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SHORT COMMUNICATION

Feasibility of cattle urine as nutrient medium for the microalgal biomass production

A. Suresh[1,2,*], S. Tamilvanan[1], K. Harini[1], H.V. Sevanthi[1], R. Deepan Guna[1], R. Mahalakshmi[1], S. Suriyapriya[1], D. Sharmila[1], M. Thenmozhi[1]

[1] *Department of Biotechnology, School of Life Sciences, Vels Institute of Science, Technology and Advanced Studies, Chennai-600117, Tamil Nadu, India*

[2] *Department of Biotechnology, College of Biological and Chemical Engineering, Addis Ababa Science and Technology University, Addis Ababa-16417, Ethiopia*

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ABSTRACT

The nutrient medium used for the cultivation of microalgae adds more cost to its value-added product as well as the commercial scale application. Therefore in this study, focused feasibility of cattle urine as a cheap source of nutrients for microalgal growth, because, it contains various minerals and economical which may support the growth of microalgae and reduce the medium cost. To check this, fresh cattle urine was collected, characterized, diluted and inoculated microalgae species *Oscillatoria*-SRA (Stagnant rainwater algae), *Oscillatoria*-CWA (Cooum waste algae), *Chlorella* and *Synecocystis* separately and incubated under fluorescent light with 8 hours light and 16 hours dark cycle. The biomass was quantified after 15 days and found out variation in biomass quantity in all microalgae isolates. The maximum of 2.6 g/L biomass was produced in *Chlorella* sp., at 10% urine, followed by *Synechocystis* sp., (2.25 g/L in 10% urine), *Oscillatoria* sp.,-SRA (1.3 g/L in 5% urine) and *Oscillatoria* sp.,-CWA (0.3 g/L in 1% urine). Moreover, lipid quantity was shown at the maximum of 12% dry weight in *Oscillatoria* sp-SRA., trailed by the 10% in *Chlorella* sp., 7% in *Synecocystis* sp., and the least of 5% in *Oscillatoria* sp-CWA. This study divulged that cattle urine alone is being able to support microalgae growth at a significant amount, thus convalescing industrial production of microalgae ultimately will reduce the cost of microalgal value-added products.

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*Corresponding Author:
Email: *blueyellowsnu@gmail.com*
Phone: +251902643433
Fax: +251902643433

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INTRODUCTION

Value added products (food, feed, fuel, pigments, bioremediator and neutraceuticals) from microalgae are a new promising industry because of their high photosynthetic efficiency and ability to grow in extreme environmental conditions (Bilal *et al*., 2017). Lately, a number of studies are being carried out to develop cheap techniques to produce useful products from microalgae not only due to its ability to produce value-added chemicals, but also for the lack of competition with food, water and land, also contribute to CO_2 mitigation (Chisti, 2008). Although, extensive work is being achieved on the production of biochemicals from algae and its biomass, (single-cell protein, animal feed, biodiesel, pigments and drugs) but still costlier when compared to the normal plant products, which obstruct the large-scale applications of algae industry (He *et al*., 2013). Since media composition requires the expensive operation cost and therefore limits its availability at a larger scale as well as boosting its final product cost (Sharma and Rai, 2015). Sustainability issues impact on the cultivation of algal biomass for its importance, on one hand, the nitrogen fertilizer manufacturing process is energy-demanding and causes greenhouse gas emissions, on the other hand the world's phosphorus reserves are depleting due to phosphate rock mining (Dawson and Hilton, 2011; Hilton and Dawson, 2012; Canter *et al*., 2015). The world, inorganic fertilizer (NPK) consumption in the year 2017 was 197 million tons with a division of 60% N, 23% P, and 17% K (Alexandratos and Bruinsma, 2012). The estimation for fertilizer consumption in the year 2050 has been projected as 263 Mt and will be elevated price (FAO, 2015). Hence, there is a need to use cost-effective nutrient media for the sustainable agriculture industry. Cattle urine is providing nutrients to plants at low prices, thus reducing the cost of production owing to the inexpensive in comparing with inorganic fertilizers moreover it's a renewable source. Therefore, it is considered and preferred for choice for plant nutrition (Jandaik *et al*., 2015; Singh *et al*., 2018). Cattle urine contains a rich source of macro and microelements, an average 5.5 gN/L (Shephered *et al*., 2017) besides cattle urinate at an average of 10 times/day with ~1.8L each time (Misselbrook *et al*., 2016), therefore ~100gN/cattle/day. Meantime world cattle population currently reached ~1 billion (Global cattle population, 2018), which produces 0.1 million tons of nitrogen/day, the however world used 0.32 million tons N/day in 2017 (Alexandratos and Bruinsma, 2012) and this can be replaced by 4 days of cattle urine and the same for other nutrients (in particular phosphorous and potassium) as well. Obviously, these nutrients are recycled for agriculture, the artificial fertilizer production could be reduced significantly, in addition culturing microalgae on the urine is that microalgae removes nutrients and clean it for recycling purposes and also yield high biomass (Chang *et al*., 2013). Recently, growing of microalgae in human urine has gained more interest, (Jaatinen *et al*., 2016; Torres *et al*., 2014; Tuantet *et al*., 2014; Zhang *et al*., 2014) yet not much scientific evidence on cattle urine (Sharma and Rai, 2015). Therefore, in this research, focused feasibility of cattle urine as a cheap source for nutrient media for microalgae, which reduce the growth medium cost, eventually this will bring down the cost of microalgal value-added product and make sustainable production. To explore this, the cattle urine was collected and used for the microalgal growth at various dilutions and checked for the biomass production and lipid content. The overall findings of this study could be very helpful to the scientific society and the algal industry to reduce the nutrient medium-cost eventually reduces the cost of microalgal value-added products. This study has been carried out in Vels Institute of Science, Technology and Advanced Studies, Chennai, India in 2018.

