A PROJECT WORK

ON

"DETERMINATION OF EDIBLE PROPERTIES OF OIL IN THE SEEDS OF *Echinochloa colona*"

PAPER: 496B

SUBMITTED BY

Sourav Das

ROLL- PG/VUEGS08/ZOO-IVS NO- 0026

FOR PARTIAL FULFILLMENT OF M.SC IN ZOOLOGY

EGRA SSB COLLEGE, VIDYASAGAR UNIVERSITY

UNDER THE SUPERVISION

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CERTIFICATE OF COMPLETION OF PROJECT WORK

This is to certify that Mr. Sourav Das, a student of the MSc IV Semester Zoology program at Egra Sarada Shashi Bhusan College, has successfully completed his project work entitled "DETERMINATION OF EDIBLE PROPERTIES OF OIL IN THE SEEDS OF *Echinochloa colona*" under my supervision.

During his MSc in Zoology program, Mr. Sourav Das displayed exemplary dedication, diligence, and passion for the subject matter. Throughout the duration of the project, he exhibited strong research skills, critical thinking abilities, and demonstrated a comprehensive understanding of the concepts and theories relevant to the field of Zoology.

His project work showcased originality and intellectual curiosity, as he conducted an in-depth analysis of the subject matter. Mr. Sourav Das demonstrated exceptional competence in the design, execution, and data analysis of his research, highlighting his strong grasp of scientific methodologies.

His commitment to the project was commendable, and he displayed exceptional time management and organizational skills in completing the project within the designated timeframe.

Based on his impressive performance, I have no doubt that Mr. Sourav Das will make significant contributions to the field of Zoology in the future.

I, therefore, recommend and endorse his project work for the fulfillment of his MSc in Zoology. I wish him all the best for his future endeavors.

Congratulations, Mr. Sourav Das, on this remarkable achievement!

Sincerely,

Declaration

I hereby declare that this dissertation entitled "DETERMINATION OF EDIBLE PROPERTIES OF OIL IN THE SEEDS OF *Echinochloa colona*" was carried out by me for the degree of M.Sc in Zoology, under the supervisions of “Dr. Dipak kr. Tamili”, Hon’ble Pricipal; “Dr. Sudipta Kumar Ghorai”, Associate Professor, PG department of Zoology, “Dr. Nirmal Kr. Hazra” Associate Professor, Department of chemistry, Egra SSB College.

The interpretation put forth are based on my reading and understanding of the original texts and they are not published anywhere in the form of books, monographs or articles. The other books, articles and websites, which I have made use of are acknowledged at the respective place in the text for the present work, which I am submitting to the Egra SSB college under Vidyasagar University, no degree or diploma or distinction has been conferred on me before, either in this or in any other University/Institute.

Place : Egra ……

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Registration No – 1081796 of 2018 – 2019

Acknowledgement

I would like to express my deep and sincere gratitude to my supervisors, “Dr. Dipak kr. Tamili”, Hon’ble Pricipal; “Dr. Sudipta Kumar Ghorai”, Associate Professor, PG department of Zoology, “Dr. Nirmal Kr. Hazra” Associate Professor, Department of chemistry, Egra SSB College, for giving me the opportunity to do the project work and providing his invaluable guidance throughout the duration of my dissertation work. It was a great privilege and honor to study under his guidance.

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Finally I would like to thanks my parents and my friends for generosity with which they have accepted to shoulder their burden of hectic schedule during the work and extended their best possible cooperation so that I could devote myself to the project work.

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CONTENTS

ABSTRACT

Echinochloa colona belongs to the family Poaceae. It is used as bioherbicide for its phytotoxic effect and also used as gluten free grain for gluten sensitive patient. The project work is based on to collect the oil from its seeds and to determine the edible properties like acid value, saponification value and iodine value. The study is also designed to compared these edible properties with other oils, which are commonly available in the market. The acid value of this oil is 1.91mg KOH/g, which is in the range comparing with other edible oils. The saponification value and iodine values are respectively 188 mg KOH/g and 63g/100g oil which are also similar with other edible oils. The yield of this oils is 100ml/kg, which is much lower than the other oil seeds. This low quantity oil problem could be solved by producing hybrid seeds, applying biotechnology in future. Acid value, saponification value and iodine value are three important parameter of any edible oil. Though these three nutritional parameter are range able in the oil, so the seeds of *Echinochloa colona* can be used as alternative oil source.

