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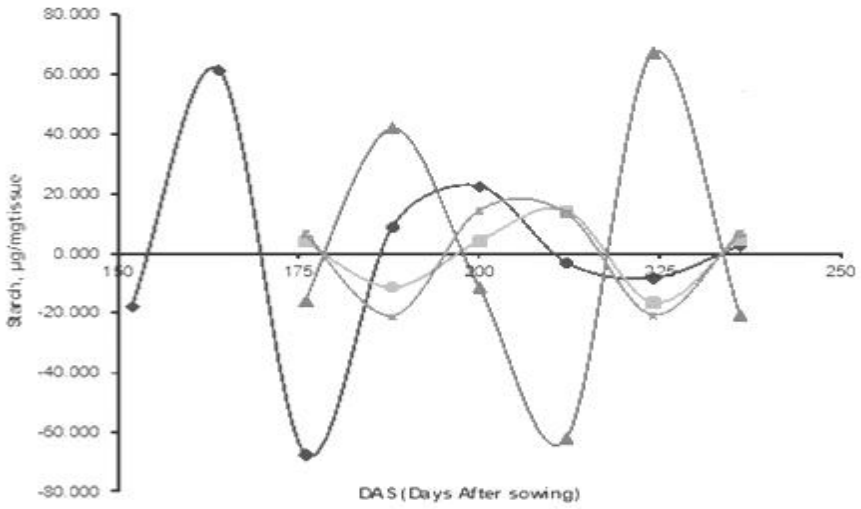

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RESEARCH ARTICLE

ESTIMATION OF STARCH CONTENT IN THE TREATED PERISPERM OF EURYALE FEROX SALISB. (MAKHANA) DUE TO POLYNOMIAL REGRESSION FIT EQUATION

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ABSTRACT

Euryale ferox Salisb. (fox nut / makhana / gorgon plant) belonging to water lily family viz, Nymphaeaceae, is one of the important cash crops of North Bihar, particularly in the districts of Darbhanga, Madhubani, Saharsa, Purnea and Katihar. Polynomial Regression is a form of linear Regression in which the relationship between the independent Variable x and the dependent Variable Y is modelled as an nth degree polynomial. Polynomial regressions, also known as Polynomial least squares fittings.Starch was the higest content of *Euryale ferox*, its structure and characteristics were critical in processing. Edible perisperm of makhana costitutes 80% starch .Nath & Chakraborty (1985) reported 77% starch in the perisperm. Starch content in the kernel of over – mature fruits (236 DAS,day after sowing)) in comparison to 1/3rd mature (176 DAS) ones is about 0.96 : 1: 1-fold respectively under conditions of 0.0001% , 0.001%, & 0.01% kinetin after 1min and the ratio is about 1: 1.26: 1.46-fold in 5min treatment, the higher concentration of treatment, the higher amount of starch content is produced.

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INTRODUCTION

The main edible portion of Makhana is its white perisperm inside the seed which is consumed mainly in its popped form either as snacks or as desserts (Payas). Euryale ferox (Makhana) is the foremost aquatic macrophyte grown as cash crop in the non – calcareous Kosi- Kamala belt (Jha, 2002).Starch was the highest content of *Euryale ferox* its structures and characteristics were critical in the processing. Some characteristics of *Euryale ferox* starch were compared with potato starch and corn starch, including physical and chemical properties, molecular structure and rheological properties of starch. *Euryale ferox* starch contained of 11.79% of water, 0.04% of fat, 0.07% ashes. *E.ferox* have comparatively lower nutrient values but presence of high ascorbic acids in perisperm (101 mg / 100g fresh wt), phenols (0.28per 100g fresh wt of endosperm) and phytosterol (0.16 per 100 g fresh wt. of perisperm) may be responsible for its medicinal properties. Makhana is best grown in age – old perenial water bodies with a rich mucky bottom providing nutrients to the plants. Growth of plants is not proper in freshly excavated ponds or water area because they lack the highly nutritive mucky bottom (Thakur, 1978). Fish farmers of the banper subcaste are skilled in harvesting Makhana seeds from the pond bottom (Jha, 2002). Read (1946) reported biochemical composition of *E.ferox* containing carbohydrate (75.7%), Protein (9.9%), fat (0.3%), and ash (0.6%). According to Phang (2002), protein of Arthrospira (=Spirulina), a non – conventional aquatic source of nutrition, contains isoleucine (3.5- 4.1%), leucine (5.4- 5.8%), lysine (2.9- 4.0%), methionine (3.5- 4.1%), phenylalanine (2.8- 4.0%), threonine (3.2- 4.2%), tryptophan (0.91- 1.1%) and valine (4.0- 6.0%). The seeds are mostly used as stomachic, for articular pains, micturition and for seminal loss (Roi, 1950). Because of its less fat contents it is ideal for invalids. Also, these are used as tonic for seminal organs (Crevost *et al.*, 1920), as well as remedy for diseases of the spleen and gonorrhea. Jha (1987) reported that net protein utilization (NPU 49.3), true digestibility (TD 89.6) and apparent digestibility (AD 69.1) of makhana were comparable to the values of most cereals. The above value were lower when compared to soyabean, egg and human and cow milk (Jha, 1991).

