Pak. J. Bot., 46(2): 465-470, 2014.

AUTO TOXICITY OF *AVICENNIA MARINA* (FORSK.) VIERH IN PAKISTAN

KANWAL NAZIM[1*], MOINUDDIN AHMED[2], SYED SHAHID SHAUKAT[2], MUHAMMAD UZAIR KHAN[1] AND SYED SADRUDDIN HUSSIAN[2]

[1]*Marine Reference Collection and Resouce Centre, University of Karachi*
[2]*Laboratory of Dendrochronology and Plant Ecology, Department of Botany and Gulshan-e-Iqbal, Karachi, Pakistan*
[*]*Corresponding author e-mail: nazim_kanwal@yahoo.com*

Abstract

During field survey it was observed that despite the presence of full sunlight mangrove forests show gaps or small number of stunted seedlings. These observations leaded to consider evaluation of autotoxicity of *A.marina,* therefore a glasshouse experiment was conducted to observe the possible allelopathic effects of leaf and pneumatophore extracts of *Avicennia marina* on its own seedling survival and growth. Two different types of soil with a total of five treatments of leaves and pneumatophores were used including control amid five replicates. The results showed that the extracts from *Avicennia* leaves and pneumatophores had inhibitory effects on it's own growth. The leaf extracts showed stronger allelopathic effects than pneumatophore extracts on root length and dry weight while pneumatophore extracts had greater allelopathic effects on shoot height. Therefore, it may also be concluded that besides shade, allelopathy may be responsible for lack of *Avicennia* seedlings under its own mother plant.

Introduction

The vegetational composition and population structure of a site often considerably depends on allelopathic interactions between species (Shaukat *et al*., 1983). Allelopathy is also an important ecological process in natural and managed ecosystems (Rice, 1995). According to Radosevich & Holt, (1984) the primary effect of allelopathy results from an association with plant litter in or on the soil. Aldrich, (1984) stated that allelochemicals must be concentrated in the leaves, stem or roots rather than in the fruit or flowers. Putnam, (1985) reported that allelochemicals are present in virtually all plant tissue, i.e. leaves, fruit, stems, and roots. Allelopathy involves the production of allelochemicals by a plant that can be harmful or beneficial to other plants or may be to itself (autoallelopathy). Shaukat *et al*., (2003) explained that the allelopathic interactions involve the production and release of chemical substances (allelochemicals) by certain, plants that inhibit the growth and development of the individuals of the neighboring species and sometimes their own (autoallelopathy).

The shoreline of Pakistan is approximately 1050 km long and 40-50 km wide. It is dominated by the grey mangrove *Avicennia marina* which has an extensive system of aerial roots or pneumatophores, having pores or lenticels through which gaseous exchange occurs. Leaves are opposite, glossy green above and pale grey below having salt secreting glands. Mangrove ecosystem, like other ecosystems exchange materials and energy between the organisms and their environment. Historically, more than 160,000 ha of the Indus Delta were covered with the mangroves which have reduced to 74,000 ha as per recent studies by WWF-Pakistan. The survival of these forests is largely associated with inundation frequency, wave action, duration, prevalence of certain environmental conditions, ecological resources and anthropogenic disturbances. They stresses are often chronic and continuous for the development of the mangrove ecosystem, any recovery won't occur because these are always being there. Field observations showed that whenever there was accumulation of leaf litter or high density of pneumatophores, the mangrove seedlings were either completely absent or if there were a few seedlings in the vicinity, they exhibited stunted growth. It was also examined during field survey that mangrove seedlings did not grow under shade (Kathiresan & Rajendran, 2002) they require direct sunlight for their establishment. Lack of mangrove seedlings under mangrove trees were reported by various workers Smith, (1992); Feller & McKee, (1999); Clarke & Kerrigan, (2000). Big gaps are also observed in mangrove ecosystem due to the death of mature trees or according to Feller & McKee, (1999); Clarke & Kerrigan (2000) windstorms, lightning, cyclones, pathogens and wood borers are the instinctive disorders that form gaps in mangroves. These gaps also show no seedlings or small number of stunt seedlings despite the presence of full sunlight. These observations leaded to consider evaluation of autotoxicity of *A.marina*. This phenomenon may play an important role in mangrove ecosystem which may have been given no attention in Pakistan. Therefore, the purpose of the present study was to determine whether pneumatophores and leaves have allelopathic effects against seedlings of *Avicennia marina.*