Key Words: *Echinochloa colona,* bioherbicide, phytotoxic, gluten, saponification, edible, biotechnology, nutritional.

INTRODUCTION

Echinochloa colona belongs to the family Poaceae commonly known as "awnless barnyard grass" is a type originating from tropical Asia and distributed throughout India (Borkar *et al*., 2015). It is a useful bio herbicide for controlling agricultural weed because of its phytotoxic effects (Gomma *et al*., 2012). This grass produces gluten free grain which play an important role for gluten sensitive patients (Murugesan *et al*., 2015). This study was designed to isolate the oil from *Echinochloa colona* seeds and to characterize its edible properties. Edible oils are vital stuff in our day-to-day life which not only provide energy but also give many essential fatty acids and many fat-soluble vitamins (Zahir *et al*., 2014). In our daily requirements of calories nearly 10% comes from saturated fatty acids and 20-30% comes from polyunsaturated and mono unsaturated fatty acids. Little amount of saturated fatty acids are common in diet and significant relation found between high consumption of saturated fat and blood LDL concentration. High consumption of saturated fat can be a risk factor for cardiovascular diseases. The compositional of edible oils were monitored by different physical and chemical properties (Ceriani *et al*., 2008). The physiological parameters include acid value, saponification number, iodine value and many others. These values define the quality of the oil. Break down triglycerides of oil are converted into fatty acids and glycerol which increases the acid value. High acid value indicates that oil is old or rancid (Borkar *et al*., 2015). Oil which has high acid value can damage human health. Higher saponification value indicates that the chain length of fatty acid is shorter and the molecular weight of fatty acid is also low. By the study of iodine value, the degree of unsaturation in oil or fat could be measured (Ekwu *et al*., 2004). By studying these physiological parameters edible properties of *Echinochloa colona* seeds oil can identify.

AIMS AND OBJECTIVE

Objectives of this work are to -

1. Isolate the oil from *Echinochloa colona* seeds.
2. Determine the acid value of the oil.
3. Determine the saponification value of oil.
4. Determine the iodine value of oil.
5. Compare the nutrient value with other edible oil.

REVIEW OF LITERATURE

- The study by Peerzada *et al*., (2016) focused on different characteristics of *Echinochloa colona* like short seed dormancy, prolific seed production ability to germinate under different variety temperature, allelopathy effect and resistance against different types herbicides.
- The resistance of *E. colona* studied by Alarcon-Riverte *et al*., (2013). The method of this paper includes glyphosate response, shikmic acid bio assay and EPSP gene sequencing.
- The paper by Chauhan *et al*., (2009) focused on the germination of *E. colona* seeds. This work tells about the factor like light, depth of soil how to effect the germination process by *E colona* seeds.
- The response *E. colona* to water stress, the work is done by Chauhan *et al.*, (2010). This reveals the growth and response of this grass to water stress.
- The study by Rout *et al*., (2000) focused on how chromium and nickel affect on the germination and growth of *E. colona* population. This work shows that the growth of *E. colona* in chromite mine spoils, because of metal tolerance. This finding helps in revegetation in the mine wastes.
- The paper by Borkar *et al*., (2015) focused on antioxidant effect and characterization of different bioactive compounds which are isolated from *E. colona*. The used ethanolic extraction process to isolate the bio-active compounds. The conclusion of this study reveals that the plant is principal source of presumed antioxidant.
- Recent studies, Trung and Quang (2022) shows that the efficiency of phytoremediation by *E. colona*, can increase in presence of phosphorus solubilizing bacteria (PSB). This study reveals how PSB enhance Pb^{2+} uptake by *E. colona* grass.
- Ajaib *et al*., (2013) reveals how the extract from this grass active against gram positive bacteria (*Staphylococcus pneumoniae, Streptococcus pneumonia*) and gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*)
- The study by Refatti *et al*., (2019) focused on resistance of E. colona to Cyhalofop-Butyl due to increasing temperature and high concentration of CO_2.
- The paper by Pan *et al*., (2019) confers the Glyphosate resistance in E. colona. This project confirms this resistance property because the presence of Aldo keto reductase (AKR), metabolizes glyphosate.