MATERIAL AND METHODS

The fruit samples were collected at eight different stages of their maturation and development. The first collection of fruit samples of Makhana (*Euryale ferox* Salisb.) was done at immature stage (i.e 152 DAS) in the year 2011. Subsequent fruit samplings were made at regular interval of 12 days i.e at 1/4th mature stage (164 DAS) , 1/3rd mature stage (176 DAS) , ½ mature stage (188 DAS) , 2/3rd mature stage (200 DAS) , 3/4th mature stage (212 DAS) , fully mature stage (224 DAS) and finally at the over- mature stage (236 DAS) stage . The

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fruits were treated with three different concentrations i.e 0.0001%, 0.001% and 0.01% of kinetin at the six stages of fruit maturation and development. Thereafter, chemical treatment was made at beginning from 1/3rd mature (176 DAS) to over mature stage (236 DAS).

Table / Figure No.2E: Polynomial Regression Fit on the basis of the Equation Y = a + bX + cX^2 + dX^3

DAS(X)	Perisperm of *Euryale ferox* Salisb.			
	Starch (Mean), µg/mg tissue			
	Control (Y1)	Kinetin Treatment for 1 Minute		
		0.0001% (Y2)	0.001% (Y3)	0.01% (Y4)
152	686.30			
164	491.48			
176	220.28	223.22	600.27	349.90
188	251.20	249.95	625.90	430.60
200	280.85	278.27	654.20	461.73
212	297.62	304.18	675.31	437.53
224	325.90	324.63	741.92	454.95
236	325.90	465.75	332.50	703.55
Y = a + bX + cX^2 + dX^3				
a	30510.20281	-24584.14298	93462.63428	-74347.83927
b	-441.36570	373.50280	-1415.21890	1113.62750
c	2.13013	-1.87433	7.15182	-5.51432
d	-0.00340	0.00314	-0.01198	0.00908
DAS(X)	*Predicted Y1*	*Predicted Y2*	*Predicted Y3*	*Predicted Y4*
152	704.006			
164	429.796			
176	287.577	219.081	616.017	342.884
188	242.119	261.348	583.713	451.728
200	258.191	274.063	665.479	447.378
212	300.562	289.797	737.127	423.979
224	334.002	341.117	674.464	475.677
236	323.279	460.594	353.301	696.614
DAS(X)	*Residuals Y1*	*Residuals Y2*	*Residuals Y3*	*Residuals Y4*
152	-17.706			
164	61.684			
176	-67.297	4.139	-15.747	7.016
188	9.081	-11.398	42.187	-21.128
200	22.659	4.207	-11.279	14.352
212	-2.942	14.383	-61.817	13.551
224	-8.102	-16.487	67.456	-20.727
236	2.621	5.156	-20.801	6.936

