Pakistan Journal of Nematology (2016) 34 (1): 91-100

ISSN 0255-7576 (Print)
ISSN 2313-1942 (Online)
www.pjn.com.pk

http://dx.doi.org/10.18681/pjn.v34.i01.p91

Evaluation of marine red alga *Melanothamnus afaqhusainii* against *Meloidogyne incognita*, fungus and as fertilizing potential on okra

A. M. Khan[1,†], S. Naz[1] and M. Abid[2]

[1]*Department of Chemistry, Federal Urdu University of Arts, Science and Technology, Gulshan-e-Iqbal Campus, University Road, Karachi-75300, Pakistan*
[2]*Department of Botany, Federal Urdu University of Arts, Science and Technology, Gulshan-e-Iqbal Campus, University Road, Karachi-75300, Pakistan*

[†]Corresponding author: dr.abdulmajeedkhan@fuuast.edu.pk

Abstract

Study was conducted to evaluate seaweed *Melanothamnus afaqhusainii* using its powder and different extracts including *n*-hexane, DMSO and water extract against naturally occurring soil nematode namely *Meloidogyne javanica* anti fungal activity against *Fusarium moniliforme* and *Rhizoctonia solani* and fertilizing potential on okra *Abelmoschus esculentus*. It was observed that the soil sample which was mixed with algal powder supported the growth of the okra plant and proved that the alga under investigation possessing some level of fertilizing potential.

Keywords: Red seaweed, okra, root-knot nematode, antifungal activity, nematicidal activity.

The gradual change in the environment, biodiversity, and soil fertility attracted the researchers to develop the chemical substances to increase the long term fertility of the soil. In this regard, synthetic fertilizers are playing a vital role for the promotion of agriculture. In general, the fertilizers are classified as synthetic and natural fertilizers. The synthetic fertilizers are prepared from urea on commercial scale using other ingredients like potash, lime and phosphate rocks. The natural fertilizers are derived from live stocks including biowastes, wastewater, algae etc.

The literature survey showed that seaweeds possess a potential for the production of commercially important products including biofuels, fertilizers, pharmaceutically important drugs, cosmetics and others (Khan, 2000; Rizvi & Shameel, 2006; Thirumaran *et al*., 2009). In Pakistan, different coastal areas like Buleji, Manora, Gidani, Paradise point are rich in various seaweeds. The availability of sea weeds in bulk amount attracted the researchers to investigate them for their nematicidal activity (Atta-ur-Rahman *et al*., 1997; Andrews, 2013; Haq *et al*., 2011). Literature showed paucity of research work from the coast of Pakistan which stimulated us to investigate marine red alga *M. afaqhusainii* which is available in bulk amount on the coasts.

Melanothamnus afaqhusainii is a marine reddish brown alga that belongs to the family *Rhodomelaceae* found on the rocks where the water waves can reach regularly. Some of the distinguish characteristics of the morphology of this alga are: it has cylindrical shape, its length is 36 cm, normally its growth appears during September to April (Shameel, 1999; Khan, 2000; Khan *et al*., 2012, Khan & Hussain, 2015). Some related work on this alga under different conditions is also reported in literature (Atta-ur-Rahman *et al*., 1997; Rizvi, 2010; Sultana *et al*., 2011; Sultana *et al*., 2013).

Materials and Methods

Collection and Extraction: The alga was collected from Buleji coast of Pakistan and identified by Prof. Dr. Mustafa Shameel (late), Department of Botany, University of Karachi. Its voucher specimen number (KUH-SW-R1461) was deposited in the herbarium of the same department. After the collection, the unwanted material was removed by washing with fresh water and then it was spread under shadow for smooth drying for about two weeks. Thereafter, the alga was converted into the powder form mechanically. Firstly, the powder was soaked in *n*-hexane (3litres) for a week then it was decanted and concentrated by rotary evaporator under reduced pressure. The whole process repeated thrice that yielded oily content (85 g). The residue (100 g) was soaked in DMSO (300 ml) for a week. The DMSO extract was decanted and concentrated that yielded 25 g DMSO extract. Finally, the residue (100 g) was soaked in water (500 ml) for a week and then decanted. The water extract was concentrated at water bath at 100 ^{o}C that yielded 45 g of water extract.

