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Allelopathic potential of leaves of *Lantana camara* L. for purification of waste water of Amani Shah Nala, Jaipur

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ABSTRACT

Plant extracts of various parts viz., seeds, leaves, bark, roots and seeds have been used for water purification for many centuries. According to UNICEF about 15 % population of the world is facing safe drinking water problem and 5 million people died once a year (WHO, 2006). Different plant parts of *Strychnos potatorum*, *Tamerindous indica*, *Cyamopsis psoraloides*, *Hibisicus sabdariffa*, *Trigonella foenum*, and *Lens esculenta* have been conducted using raw water with turbidity that ranged from 50 to 7500 NTU (Nephelometric Turbidity Units). The activity of *Lantana* leaves were tested by two experiments. In experiment 1 colony count and turbidity methods was followed whereas in experiment 2 only colony count method was followed. The increasing concentration from 1-10 reduced the bacterial growth and turbidity also decreased with increase the concentration.

Introduction

Lantana camara L. (family Verbenaceae) is popular ornamental garden plant (Ghisalberti, 2000) and commonly called as Wild sage and Sleeper weed. It is native to tropical and sub-tropical areas of America (Palmer and Pullen, 1995). It is considered as the ten most toxic (Holm and Herberger, 1969) and worst weed (Holm *et al*., 1977; Cronk and Fuller, 1995) in the world. It has been used for various disorders (Day *et al*., 2000). In China it has been used for ecological pest management and pests are controlled by incorporation into herbicide made out of this plant (Graff, 1986; Kong *et al.*, 2007).

In the reports of UNICEF (2009) about 15 % population of the world is facing safe drinking water problem. Around 5 million people died once a year (WHO, 2006). Natural plant extracts derived from seeds, leaves, bark, roots and seeds have been used for water purification for many centuries. Plants like *Strychnos potatorum* (Shultz and Okun, 1984; Sanghi *et al.* 2006) and Zea mays was used as a settling agent by sailors in the 16th and 17th centuries. Previous studies reveal that plant extracts such as nirmali tree (*S. potatorum*), tamarind tree (*Tamerindous indica*), guar plant (*Cyamopsis psoraloides*), red sorella plant (*Hibisicus sabdariffa*), fenugreek (*Trigonella foenum*) and lentils (*Lens esculenta*) have been conducted using raw water with turbidity that ranged from 50 to 7500 NTU (Shultz and Okun, 1984). Bacterial presence has been removed from range of 90–99% (Madsen *et al.*, 1987).

Turbidity values as high as 270–380 NTU were reduced to around four NTU, which are within the WHO (2006) guideline value with the addition of the powder (Sutherland *et al*, 1994). Yongabi (2004) tested the coagulative and disinfective capabilities of *M. oleifera*, *J. curcas*, *Pleurotus tuberregium sclerotium* and *H. sabdariffa* against alum on wastewater samples. The number of total bacterial counts reduced from 'too numerous to count' to 2700 colony forming units per ml with *M. oleifera* powder, which accounted for a 66% greater reduction than alum. In particular, Yongabi (2004, p. 12) claimed that *J. curcas* seeds and calyx of *H. sabdariffa* possessed both a coagulative and a disinfective ability. Ghebremichael (2004) reported that the sludge produced from *M. oleifera* coagulated turbid water is only 20–30% that of alum. Litherland (1995), Sanghi *et al.* (2006) and Katayon *et al.* (2006) reported that the residue of alum in water may be carcinogenic (Sanghi *et al.*, 2006).

The plant extracts of *M. oleifera* and *S. potatorum* have been used for water purification in Malawi (Jahn, 1986; Muyibi and Evison, 1995; Sanghi *et al*., 2006).

Fig. 1. Amani Shah Nala, Near Maharani Farm, Jaipur, India

Material and method

Collection of sample: The polluted water sample was collected from the Amani Shah Nala, near Maharani Farm, Jaipur in the month of January, 2012. The water sample was collected in the sterilized glass bottles. These sample bottles were rinsed three times with source water to minimize the risk of external contamination before sampling (Paqualab Manual, 2005, p. 17).