Materials and Methods

A pot experiment was conducted in a greenhouse at Federal Urdu University during July, 2009. The mature green leaves and pneumatophores of *Avicennia marina* were collected from the mangrove swamps at Sandspit, Karachi coast. It is a sandy place located in the southwest of Karachi city and stretches about 20km along the Arabian sea coast. The collected leaves were air-dried for 3 to 4 days at about 28±2ºC. The mature pneumatophores which were collected from the ground level, were washed to remove the adhering soil, algae and barnacles, subsequently they were air-dried. The leaves and the pneumatophores were ground with the help of electrical grinder. Mud samples were collected from Sandspit Karachi coast 24°84 N° latitude 66.89 E° longitude while soil (loamy sand) samples were collected from Federal Urdu University. This experiment was set up based on factorial design with two factors namely, the soil type and the extract type. A total sixty pots were prepared with five replicates for each treatment including controls. The powder of pneumatophores (10g/l and 20g/l) and leaves (10g/l and 20g/l) were soaked in water for three days. A single seed (propagule) of *Avicennia marina* was sown in each pot. The soil in the pots was kept moist with fresh water throughout the duration of experiment. Survival was recorded on alternate days and it was completed at 15 days after sowing. The shoot length and number of leaves were recorded every month. The plants were terminated after 12 weeks. The number of roots, fresh weight of roots and shoots were recorded. The shoots and roots were dried for 24h in an oven at 80^{o}C and subsequently dry weights of roots, dry weights of shoots and root shoot ratio were determined. The data were subjected to factorial ANOVA and logistic curves were fitted, using computer software Costat and Curve fit respectively. The logistic curves were fitted to the data to describe the relationship between plant response and time by using the following equation:

$$Y = \frac{a}{(1 + b^{*} \exp(-xc))}$$

Results

Effects of treatments on final shoot length, root length, number of roots; fresh shoot weight, fresh root weight, dry weight of shoot, dry weight of root and root shoot ratio were recorded and summarized (Tables 1, 2 and 3).

The final survival percentage remained unaffected and 100% survival was recorded in all treatments (i.e., extracts) and controls (Table 1). However, the time of emergence was markedly delayed compared to control. Every logistic curve has a single inflection point which separates the curve into two equal regions of opposite concavity. It was obvious that treatments inhibited the shoot length in both sand and loamy sand soil compared to controls. As shown by the S-shaped curve (Figs. 1 to 2), the characteristic concave shape in the graph of a logistic function shows that initial exponential growth is followed by a period in which growth slows down and then tends to level off, approaching (but never attaining) a maximum upper limit. Figures 3 and 4 showed that among all the treatments number of leaves were higher in control in both sand and loamy sand, though no significant difference was found.

Table 1 showed that the treatments, soil types and their interactions were significant ($F=138.84$ $p<0.001$, $F=199.94$ and $F=67.09$ at $p<0.001$ respectively). The number of leaves was also significantly affected by treatments and their interactions ($F=10$ and $F=13.66$ at $p<0.001$) respectively. The results of FANOVA of the number of roots and root length showed reduction compared to control, however treatments in general had no significant effect on these two parameters. The soil type significantly affected the number of roots and length of roots ($F=25.66$, $p<0.001$ and $F=11.95$, $p<0.01$ respectively). The results disclosed that the root length was maximum in controls 20.5±0.51 in sand and 15±2.08 in 20% pneumatophores in loamy sand. The least root length 14.16±0.83 was found in 20% Pneumatophores in sand. Soil type had a significant influence on the activity of shoot and root length ($p<0.001$). The reduction was more pronounced in loamy sand compared to sand. The shoot and root growth were more or less equally affected by the amendments.

