Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231-6345 (Online)
An Online International Journal Available at http://www.cibtech.org/jls.htm
2013 Vol. 3 (1) January-March, pp.252-255/Mehar and Shaik

Research Article

TESTING THE ALLELOPATHIC EFFECT OF AQUEOUS EXTRACT OF LEAVES OF *PROSOPIS JULIFLORA* DC ON THE GROWTH OF BGA

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ABSTRACT
Prosopis juliflora DC is an allelopathic plant, which influences the growth of the plants and microbes in its vicinity by releasing chemicals. We tested allelopathic potential of extract prepared from the mature leaves of *P. juliflora* on the growth of the two species of plant associated and plant growth promoting algae in different concentrations (0.1%, 1%, 2.5%, 5%, 10%, 15%, 20% and 25%). When these were tested we found that the higher concentrations (which are not possible in the nature) of the extracts were inhibitory for the growth of both the species A (5%, 10%, 15%, 20% and 25%) and B (15%, 20% and 25%), whereas, the lower concentrations (0.1%, 1%, 2.5% for sps "A" and 0.1%, 1%, 2.5%, 5%, 10% for sps "B") were not inhibitory. Results suggest that, the extracts of the plant in natural conditions, and in realistic concentrations are not inhibitory for the growth of the algae tested.

Key Words: *Allelopathy, Allelochemicals, Algae, Prosopis Juliflora*

INTRODUCTION
Allelopathy is a plant-plant and plant-microbe interaction, by releasing chemicals into their surrounding environments (Mollisch 1937 and Rice 1984). There are several plant species which are famous for their allelopathic potential such as *Prosopis juliflora* (Al-Humaid and Warrag 1997; Noor *et al.*, 1995; Sankhla 1965 and Warrag 1995), *Lantana camara, Tamarindus indicus, Datura metal* and so on. These are reported to inhibit the growth of the plant by releasing allelochemicals. Some of the allelochemicals interfere with the germination process and cause the failure of germination, while some interfere with the growth of radical and plumule (Inderjit and Bhowmik 2004 and Muscolo *et al.,* 2001 and Reigosa *et al.*, 1999). Some of the chemicals when released into the soil are reported to influence physicochemical properties of soil (Inderjit and Bhowmik, 2004). This alters the composition and activity of soil microflora, such as plant growth promoting bacteria and fungi. In submerged cultivation of rice, the addition of litter could influence the growth and activity of aquatic algae, more importantly the blue green algae which are vital for the functioning of the system, due to the pivotal role they play in the nutrient cycling. Detrimental influence of the litter on BGA is gratuitous for the cultivation of rice crop which makes the major food crop of Andhra Pradesh. Therefore, the present study was undertaken to study the effect of litter on the growth of BGA isolated from rice fields in Tirupati, Andhra Pradesh.

MATERIALS AND METHODS
Collection of plant material and extract preparation
Matured leaves of *P. juliflora* DC were collected from the trees growing in the campus of Sri Venkateswara University, Tirupati, Andhra Pradesh which is present at the longitudes and latitudes of $13^0$40'N and $79^0$20'E, and used as the source allelopathic plant material.
The leaves were shade dried for one week, powdered and sieved through 2mm sieve. The fine powder was liquefied with distilled water (10grams of powder in 200ml of distilled water). The mixture was incubated for 2 days, than filtered through Watsman No. 1 filter paper, and used as stock solution. The stock material was tested for its pH, EC and polyphenol content.
Different concentrations of the stock extracts were prepared *viz.*, 0.1%, 1%, 2.5%, 5%, 10%, 15%, 20% and 25% by diluting with distilled water.

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Preparation of paper discs

The above diluted extracts were used to prepare paper discs. The Watsman no.1 filter paper was made into discs. Each of the discs was made to contain 1ml of the extract. This was done by adding 25 µL of the extract and then drying, the sequence was repeated till the disc contained 1ml of the extract. Discs which served as control were similarly wetted with distilled water for the equivalent volume.

