., of laying, EN3 = egg number at 240 d., of laying, EW3 = average egg weight at 240 d., of laying, EN4 = egg number till 52 wk. of age, EW4 = average egg weight till 52 wk. of age, EM = egg mass till 52 wk. of age

Table 3 : Test the significance of source of variations from line x tester analysis for some egg production traits

S.O.V	d.f	Traits									
		A.S.M	EN 1	EW 1	EN2	EW 2	EN3	EW3	EN 4	EW 4	EM
Bet. Rep.	4	**	**	**	NS	**	**	**	**	**	**
Bet. Genotypes	13	**	**	**	**	**	**	**	**	**	**
Bet. Total Parents	5	**	**	**	**	**	**	**	**	**	**
Bet. Total Hybrids	7	**	**	**	**	**	**	**	**	**	**
Parents vs. Hybrids	1	**	**	**	**	**	**	**	**	**	**
Bet. Lines	3	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Bet. Testers	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Bet. Line x Tester	3	**	**	**	**	**	**	**	**	**	**
Error MS	1177	69	36	6	98	6	209	4	337	6	2

A.S.M = age at sexual maturity, EN1= Egg number at the first 90 d., of laying, EW1 = early egg weight at the first 90 d., of laying, , EN2 = egg number at 180 d., of laying, EW2 = average egg weight at 180 d., of laying, EN3 = egg number till 240 d. of laying, EW3 = average egg weight till 240 d. of laying, EN4 = egg number till 52 wk. of age, EW4 = average egg weight till 52 wk. of age, EM = egg mass till 52 wk. of age.

Table 4 : General combining ability effects (GCA) of lines and testers for some egg production traits

Parents	Traits									
	A.S.M	EN 1	EW 1	EN2	EW 2	EN3	EW3	EN 4	EW 4	EM
Lines (Males)										
Baheij (Bj)	76.7 NS	-6.2 NS	-16.2 NS	-39.5 NS	-11.0 NS	-56.5 NS	-9.5 NS	-143.8 NS	-10.2 NS	-9.6 NS
Matrouh (Mt)	-30.0**	-17.5 NS	1.8*	-52.1 NS	-4.1 NS	-49.5 NS	-7.8 NS	-40.0 NS	-9.6 NS	-4.0 NS
Silver Montazah (SM)	-12.4**	20.4**	2.1**	74.4**	3.7**	59.8**	6.5**	142.1**	10.9**	9.8**
Golden Montazah (GM)	-34.3**	3.3*	12.2**	17.2**	11.4**	46.1**	10.8**	41.8**	8.9**	3.8**
S.E. GCA Lines	1.8	1.3	0.5	2.2	0.5	3.2	0.4	4.1	0.5	0.2
Testers (Females)										
Lohman Brown (LB)	33.0NS	-2.0 NS	2.4*	-35.1 NS	2.1*	-22.7 NS	3.1**	-51.0 NS	5.7**	-1.4 NS
Lohman Select. Leghorn (LSL)	-33.0**	2.0 NS	-2.4 NS	35.1**	-2.1 NS	22.7**	-3.1 NS	51.0**	-5.7 NS	1.4 NS
S.E. GCA Testers	2.6	1.8	0.7	3.1	0.7	4.5	0.6	5.8	0.7	0.4

A.S.M = age at sexual maturity, EN1= Egg number at the first 90 d., of laying, EW1 = early egg weight at the first 90 d., of laying, , EN2 = egg number at 180 d., of laying, EW2 = average egg weight at 180 d., of laying, EN3 = egg number till 240 d. of laying, EW3 = average egg weight till 240 d. of laying, EN4 = egg number till 52 wk. of age, EW4 = average egg weight till 52 wk. of age, EM = egg mass till 52 wk. of age, S.E. = stander error for general combining ability, *, ** = Significant at 0.05 and 0.01 probability level respectively. NS. = Non-significant at 0.05 probability level.

Table 5 : Specific combining ability (SCA) estimates for some egg production traits from line x tester analysis

F1 Hybrids	Traits									
	A.S.M	EN 1	EW 1	EN2	EW 2	EN3	EW3	EN 4	EW 4	EM
Bj x LB	95.4NS	-1.7 NS	13.7**	-18.7 NS	4.1**	-19.0 NS	-3.4 NS	-39.1 NS	-4.2 NS	-2.8 NS
Bj x LSL	-95.4**	1.7 NS	-13.7 NS	18.7**	-4.1 NS	19.0**	3.4*	39.1**	4.2**	2.8*
Mt x LB	-29.9 **	-8.6 NS	-4.1 NS	-23.3 NS	-3.2 NS	-19.6 NS	0.5 NS	-14.2 NS	1.4 NS	-0.6 NS
Mt x LSL	29.9NS	8.6**	4.1**	23.3**	3.2*	19.6**	-0.5 NS	14.2 NS	-1.4 NS	0.6 NS
SM x LB	-23.3 **	6.1*	-5.3 NS	24.4**	3.3*	33.5**	9.8**	43.1**	10.1**	4.3*
SM x LSL	23.3NS	-6.1 NS	5.3**	-24.4 NS	-3.3 NS	-33.5 NS	-9.8 NS	-43.1 NS	-10.1 NS	-4.3 NS
GM x LB	-42.2 **	4.2 NS	-4.3 NS	17.6**	-4.2 NS	5.2 NS	-7.0 NS	10.2 NS	-7.3 NS	-0.9 NS
GM x LSL	42.2NS	-4.2 NS	4.3**	-17.6 NS	4.2**	-5.2 NS	7.0**	-10.2 NS	7.3**	0.9 NS
S.E.	3.7	2.6	1.0	4.4	1.0	6.4	0.9	8.2	1.1	0.5

A.S.M = age at sexual maturity, EN1 = Egg number at the first 90 d., of laying, EW1 = early egg weight at the first 90 d., of laying, , EN2 = egg number at 180 d., of laying, EW2 = average egg weight at 180 d., of laying, EN3 = egg number till 240 d. of laying, EW3 = average egg weight till 240 d. of laying, EN4 = egg number till 52 wk. of age, EW4 = average egg weight till 52 wk. of age, EM = egg mass till 52 wk. of age, S.E. = stander error for specific combining ability, *, ** = Significant at 0.05 and 0.01 probability level respectively. NS. = Non-significant at 0.05 probability level.

Table 6 : General combining ability variances (σ^2GCA) from lines and testers, specific combining ability variance (σ^2SCA) from line x tester, additive (σ^2A) and dominance (σ^2d) mean squares for some egg production traits

Traits	σ^2GCA Lines (L)	σ^2GCA Testers (T)	σ^2SCA (L x T)	σ^2A	σ^2d
A.S.M	-3814420.6	-1907045.0	7629911.2	-3051470.4	30519644.7
EN 1	-159873.6	-79960.9	319842.9	-127908.5	1279371.6
EW 1	-293454.1	-146738.6	586963.2	-234767.9	2347852.9
EN 2	-1301886.1	-650787.1	2605100.1	-1041446.5	10420400.4
EW 2	-297264.5	-148639.9	594565.8	-237814.7	2378263.2
EN 3	-2283164.3	-1141754.4	4567802.1	-1826600.3	18271208.5
EW 3	-300680.2	-150346.5	601401.2	-240546.7	2405604.9
EN4	-4088800	-2044832.7	8183431.3	-3271213	32733725
EW4	-301570.7	-150785.3	603193.0	-241256.5	2412772.2
EM	-11580.8	-5796.9	23190.5	-9267.2	92762.0

σ^2GCA Lines = general combining ability mean square from lines, σ^2GCA Testers = general combining ability mean square from testers, σ^2SCA $_{(L \times T)}$ = specific combining ability mean square from line x tester analysis, σ^2A = additive genetic variance, σ^2d = dominance variance, A.S.M = age at sexual maturity, EN1 = Egg number at the first 90 d., of laying, EW1 = early egg weight at the first 90 d., of laying, , EN2 = egg number at 180 d., of laying, EW2 = average egg weight at 180 d., of laying, EN3 = egg number till 240 d. of laying, EW3 = average egg weight till 240 d. of laying, EN4 = egg number till 52 wk. of age, EW4 = average egg weight till 52 wk. of age, EM = egg mass till 52 wk. of age.