- The study Samantary *et al*., (2001) focused on chromium and nickel tolerance of E. colona. This project will help in phytoremediation because of its metal tolerance activity.
- The allelopathy effect of E. colona on the seedling growth and seed germination was studied by Chopra *et al*., (2017). The allelopathy effect studied on rice and soyabean.
- Phytotoxic effect of E. colona studied by Gomma *et al*., (2012). The study resulted that CH_2Cl_2 and ticrin can be used as bioherbicide for controlling the weed in agricultural field.
- Propanil resistance in E. colona population studied by Fischer *et al*., (1993). This study reveals that in the presence of propanil the reproductive fitness of E. colona is reduced.
- Acylamidase and propanil resistance in E. colona grass were studied by Lea *et al*., (1994).

MATERIALS AND METHODS

1. Seed material

Echinochloa colona seeds were brought from a shop in Digha region (21˚68′ 10"N, 87˚56′26"E) located in Purba Medinipur, West Bengal, India. The seeds were washed out to remove the impurities and also dried.

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Fig.1: a. *Echinichloa colona* plant. b. Seeds of *Echinochloa colona*.

2. Oil extraction

Dried seeds were crushed in mixer. The crushed seeds were put into bags, made of filter paper. The bags are put into soxhlet chamber. 100ml of selected solvent (Petroleum ether) was put in to the round bottom flask. The soxhlet extractor was then connected to the distillation apparatus, and the distillation process was started to obtain the desired sample. After completed the process the round bottom flask was placed into rotavapor to remove the solvent from the sample. The extracted oil which was collected after removing solvent, used for further analytical process.

Fig. 2: a. Seeds are crushed in mixture. b. Crushing seeds are put into filter paper bags. c. Soxhlet apertures.

3. The analytical method

3.1 Acid value

1.0g of oil sample was weighed and dissolved into 50ml of neutralize alcohol. 2 to 3 drops of phenolphthalein indicator were added and titrated against 0.1N potassium hydroxide (KOH) solution until the pale pink colour appeared. The acid value is calculated by using this formula:

Acid value = (V x 0.00561 x 1000)/weight of sample

V: Volume of 0.1N KOH consumed by oil.

3.2 Saponification number

N/2 Alcoholic KOH solution was prepared by dissolving 7.0 g of KOH in 20 ml of distilled water and 230 ml of ethanol (95%). the solution was kept for 24 hours and filtered. The alcoholic KOH solution was standardized against N/2 oxalic acid solution. 1g oil was weighed and put in to 250 ml of flat bottom flask. 25 of ml N/2 alcoholic KOH was added to the sample and heated for 30-40 minutes until the sample was fully dissolved. The solution was allowed to cool to room temperature. Two drops of phenolphthalein indicator was added to the sample and titrated against the HCl solution till the disappearance of pink colour. With the same time and condition a blank was also determined [7].

Saponification number = (P - Q) x N x 56.1/Weight of oil in gram

P: ml of HCl required by blank

Q: ml of HCl required by sample

N: Normality of HCl

3.3 Iodine value

Iodine value of oil sample was determined by Wij's Method. Wij's solution was prepared by dissolving 16.2 g of iodine monochloride (ICl) in a 1L volumetric flask mixed with glacial acetic acid. 1g of oil was dissolved in 15 ml carbon terachloride. Then 25 ml of Wij's solution, 100 ml of water and 15 ml of 10% potassium iodide was added to the solution and kept for 30 minutes in dark place. The solution was titrated against standardized thiosulphate solution till the straw colour appeared. Then 1ml of starch indicator was added and titration was continued till the disappearance of blue color. In a same time, a blank solution was also determined.

$$\text{Iodine value} = \frac{(\text{Titer value of blank} - \text{titer value of oil samples})\ \text{ml} \times 0.01269 \times 100}{\text{weight of oil sample(g)}}$$

Fig.3: Different experiments performed in laboratory.

