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Algal inhibitory efficiency of secondary metabolites of *Tamarindusindica* and *Azadirachtaindica* – A comparative pilot scale study

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Abstract**—** *This study includes isolation of oil producing algae (Anabaena, Nostoc. Spirulina, Diatom, Volvox, Spirogyra) and subjecting the mixture of algae to the sun dried pulp extract of Tamarindusindica to observe inhibitory effect of secondary metabolites on algal growth. The comparative analysis of inhibiting efficiency was done between the extracts of Tamarindusindica and Azadirachtaindica which proved that the secondary metabolites of Azadirachtaindica are more efficient than the secondary metabolites of Tamarindusindicain inhibiting the growth of algae. 100% and 75% concentration of the crude extracts were used to evaluate the inhibitory effect. Contamination from bacteria and fungi was prevented by maintaining the pH at 8.5. The extract of the sun dried pulp of Tamarindusindica showed inhibitory activity against the above mentioned species of algae.*

Keywords**— Algal inhibition, Tamarindusindica, Azadirachtaindica.**

I. INTRODUCTION

Accelerated eutrophication has been one of the most widespread environmental problems (UNEP). Eutrophication is the enrichment of water by nutrient salts (Nitrate, Phosphate, Potassium) that causes structural changes to the ecosystem such as increased production of algae and aquatic plants, depletion of fish species, general deterioration of water quality and other effects that reduce and preclude use (OECD, 2005). All water bodies are subjected to slow eutrophication but in recent years eutrophication has been accelerated due to anthropogenic activities (Hu *et al*. 2008).

Human development and associated increasing population growth in the watershed area underlie many of the environmental problems occurring in fresh water, transitional (e.g. estuaries and lagoons) and coastal ecosystems (OECD, 2005). Nutrient enrichment (N, P, and K) is one of the most prominent consequences directly related to human activities (Paerl 2006). The nutrient composition has been one of the main factors in excessive proliferation of algae in aquatic ecosystems (Paerl& Huisman 2009). Algal growth promoted by these salts can clog the gills of fish, in addition to anoxic water conditions and death of aquatic life forms (Najem*et al*. 2011). The risk to water quality deterioration is aggravated by the co-dominance of bloom-forming members of the green algae (Chia *et al*. 2016).

Algae like Chlorophytes on their own are not considered being a nuisance, however, nutrient-enriched conditions favor the excessive proliferation of members of this group (Paerl*et al.* 2001). Many types of researches were carried out to control the algal growth by mechanical, physical and chemical methods in addition to bio manipulation (Tesson*et al*. 2014, Zhao *et al*. 2018). All these approaches were unsatisfactory and hence extracts of bioactive compounds from plants that inhibit or prevent algal growth have been in use (Ghorbanian*et al*. 2008). The secondary metabolites of these plants are known to contain antimicrobial properties (Wallace. 2004). Most of the phytochemicals from plant sources such as polyphenols and flavonoids have been reported to have a positive impact on health and cancer prevention (Venugopal*et al.* 2012).

The excessive production of oxygen radicals during algal metabolism is known, especially when they are exposed to

stress conditions (Zhang *et al*. 2013). The presence of bioactive secondary metabolites in plants induce high production of compounds like nitric oxide and H2O2, which have the potential to inhibit antioxidant enzyme activity (Clark *et al*. 2000; Qiao*et al*. 2014). The mechanism of action of these phytochemical extracts may be via lysing the cell, increasing permeability of the cell wall and membrane, inhibition of protein and DNA synthesis and/or by inhibiting the transport of nutrient across the cell wall or membrane (Stewart *et al*. 1979).

Tamarindusindica is a medicinal plant belonging to the family Fabaceae. It has been used as a medicinal plant for centuries; its fruits being the most valuable part. It contains majorly flavonoids, alkaloids and polyphenols (Arranz*et al*. 2010) and has exhibited an inhibitory effect against various organisms (Okoh*et al*. 2017).