Materials: A number of materials including red alga, fungi, waste water, distilled water, *n*-hexane, DMSO, alcohol, okra plant seeds, nematode, potato dextrose agar, infested soil, microscope, autoclave, incubator, beakers, plants pots, cavity box, filter paper, wire loops and petri dishes were used for the experimental purpose.

Antifungal activity: A Potato dextrose agar (PDA) was selected for the growth of fungi (Bonzi1 *et al*., 2012). All the glass wares and materials were sterilized before the determination of antifungal activity. The streaking method was used to obtain the pure culture of *Fusarium moniliforme* and *Rhizoctonia solani* (Demirci *et al*., 2011, Fig. 1).

Disc diffusion method: The antifungal activity was determined by Disc Diffusion Method (Kositchaiyong & Sombatsompop, 2012). The freshly prepared cultures of *F. moniliforme* and *R. solani* were placed in the centre of the Petri dish. The sterilized filter paper discs were soaked in *n*-hexane (0.5%), DMSO (1%) and water (0.25%) extracts for few seconds. After drying at room temperature these disks were placed in the corners of all petridishes and finally incubated at 37 ^{o}C for 24, 48 and 72 hours. At the end, the zones of inhibitions were determined (Fig. 2 and 3).

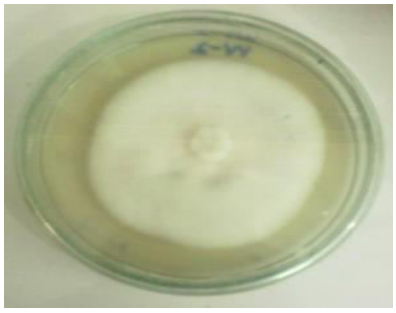
Nematicidal activity (*in vitro*): Different concentrations of *n*-hexane, DMSO and water extracts of *M. afaqhusainii* were studied against *M. javanica*. Hussey and Barker's method was used to isolate the eggs of *M. javanica* from *Solanum melongena* roots (Wiratno *et al*., 2009). The nematicidal activity of the algal extract was determined by a hatching test (Khalil & Badawy, 2012). To perform this test, 1 ml of each extract was separately taken in glass cavity block and placed at room temperature. Now, hatched eggs were examined under microscope (X6 107BN Nikon, made in China, Fig. 4) and counted after 24, 48 and 72 hours.

Mortality test: The *M. javanica* eggs which were obtained from the brinjal roots were put in distilled water and incubated at 27°C for 48 hours. After the performance of the hatching test the juveniles were collected in distilled water. About 30 to 45 juveniles/ml and algal extract (1 ml) were taken in the glass cavity block (0.25 mm diameter) and placed at 27^{o}C (room temperature) for 24 hours. Now, each sample was replicated 3 times. The control was used to compare the activity of the samples. The composition of the control was distilled water (1 ml), different solvents (1 ml each separately) and nematode suspension (1 ml). On the other hand, the sample contains algal extract (1 ml) and nematode suspension (1 ml).

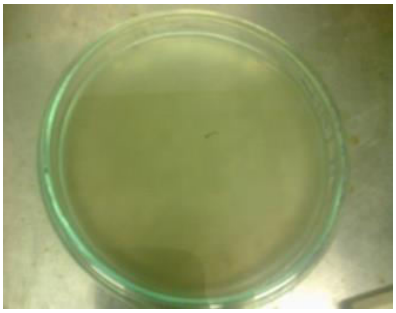
The killed juveniles were counted by microscope (X6, 107BN Nikon, made in China, Fig. 5) after 24, 48 and 72 hours. The % mortality was calculated by the following formula (Rizvi & Shameel, 2006):

$$\text{Mortality (\%)} = \frac{\text{Number of dead nematodes}}{\text{Total number of nematodes}} \times 100$$