The allelopathic effect of leaves and pneumatophores was found to be stimulatory regarding the fresh and dry weight of shoot and root of *Avicennia* seedlings (Table 2). The fresh weight of shoot and root was significant at $p<0.01$ whereas the dry weight of shoot and root was significant ($F=17.61$ and $F=17.4$ at $p<0.001$). Soil type was not statistically significant on fresh and dry weight of shoot and root. The interaction between treatment and edaphic factors in dry weight of shoot was significantly different ($F=4.26$, $p<0.05$). The fresh weight of shoot and root were maximum in control 4.6±0.29 g and 3.27±0.38 g respectively in mud compared to loamy sand. Fresh weight of shoot and root were reduced by 3.47±0.26 and 1.60±0.41 of control by 20% leaf extract. The results clearly showed that fresh and dry weight of shoot and root had no inhibitory effect of mud and soil. The results showed that the dry weight of shoot was maximum in controls 3.32±0.17g in mud while dry weight of root was found highest 2.24±0.06g in loamy sand compared with other treatments (Table 3). However, soil type did not significantly suppress the fresh and dry weights of shoot, root and root shoot ratio.

Discussion

This study provides evidence on the autoallelopathic potential of *Avicennia marina*. The allelo-chemicals released from different concentrations of leaves and pneumatophores have harmful effects as exhibited by the delay in emergence, number of leaves and reduction in shoot and root growth. In the present study, response indices revealed that the inhibition of growth parameters of seedlings was more pronounced than the influence on seed emergence. To the best of our knowledge no previous report exists on the autoallelopathy of *A.marina*, hence from preliminary screening it was found that leaf and pneumatophores extracts had no allelopathic effect on seed emergence. It is suggested that the allelochemicals, present in the extracts were not harmful for the growth and development of the embryo. The inhibitory effect of leaf and pneumatophores on seedling growth was due to the presence of growth inhibitors (allelochemicals) in the extracts. It is obvious that mangrove seedlings, which are existing in fully exposed areas, often fail to develop in spite of the fact that all other environmental factors appear favorable (Kathiresan & Rajendran, 2002). During surveys many times it was observed that mangroves in early stages of development experience higher mortality rates and are at higher risk compared to the large trees. The results explained that besides other factors this suppression was possibly due to the presence of allelochemicals in leaves and pneumatophores of *Avicennia marina* derived from rain falling through the tree canopy and would restrict upward growth of plants and drastically limit their growth. Leaf litterfall and the decaying pneumatophores may produce different chemical compounds that inhibit the growth of the same species at a certain concentration.

Table 1. Factorial analysis of variance (FANOVA) on effect of treatments on overall growth of mangrove species *Avicennia marina*.

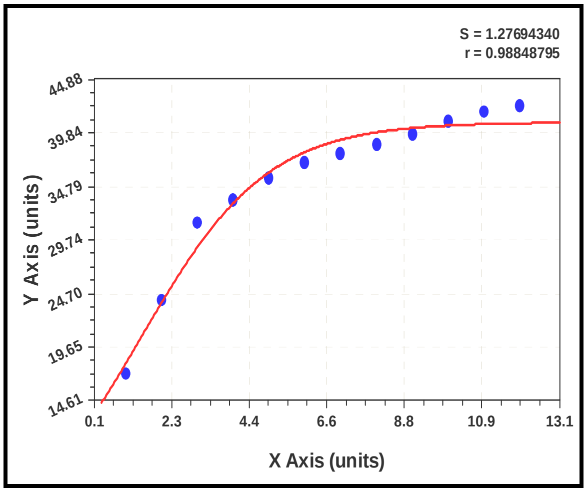
Mean effects	F-values of means								
	Shoot length (cm)	Number of leaves	Root length (cm)	No. of roots	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	R/S dry weight ratio (g)
Treatments	138.84***	10***	2.73 ns	1.33 ns	4.74**	6.84**	17.61***	17.4***	1.74 ns
Edaphic factors	199.94***	385.33**	25.66***	11.95 **	0.001ns	0.07ns	3.39ns	0.01ns	0.93 ns
Interaction treatment x Edaphic factors	67.09***	13.66***	2.91*	3.87 *	0.168ns	0.05ns	4.26*	0.47ns	0.99 ns

Table 2. Mean values of treatments on overall growth of mangrove species *Avicennia marina*.