MATERIALS AND METHODS

Collection and characterization of cattle urine

Five liters of fresh cattle urine (from 5 different cattle's) were collected at the early morning from the cattle shelter in Tiruvannamalai region, Tamil Nadu and stored at 4°C. The mixed cattle urine was analyzed physicochemical parameters (Table 1) in Chennai testing laboratory private limited, Chennai, Tamil Nadu.

Microalgae cultures

Four microalgae cultures were used, two species from *Oscillatoria* (SRA-stagnant rainwater algae and CWA-Cooum waste algae), one from *Chlorella* (GSA-Goat shed algae) and *Synecocystis* (AnA-Anakaputur algae) (Suresh *et al*., 2018). The collected algal species were subcultured in 200 ml of Himedia (M342) algal culture broth and incubated under white light around 4000 lux at 25°C with 16 hours light and 8 hours dark cycle.

Growth of microalgae on cattle urine

Ten days grown microalgal cultures were taken and rinsed with sterile deionized water (twice) and blended smoothly with pestle and mortar. Then smaller amounts equal to 100 mg dry weight of homogenized culture were introduced into the test tubes which contains 10 mL of sterile cattle urine with varied dilution (0%, 1%, 5%, 10% and 15%). Then inoculated tubes were incubated as mentioned previously. All the experiments were performed in duplicate.

Quantification of microalgae growth and lipid

Microalgal biomass were collected after 15 days of growth by vortex and scratching method and filtered using pre-weighed Whatman No.1 filter paper. Then filter paper was rinsed with distilled water and dried at 60°C in a hot air oven for 12 h then weighed and calculated the dry weight and specific growth rate (μ) as per Sayadi *et al.* (2016). The lipid was extracted with chloroform; methanol (2:1 v/v) and estimated as per Suresh *et al*. (2013).

RESULTS AND DISCUSSION

Characterization of cattle urine

Cattle urine was characterized (Table 1) and contained 21.6 g/L dissolved solids with a conductivity of 30 mS/cm, which indicates that the huge amount of dissolved minerals. The maximum of 6.6 g/L potassium was observed, followed by Cl (6.3 g/L) and Na (2.4 g/L). In the case of nitrogen, 1.8 g/L, whereas phosphorous shown only 0.015 g/L. The least amount of 0.07 mg/L Cu was noticed while nitrate was below detectable limits (0.10 mg/L). A great variation was observed in the composition of cattle urine with other countries such as Brazil shown 12.6, 2.6 and 1.7 g/L N, K and Cl, respectively (De Oliveria *et al*., 2009), whereas in New Zealand, 7.7, 9.4 and 0.3 g/L of N, K and P, respectively (Ledgard *et al*., 1982). These great differences were attributed to the many factors such as collection time, types of feed used, climate, and species. However, this data indicate that the cattle urine encompasses many minerals which can support the microalgae growth.