Collection, isolation and maintenance of algae

Water sample containing algae was collected from nearby fields and used for the isolation of the algal species. The BG-11 media was used for the culturing algae. 150ml of the autoclaved media was taken into sterilized conical flasks (3 numbers) and 5ml of the pond water was transferred into it. The conical flasks were kept in incubator at 25^0C temperature, with favorable light intensity for 15 days to grow algae. After incubation 2 species of the algae were isolated from the algal mixture that grew in the flasks, and transferred to other conical flasks with fresh media. The algae were sub-cultured every 15^{th} day into fresh media.

Setup of the Experiment

For the algal growth assay the method proposed by Tsuda et al., (2005) was used with some modifications. Two layers of algal media (with agar) were prepared in the petriplates i.e. upper layer contained algal culture and the lower one without the algae. Paper discs were placed onto the surface of solidified media. Each petriplate contained two paper discs, one of which was extract treated and the other one was the control (Fig. 1). The set up was triplicated and arranged in randomized block design. All the petriplates were kept for incubation for a period of 7 days. After 7 days, the zone of inhibition was measured and recorded.

RESULTS AND DISCUSSION

The polyphenol content, pH and EC of the stock extract are given in the Table 1.

The lower concentrations of the extracts did not inhibit the algae. Species A, was inhibited by the concentrations starting from 5% (5%, 10%, 15%, 20% and 25% extracts; table 2), whereas, species B was inhibited by higher concentrations of the extract only, starting from 15 % (15%, 20% and 25% extracts). 25% extract addition, which caused maximum inhibition of Species "A" (Table 2), had minimum effect on the Species "B" (Table 3). It shows that the different algae at different concentrations act differently.

As the growth of the plant not only depends on the soil fertility, it is influenced by many other factors including the biota in the vicinity of the plant. Soil microflora such as BGA fix the nitrogen form air, and make it usable for the plants. When chemical fertilizers or organic manure is added to the soil, care needs to be taken to ensure that it has no detrimental effect on the microflora of the rhizosphere. Tsuda et al., (2005) reported that the allelochemicals from 21 different plant species inhibited the growth of the Cyanobacteria, Mycrocystis. According to Gross (2003), the leaf litter may strongly influence algal communities especially in shallow lakes and reservoirs. In some shallow lakes, large macrophyte populations can suppress water blooms in spite of eutrophication (Scheffer et al., 2001 and Takamura et al., 2003). The underlying reason in all these cases is proposed to be the release of allelochemicals from the litter which influences the growth of the target species (Morris et al., 2003). Newman and Barrett (1993) and Ridge et al., (1999) demonstrated the algicidal effect of leaf litter and straw.

Both the species we tested had no inhibition zone at the lower concentrations of leaf extracts. But the interesting observation was the zone of inhibition, which was visible during the early periods of incubation, indicating the suppression of the growth of algae by the extract; it diminished in the later course of incubation. Finally the algal growth could be seen within the inhibition zone during the final stages.

The present attempt, suggests that the leaf extracts of *P. juliflora* have no inhibitory affects on the growth of algae in treatments with realistic concentrations (concentrations which can be encountered, when litter is added in the fields), and slightly at higher concentrations. The algae develop tolerance against the alllelochemicals in due course of time and starts to make use of the nutrients present in the extract for its growth.