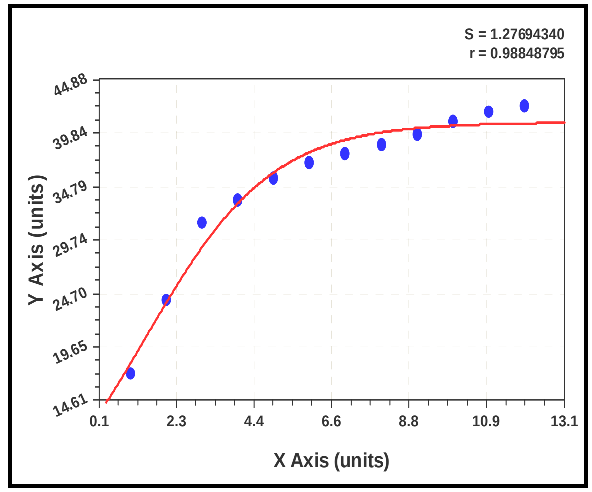
Treatments	Mean of equal number of plants								
	Number of leaves	Shoot length (cm)	Root length (cm)	Number of roots	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	R/S dry weight ratio (g)
Control	a 10 ± 0.76	a 41.96 ± 0.31	a 17.08 ± 1.68	a 8±0.88	a 4.53 ± 0.18	a 3.23 ± 0.23	a 3.18 ± 0.11	a 2.325 ± 0.11	a 0.72 ±
Leaf 10%	b 8 ± 0.75	c 35.34 ± 1.70	b 13.33 ± 1.55	a 9±0.52	b 3.75 ± 0.16	b 1.9 ± 0.27	a 2.88 ± 0.19	b 0.98 ± 0.12	a 0.61 ± 0.05
Leaf 20%	b 9 ± 0.50	c 35.03 ± 0.43	ab 15.46 ± 1.18	a 8±0.70	b 3.51 ± 0.15	b 1.65 ± 0.07	a 2.84 ± 0.17	b 0.72 ± 0.09	a 0.25 ± 0.03
Pneumatophore 10%	a 9 ± 0.84	c 35.3 ± 1.02	a 16.33 ± 1.19	a 9±1.12	b 3.53 ± 0.29	b 1.7 ± 0.31	b 2.37 ± 0.09	b 0.89 ± 0.25	a 0.37 ± 0.10
Pneumatophore 20%	b 9 ± 0.67	b 36.97 ± 1.41	ab 14.58 ± 1.02	a 7±0.65	b 3.45 ± 0.09	b 1.83 ± 0.19	c 2.03±0.11	b 0.77 ± 0.14	a 0.37 ± 0.06
LSD	**0.38**	**0.73**	**2.62**	**1.71**	**0.6**	**0.74**	**0.32**	**0.47**	**0.43**

Table 3. Mean values of soil type on overall growth of mangrove species *Avicennia marina*.

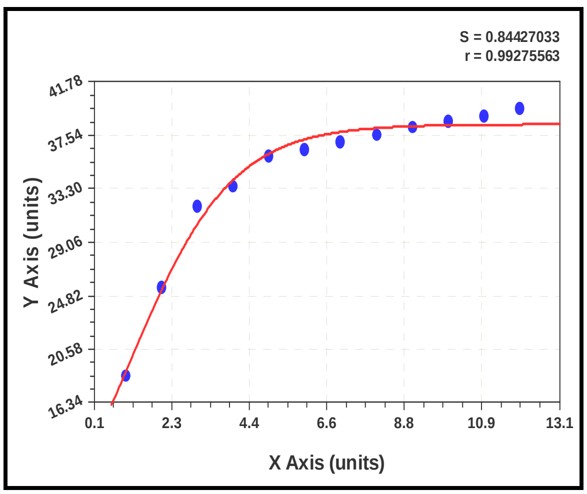
Treatments	Mean of equal number of plants								
	Number of leaves	Shoot length (cm)	Root length (cm)	Number of roots	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry Weight (g)	R/S Ratio (g)
Soil	b 8 ± 0.64	b 35.35 ± 1.63	b 13.34 ± 0.68	a 7 ± 0.43	a 3.75 ± 0.14	a 2.03 ± 0.21	a 2.57 ± 0.08	a 1.29 ± 0.16	a 0.42 ± 0.05
Mud	a 10 ± 0.57	a 38.49 ± 1.72	a 17.37 ± 0.69	b 9 ± 0.45	a 3.76 ± 0.16	a 2.09 ± 0.20	a 2.75 ± 0.17	a 1.14 ± 0.19	a 0.4 ± 0.05