Many studies have been done to prevent the growth of algae using the secondary metabolites of various medicinal plants in addition to various physical as well as chemical methods. However, these chemical methods have been causing harmful effects on the environment.

This study explores the potential of *Tamarindusindica* extract to inhibit the proliferation of algae in an environmentally friendly way and compare its efficiency of inhibition with *Azadirachtaindica*leaf extract.

II. MATERIALS AND METHODS

SAMPLE – Algae (lake water), *Tamarindusindica*(fruit), *Azadirachtaindica*(leaves).

GLASSWARE REQUIRED – Petri plates, conical flasks, beakers, glass slides, pipettes.

Instruments / apparatus – Compound microscope, autoclave, centrifuge, colorimeter, Soxhlet apparatus, cork borer, micropipettes.

Chemicals used - Methylene blue, Safranin, 1N NaOH, Ethanol, 0.1 N HCl, acetone, Triple distilled water, conc.HCl, conc. H_2SO_4.

Other Requirements – Forceps, needles, pH paper, centrifuge tubes, plastic trays, sample bottles, spatula, blotting paper, filter paper, cotton, muslin cloth.

Sampling of Algae

The water sample was collected from Kempabudi Lake, Chamarajpet around the month of August 2018.

Isolation and growth of algae

The collected sample was inoculated into Algae Culture Broth. After a significant amount of growth, the algae mixture was sub cultured on agar plates in the same Algae Culture media.

The microscopic view of the algal mixture showed the following species of algae.

S. no.	ALGAE
1.	*Anabena*
2.	*Nostoc*
3.	*Spirulina*
4.	*Spirogyra*
5.	*Volvox*
6.	*Diatom*

Identification of algae

The algal isolates that were obtained did not represent the whole algae in the collected samples in this study. Some algae need typical media with typical environmental factors to be grown which were different from those utilized in this study (Abedin and Taha, 2008).

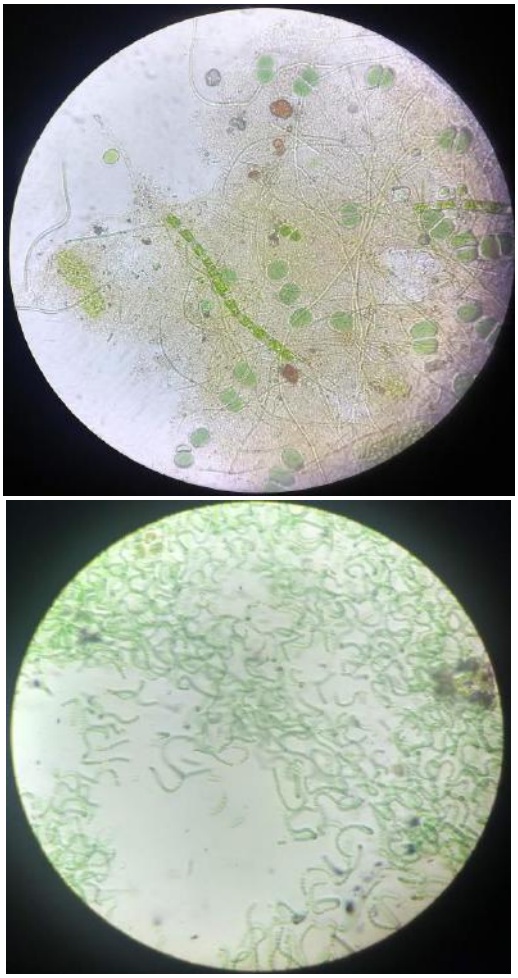

Fig.1: Microscopic views of algae mixture.

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Control of contaminants

Growth of contaminating bacteria and fungi were prevented by maintaining the pH of the media at 8 - 8.5.

Extraction of *Tamarindusindica*pulp

The fruit of the *Tamarindusindica* was collected from a nearby botanical garden and was sun dried. The dried pulp was administered into the Soxhlet apparatus and the extract was obtained using solvent extraction method by using ethanol.

The extract was subjected to drying to evaporate the ethanol.