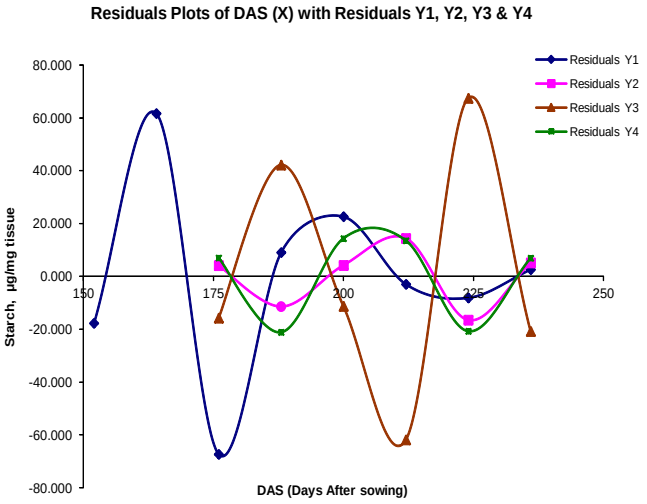
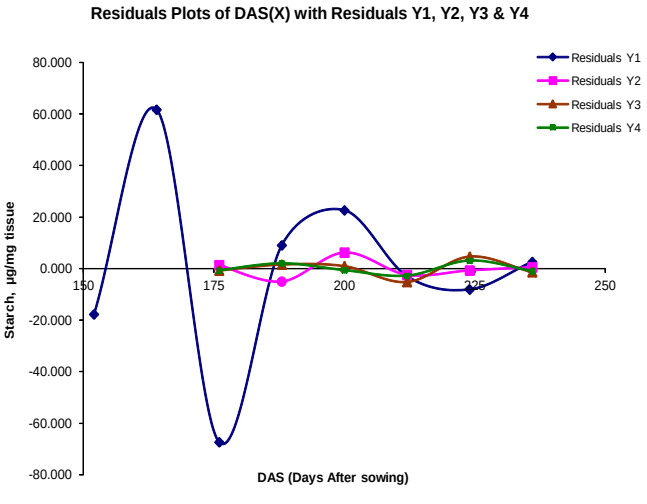


Table / Figure No.2F: Polynomial Regression Fit on the basis of the Equation Y = a + bX + cX^2 + dX^3

DAS(X)	Perisperm of *Euryale ferox* Salisb.			
	Starch (Mean), µg/mg tissue			
	Control (Y1)	Kinetin Treatment for 5 Minute		
		0.0001% (Y2)	0.001% (Y3)	0.01% (Y4)
152	686.30			
164	491.48			
176	220.28	393.95	344.13	198.23
188	251.20	421.33	373.37	222.12
200	280.85	449.63	401.67	250.43
212	297.62	449.47	421.63	279.67
224	325.90	459.68	449.93	307.97
236	325.90	477.78	449.93	307.97
Y = a + bX + cX^2 + dX^3				
a	30510.20281	-7406.30484	3257.48168	7018.98303
b	-441.36570	109.98797	-49.52295	-106.67531
c	2.13013	-0.51589	0.26917	0.54552
d	-0.00340	0.00081	-0.00046	-0.00091
DAS(X)	*Predicted Y1*	*Predicted Y2*	*Predicted Y3*	*Predicted Y4*
152	704.006			
164	429.796			
176	287.577	392.555	344.867	198.975
188	242.119	426.385	371.773	220.100
200	258.191	443.363	400.687	251.059
212	300.562	451.895	426.790	282.451
224	334.002	460.389	445.261	304.875
236	323.279	477.254	451.281	308.930
DAS(X)	*Residuals Y1*	*Residuals Y2*	*Residuals Y3*	*Residuals Y4*
152	-17.706			
164	61.684			
176	-67.297	1.395	-0.737	-0.745
188	9.081	-5.055	1.597	2.020
200	22.659	6.267	0.983	-0.629
212	-2.942	-2.425	-5.160	-2.781
224	-8.102	-0.709	4.669	3.095
236	2.621	0.526	-1.351	-0.960



For the purpose of chemical treatment the fruits while intact on the plants were dipped for one minute and five minute separately in each of the solutions of three different concentrations i.e. 0.0001%, 0.001% and 0.01% of Kinetin. All such treated fruits were properly tagged mentioning the concentration of treated hormone with date of chemical treatment and the fruits were picked after 12 days of chemical application. However, no chemical treatment was made in the fruits at immature (152 DAS) and 1/4th mature (164 DAS).