Table 7 : Contribution of lines, testers, and lines x testers mean squares to the total variance for some egg production traits

Traits	Contribution (%)		
	Lines	Testers	Lines x Testers
A.S.M	**0.014**	**0.008**	**99.98**
EN 1	**0.032**	**0.001**	**99.97**
EW 1	**0.010**	**0.001**	**99.99**
EN 2	**0.05**	**0.03**	**99.92**
EW 2	**0.0064**	**0.0004**	**99.99**
EN 3	**0.033**	**0.006**	**99.96**
EW 3	**0.007**	**0.001**	**99.99**
EN4	**0.07**	**0.02**	**99.91**
EW4	**0.009**	**0.003**	**99.99**
EM	**0.126**	**0.005**	**99.87**

A.S.M = age at sexual maturity, EN1 = Egg number at the first 90 d., of laying, EW1 = early egg weight at the first 90 d., of laying, , EN2 = egg number at 180 d., of laying, EW2 = average egg weight at 180 d., of laying, EN3 = egg number till 240 d. of laying, EW3 = average egg weight till 240 d. of laying, EN4 = egg number till 52 wk. of age, EW4 = average egg weight till 52 wk. of age, EM = egg mass till 52 wk. of age.

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Quality and Safety of *Citrus Sinensis* Coated with Hydroxypropylmethylcellulose Edible Coatings Containing *Moringa Oleifera* Extract Stored at Ambient Temperature

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Abstract - Edible coatings based on hydroxypropylmethyl-cellulose(HPMC) with and without an aqueous extract of Moringa leaves, were developed and applied to orange, in order to improve quality and shelf life during storage, while taking advantage of the beneficial health properties of Moringa. Weight loss, firmness and ascorbic acid content of uncoated and coated samples were determined throughout ambient storage. The two experimental coatings were: HPMC without crude extract of Moringa oleifera and HPMCME mixed with 75mg/ml of crude extract of Moringa oleifera . Four hundred and eighty (480) orange fruits were stored for seven weeks at ambient temperature of 27±3oC and relative humidity of 50- 65%. The overall result showed that polysaccharides coating from (HPMC) and (HPMCME) is effective in extending the shelf-life of orange fruits when compared to untreated in the following order:(HPMCME) > (HPMC) >Control.

Keywords : *Edible coatings , Moringa oleifera , Orange, Hydroxypropylmethylcellulose.*

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Quality and Safety of *Citrus Sinensis* Coated with Hydroxypropylmethylcellulose Edible Coatings Containing *Moringa Oleifera* Extract Stored at Ambient Temperature

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Keywords : Edible coatings , Moringa oleifera , Orange, Hydroxypropylmethylcellulose.

I. Introduction

The sweet orange (*Citrus sinensis* (L.) Osbeck), is the most commonly grown tree fruit in the world (Morton, 1987).Citrus fruits are produced all around the world and world citrus production in selected major producing countries in 2005/2006 is 72.8 million metric tons. Citrus fruits are said to be the first crops in the international trade in terms of values (CIAC, 2002).

Moringa oleifera is considered one of the world's most useful trees, as almost every part of the tree can be used for food or has some other beneficiary property. Moringa is a special food for the tropics, because the tree is in full leaf at the end of scarce (Iwu, 1993). It is available all year round. Almost all parts are used as food and forage for livestock (Ram, 1994). The part (leaves, fruits, flowers and immature pods) are edible and form part of traditional diet in many countries of the tropics and subtropics (Odee, 1998).

Edible coatings have long been known to protect perishable food products from deterioration by retarding dehydration, suppressing respiration, improving textural quality, helping retain volatile flavor compounds and reducing microbial growth (Debeaufort, Quezada-Gallo, & Voilley, 1998). Specially formulated edible coatings may provide additional protection against contamination of microorganism while serving the similar effect as modified atmosphere storage in modifying internal gas composition (Park, 1999). According to their components, edible films and coatings can be divided into three categories: hydrocolloids (proteins and polysaccharides), lipids, and composites. Antioxidants, flavors and pigments, vitamins, and antimicrobial agents can be successfully incorporated into edible coatings to improve their functional properties. In the literature, several reviews reported on the efficacy of films and coatings containing antimicrobials to control microbial growth on fruits and vegetables (Ayala-Zavala et al., 2008; Cagri et al., 2004).

The polysaccharides as coating materials for fruits have been used extensively in the past few years. These natural polymers, in addition to the above mentioned benefits, present advantages due to their availability, low cost, and biodegradability. The latter in particular is of great interest, as it leads to a reduction in the large quantities of non-biodegradable synthetic packaging materials (Zhou *et al.*, 2008). In addition, their physico-chemical properties can also be improved by modifying them. Cellulose is a naturally occurring polymer which is found abundantly and is usually present as a linear polymer of anhydroglucose (Kester and

Fennema, 1986). At a molecular level, cellulose is a simple linear polymer comprising of β-[1,4] linked D-glucose molecules. Because of its regular structure and array of hydroxyl groups, it tends to form strong hydrogen-bonded crystalline microfibers, which are insoluble in several solvents (Zugenmaier, 2006).

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Several cellulose derivatives such as methyl cellulose (MC), carboxymethyl cellulose (CMC), hydroxypropyl cellulose (HPC), and hydroxypropylmethyl cellulose (HPMC) are widely produced commercially (Olivas and Barbosa- Canovas, 2005). These edible coatings have been applied to a variety of fruits to provide moisture, oxygen and carbon dioxide barriers, and to improve adhesion of coating formulations (Kester and

Fennema, 1986). Coatings and films that are based on these cellulose ethers are generally transparent, flexible, odourless, tasteless, water-soluble, and resistant to O_2 and CO_2 (Nisperos-Carriedo *et al.*, 1992).

The aim of this work was to analyze the effect of hydroxypropylmethylcellulose incorporated with Moringa aqueous extract on the development of the physicochemical properties stored at ambient temperature.