Microalgae growth and lipid production in cattle urine

Microalgae has a lot of potentials to solve world problems (hunger, fuel, disease, pollution), however, its product is costlier than the plant source due to its production cost especially nutrients. Therefore, in this study used cheap cattle urine as a nutrient for the microalgal isolates, which are indigenous and naturally adapted to the urine wastewater (Suresh *et. al*., 2018), hence expected high utilization of nutrients from cattle urine and the result would be high biomass. Fig. 1 shows the microalgae growth in cattle urine at varying concentration in deionized water and it disclosed that the *Chlorella* sp., and *Synecocystis* sp., shown the abundant growth at 5 to 10% cattle urine in compare with the other two *Oscillatoria* sp.

Within the two *Oscillatoria* sp., SRW has grown more than the *Oscillatoria* sp., of CWA sample, it was unexpected because the Cooum River contains more urine than the stagnant rainwater. This result might be a slow growth rate of CWA, but in this study microalgae were incubated only for 15 days and it would be better growth if more days of incubation. Fig. 2 depicts the growth of microalgal isolate *Oscillatoria* sp., (SRA) in agar plate with different concentration of cattle urine and visibly indicated that the 5% of cattle urine supported the microalgal growth significantly, whereas 10% exhibited insignificant growth and a

Table 1: Physicochemical characterization of cattle urine

Parameters	mg/L
pH at 25°C	7.9
Alkalinity as $CaCO_3$	7900
Conductivity	30120 µS/cm
TDS	21610
TN	1848
NO_3	*BDL (DL=0.10)
K	6630
Chloride	6362
Na	2420
Ca	752
S	198
Mg	55
P	14.8
Mo	7.37
Al	4.22
Fe	3.4
Zn	0.18
Co	0.16
Mn	0.12
B	0.12
Cu	0.07

*BDL; Below detection limit, DL; Detection limit

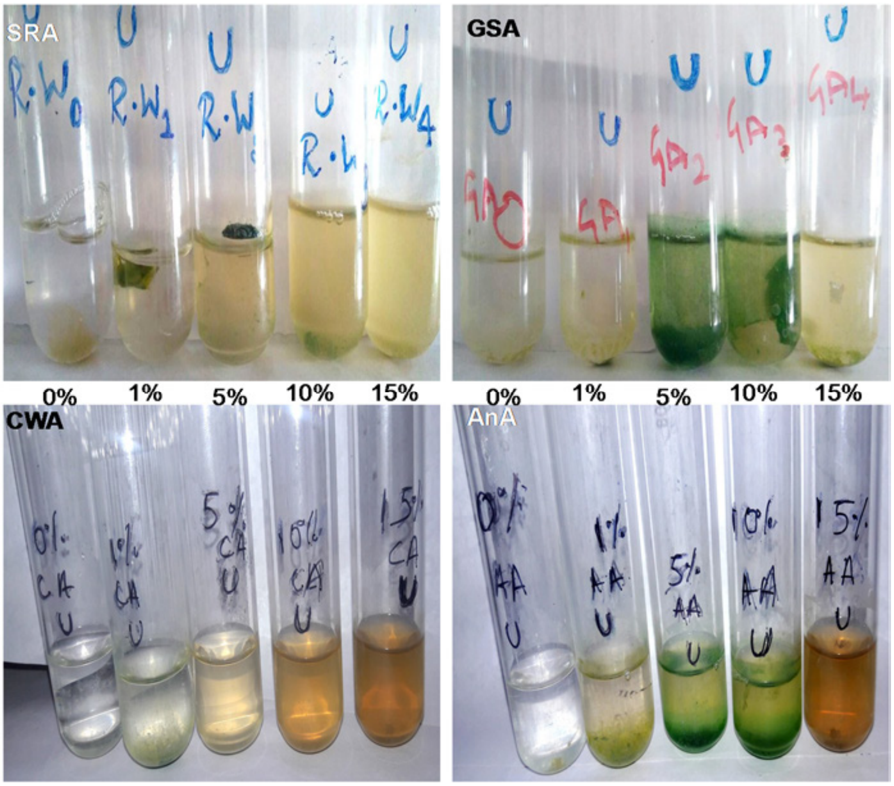

Fig. 1: Growth of microalgal isolates in cattle urine at varying concentration for 15 days under white light. *Oscillatoria* sp., SRA (stagnant rainwater algae); *Chlorella* sp., GSA (Goat shed algae); *Oscillatoria* sp., CWA (Coovam water algae); and *Synechocystis* sp., AnA (Anakaputur algae)