(a) Inoculation of *F. Moniliforme*



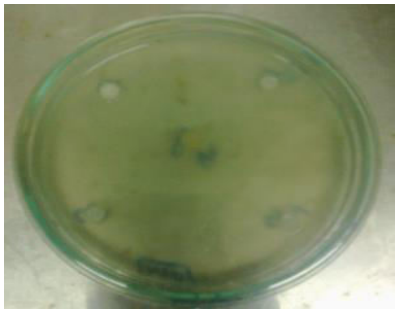
(b) Growth of *F. moniliforme* after 72 hours



(c) Inoculation of *R. solani*

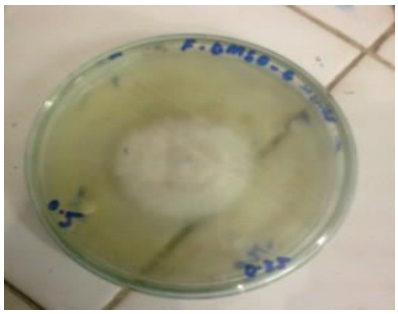
(d) Growth of *R. solani* after 72 hours

Fig. 1. Pure culture preparation of *F. moniliforme* and *R. solani* on potato dextrose agar media

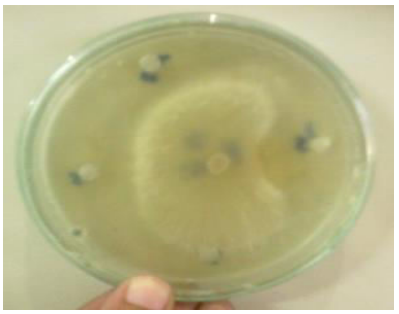


(a) Inoculation of *F. moniliforme*

(b) Zone of inhibition after 24 hours

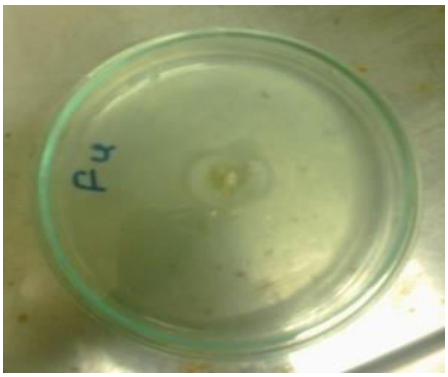


(c) Zone of inhibition after 48 hours

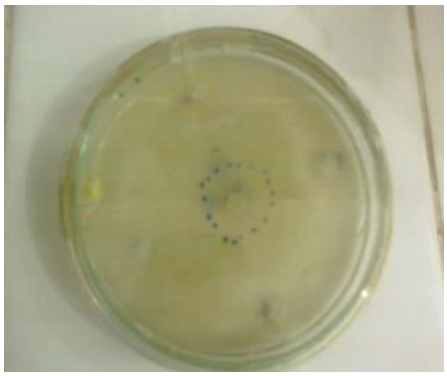


(d) Zone of inhibition after 72 hours

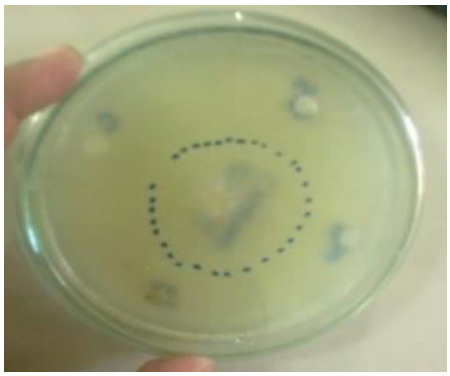
Fig. 2. Determination of antifungal activity by disc diffusion method.



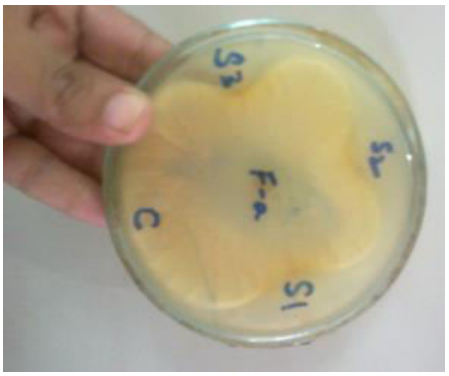
(a) Inoculation of *R. solani*



(b) Zone of inhibition after 24 hours



(c) Zone of inhibition after 48 hours



(d) Zone of inhibition after 72 hours

Fig. 3. Determination of antifungal activity of different extracts by Disc Diffusion Method.