II. Materials and Methods

a) Source of Materials

Freshly harvested oranges were procured from the local market of Ilorin, kwara state, Nigeria. They were selected on the basis of sized, color and absence of external injuries. Fresh leaves of *Moringa oleifera* was obtained from Nigeria stored products research institute(NSPRI) garden.

b) Prepration of aqueous extract Moringa oleifera (AEMO)

Moringa oleifera leaves was dried in an open laboratory and ground into very fine powder using an electric blender (supermaster ®, Model SMB 2977, Japan). The powder was further sieved to pass through 1 mm^2 perforations. The powder was then packed in plastic containers with tight lids and stored in a refrigerator at 4°C prior to use. Fifty grams of the dried *Moringa oleifera* leaves was weighed and introduced into conical flask containing 250ml of distilled water. The conical flask was then covered with aluminum foil and placed on mechanical shaker. The suspensions was then shaked for 48hours at 190rev.per.min. The extract was decanted and passed through different clean muslin cloth and later filtered with whatman fitter paper. The filtrate obtained was evaporated to dryness at 50^0c and the residues obtained are kept in an aluminum foil.

c) Reconstitution and sterilization of extract

The dried residue was weighed into McCartney bottles and appropriate volume of distilled water was added to make a stock solution of 75mg/ml, for example 750mg in 10mls of distilled water. The stock solution was then sterilized using 0.65 membrane filter by suction pump. The sterilized extract were stored inside McCartney bottle and kept in a refrigerator.

d) Surface preparation of the oranges

The primary purpose of surface preparation was to remove all contaminants that would hinder proper coating adhesion and to render a sound clean substrate suitable for firm bonding. The surface should be in paint ready condition. Mold, mildew and/or algae should be removed and sterilized with a 25% hypochlorite solution (1 gallon household bleach to 3 gallons water). The oranges will be soaked in the 25% hypochlorite solution for two minutes.

e) Film-forming dispersions methodology

1. Preparation of edible coatings HPMC

Hydroxypropylmethylcellulose **(HPMC)** coatings (3%) was prepared by dissolving 6.0 g of hydroxypropylmethylcellulose powder (Hangzhou Hongbo Chemical Co. Ltd, China) in 200 ml of water ethyl alcohol mixture (3:1L/L) at 80°C and stirred for 10 min by using magnetic stirrer. Ethyl alcohol was used to reduce drying time and obtain a transparent and shinny coating. 2% propylene glycol was also added in the formulation as plasticizer.

2. Preparation of edible coatings HPMCME:

75mg/ml of **(AEMO)** was added to the resulting solution of HPMC coatings. The mixtures was emulsified at room temperature using a rotor stator homogenizer ultraturrax (DI25 Yellow Line, IKA®, Germany) at 13,500rpm for 4min and then degassed at room temperature by means of a vacuum pump.

f) Application of the coatings

Oranges fruits were dipped in the film forming dispersions for 1min. Afterwards, they were hung up and dried at room temperature with natural convection for 2–3 h and then stored in perforated basket at 27±3°C and 50–60% R.H.

g) Treatments

T_o (control):- T_0 was selected as the control (untreated oranges)

T_1 Oranges was coated with hydroxypropyl-methylcellulose in addition to 75% *Moringa oleifera* (HPMCME)

T_2 Oranges was coated with hydroxypropyl-methylcellulose without 75% *Moringa oleifera* (HPMC)

The treated and untreated will be packed in small plastic basket and each basket contain 20 orange fruits. The basket will be stored at ambient temperature (27+2°C) and at 50-60% relative humidity .Physiochemical analysis will be carried out from 1-7weeks of coating.

Firmness:- Firmness was measured as the maximum penetration force (N) reached during tissue breakage, and determined with a 5 mm diameter flat probe. The penetration depth was 5 mm and the cross-head speed was 5 mm s^{-1} using a TA-XT2 Texture Analyzer (Stable Micro Systems, Godalming, UK), MA.

Oranges were sliced into halves and each half was measured in the central zone.

Ascorbic acid: - Ascorbic acid content was measured using 2, 5-6 dicholorophenol indophenols' method described by A.O.A.C 1994.

Percentage weight loss:- The water content of the orange fruit was determined using the Equation (1).Water content(%) $100 \times \frac{(M_1 - M_2)}{M_1}$

Where: M_1 = Mass of sample before drying in g. M_2 = Mass of sample after drying, in g.

h) Statistics

The results of this investigation are means of seven measurements. To verify the statistical significance of all parameters the values of means ± S.E. were calculated. SPSS software (version 12.0, SPSS Inc., US) was used for all statistical analysis for Analysis of variance. The significance level used was 0.05.

III. Results and Discussion

a) Weight loss

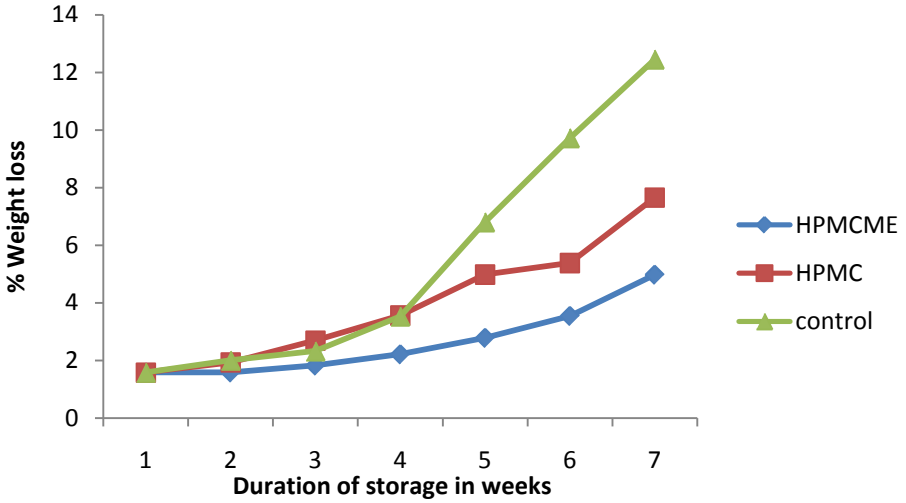

Fig1 : Effect of edible coatings from HPMCME and HPMC on % weight loss of orange stord at amblent temp

Weight loss which occurs in orange fruit during ripening is due to both two transpiration and respiration. Transpiration is associated with water vapour pressure of the surrounding atmosphere and the orange surface. Weight loss contributed by respiration is a result of carbon atoms, in the form of carbon dioxide molecules, leaving the fruits (Park, 2000).

The mean±SE value for the weight loss of HPMCME and HPMC were 40.58±0.47 and28.56± 0.98 while the mean±SE value for the weight loss of uncoated oranges was 26.71±1.04.

The result was most likely due to the fact that edible coatings based on hydrocolloids, because of their hydrophilic natures have poor barrier properties towards water vapour transition (Olivas and Barbosa-Canovas, 2005). Because of the regular structure and array of hydroxyl groups of cellulose derivatives, they tend to absorb and form strong hydrogen- bonds with water molecules of fruits and environment (Togrul and Arsalan, 2004).. Banks (1984), Maftoonazad and Ramaswamy (2005) and Navarro-Tarazaga *et al.*, (2008) found that edible coatings based on Na-CMC, HPMC and MC effectively decreased weight loss of banana, mandarin and avocado, respectively.