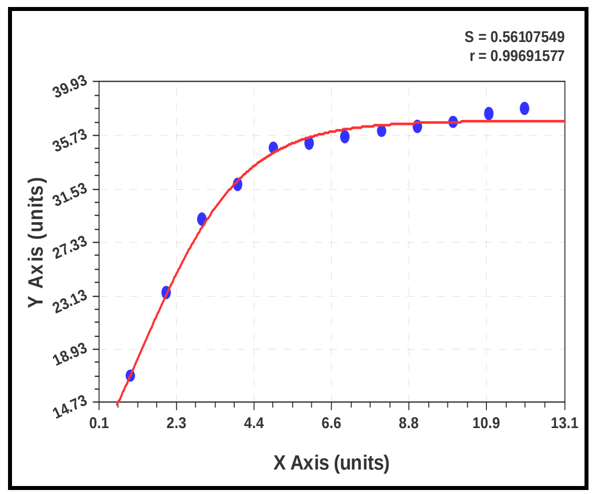
a. Control



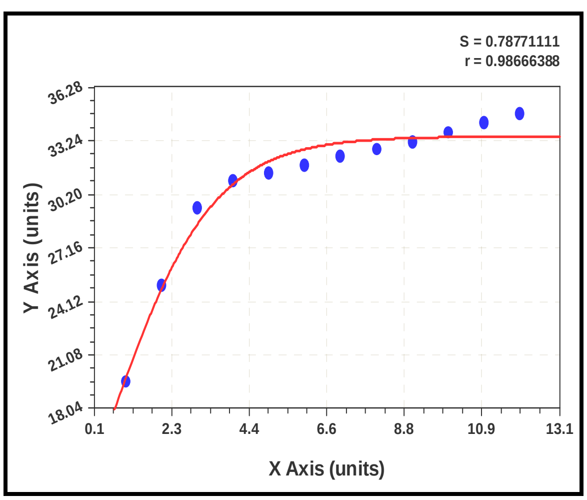
a. Control



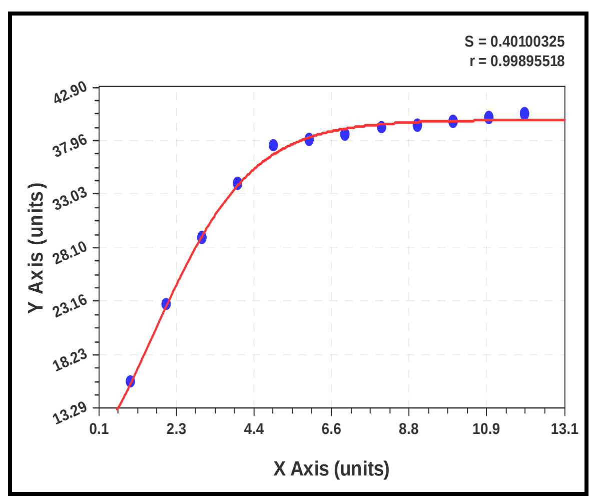
b. Leaf 10%



b. Pneumatophores 10%



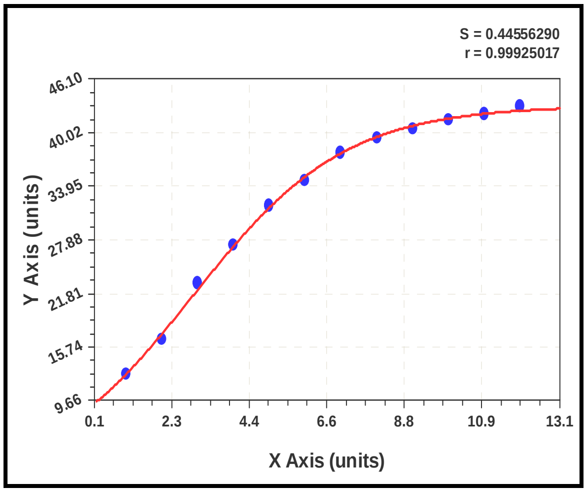
c. Leaf 20%



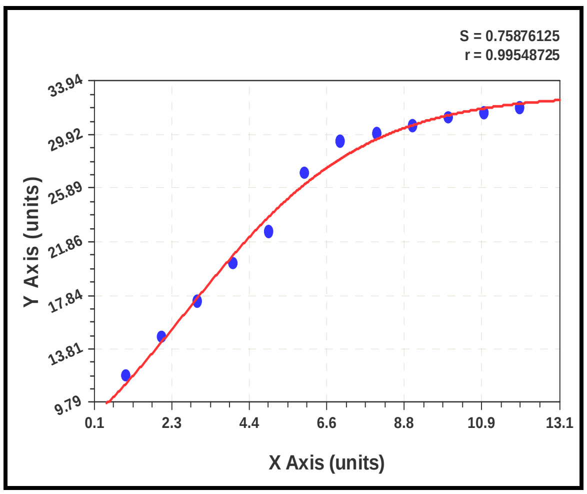
c. 20% Pneumatophores

Fig. 1. Allelopathic effect of different concentrations of leaves over the height of *Avicennia marina* in mud.

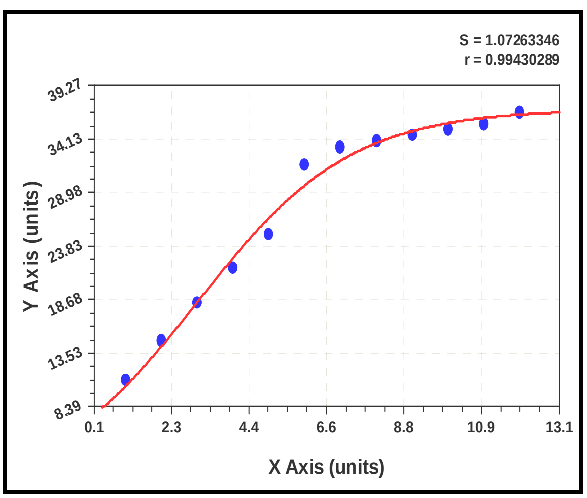
Fig. 2. Allelopathic effect of different concentrations of Pneumatophores over the height of *Avicennia marina* in mud.