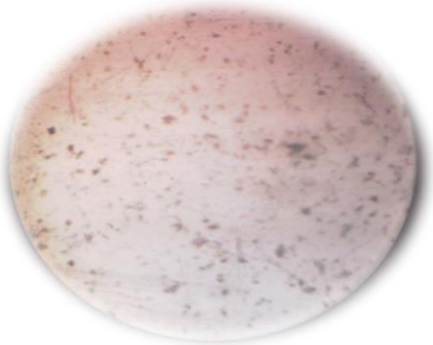


Fig. 4. Microscopic examination of the eggs of *M. javanica*

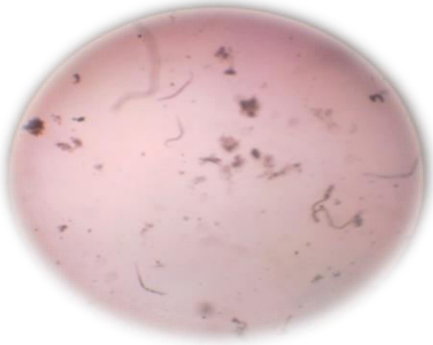


Fig. 5. Microscopic examination of *M. javanica* juveniles obtained from brinjal plants.

***In vivo* nematicidal activity:** For the determination of the seeds germination rate, 17 seeds of okra plant were placed on wet filter paper in a petridish. It was incubated at room temperature (32 ^{o}C) for seven days (168 hours) (Fig. 6). After the experimental time, the rate of germinated seeds is counted by using the following formula:

$$\text{Rate} = \frac{\text{No. of seeds germinated after week}}{\text{Total number of seeds}} \times 100$$

Seaweed's effect on the plants growth: The refined algal powder (20 g) was mixed with the soil taken in fibre cups (250 ml capacity) using different concentrations (*i.e.* 0.25%, 0.50% and 1.0%) and placed all the pots in the open atmosphere for two weeks along with the control (only soil /infested soil). All the pots were watered regularly for two weeks then five seeds of okra plant were sown in each pot and placed all the pots in the green house for germination. After the appearance of the growth, the eggs of *M. javanica* (1000 eggs/8 ml) were injected in the roots of plants and monitored the growth for 45 days (Fig. 7). After 15 days, the Invasion Test was performed to determine the number of motile and immotile nematodes. The roots were washed and examined for their galls after 15, 30 and 45 days. The egg-masses, gall number and juvenile numbers were determined along with the determination of average of shoot, root length and weight of shoot and root (Fig. 8-10)

Results and Discussion

The continuous decline in the fertility of the soil resulted in a rapid loss of biodiversity and badly affected the agriculture around the globe that stimulated the researchers to develop the alternative, sustainable and potential fertilizers that could effectively overcome the challenges faced by the entire field of agriculture. In this regards, this research article is particularly based on the evaluation of marine red alga *M. afaqhusainii* for its potential towards agriculture. Both the fungi showed prominent growth without the addition of the algal extract. The study showed that different extracts of this alga displayed the significant inhibitory effect on the growth of *F. moniliforme* and *R. solani* (Table 1).

This study evidenced that the algal powder and extracts possessing a potential to inhibit the growth of nematodes and provide the feasible environment for the growth of the plant. In this experimental study, algal powder, *n*-hexane, DMSO and water extracts were used for the determination of nematicidal activity against *M. javanica*. The hatching test was also performed where algal DMSO extract showed 13% and water extract showed 18% nematicidal activity after 72 hours (Table 2).