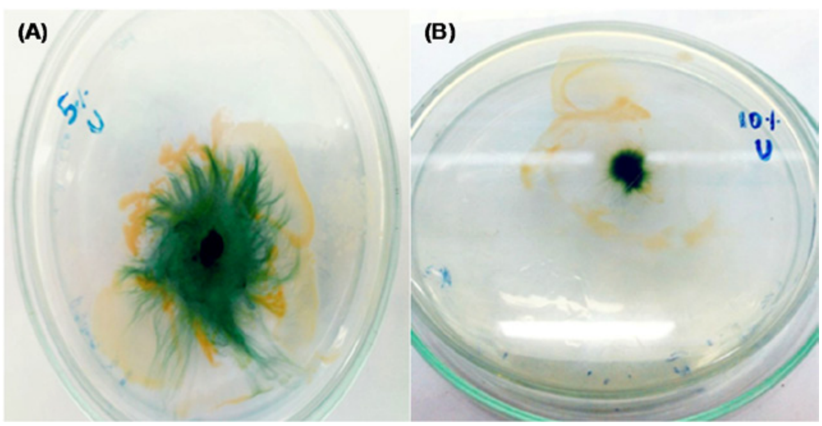

Fig. 2: Growth (10days) of *Oscillatoria* sp., (SRA) in agar medium containing varied dilution of cattle urine (A; 5%, B; 10%)

similar effect was noticed in broth medium (Fig. 1).

However, Fig. 3 explained that the 10% cattle urine supported the growth of *Chlorella* sp., up to a maximum of 2.6g/L, and it reduced to 0.5g/L in 15% cattle urine. The same pattern was observed in *Synecocystis* sp, where reached its maximum growth of 2.25g/L at 10% cattle urine and reduced same as *Chlorella* sp. While species of *Scenedesmus*

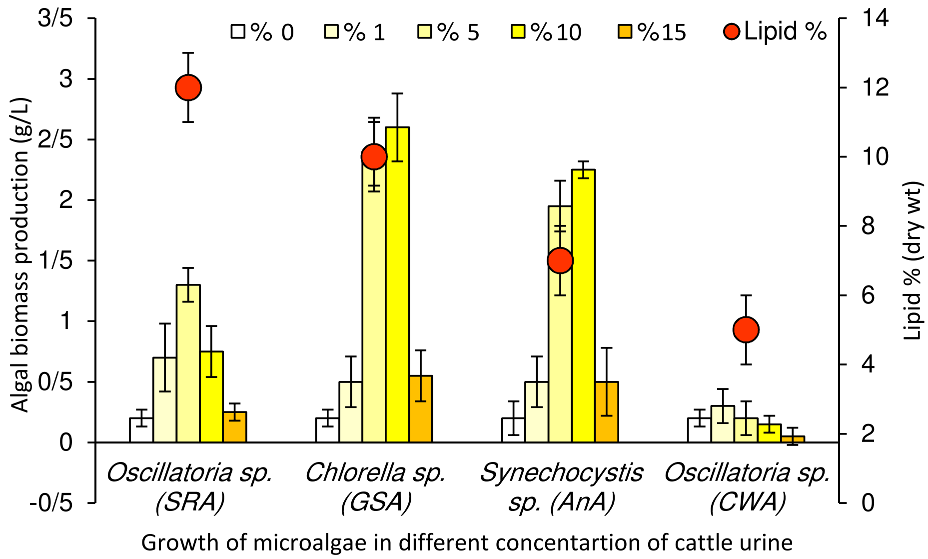

Fig. 3: Biomass production and maximum lipid percentage of microalgal isolates cultured in cattle urine at varied dilutions

Table 2: Comparison of microalgae biomass production using urine as nutrient source