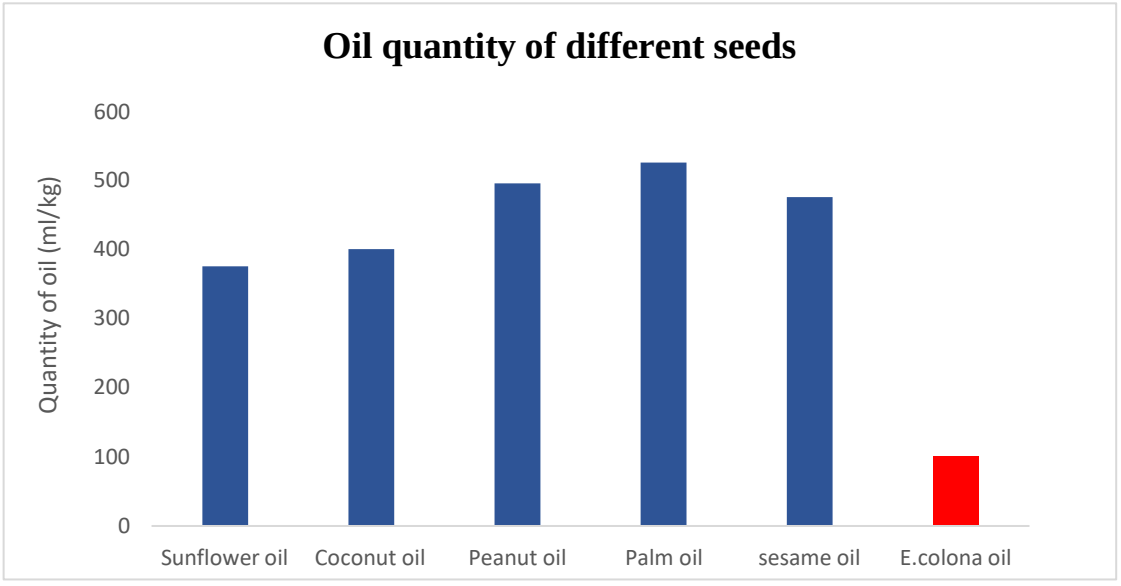
RESULT

1. Oil quantity

Oil quantity from different seeds depend on various things like type of seeds, oil extraction method and many others. The oil quantity of oil is much more lower in the seeds of *E. colona*. 1kg of E. colona seeds give about 100 ml oil. The graph below shows a comparison of oil quantity with other seeds, available in the market.

Sl. No.	Oil	Quantity
1	Sunflower oil	300-450
2	Coconut oil	350-450
3	Peanut oil	440-550
4	Palm seed oil	500-550
5	Sesame oil	450-500

Table1: Some standard values of edible oils. The acid values of *Echinochloa colona* seeds oil is 1.91 which is little bit higher than mustard oil, soya oil and coconut oil but less than PKO and palm oil (Source: https://piteba.com/en/content/50-How-much-oil-can-press-from-kilo-oil-seeds-nuts).