a. Control

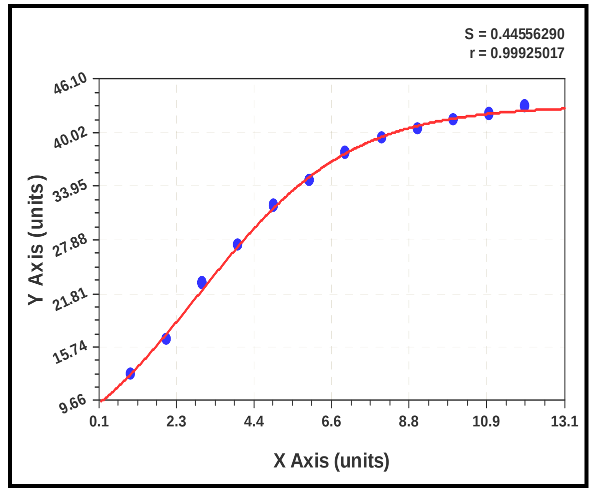


b. Leaf 10%

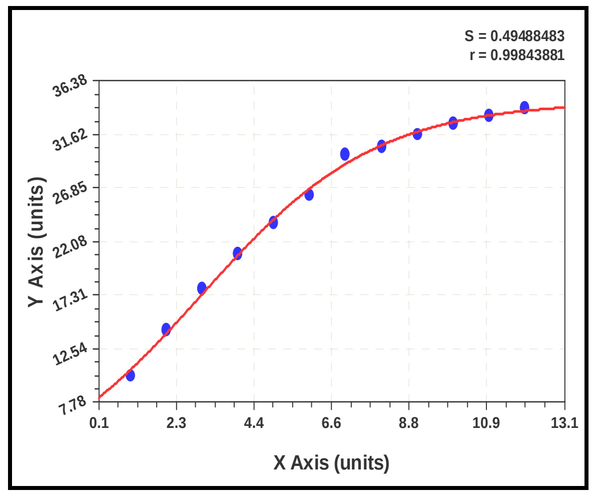


c. Leaf 20%

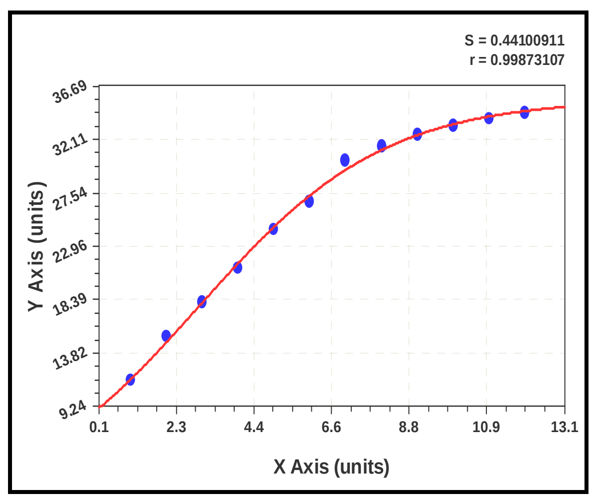
Fig. 3. Allelopathic effect of different concentrations of leaves over the height of *Avicennia marina* in soil.



a. Control



b. 10% Pneumatophores



c. 20% Pneumatophores

Fig. 4. Allelopathic effect of different concentrations of Pneumatophores over the height of *Avicennia marina* in soil.

Avicennia marina releases some toxic compounds i.e. phenols, tannins, azadirachtin, and ricinine (Tariq *et al*., 2007). According to Alam & Islam, (2002) these compounds are volatile in nature and are considered a threat to the marine environment. The toxic nature of phenolic compounds with reference to other plants has been reported by several workers (Stowe *et al*., 1987; Blum, 1996; Inderjit, 1998; Burhan & Shaukat, 2000). The differential effect of the different concentrations of the extracts on the early seedlings growth on its own test species presumably because of these different phenolic compounds and possibly other inhibitors might have variable effects. According to Kathiresan & Subramonia (1989) the seedlings of other mangrove species *Rhizophora mucronata* established poorly at stagnant pool due to leachates from the litter which may interfere with the growth of its seedlings.

It was found that in general the inhibitory allelopathic impact of leaf extract was more pronounced than that of pneumatophores may be due to an osmotic effect which may have been involved in this process (Clarke and Hannon, 1971). These findings were supported by Rajangan, (1984) who recorded inhibitory effect of aqueous extracts from fresh mangrove leaves on rooting of some crops. The results suggested that the different concentrations of treatments did not pose any toxicity on the number of roots however; the soil types and their interactions have adverse effect on the root growth and the number of roots. Kathiresan & Subramonia, (1989) recorded the inhibitory effect of leachates from leaf