a) Firmness

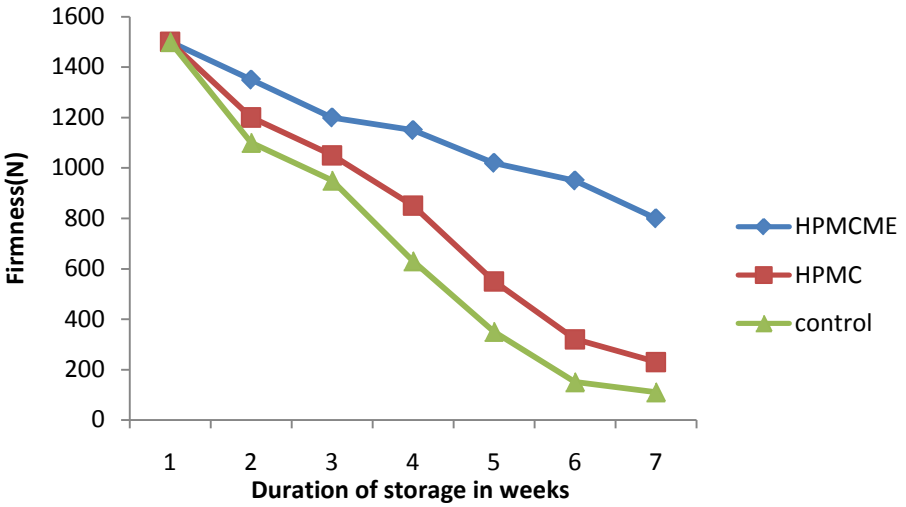

Fig 2 : Effect of edible coatings from HPMCME and HPMC on firmness of orange at ambient temp

In general, the firmness of orange fruit during ripening decreases. Softening in orange fruit may be largely due to the breakdown of starch to form sugars since starch granules could have a structural function in the cells (Cano *et al*., 1997). This could possibility be due to the breakdown of celluloses and other non pectic polysaccharides under the catalysis of cell wall hydrolysis enzymes (Wills *et al*., 1998). Figure 2 shows the firmness of coated and control oranges samples. As clearly shown in Figure 2, all edible coatings significantly ($P < 0.05$) retard the changes in orange firmness as compared to control samples. The effects of HPMCME and HPMC coatings on firmness of orange fruits stored at ambient temperature are shown in Fig.1 above .The mean ± SE values for the firmness of coated HPMCME and HPMC oranges were 7970±88.94 and 5700± 177.76 respectively while the mean±SE value for the firmness of uncoated oranges was 4790±197. Navarro-Tarazagav *et al*. (2008) also found that by increasing the concentration of fatty acids in edible coating based on HPMC, the weight loss of coated mandarins decreased but permeability of coating towards O_2 and CO_2 increased.

b) Ascorbic acid (AsA)

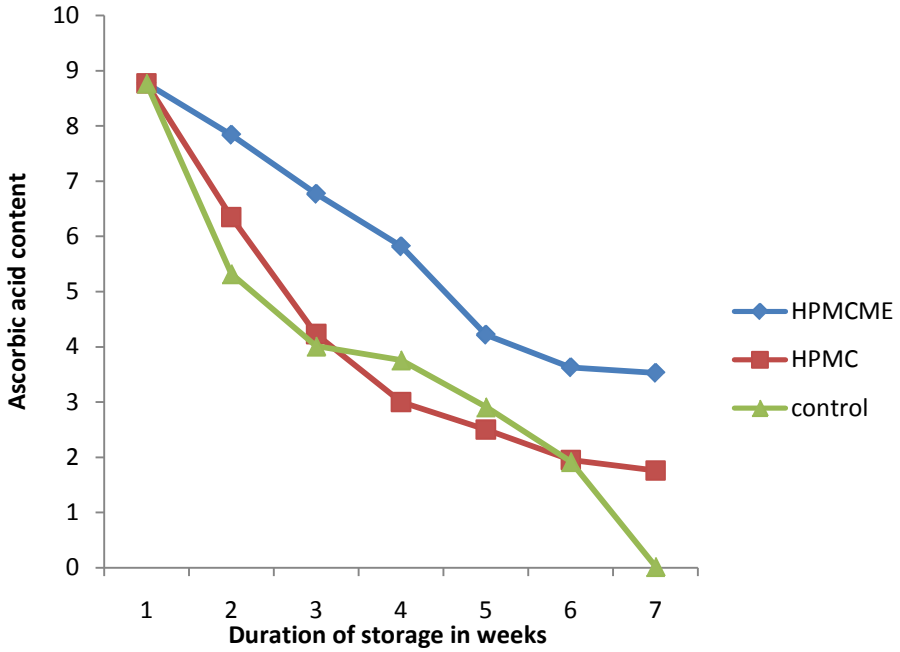

Fig 3 : Effect of edible coatings from HPMCME and HPMC on ascobic acid content of orange stored at ambient temp

The AsA content in the orange of storage at ambient temperature shown in Figure 3 above. The mean±SE value for the coated HPMCME and HPMC on vitamin C were 18.48 ± 0.47 and 27.77±0.81 for coated oranges while The mean±SE value for the vitamin C for uncoated oranges was 38.40±1.61.

The AsA content of coated HPMCME and HPMC treatment decreased gradually with prolonged storage time. Throughout the storage period, there were significant differences between control, coated HPMCME and HPMC ($P < 0.05$). The decrease in AsA level was associated with a reduced capability of preventing oxidative damage and with the incidence of physiological disorders during storage (Lin et al., 2008). AsA in a higher level in coated HPMCME and HPMC fruits than in control, similar to that reported by Dang et al. (2010), who found that the content of AsA of CA-coated fruits was higher than that of the control.

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Breeding and Productive Performance of Three Breeds of Rabbit in South-West Nigeria

By Dr. O. Olowofeso, A. J. Adejuwon, V. A. Ademokoya & S. O. Durosaro
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Abstract - Three breeds of rabbit-Giant Flemish (GF), Chinchilla (CH) and Rex (RX) were procured from a reputable farm in South-West Nigeria for crossbreeding to determine the productive performance of each breed and with the aim of selecting the most superior genotype(s) for increased rabbit production. Animals were paired and the pairing resulted into six genetic groups. A total of twenty-one animals (18 does and 3 bucks) were involved. Data on productive traits such as litter size at birth and at weaning, litter weight at birth and at weaning, gestation length in each female breed, percentage mortality during pre-weaning in each group and percentage survivability till weaning were collected for each crossing. Results shows that mean litter size at birth in the genetic groups ranged from 1.67±0.33 to 4.00±0.00 and mean litter size at weaning was between 1.67±0.33 and 3.00±0.58 in groups CH vs. RX and RX vs. GF, respectively. Mean litter weight at birth was between 39.18±0.74 and 43.56±0.88 g. In the genetic groups with the exception of CH vs. RX, mean litter size at birth (MLS@B) was greater than mean litter size at weaning (MLS@W). Gestation length of the breeds of rabbit used ranged from 29.67±0.66 to 30.33±0.67 days. Genetic group CH vs. GF has the highest mean litter weight at birth and highest mean litter weaning weight.