Evaluation of the inhibitory effect of the pulp extract

Wells were cut into the algae cultured plate and the extract was administered into the wells. Inhibitory effects of *Tamarindusindica* pulp extract was tested against the mixture of algae depending on the diameters (mm) of inhibition zones through the agar-well diffusion method.

Extraction of *Azadirachtaindica* leaves

The leaves of the *Azadirachtaindica* were collected from a nearby botanical garden and were sun-dried. The dried leaves were administered into the Soxhlet apparatus and the extract was obtained using solvent extraction method by using ethanol.

The extract was subjected to drying to evaporate the ethanol.

Evaluation of the inhibitory effect of the leaf extract

Wells were cut into the algae cultured plate and the extract was administered into the wells. Inhibitory effects of *Azadirachtaindica* leaf extract was tested against the mixture of algae depending on the diameters (mm) of inhibition zones through the agar-well diffusion method.

III. RESULTS

The zone of inhibition was observed on day 2 of administration. The diameter of the zone of inhibition continued to increase until the fifth day. After the fifth day, there was no increase in the diameter. On reducing the concentration of the extract administered, the diameter decreased in direct proportion. On comparison with *Azadirachtaindica* leaf extract, the diameter (in mm) observed was larger for *Azadirachtaindica* than *Tamarindusindica*.

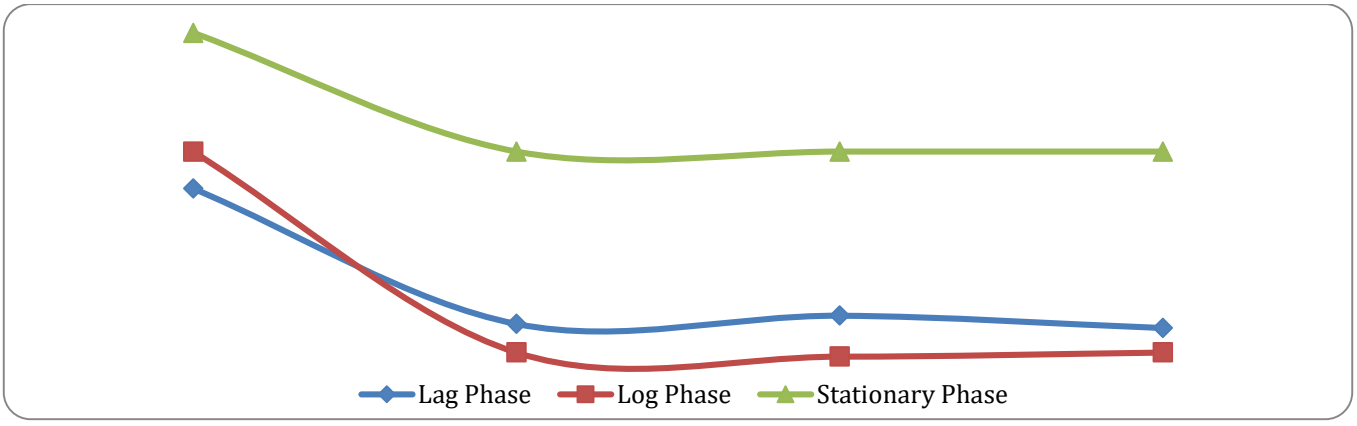

Fig. 2: Growth curve of algae in broth media.