litter of mangrove on root growth but found no significant effect on the number of roots. This study provided the evidence about the inhibitory effect of the treatments on dry weight of root rather than shoot. The reduction in seedling dry weight may be due to adsorption of leachates near the soil surface, allowing only limited contact with roots of emerging seedlings. Barnes & Putnam, (1986) stated that the roots are in close proximity to soil would therefore have a greater probability of coming into contact with allelopathic compounds. The location in relation to growing roots appear to be an important factor in the allelopathic interaction (White *et al.,* 1989). Most of the allelopathic compounds such as alkaloids, phenols and various common derivatives are absorbed by roots (Winter, 1961) rather than other vegetative parts.

The phytotoxicity of *Avicennia marina* varied in the two soil types that differed in soil texture. The greatest activity was recorded in sandy soil this is presumably due to the fact that this type of soil has a very small proportion of colloidal material and hence the adsorption of allelochemicals is negligible (Shaukat *et al*., 2003).

Conclusion

On the basis of short term experiment it is concluded that chemical released from *Avicennia marina* though allow germination of seeds, diminished the height, number of leaves, fresh and dry weight of shoot and roots of its own seedlings through the process of allelopathy. Therefore, it may also be concluded that besides shade, allelopathy may be responsible for lack of *Avicennia* seedlings under its own mother plant. After the natural or accidental death of mature trees, gap is created. These weak seedlings gradually become weaker by the time and finally eliminated from the canopy of the mature trees. It is anticipated that since soils of these gaps are also rich in allelopathic chemicals no seedlings survived in these gaps for a long time despite the enough sun shine. However, long term studies are required to understand this mechanism in mangrove ecosystem.