Keywords : *Breeding, hierarchic design, Nigeria, performance, rabbit.*

GJSFR-G Classification: *FOR Code: 060411*

Strictly as per the compliance and regulations of:

Breeding and Productive Performance of Three Breeds of Rabbit in South-West Nigeria

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Abstract - Three breeds of rabbit-Giant Flemish (GF), Chinchilla (CH) and Rex (RX) were procured from a reputable farm in South-West Nigeria for crossbreeding to determine the productive performance of each breed and with the aim of selecting the most superior genotype(s) for increased rabbit production. Animals were paired and the pairing resulted into six genetic groups. A total of twenty-one animals (18 does and 3 bucks) were involved. Data on productive traits such as litter size at birth and at weaning, litter weight at birth and at weaning, gestation length in each female breed, percentage mortality during pre-weaning in each group and percentage survivability till weaning were collected for each crossing. Results shows that mean litter size at birth in the genetic groups ranged from 1.67±0.33 to 4.00±0.00 and mean litter size at weaning was between 1.67±0.33 and 3.00±0.58 in groups CH vs. RX and RX vs. GF, respectively. Mean litter weight at birth was between 39.18±0.74 and 43.56±0.88 g. In the genetic groups with the exception of CH vs. RX, mean litter size at birth (MLS@B) was greater than mean litter size at weaning (MLS@W). Gestation length of the breeds of rabbit used ranged from 29.67±0.66 to 30.33±0.67 days. Genetic group CH vs. GF has the highest mean litter weight at birth and highest mean litter weaning weight. Based on the results of this study, it was concluded that genetic groups CH vs. GF, GF vs. RX, and GF vs. CH be considered for increased rabbit production in South-West, Nigeria.

Keywords : Breeding, hierarchic design, Nigeria, performance, rabbit.

I. Introduction

A mating design often encountered in breeding programme is the hierarchic design in which several females are mated to one male only. In hierarchic design, several progeny can be generated and such progeny are very easy to measure to provide useful data. With the increase in human population especially in developing country like Nigeria, the supply of enough animal protein from the five principal livestock species (cattle, sheep, goats, swine and poultry) had become impossible, hence the interest in microlivestock such as rabbit because its production has enormous potential in alleviating the problem of animal protein supply in developing economy according to Cheeke (1986); Biobaku and Dosunmu (2003); Fayeye and Ayorinde (2003). The important attributes of rabbits as microlivestock include small body size, short generation interval, ability to utilize less competitive feeds, rapid growth, potentials for genetic improvement and production of high quality meat and useful by-products (Cheeke, 1986; Egbo *et al.,* 2001; Herbert, 2011). Apart from high temperature usually above 30^{0}C which may impose undue stress on the animal, rabbit production can significantly contribute to man's need at all times. Genetic improvement of rabbit is important in order to increase their contribution to the much needed animal protein in Nigeria. A prerequisite for this improvement is the knowledge of their breeding pattern and the ability to select for highly prolific individuals. In searched literature, litter growth and weaning characteristics in two generations of straightbred and crossbred rabbits have been reported by Fayeye and Ayorinde (2003, 2010). However, because of inbreeding depression which is often associated with purebreeding activities in livestock production enterprise, it is desirable to encourage crossbreeding among breeds so as to exploit the full potentials of different breeds. Crossbreeding according to Nofal *et al.* (1997) and Oseni *et al.* (1997) is one of the fast tools offered to the livestock breeders to improve many traits in farm animals. Rabbits because of its enormous benefits associated with its production and with the belief that the microlivestock will certainly bridge the animal protein gap been experienced by man, it is imperative to give available rabbit breeds the needed attention just like other animal genetic resources so as to have more animal products that could supply the immediate needs of man.

In South-West Nigeria, three rabbit breeds available in most farms are the Giant Flemish, Chinchilla and the Rex. Other breeds abounds in some well organised farms in this part of the country include the New Zealand White, Californian White, etc. The productive performance of New Zealand White and Californian White in particular has been studied by Lukefahr and Hamilton (1997) and straight breeding as well as crossbreeding of these same breeds of rabbits have been reported by Fayeye and Ayorinde (2003); Odeyinka *et al.* (2008).

However and to our own knowledge, productive performance of Giant Flemish, Chinchilla and Rex breeds of rabbit involved in crossbreeding activities have not been reported. The objective of this study was to attempt several crossings involving these three breeds of rabbit so as to know the superior genotype(s) and their productive performance before intense

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selection and breeding programmes on them can be initiated.

II. Materials and Methods

a) Experimental site, sample size and animal management

A breeding farm in Camp, Odeda Local Government Area of Ogun State, Nigeria was used. Odeda Local Government Area shares boundary with Abeokuta South, Abeokuta North, Obafemi-Owode Local Governments and Oyo State in the South-West, East, and North respectively. The climate in the area is tropical with maxima rainfall from April-July and September-October. The average temperature in the area is about 32°C, but relative humidity can be as high as 95%. The study reported herein was carried out between January and April 2011. A total of twenty one rabbits (eighteen does and three bucks) of three different breeds were procured from a private farm in Ibadan, Oyo State, Nigeria. They were thereafter conveyed to Abeokuta.

The breeds of rabbit used in this study were Giant Flemish, Chinchilla and the Rex, respectively. The ratio of does to buck procured in each breed was 6:1. The dimension of each hutch used was 70 x 60 x 50-cm. Before taken the does to the bucks, the animals were allowed two weeks of adjustment period to the experimental site. Buck of each rabbit breed was kept in individual hutch and rabbit does were taken to the bucks. Six does of the same breed were mated to a buck. After mating, the does were taken to their designated hutches. Commercial diet and quality drinking water were provided *ad libitum* to the rabbits. Diet requirement was in accordance with what was suggested by Ouyed and Brun (2008) for these microlivestock. For increased libido, normal erection and ejaculation by the bucks, a drug called *Viagra* was procured from a pharmacy shop in Abeokuta, Nigeria and 0.75 mg of the drug was dissolved in the drinking water of the bucks at the same time. Pregnancy determination in the does was by abdominal palpation only. Pregnant does were allowed to kindle without any interference and kittens produced were nursed by the does for four weeks before they were weaned.

b) Mating patterns in this study

The three breeds of rabbit used were Giant Flemish (GF), Chinchilla (CH) and Rex (RX). The breeds were paired and the pairing resulted into six different combinations represented in Table 1.

c) Data collection and statistical analysis

Litter size at birth and at weaning as well as litter weight at birth and at weaning, gestation length in each female breed, percentage mortality during pre-weaning in each genetic group and percentage survivability till weaning were considered for each crossing. Descriptive statistics were carried out on the data generated and results were expressed as means and their standard error of means. To ascertain whether there are differences in means, Duncan Multiple Range Test (DMRT) was carried out with all the means. For percentage survivability (%S), the method used was similar to that of Odeyinka *et al.* (2008). In this study, %S was depicted as: %S = (100MLS@W)/MLS@B, and percentage mortality was obtained as: 100% - %S, where MLS@B = Mean litter size at birth and MLS@W = Mean litter size at weaning, respectively.

III. Results and Discussion

The productive performance or traits of the three breeds of rabbit used in several crossings are shown in Table 2. In this study, the mean litter size at birth in all the genetic groups ranged from 1.67±0.33 to 4.00±0.00 and mean litter size at weaning was between 1.67±0.33 and 3.00±0.58 in genetic groups CH vs. RX and RX vs. GF, respectively (Table 2).

The highest mean litter weight at birth was 43.56±0.88 and the least was 39.18±0.74 in genetic groups CH x GF and GF x CH. Similarly, the highest mean litter weight at weaning was also obtained in genetic group CH x GF. The least value for this trait was observed in mating pattern that involved Rex buck with Giant Flemish does. Gestation length of rabbit does involved in the breeding activities were almost same. Percentage survivability was very high in all the genetic groups. In this study, highest percentage mortality of 30.03% was observed in genetic group GF x RX (Table 2).