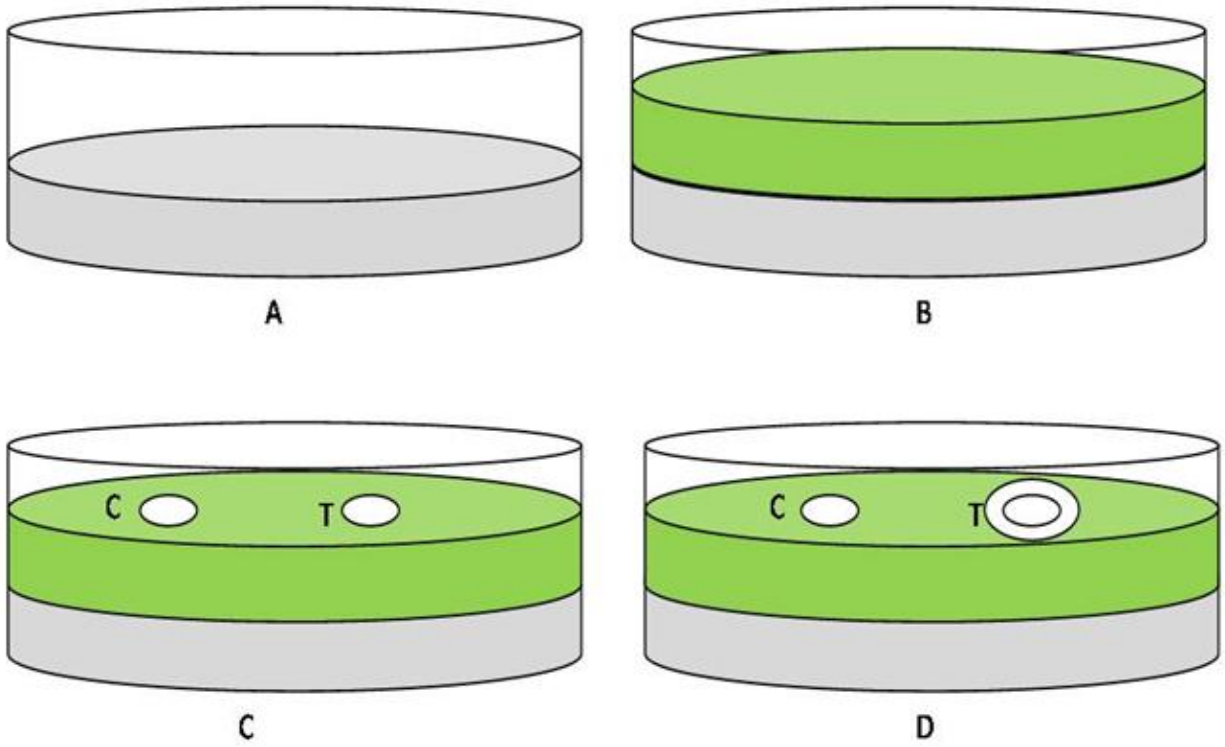

Figure 1: Set up of the Experiment
A- petriplate with BG-11 media bottom layer, B- algae+media over the bottom layer,
C- placing the paper discs (C= control and T= treatment), D- zone of inhibition visible around the treatment disc

Table 1: Polyphenol content, pH and EC of the Stock

S. No.	Parameter	Value
1.	Polyphenol Content	3.50
2.	pH	9.05
3.	EC	0.18

Table 2: Zone of inhibition of algae (Species "A") when exposed to paper discs prepared with leaf extracts

S. No.	Treatments	Zone of Inhibition(sps a) Diameter (mm)
1.	0%	0
2.	0.25%	0
3.	0.50%	0
4.	1%	0
5.	2.50%	0
6.	5%	13.5±3
7.	10%	17.5±1.9
8.	15%	17.5±1
9.	20%	22±2.8
10.	25%	24±1.6

Table 3: Zone of inhibition of algae (Species "B") when exposed to paper discs prepared with leaf extracts

S. No.	Treatments	Zone of Inhibition Diameter (mm)
1.	**0%**	0
2.	**0.25%**	0
3.	**0.50%**	0
4.	**1%**	0
5.	**2.50%**	0
6.	**5%**	0
7.	**10%**	0
8.	**15%**	17.33±1.1
9.	**20%**	20.00±5.3
10.	**25%**	12.67±1.2

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