Impact of aqueous leachate of *L. camara* leaves (Exp. I)

Fresh leaves of *L. camara* collected from the Department of Botany, University of Rajasthan, Jaipur were cut into small pieces (2-3cm). Five concentrations (1, 2, 3, 5 and 10%) of leachates were prepared by adding 10, 20, 30, 50 and 100 g fresh leaves in 1 liter polluted water and kept for 48 h and referred to

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as treated polluted water. After 48 h, it was filtered through 3 layers of muslin cloth. These samples were serially diluted to 10^{-2} and 10^{-5} times and 10^{-5} was used for plating on nutrient agar for counting the bacterial colonies by colony count method. Five replicates were used for each treatment.

Further, nutrient broth was also inoculated using various concentrations of treated polluted water and optical densities were measured. In both the cases, untreated polluted water without plant material was used as control.

(ii) Impact of aqueous extracts of *L. camara* leaves (Experiment II)

The extracts of various concentrations of *Lantana camara* ranging from 1-10%, were prepared by boiling fresh leaves in distilled water for 20 minutes. It was filtered through three layers of muslin cloths. Each 100 ml extract of each concentration was inoculated with 1 ml of polluted water sample and was kept for 24 h at 37^{o} C in BOD incubator and plated on nutrient agar media for counting bacterial colonies after 24 h. The untreated water without plant material was used as control. Five replicates were used for each treatment.

(A) Colony Count Method: Treated polluted water (1ml) of each concentration was transferred into sterilized Petri Plates. Autoclaved luke warm nutrient agar media was poured into Petri Plates containing treated polluted water. Petri Plates were gently swirled on the surface of the laminar bench to mix the inoculum properly. In control, untreated polluted water was used. After solidification of media, plates were placed in inverted position for 24 h at 37^{o} C in BOD incubator. The bacterial colonies appeared were counted after 24 h. The experiments were performed in five replicates.

(B) Optical Density Method: Autoclaved nutrient broth media (10 ml) was poured into glass test tubes. These test tubes were inoculated with 100µl (0.1ml) treated polluted water. These tubes were incubated for 24 h in BOD incubator at 37^{o} C. After incubation period optical density was measured (CARL ZEISS, JENA, DDR, VSU, 2-P spectrophotometer).

Results

Allelopathic impact of leaves of *L. camara* on microbes present in polluted water

Aqueous leachate of *L. camara* leaves strongly significantly inhibited the number of bacterial colonies at each concentration ranging from 1 to 10 in all the diluted polluted water samples (10^{-2} and -10^{-5}). Maximum numbers of colonies were observed in control (98.2±27.9) and only 1.5±0.94 colonies were found at higher concentration (Exp.1, Fig.2). In control 60.6 ±24.26 number of bacterial colonies was observed (Fig. 3). The results reveal that increasing concentration of extract from 1-10% also reduces the bacterial growth of polluted water.

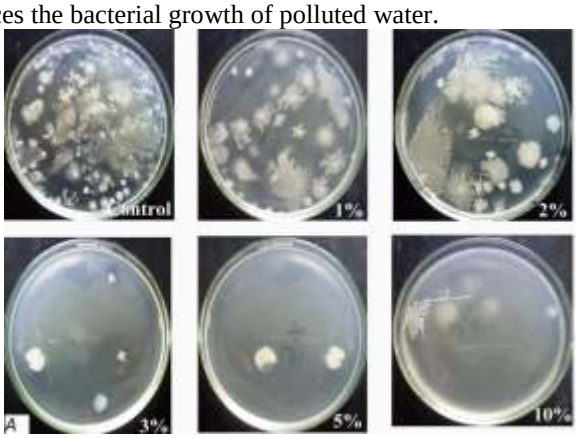

Fig. 2. Allelopathic impact of aqueous leachate of *L. camara* leaves (Colony count method, Experiment 1)

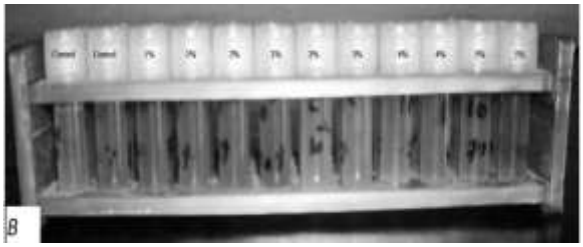

Fig. 2. Allelopathic impact of aqueous leachate of *L. camara* leaves (Optical Density Method, Experiment 1)