Apart from CH vs. RX, where mean litter size at birth and at weaning were low, all other values obtained for these economically important traits were consistent with values reported by Sorensen *et al.* (2001) and Odeyinka *et al.* (2008) for some breeds of rabbit. Mean litter weight at birth reported in literature oscillates between 38.95 and 42.31 g (Fayeye and Ayorinde, 2010). In the six mating patterns carried out in this study, the mean litter weight at birth was between 39.18±0.74 and 43.56±0.88 g for groups GF vs. CH and CH vs. GF, respectively. These values were consistent with values reported in literature. Apart from the genetic group CH vs. RX, where MLS@B and MLS@W are equal because of lack of pre-weaning mortality, in other groups, MLS@B was greater than MLS@W. A similar situation (i.e. LSB > LSW) in breeds of rabbit had been reported by Sorensen *et al.* (2001).

Gestation length of the breeds of rabbit used was not significantly ($P>0.05$) different among the genetic groups. The range of values for this trait in all the groups was from 29.67±0.66 to 30.33±0.67 days (Table 2). These values corresponds favourably to the 28.10 to 30.40 days and 29.92 days reported in southern and northern Nigeria by Odeyinka *et al.* (2008);

Akpa and Alphonsus (2008) for some breeds of rabbit. Also, the gestation length of the breeds of rabbit used was within the range of length of gestation reported by Sorensen *et al.* (2001). However, the gestation length for the group of rabbits used in this study was slightly lower that the values reported by Fayeye and Ayorinde (2010). The slight variation in values might be as a result of breed differences, feeding regime and other management strategies involved.

In this study, genetic group CH vs. GF has the highest mean litter weight at birth and highest mean litter weaning weight. This may be due to good mothering ability of the Giant Flemish does to their kittens or may be because they were able to acclimatise to the experimental site better than other breeds of rabbit used. Therefore, this genetic group may be selected for increased rabbit production in South-West Nigeria. Percentage survivability of the progeny of this particular genetic group till weaning was about 78%. In group CH vs. RX, mean litter size at birth and weaning were very low, but all the kittens produced survived and hence percentage survivability was 100%. In the absence of other genetic groups used in this study, rabbit breeders and farmers alike can also invest on this group and subsequently cross them with more prolific breeds for better performance. Apart from genetic group CH vs. RX where percentage mortality was zero, it was 18.26 to 30.03% in all other groups unlike the 16 to 19% previously reported by Sorensen *et al.* (2001) for breeds of rabbit. This therefore means that kittens of breeds of rabbit needs proper handling, balanced diet, sound medication and favourable production environment at all times to lessen this high mortality percentage.

IV. Conclusion

Based on the results of this breeding experiment, it was concluded that genetic groups CH vs. GF, GF vs. RX, and GF vs. CH be considered for increased rabbit production in South-West, Nigeria. Where resources are available, the mating patterns described in this study may be carried out with more animals and for prolong period too.

Table 1 : Six mating patterns involving three breeds of rabbit in South-West, Nigeria

No. of crossing	Sex		Resulting genotype or genetic group*
	Buck	Doe	
I	GFx	RX (3)	GFRX
II	RX x	GF (3)	RXGF
III	GF x	CH (3)	GFCH
IV	CH x	GF (3)	CHGF
V	RX x	CH (3)	RXCH
VI	CH x	RX (3)	CHRX

** GF = Giant Flemish, CH = Chinchilla and RX = Rex breeds of rabbit; Number in parenthesis represents number of does used in this study.*

Table 2 : Summary statistics of the productive performance of three breeds of rabbit in six mating patterns in South-West Nigeria*

Mating pattern or genetic group	Productive performance of three breeds of rabbit in six different mating patterns						
	Mean litter size at birth (MLS@B)	Mean litter size at weaning (MLS@W)	Mean litter weight at birth (MLW@B) (g)	Mean litter weight at weaning (MLW@W) (g)	Mean gestation length (GL) (days)	% Survivability (%S)	% Mortality (%M)
GF x RX	3.33±0.34	2.33±0.33	42.10±0.92	222.86±3.59	30.33±0.67	69.97	30.03
RX x GF	4.00±0.00	3.00±0.58	41.42±0.89	207.78±5.96	30.33±0.67	75.00	25.00
GF x CH	3.67±0.33	3.00±0.58	39.18±0.74	222.22±7.95	30.00±0.58	81.74	18.26
CH x GF	3.00±0.00	2.33±0.33	43.56±0.88	271.43±40.08	29.67±0.66	77.67	22.33
RX x CH	4.00±0.00	3.00±1.00	40.92±1.11	214.44±7.09	29.67±0.66	75.00	25.00
CH x RX	1.67±0.33	1.67±0.33	40.60±1.15	216.00±6.48	30.33±0.67	100.00	0.00

** Abbreviations used in this table are as defined within text above.*

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Effects of Edible Coatings from Aloe Vera Gel on Quality and Postharvest Physiology of Ananas Comosus (L.) Fruit During Ambient Storage

By Adetunji C. O, Fawole O.B, Arowora K.A, Nwaubani S.I, Ajayi E. S, Oloke J. K, Majolagbe O. N, Ogundele B. A, Aina J. A, Adetunji J. B

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Abstract- Pineapple (Ananas comosus (L.) Merr.) is an important fruit crop grown in Nigeria. Extension of the shelf life of pineapple fruits continues to be a challenge in Nigeria. The search for safe, healthy and environmental friendly treatments has led to increased interest in research into edible and biodegradable films and coatings. The aim of this study was to evaluate the effect of Aloe vera gel as an edible coating on weight loss, ascorbic acid, pH and firmness in order to extend the shelf-life of pineapple stored at ambient temperature of (27+2oC) and relative humidity of 55-60% for seven weeks. The above parameters which are related to post-harvest quality loss were however significantly controlled in the pineapple coated with A. vera gel. The storability of pineapple fruits was extended by seven weeks. It was concluded that A. vera gel used as a coating for pineapple could serve as an alternative to post-harvest chemical treatments.

Keywords : *Pineapple, Edible coating , Aloe vera gel, biodegradable films.*

GJSFR-G Classification: *FOR Code: 060199*

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I. Introduction

Postharvest losses of tropical fruits are a serious problem because of rapid deterioration during handling, transport and storage (Yahia, 1998). Edible coatings are thin films that improve produce quality and can be safely eaten as part of the product and do not add unfavourable properties to the foodstuff (Baldwin, 1994; Ahvenainen, 1996). Edible coatings provide a barrier against external elements and therefore increase shelf life (Guilbert *et al.*, 1996) by reducing gas exchange, loss of water, flavours and aroma and solute migration towards the cuticle (Saltveit, 2001). The first kind of edible coatings were water–wax microemulsions, used since the 1930s to increase brightness and colour in fruits, as well as fungicide carriers. Water loss is another problem that can be controlled with edible wax coatings (Debeaufort *et al.*, 1998). Edible waxes can also offer protection against cold damage under storage (Nussinovitch & Lurie, 1995). Nowadays, an edible coating is made of polysaccharides, proteins and lipids (Guilbert *et al.*, 1996) and resins as well (Baldwin *et al.*, 1995).

Pineapple (*Ananas comosus* (L.) Merr.) is an important fruit crop grown in many tropical and subtropical countries. Fresh pineapple fruit is perishable (Chen and Paull, 2001; Avallone *et al.*, 2003; Soares *et al.*, 2005; Wilsonwijeratnam *et al.*, 2005; Ko *et al.*, 2006).