Estimation of Starch **-** The estimation of starch content was made with with the help of Anthrone method (Mc Cready *et al*. 1950). In a centrifuge tube 0.5 ml (100mg / ml GDW) tissue homogenate was taken and then 1 ml each of 10% $ZnSO_4$ and 0.5N NaOH was added and mixed. The mixture was subjected to centrifugation at 3000rpm for 20 minutes. The aliquot was collected in another tube (for being used in the estimation of total sugar) whereas the precipitate was left overnight in an inverted position to dry. Next day, 2ml of 72% perchloric acid was added to the fully dried precipitate in order to dissolve starch. Then 0.5 ml starch solution was taken in another test tube and the volume was made 2 ml by addition of G.D.W. Thereafter, 8ml anthrone reagent in 80% sulphuric acid was added to the above suspension and mixed. The tube was then heated for 7 mints at 90C in water bath and then on it was cooled in running tap water. The colour intensity was read at 625nm against the reagent blank. The amount of starch in the tissue was determined with the help of standard curve of starch by using the conversion factor of 0.9 (Snell & Snell , 1953)

RESULT AND CONCLUSION

The biochemical investigations in perisperm (seed) of Makhana (*Euryale ferox* Salisb.) both the treated fruits and control ones were made for the metabolites like starch . The experimental value of starch under conditions of both control and chemical treatment as well as the predicted / theoretical values on the basis of Polynomial Regression Fit Equation Y= a+ bX+ Cx^$_2$ + dX^$_3$ in the perisperm during fruit development due to the effect of Kinetin treatment (0.0001%, 0.001%, 0.01%) for 1min and 5 min have been presented in Tables / Figures 2E and 2F respectively. **Perisperm: Effect of 1min kinetin treatment -** In the perisperm of 0.0001% kinetin treated fruits for 1min the starch content was lowest at 1/3rd mature stage (176 DAS), which suddenly increased 1.11- fold at over - mature stage (236 DAS).

In the perisperm of 0.001% kinetin treated fruits for 1min the starch content was low at 1/3rd mature stage (176 DAS) which increased 1.04- fold at fully mature stage (224 DAS) and thereafter it suddenly decreased at over - mature stage (236 DAS) .

In the perisperm of 0.01% kinetin treated fruits the starch content was low at 1/3rd mature stage (176 DAS) which increased considerably 1.30-fold at fully mature stage (224 DAS) and thereafter it increased suddenly 1.54-fold at over - mature stage (236 DAS).

Perisperm : Effect of 5min kinetin treatment **-** In the perisperm of 0.0001% kinetin treated fruits for 5min the starch content was low at 1/3rd mature stage (176 DAS) which increased suddenly at ½ mature stage (188 DAS) and thereafter it increased 1.21- fold in over - mature fruits (236).

In the perisperm of 0.001% kinetin treated fruits for 5min the starch content was low at 1/3rd mature stage (176 DAS) which increased 1.3 0-fold in over - mature fruits (236 DAS).

In the perisperm of 0.01% kinetin treated fruits for 5min the starch content was low in 1/3rd mature stage (176 DAS) which continuously increased 1.55- fold at fully mature stage (224 DAS) and thereafter it similar condition in 1-fold in over- mature fruits (236 DAS) increased in over-mature fruits (236 DAS).

In the perisperm of 0.01% GA_3 treated fruits for 1min the starch content declined considerably 1.11- fold at 1/3rd mature (176 DAS) and thereafter it increased considerably in the continuous manner upto 1.38-fold in over - mature fruits (236 DAS).

Tables / Figures 2E and 2F representing Polynomial Regression Fit Equation of Protein content of *Euryale ferox* Salisb.

DISCUSSION

There is an increase in starch content at over – mature (236 DAS) stage as compared to 1/3rd mature stage (176 DAS) in 0.01% kinetin treated fruits (Perisperm) after 1 min and 5 min which is about 20%. However, there is an increase in starch content at over – mature stage (236 DAS) as compared to 1/3rd mature stage in 0.01% kinetin treated perisperm after 1min and 5 min. The changes in starch content in the kernel both in control fruits as well as in 0.0001%, 0.001% & 0.01% Kinetin treated fruits exhibit significant variation during course of fruit maturation. Starch accumulation is a general feature of developing seeds (Kramer & Kozlowski 1979, Singh & Jambunathan 1984). Amylose content of most reserve starches as in maize (Baba *et al*., 1981), Pea (costers & Bill aderis 1982) and barley (Young *et al*., 1986), increases with increasing age of the tissue examined.

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