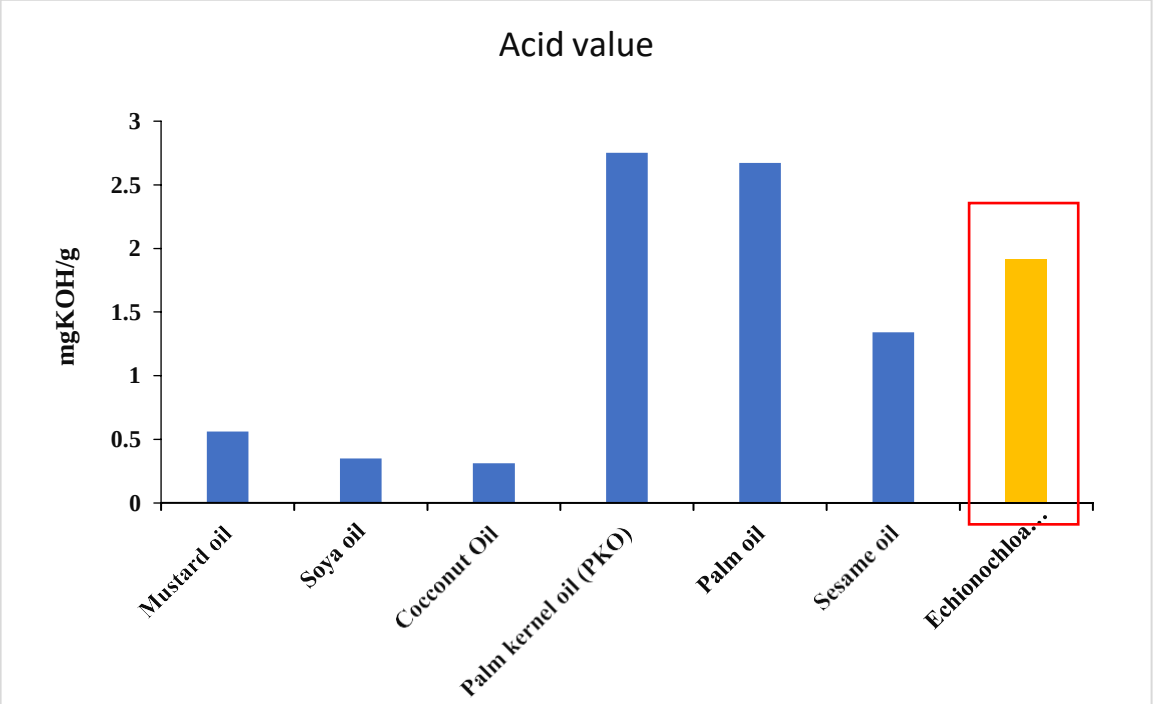


2. Acid value

Acid value of any oil indicates the amount of free fatty acid present in oil. It also defined the number of mg KOH require to neutralize the free fatty acid present in one gm of oil. The amount of free fatty acid present in the oil indicates the age and quality of oil. The relation between acid value and oil quality is inversely proportional to each other.

Sl. No.	OIL	ACID VALUE (mg KOH/g)
1.	Mustard oil	0.56
2.	Soya oil	0.35
3.	Coconut oil	0.31
4.	Palm kernel oil (PKO)	2.75
5.	Palm oil	2.67
6.	Sesame oil	1.34
7.	*Echinochloa colona* oil	1.91

Table 2: Comparative study of the physiochemical characterization and quality of edible vegetative oils. (Source: Ichu CB and Nwakanma HO, 2019).

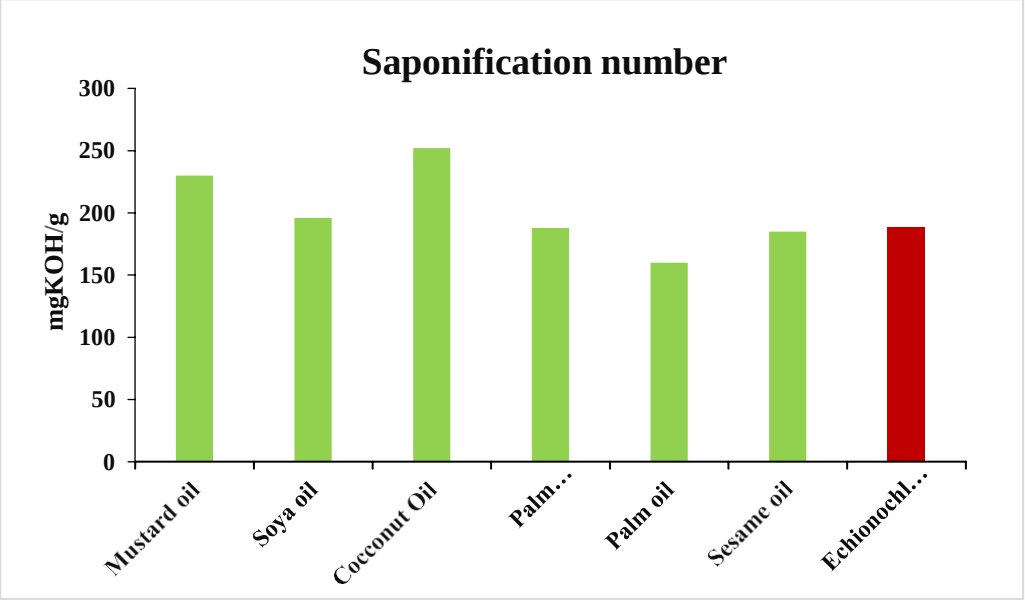


3. Saponification Value

Saponification number informs about the average chain length. Through this one can understand the molecular weight of fatty acid. In other way we can say saponification number is the number of milligram of KOH required to neutralize the fatty acid resulting in complete hydrolysis of 1 g of fat or oil. Long chain fatty acid chain indicates less acidity so that KOH utilization become lesser. If the average chain length of the fatty acid or oil becomes shorter the saponification number become higher.