Microalgae	Urine concentration (v/v)	Biomass production	Reference
Chlorella sp.,	10% (cattle urine)	2.6 g/L	This study
Synecocystis sp.,	10% (cattle urine)	2.25 g/L	
Oscillatoria sp.,(SRA)	5% (cattle urine)	1.3 g/L	
Oscillatoria sp.,(CWA)	1% (cattle urine)	0.3 g/L	
Chlorella pyrenoidosa	7.5% (cattle urine)	1.93 g/L	Sharma and Rai, 2015
	10% (cattle urine)	1.8 g/L	
	12.5% (cattle urine)	0.6 g/L	
Chlorella vulgaris	1% (human urine)	0.6 g VSS/L	Jaatinen *et al*., 2015
Chlorella sorokiniana	1% (human urine)	1 g/L	Tuantet *et al*., 2014
	20% (human urine)	8.1 g/L	
Chlorella vulgaris	2% (human urine)	1.474*	Torres *et al*., 2014
Secendesmus sp	2% (human urine)	0.913*	
Spirulina platensis	0.83% (human urine)	0.81 g/L	Chang *et al*., 2013
Spirulina platensis	0.6% (human urine)	2.32 g/L	Lun and Cheng, 2006
	0.6% (Synthetic human urine)	2.4 g/L	
Scenedesmus acuminatus	2% (human urine)	0.16 g/L	Adamsson, 2000

*Average OD (600 and 750nm) value increased from 0.1

(Adamsson, 2000) and *Spirulina* (Lun and Cheng, 2006) have been studied earlier and found to be incapable of growing in more concentrated urine which was concordant with this study. Similarly, Sharma and Rai (2015) found that more than 7.5% of cattle urine reduced the microalgal growth. Moreover, unionized ammonia (as NH_3) can be lethal to microalgae as shown by Adamsson (2000) and reported diminished growth of microalgae when ammonium concentration increased while adding more cattle urine (2% urine was used). However, in this study, the microalgal growth was not reduced bellow 2% cattle urine and signifying that biomass production sustained even with increasing ammonium concentration in the 10% of cattle urine. This result might be because of those microalgae used for this study were collected from a urine contaminated site and adapted for more urine concentration.

Unexpectedly, the CWA *Oscillatoria* sp., did not grow at 5% cattle urine and more, which shown 0.3g/L in 1% urine. In other hand SRA *Oscillatoria* sp., grown at the maximum of 1.3g/L at 5% urine, however, the growth reduced in 10% and 15% urine. The maximum specific growth rate (μ) of 0.243 per day was noticed in Chlorella sp., in 10% cattle urine, followed by Synechocystis (0.231/day in 10% cattle urine), *Oscillatoria* sp.,-SRA (0.186/day in 5% cattle urine) and least amount of 0.076/day in *Oscillatoria* sp.,-CWA in 1% cattle urine, respectively. These data clearly indicate that the cattle urine absolutely supports the growth of microalgae, however, each microalgae has the optimum dose of urine as well as the source of urine (Table 2). Many studies have proven that the human urine was supporting the microalgae, however, there was optimum concentration, which falls between 5-10% of urine, which is in accordance with this study with cattle urine. Moreover, for plant growth also falls under this 10-15% concentration of cattle urine (Singh *et al*., 2018; Jandaik *et al*., 2015). Fascinatingly, the *Oscillatoria* sp., from SRA contained the maximum lipid (12%) than the *Chlorella* sp., (10%) which was shown maximum biomass. The least lipid concentration (5%) was found in *Oscillatoria* sp from CWA, and *Synecocystis* sp., exhibited 7% lipid in dry wt of algae. In contradicting to our study previous studies observed high lipid production in *Chlorella* sp., at 33% lipid in 10% cattle urine, while 23% lipid in 7.5% cattle urine and this amount were higher than the same microalgae which grown in Fogg's medium (standard microalgae growth medium), which accumulated only 7% lipid (Sharma and Rai, 2015).