The fruit is known for its nutritive and health promoting properties. It is commonly used as table fruit or in desserts. The shelf life of ripe pineapple is short and limited to 4-6 days (Hajare *et al.*, 2006). Fresh pineapple contains thick, thorny inedible peel and a large crown, which consumes storage space and also results in higher transportation costs (Fernandes *et al*, 2006)

Currently, there is an increasing interest in the use of *Aloe vera* gel in the food industry, being used as a source of functional foods in drinks, beverages and ice creams (Moore and MacAnalley, 1995). Nevertheless, processing techniques used to obtain *A. vera* gel are very important to ensure the product quality and to maintain almost all the bioactive components (He et al., 2005).Recently, Adetunji *et al* .,2012 discover that *Aloe vera* gel could prolong the shelf life of citrus stored at ambient condition for seven weeks while it maintain all the good qualities of oranges. The aim of this work was to study the effect of *A. vera*, applied as an edible coating, on the change in physicochemical parameters and shelf life in Pineapple, related to fruit quality during ambient storage for a period of seven weeks.

II. Materials and Methods

a) Preparation of edible coatings

i. Preparation of Aloe vera gel

Matured leaves of *Aloe vera* plant was harvested and washed with a mild chlorine solution of 25%. *Aloe vera* gel matrix was then separated from the outer cortex of leave and this colorless *hydroparenchyma* was ground in a blender .The resulting mixture was filtered to remove the firbres. The liquid obtained constituted fresh *Aloe vera* gel. The gel matrix was pasteurized at 70°C for 45min. For stabilized the gel was cooled immediately to an ambient temperature and ascorbic acid (1.9 - 2.0g L^{-1})was then

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added citric acid (4.5 - 4.6gL^{-1}) was added to maintain the pH at 4.The viscosity of the stabilized Aloe vera gel and it coating efficiency was improved by using 1% commercial gelling agent and was used as coating agent .It was later be stored in brown Amber bottle to prevent oxidation of the gel. Adetunji *et al* (2012).

a. Materials

Freshly harvested Pineapple were procured from the local market of Ilorin, kwara state, Nigeria. They were selected on the basis of sized, color and absence of external injuries. Fresh leaves of *Aloe vera* was obtained from Nigeria stored products research institute garden.

b. Surface preparation of the pineapple

The primary purpose of surface preparation was to remove all contaminants that would hinder proper coating adhesion and to render a sound clean substrate suitable for firm bonding. The surface should be in paint ready condition. Mold, mildew and/or algae should be removed and sterilized with a 25% hypochlorite solution (1 gallon household bleach to 3 gallons water). The Pineapple will be soaked in the 25% hypochlorite solution for two minutes.

ii. *Treatments*

T_o (control):- T_0 was selected as the control (untreated Pineapple)

T_1 Pineapple was coated with *Aloe vera* gel.

The treated and untreated Pineapple were packed in small plastic baskets and each basket contained 20 Pineapple fruits. The baskets were stored at ambient temperature (27+2°C) and at 50-60% relative humidity .Physiochemical analysis were carried out from 1-7weeks after coating.

a. Weight loss

To evaluate weight loss, separate samples in 3 replicates of each treatments were used. The same samples were evaluated for weight loss each time at weekly intervals until the end of experiment. Weight loss was determined by the following formula:

$$\text{Weight loss (\%)} = [(A-B)/A] \times 100$$

where A indicates the fruit weight at the time of harvest and B indicates the fruit weight after storage intervals. (A.O.A.C., 1994)

b. Firmness

Firmness was measured as the maximum penetration force (N) reached during tissue breakage, and determined with a 5 mm diameter flat probe. The penetration depth was 5 mm and the cross-head speed was 5 mm s^{-1} using a TA-XT2 Texture Analyzer (Stable Micro Systems, Godalming, UK), MA. Pineapple were sliced into halves and each half was measured in the central zone.

c. pH

After firmness analysis, oranges were cut into small pieces and homogenized in a grinder, and 10 g of ground Pineapple was suspended in 100 ml of distilled water and then filtered. The pH of the samples were assessed using a pH meter (pH-526; WTW Measurement Systems, Wissenschaftlich, Technische Werksta¨tten GmbH, Wellhelm, Germany)

d. Ascorbic acid

Ascorbic acid content was measured using 2,5-6 dicholorophenol indophenols' method described by A.O.A.C 1990.

b) Statistics

The results of this investigation are means of seven measurements. To verify the statistical significance of all parameters the values of means ± S.E. were calculated. SPSS software (version 12.0, SPSS Inc., US) was used for all statistical analysis for Analysis of variance. The significance level used was 0.05.

c) Firmness

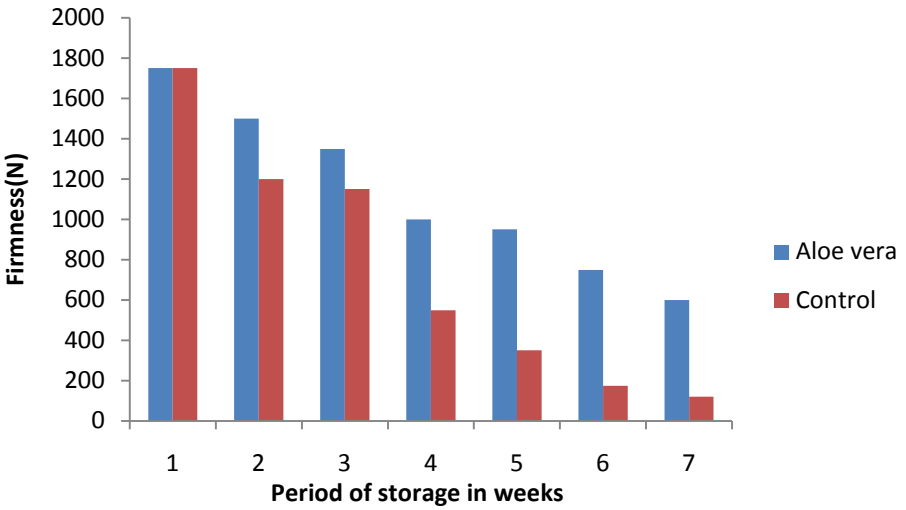

Fig 1 : Effect of Aloe vera gel coatings on Firmness of pineapple at ambient temp

Firmness is an important factor that influences the consumer acceptability of fresh-cut fruits and it is related to water content and metabolic changes (Rojas-Grau *et al.*, 2008).

The mean±SE value for the firmness was 7900±157.43N and while the mean±SE value for the of uncoated was 5295±233.11N. The analysis of variance shows that edible coating from *Aloe vera* gel on firmness of pineapple were significant ($p<0.05$) compared to the uncoated.