Sl. No.	Oil	Saponification value (mg KOH/g)
1.	Mustard oil	230
2.	Soya oil	196
3.	Coconut oil	252
4.	Palm kernel oil (PKO)	188
5.	Palm oil	160
6.	Sesame oil	185
7.	*Echinochloa colona* oil	188

Table 3: standard values of saponification number of some edible oils. The saponification number of *Echinochloa colona* oil is 188 mg KOH/g which value is similar to palm kernel oil and near to sesame oil (Ichu CB and Nwakanma HO, 2019).

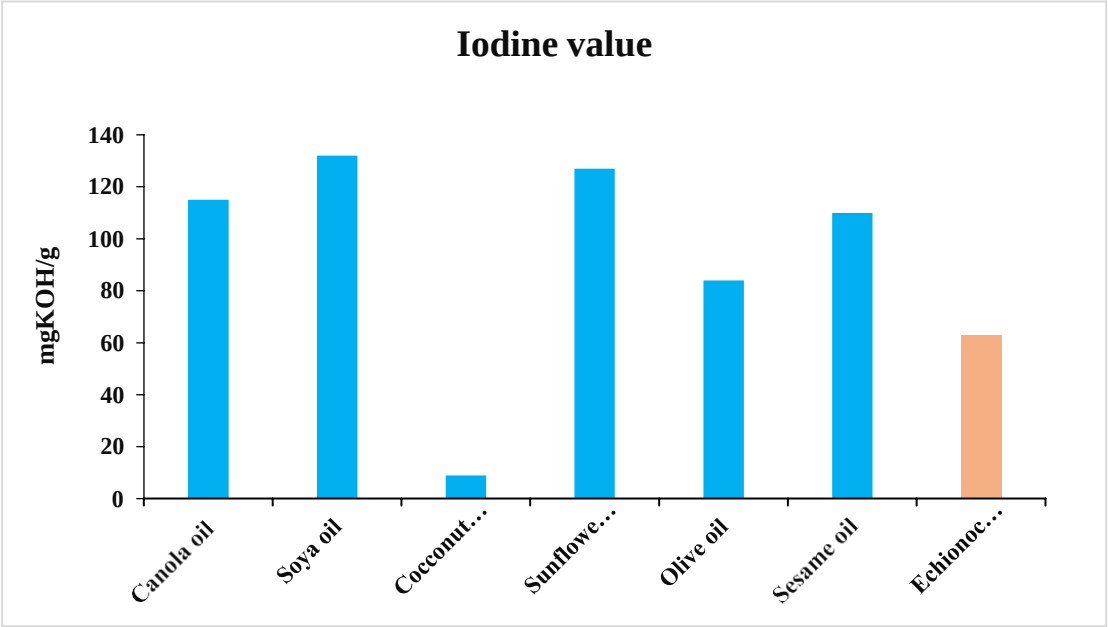


4. Iodine value

Iodine value number is measured as the number of grams of iodine taken up by 100g of oil or fat. Iodine value or iodine defines the degree of unsaturation in any oil.

Sl. No.	Oil	Iodine value
1	Canola oil	115
2	Soya oil	132
3	Coconut oil	9
4	Sunflower oil	127
5	Olive oil	84
6	Sesame oil	110
7	*Echinochloa colona* oil	63

Table 4: shows standard iodine value of some edible oils. Iodine value of *Echinochloa clona* oil is 63mg KOH/g which is much more higher than coconut oil but less than other oils like canola, soya, sunflower, olive and sesame oil (Seneviratne *et al*., 2016).



DISCUSSION

The composition of any edible oils monitored by different types of physical and chemical properties. Acid value, saponification value, iodine value are three important parameters of an edible oil. So, I have measured these three values and compared these values with commonly available other seed oils in the market. These three values are range able comparing to other available edible oils. *Echinochloa colona* completes its life cycle under 42 to 64 days which is much more faster than mustard, canola and other oil seeds. It produces 2000 to 40000 seeds in a plant. The seeds cost around 20 to 30 rupees per kg which is much more cheaper in price comparing to other oil seeds. Applying biotechnology, can introduce hybrid seeds that can gives higher quantity of oil. If oil contains any kind of unsaturated fatty acid, which is beneficial to human health, could be studied later.

CONCLUSION

The oil quantity from *E. colona* seeds is much more lower than the other oil seeds which are commonly available in the market. But the nutritional parameters of this oil are in range, comparing with other edible oils. The price of the seeds of *E. colona* is lesser than other oil seeds. Because of these reasons ,the seeds can be used as alternative oil source.

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