References

Alam, S.M. and E.U. Islam. 2002. Effect of aqueous extract of Leaf, stem and root of nettle leaf goose foot and NaCl on germination and seedling growth of rice. *PJST*., 1(2): 47-52.

Aldrich, J.D. 1984. Weed-crop ecology: Principles and practices. *Breton Publishers*, 215-241.

Barnes, J.P. and A.R. Putnam. 1986. Evidence for allelopathy by residues and aqueous extracts of rye *(Secale cereale). Weed Science*, 34: 384-390.

Blum, U. 1996. Allelopathic interactions involving phenolic compounds. *Korean J. Bot*., 28: 259-267.

Burhan, N. and S.S. Shaukat 2000. Effect of phenolic compounds on germination and seedling growth of some crop plants. *Pak J. Biol. Sci*., 3: 269-274.

Clarke, P.J. and R.A. Kerrigan. 2000 Population structure and community composition of forest gaps in tropical mangroves. *Biotropica*, 32: 642-652.

Clarke, T. N and N. J. Hannon. 1971. The mangrove swamp and salt marsh communities of the Sydney district. III. Plant growth in relation to salinity and waterlogging. *Journal of Ecology*, 58: 351-369.

Feller, I.C. and K.L. McKee. 1999. Light-gap creation in a Belizean red-mangrove forest by a wood- boring insect. *Biotropica*, 31, 607-617.

Inderjit, 1998. Influence of *Puchea lanceolata* (Asteraceae) on selected soil properties. *A. J. Bot*., 85: 64-69.

Kathiresan, K. and N. Rajendran. 2002. Growth of mangrove (*Rhizophora apiculata*) seedlings as influenced by GA3, light and salinity. *Rev. Biol. T. Trop*., 50(2): 525-530.

Kathiresan, K. and T. Subramonia. 1989. Effect of leachates from mangrove leaf litter on rooting of *Rhizophora* seedlings. *Geobios*, 16: 27-29.

Putnam, A.R. 1985. Weed allelopathy. In: *Weed physiology volume 11: Reproduction and Ecophysiology*. (Ed.): S.O. Duke. CRC Press, 131-155.

Radosevich, S.R. and J.S. Holt. 1984 Weed ecology: implications for vegetation management. New York: John Willey, 1984. 265

Rajangan, M. 1984. Studies on allelopathic effects of mangrove leaves on crop plants. Ph.D. thesis, Annamali University, India.

Rice E.L. 1995. *Biocontrol of Weeds and Plant Diseases: Advances in Applied Allelopathy*. University of Oklahoma Press, Norman, OK, USA.

Shaukat, S.S., D. Khan and S.T. Ali. 1983. Suppression of herbs by *Inula grantioides* Boiss. in Sindh desert. *Pak J. Bot*., 15: 43-67.

Shaukat, S.S., N. Munir and I.A. Siddiqui. 2003. Allelopathic responses of *Conyza canadensis* (L.) Cronquist: A cosmopolitan weed. *Asian J. Plant Sci*., 2(14): 1034-1039.

Smith, T.J. III. 1992. Forest structure. *Tropical Mangrove Ecosystems* (Eds.): A.I. Robertson & D.M. Alongi, pp. 101-136. American Geophysical Union, Washington, DC.

Stowe, L.G., B.S. Kil and Y.J. Yin. 1987. A survey of old field herbs for susceptibility to phenolic compounds. *Korean J. Bot*., 30: 11-20.

Tariq, M., S. Dawar, F.S. Mehdi and M.J. Zaki. 2007. Use of *Avicennia marina* (Forsk.) Vierh in the control of root knot nematode *Meloidogyne javanica* (Treub) chitwood on okra and mash bean. 2007. *Turk J Biol*., 31: 225-230.

White, R.H., W. Douglas and B. Udo. 1989. Allelopathic potential of legume debris and aqueous extracts. *Weed Sciences*, 37: 674-679.

Winter A.G. 1961. New physiological and biological aspects in the interrelationships between higher plants. In *Mechanisms in Biologkal Competition*, (Ed.): F.L. Milthorpe. p. 229-244.NewYork: Academican, Press.

(Received for publication 3 February 2012)