Lerdthanangkul and Krochta (1996) also made similar observations and concluded that coatings and/or films significantly affected firmness of fruits in storage. The softening process in pineapple has been reported to be dependent on the increase in polygalacturonase, ßgalactosidase and pectinmethylesterase activities Rem´on *et al.*, 2003, being responsible for fruit quality loss. *A. vera* treatment significantly reduced the firmness losses (more than 50%) during ambient storage compared with control fruits. In addition, *A. vera* gel probably had some effects on the reduction of cell wall degrading-enzymes responsible for pineapple softening. These results show beneficial effects of the *Aloe vera* coating on increasing the pineapple shelf life, since it has been postulated that fruit softening and texture changes during pineapple storage determine fruit storability and shelf life, as well as reduced incidence of decay and less susceptibility to mechanical damage (Batisse *et al.*, 1996; Vidrih *et al.*, 1998).

d) Water content

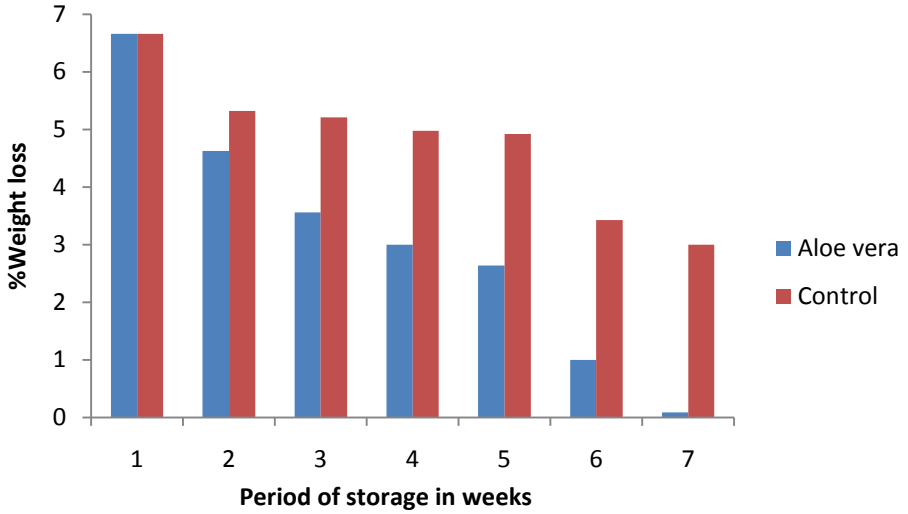

Fig 2 : Effect of Aloe vera coatings on % Weight loss of pineapple at ambient temp

The mean±SE value for the weight loss of coated pineapple was 33.52 ±0.46 % while the mean±SE value for the weight loss of uncoated pineapple was 21.58±0.83%. The analysis of variance shows that edible coating from *Aloe vera* gel on the percentage of weight loss of pineapple were significant ($p<0.05$) compared to the uncoated.

These results are in agreement with those of Mahmoud and Savello (1992) and Avena-Bustillos *et al.* (1997) who concluded that coatings and/or films significantly conserved water content.

Post harvest weight changes in fruits and vegetables are usually due to the loss of water through transpiration. This loss of water can lead to wilting and shriveling which both reduce a commodity's marketability. Edible films and coatings can also offer a possibility to extend the shelf life of fresh-cut produce by providing a semi-permeable barrier to gases and water vapor and therefore, they can reduce respiration, enzymatic browning and water loss (Guilbert, 1986; Baldwin & Nisperos-CarriedoBaker, 1995).

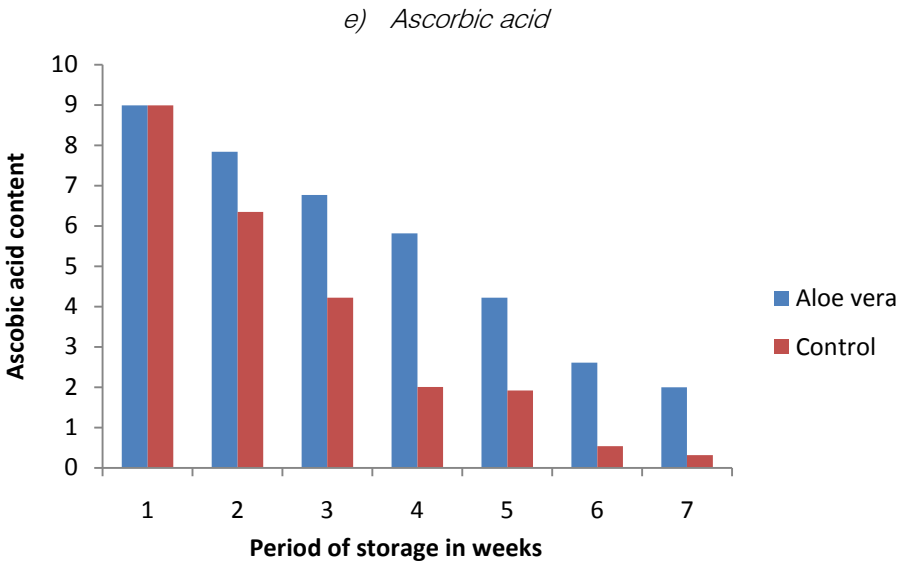

Fig 3 : Effect of Aloe vera coatings on Ascobic acid content of pinapple at ambient temp

The mean±SE value for the coated pineapple for Vitamin C was 38.25±0.99 and while the mean±SE value for the of uncoated was 24.35±1.22. The analysis of variance shows that edible coating from *Aloe vera* gel on Ascorbic acid of pineapple were significant ($p<0.05$) compared to the uncoated.

Ascorbic acid content. Ascorbic acid is lost due to the activities of phenoloxidase and ascorbic acid oxidase enzymes during storage (Salunkhe *et al*., 1991). Weichmann *et al*. (1985), while studying green bean, spinach and broccoli, postulated that the lower the oxygen content of the storage atmosphere, the smaller is the loss of ascorbic acid. The claim was that the oxidation of Vitamin C was mainly regulated by ascorbic acid oxidase and other oxidases, most of which had a low affinity for oxygen. Ascorbic acid content decreased for cherries stored at both ambient temperature and cold temperature.

Aloe vera gel coatings were effective in reducing the ascorbic acid loss for both storage conditions (Fig. 3). At the ambient temperature, the ascorbic acid contents of *Aloe vera gel* coated pineapple were significantly different from the control orange. The reduction of ascorbic acid loss in coated orange was due to the low oxygen permeability of *Aloe vera gel* coating which lowered the activity of the enzymes and prevented oxidation of ascorbic acid.

The effect of low temperature significantly reduced the ascorbic acid loss. This shows the effect of temperature on the activities of the related enzymes.

III. Conclusion

Aloe vera gel, applied as edible coating in pineapple fruit, has beneficial effects in retarding the ripening process. This treatment was effective as a physical barrier and thus reduced the weight loss during postharvest storage. In addition, *A. vera* gel delayed softening, Ascorbic acid, and maintained the quality of pineapple fruit.

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3. Submission of Manuscripts,

4. Manuscript's Category,

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The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript--must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing references at this point.

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

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than lone rationale. The author can at this moment go straight to

shortening the outcome. Sum up the study, with the subsequent elements in any summary. Try to maintain the initial two items to no more than one ruling each.

- Reason of the study - theory, overall issue, purpose
- Fundamental goal
- To the point depiction of the research
- Consequences, including definite statistics - if the consequences are quantitative in nature, account quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
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- Center on shortening results - bound background information to a verdict or two, if completely necessary
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The **Introduction** should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

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- Shield the model - why did you employ this particular system or method? What is its compensation? You strength remark on its appropriateness from a abstract point of vision as well as point out sensible reasons for using it.
- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled the declared objectives.

Approach:

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- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

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- Report the method (not particulars of each process that engaged the same methodology)
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- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

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What to keep away from

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- Skip all descriptive information and surroundings - save it for the argument.
- Leave out information that is immaterial to a third party.

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The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

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- Present a background, such as by describing the question that was addressed by creation an exacting study.
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Approach

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- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
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Approach:

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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
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Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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