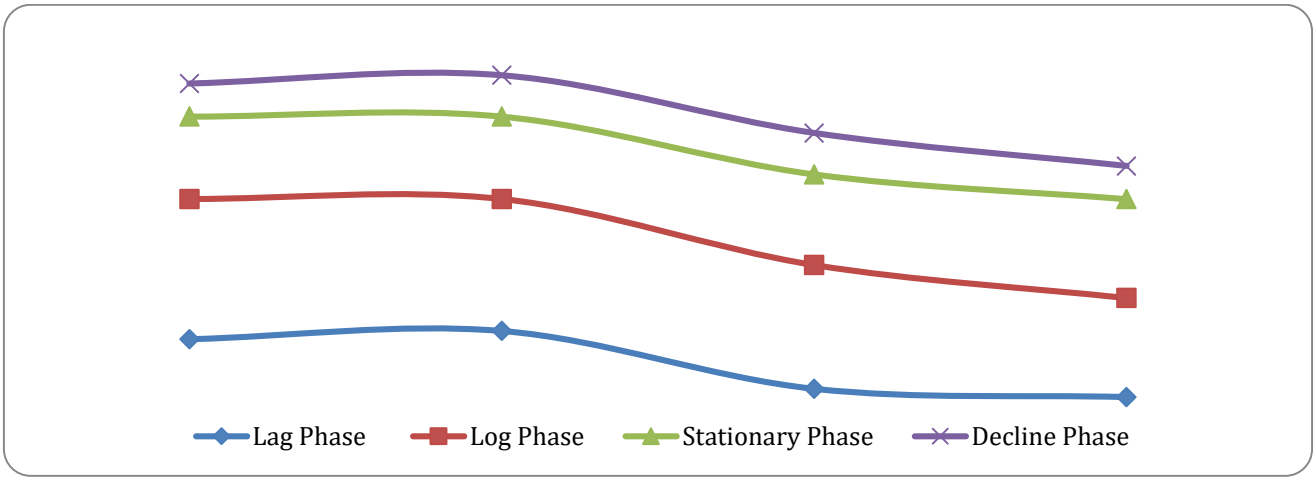

Fig. 3: Growth curve of algae in solid media.

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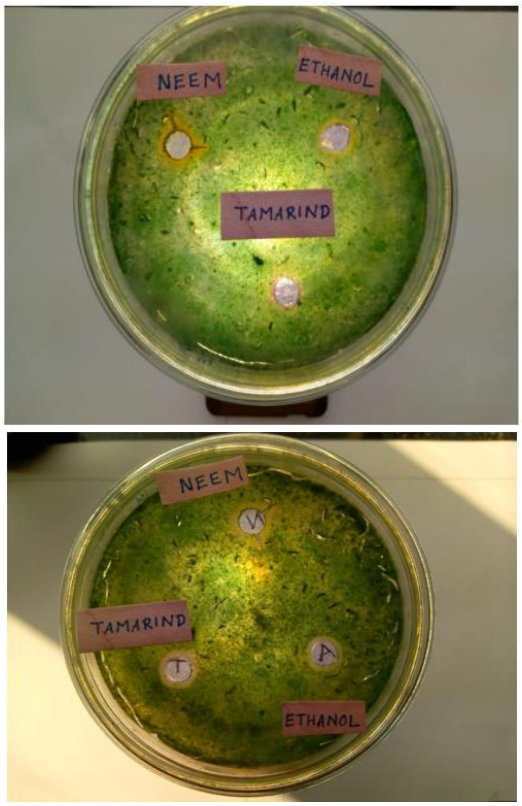

Fig.4: Inhibition zone shown by Azadirachtaindica, Tamarindusindica and ethanol on algal culture plate.

Table 1: Indicating zone of inhibition with 100%crude extract of Azadirachtaindica

Concentration	**Culture Plate**	**Volume (µL)**	**Days**	**Diameter of zone of inhibition by *Azadirachtaindica* extract(mm)**
100% Crude extract	1.	75	1	0
			2	12
			3	12
			4	13
			5	13

Table 2: Indicating zone of inhibition with 100%crude extract of Tamarindusindica

Concentration	**Culture Plate**	**Volume (µL)**	**Days**	**Diameter of zone of inhibition by *Tamarindusindica* extract(mm)**
100% Crude extract	1.	75	1	0
			2	11
			3	11
			4	12
			5	12

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Table 3: Indicating zone of inhibition with 100% absolute ethanol

Concentration	**Culture Plate**	**Volume (µL)**	**Days**	**Diameter of zone of inhibition by Ethanol(mm)**
Absolute ethanol	1.	75	1	0
			2	10
			3	10
			4	11
			5	11