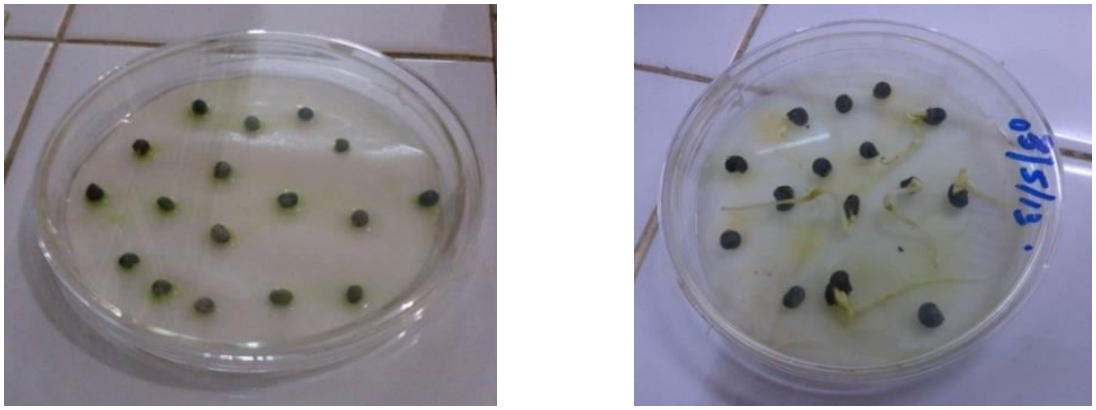


Fig. 6. Germination of okra seeds incubated at room temperature

(a)Seeds of okra plant

(b)Algal powder

(c)Process of seeds sowing

(d) Growth after two weeks

(e) Okra plants infected by nematodes

Fig.7. Pictorial representation of the nematicidal activity after two weeks (a-e)

Fig. 8. Growth of plants after two weeks.

Fig. 9. Growth of plants after four weeks

Fig. 10. Growth of plants after six weeks.

Table 1. Antifungal activity of algal water, *n*-hexane and DMSO extracts.

Fungi	Growth/ Inhibition zone (mm)	Water extract			*n*-Hexane extract			DMSO extract		
		24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
F. moniliforme	Growth zone	0.18	0.2	0.23	0.20	0.41	0.43	0.20	0.41	0.45
	Inhibition zone	0.27	0.25	0.22	0.25	0.04	0.02	0.25	0.04	0.0
R. solani	Growth zone	0.05	0.20	0.30	0.05	0.22	0.37	0.05	0.17	0.33
	Inhibition zone	0.40	0.25	0.15	0.40	0.23	0.08	0.40	0.28	0.12

Table 2. *In vitro* hatching test against algal water, *n*-hexane and DMSO extracts.

Samples	Total eggs of *M. javanica*	Eggs of *M. javanica*			Larva of *M. javanica*			% Hatching		
		24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
Control	38	38	38	37	0	0	1	0	0	3
Water extract	37	35	34	34	2	3	3	5	8	8
n-Hexane extract	44	42	41	39	2	3	5	4	7	11
DMSO extract	37	34	33	32	3	4	5	8	10	13

Table 3. *In vitro* mortality test against algal water, *n*-hexane and DMSO extracts.

Samples	Total juveniles	Live juveniles			Dead juveniles			% Mortality		
		24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
Control	37	37	36	35	0	1	2	0	2	5
Water extract	37	34	30	27	3	7	10	8	19	27
n-Hexane extract	38	30	28	27	6	10	11	16	26	29
DMSO extract	40	32	28	26	8	12	14	20	30	35

Table 4. Effect of algal powder of *M. afaqhusainii* on the growth of okra plants.