CONCLUSION

In this study, cattle urine was characterized and contains 21.6 g/L dissolved solids, 6.6 g/L potassium, 1.8 g/L nitrogen and 15 mg/L phosphorous as major nutrients. These nutrients were supporting the growth of microalgae at the sizeable amount as the observed maximum of 2.6 g/L biomass (*Chlorella* sp.,) in 10% cattle urine, followed by *Synecocystis* at 2.25 g/L. Whereas *Oscillatoria* sp., shown a maximum of 1.3 g/L in 5% cattle urine and inhibited its growth in 10% and 15% cattle urine. Moreover, suggested that each microalgae have a specific concentration of cattle urine that found out to be 5-10% v/v. Microalgae grown in cattle urine showed a decent amount of lipid accumulation at a maximum of 12% in *Oscillatoria* sp., and 10% in *Chlorella* sp. This study proved that cattle urine alone can support microalgae growth at a considerable amount and further research is needed for the specific value-added product from microalgae and its cost analysis using cattle urine as a nutrient source.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

ABBREVIATIONS

%	Percent
°C	Degree Celsius
µS/cm	Microsiemens per centimeter
Al	Aluminum
AnA	Anakaputur algae
B	Boron
BDL	Below Detection Limit
Ca	Calcium
$CaCO_3$	Calcium carbonate
Cl	Chlorine
Co	Cobalt
CO_2	Carbon dioxide
Cu	Copper
CWA	Cooum waste algae
DL	Detection Limit
Fe	Iron (Ferrum)
Fig	Figure
g	Gram
g/L	Gram per liter
gN/L	Gram nitrogen per liter
GSA	Goat shed algae
h	Hours
K	Potassium
L	Liter

mg	Milligram
Mg	Magnesium
mg/L	Milligram per liter
mL	Milliliter
Mn	Manganese
Mo	Molybdenum
mS/cm	Millisiemens per centimeter
Mt	Megatonne
N	Nitrogen
N/day	Nitrogen per day
Na	Sodium
NH_3	Ammonia
nm	Nanometer
NO_3	Nitrate
OD	Optical density
P	Phosphorous
pH	Potential of hydrogen
S	Sulfur
sp	Species
SRA	Stagnant rainwater algae
TDS	Total dissolved solids
TN	Total Nitrogen
μ	Specific growth rate
v	Volume
v/v	Volume per volume
wt	Weight
Zn	Zinc

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AUTHOR (S) BIOSKETCHES

Suresh, A., Ph.D., Assistant Professor, Department of Biotechnology, School of Life Sciences, Vels Institute of Science, Technology and Advanced Studies, Chennai-600117, Tamil Nadu, India and Department of Biotechnology, College of Biological and Chemical Engineering, Addis Ababa Science and Technology University, Addis Ababa-16417, Ethiopia. Email: *blueyellowsnu@gmail.com*

Tamilvanan, S., M.Sc., Department of Biotechnology, School of Life Sciences, Vels Institute of Science, Technology and Advanced Studies, Chennai-600117, Tamil Nadu, India. Email: *tamilvanan616@gmail.com*

Harini, K., B.Sc., Department of Biotechnology, School of Life Sciences, Vels Institute of Science, Technology and Advanced Studies, Chennai-600117, Tamil Nadu, India. Email: *harinikailash97@gmail.com*

Sevanthi, H.V., B.Sc., Department of Biotechnology, School of Life Sciences, Vels Institute of Science, Technology and Advanced Studies, Chennai-600117, Tamil Nadu, India. Email: *sevanthihv798@gmail.com*

Deepan Guna, R., B.Sc., Department of Biotechnology, School of Life Sciences, Vels Institute of Science, Technology and Advanced Studies, Chennai-600117, Tamil Nadu, India. Email: *deepanguna24@gmail.com*

Mahalakshmi, R., B.Sc., Department of Biotechnology, School of Life Sciences, Vels Institute of Science, Technology and Advanced Studies, Chennai-600117, Tamil Nadu, India. Email: *maharadjendrane1410@gmail.com*

Suriyapriya, S., B.Sc., Department of Biotechnology, School of Life Sciences, Vels Institute of Science, Technology and Advanced Studies, Chennai-600117, Tamil Nadu, India. Email: *priya26sp@gmail.com*

Sharmila, D., B.Sc., Department of Biotechnology, School of Life Sciences, Vels Institute of Science, Technology and Advanced Studies, Chennai-600117, Tamil Nadu, India. Email: *sharmisha.sai.08@gmail.com*

Thenmozhi, M., Ph.D., Assistant Professor, Department of Biotechnology, School of Life Sciences, Vels Institute of Science, Technology and Advanced Studies, Chennai-600117, Tamil Nadu, India. Email: *drmthenmozhi@gmail.com*

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