Table 4: Indicating zone of inhibition with 75%crude extract of Azadirachtaindica

Concentration	**Culture Plate**	**Volume (µL)**	**Days**	**Diameter of zone of inhibition by***Azadirachtaindica***extract (mm)**
75% (75 µL of extract + 25 µL DW)	2.	75	1	0
			2	9
			3	9
			4	9.1
			5	9.3

Table 5: Indicating zone of inhibition with 75%crude extract of Tamarindusindica

Concentration	**Culture Plate**	**Volume (µL)**	**Days**	**Diameter of zone of inhibition by***Tamarindusindica***extract (mm)**
75% (75 µL of extract + 25 µL DW)	2	75	1	0
			2	8
			3	8.1
			4	8.4
			5	8.5

Table 6: Indicating zone of inhibition with 75% absolute ethanol

Concentration	**Culture Plate**	**Volume (µL)**	**Days**	**Diameter of zone of inhibition by Ethanol (mm)**
75% (75 µL Absolute ethanol + 25 µL DW)	2.	75	1	0
			2	7
			3	7.2
			4	7.2
			5	7.3

IV. DISCUSSION

This study brought out that there is an initial lag phase in algal growth; the duration of the lag phase decreased on further sub-culturing. It was also noted that algae grow only when the substratum is not completely solid (less agar content) along with the presence of moisture.

Tamarindusindica is known to exhibit antimicrobial activity as it contains flavonoids, alkaloids and polyphenols (Arranz*et al*. 2010). Alkaloids are shown to possess some level of allelopathy on plants (Macias *et al*. 2007).

This study showed that *Tamarindusindica* extracts effectively inhibited the growth of algae. In the case of the 100% crude extract, it has been observed that the zone of inhibition was 11 mm in diameter) on day 2. The magnitude of the zone of inhibition increased on the fourth day (12 mm) and was found to remain constant after that. In the case of 75% of crude extract, the diameter of the inhibition zone was found to be 8 mm on day 2 and the magnitude increased successively up to day 5 (8.5 mm). The diameter of inhibition zones increased with the increased extract concentration. Research has shown that *Azadirachtaindica* has the ability to prevent algal growth (Chia *et al*. 2016).

In contrast with *Azadirachtaindica* leaf extract, the diameter (in mm) observed was larger in case of *Azadirachtaindica*. The zone of inhibition with 100% of crude leaf extract of *Azadirachtaindica* was 12 mm on day 2, while in case of *Tamarindusindica* it was 11 mm. The zone of inhibition with 75% of crude leaf extract of *Azadirachtaindica* was 9 mm on day 2, while in case of *Tamarindusindica* it was 8 mm. The inhibition zone in case of both remained constant after day 5.

Ethanol has been used as a control sample to eliminate experimental error (since the vaporization of ethanol hasn't been done in an ideal method). Ethanol has exhibited a smaller inhibition zone compared to *Azadirachtaindica* and *Tamarindusindica*. The diameter of the zone of inhibition with absolute ethanol was found to be 10 mm on day 2 and increased to 11 mm on day 5. The diameter of zone of inhibition with 75% ethanol was to be 7 mm on day 2 and 7.3 on day 5.

The observed result suggests that fruit extract of *Tamarindusindica*can be used as an alternative way to prevent algal bloom.

V. CONCLUSION

Solvent extract of *Tamarindusindica* is a cheap and effective alternative for the prevention of excessive algal growth which is causing disruption in the aquatic ecosystem. Its inhibitory effect was observed on day 2 and increased upto day 5. On comparison, the leaf extract of *Azadirachtaindica* proved to be more effective than *Tamarindusindica* fruit extract. The comparison was done because both are excellent medicinal plants with effective secondary metabolites. Even without the isolation of specific secondary metabolite of the *Tamarindusindica*fruit, the solvent extract proved to be very efficient. More efficiency of inhibition of algal growth might be achieved by scrutinizing the secondary metabolites of *Tamarindusindica*and administering them specifically. Further studies and investigation need to be done on the effects of the extract on other organisms.

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