The corresponding optical densities of bacterial growth in nutrient broth (24h) also confirmed the gradual declination in bacterial growth with increase in concentration from 1-10% (Table 1 and Fig. 2)

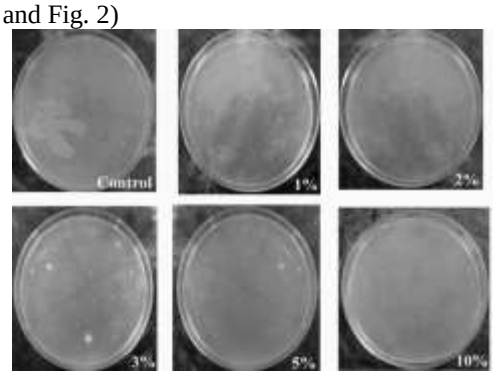

Fig. 3. Allelopathic impact of aqueous extract of *L. camara* leaves (Colony count method, Experiment 2)

Statistical analysis (Experiment 1)-The correlation coefficient (r) between different concentrations (1-10) of aqueous leachate of *L. camara* and their impact on bacterial growth of polluted water reveals a strong significant negative correlation (-0.9202). The increasing concentration of leachate reduce the bacterial growth.

The same results were obtained with extract of *L.camara* inoculated with polluted water. The same strongly inhibited the growth of bacterial colonies at each concentration. The corresponding optical densities of bacterial growth in nutrient broth (24h) also confirmed the gradual declination in bacterial growth with increase in concentrations from 1-5% concentration (Table 1).

Statistical analysis (Experiment 2)- The correlation coefficient (r) between concentrations (1-10%) of aqueous extract of *L. camara* and number of bacterial colonies present in polluted water reveals a significant negative correlation (–0.9751).

Discussion and conclusion

Today waste water problems have become a major issue for the health of society. The major sources of polluted water are food, beverage and soft drinks processing industries. These industries expel the chemical waste directly into the ponds, rivers and canals. They promote fast growth of various types of pathogenic bacteria. When these sources discharge into any kind of drinking water source it damage the water quality and cause infectious disease arose from polluted water, so pre-treatment of such water.

The study reveals that the aqueous extract and aqueous leachate of leaves of *Lantana camara* have been identified as the disinfectant and may be used as the purifying agent in industry working for the treatment of polluted water.

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Table-1: Allelopathic impact of leaves of *L. camara* on microbes present in polluted water

Polluted water samples	OD of samples at 600nm (Experiment 1)		No. of bacterial colonies			
	10^{-2}	10^{-5}	Fresh leaves of *L. camara* were added to polluted water for 48h (Experiment 1)		Sterilized Extract of *L. camara* was inoculated with 1 ml of polluted water (Experiment 2)	
			Colonies	% of Control	Colonies	% of Control
Control•	0.355±0.47	0.014±0.49	98.2±27.9	100	60.6±24.26	100
1%	0.145±1.95	0.008±0.37	35.4±9.2	36.04	37.2±7.35	61.3
2%	0.057±0.94	0.006±1.49	30.0±3.6	30.50	31.0±0.0	51.1
3%	0.035±0.48	0.005±1.30	11.6±2.1	11.80	15.3±2.12	25.2
5%	0.033±0.98	0.003±0.47	2.2±1.44	2.24	2.2±1.30	3.63
10%	0.020±0.23	0.002±0.23	1.5±0.94	1.52	1.3±0.78	2.14
r			–0.9202**		–0.9751**	

Mean ± SE, * and **- significant at 0.05% and 0.01 % level where $t = r\sqrt{n-2/1-r^2}$, r – correlation coefficient, • – polluted water sample without leaves of *L. camara* as Control, 1,2,3,5 and 10% -concentration of leaves of *L. camara* in polluted water (w/v), 10^{-2} and 10^{-3} times dilution of polluted water, OD – optical density.