Samples	Days	Buds	No. of leaves	Length (mm)		Weight (mg)		Dry weight (mg)			
				Stem	Root	Stem	Root	leaves	Stem	Root	Leaves
Control (infested soil)	15	0	4	1.61	0.41	0.49	0.07	0.46	0.13	0.08	0.11
	30	0	5	1.92	0.52	0.83	0.24	0.92	0.14	0.14	0.12
	45	1	4	2.22	0.91	1.82	0.61	1.42	0.31	0.18	0.21
Control-2 (infested soil and nematodes)	15	0	4	1.55	0.60	0.81	0.22	0.93	0.14	0.06	0.15
	30	0	5	1.97	0.92	1.53	0.46	1.86	0.24	0.12	0.32
	45	1	5	2.27	1.03	2.18	0.75	2.51	0.42	0.21	0.41
Control-3 (soil and nematodes)	15	0	4	1.47	0.43	0.52	0.14	0.43	0.12	0.04	0.08
	30	0	4	1.83	0.52	1.05	0.31	0.81	0.24	0.05	0.15
	45	1	4	2.17	0.81	1.57	0.41	1.21	0.32	0.11	0.23
Infested soil and algal powder	15	0	4	1.29	0.12	1.21	0.33	1.11	0.21	0.12	0.23
	30	1	4	1.75	0.98	1.98	0.78	1.22	0.32	0.17	0.28
	45	3	5	2.83	1.35	2.94	1.58	3.36	0.62	0.29	1.25
Infested soil, Infested soil and algal powder and nematodes	15	0	4	1.46	0.61	1.54	0.59	0.69	0.29	0.07	0.38
	30	0	4	2.14	0.73	2.11	0.94	1.39	0.32	0.19	0.72
	45	2	4	2.47	1.83	2.42	1.29	2.01	0.51	0.29	1.06
Soil, algal powder and nematodes	15	0	4	2.14	0.71	1.96	0.97	1.55	0.48	0.17	0.31
	30	2	4	5.72	1.40	2.83	1.47	2.41	0.65	0.28	0.78
	45	3	4	9.01	1.72	3.87	1.72	3.43	0.83	0.40	1.28

Percent mortality test was performed to check the dead larva in comparison with the control. The % mortality against *M. javanica* was found to be 27 %, 29 % and 35 % for water, n-hexane and DMSO extract, respectively after 72 hours (Table 3).

The fertilizing efficacy of the alga was checked by the determination of the weight of stem, roots and number of leaves and flowers (Table 4). The study showed about 59% total rate of seeds germination in comparison with the control which showed that the alga possessing a beneficial effect on the growth of the okra plant.

Conclusions

The overall production of agriculture products is gradually decreasing with increase in the population, global warming, biodiversity and global climate change that urgently demands the development of new and fascinating approaches that can increase the agriculture products by increasing the fertility of the soil through the development of most potential fertilizers. In this regards, seaweeds are also a renewable and potential viable option to generate alternative cost effective fertilizers. In this study, *M. afaqhusainii* was selected to check its potential for fertility of the soil using *M. javanica* root-knot nematode. The present investigation on this alga revealed that different extracts of this alga are active against two fungi namely *F. moniliforme* and *R. solani* and they also exhibited nematicidal activity against *M. javanica*. The algal powder also enhanced the growth of okra plant to a significant level.

Acknowledgements

The Principal Investigator is greatly thankful to the Higher Education Commission of Pakistan for the financial support under National Research Program for Universities (NRPU, No. 20–1061/R & D/07/694).

References

Andrews, S. B. (2013). *Quantifying the fertilizer value of algal meal: an evaluation of an integrated dairy-anaerobic digester-algae production facility*. M. Sc. Thesis, Oregon State University, United States.

Atta-ur-Rahman, Khan, A. M., Shabbir, M., Abid, M., Choudhary, M. I., Nasreen, A. Maqbool, M.A. Shameel, M. & Sualeh, R. (1997). Nematicidal activity of marine organisms. *Pakistan Journal of Nematology*, 15, 95-100.

Bonzil, S., Somda, I., Zida, E. P. & Sereme, P. (2012). *In vitro* antifungal activity of various local plant extracts in the control of *Phoma sorghina* (Sacc.) Boerema *et al*., and *Colletotrichum graminicola* (Ces.) Wilson, as Sorghum seed mold pathogen in Burkina Faso. *Tropicultura*, 30, 103-106.

Demirci, E., Dane, E. & Eken, C. (2011). *In vitro* antagonistic activity of fungi isolated from sclerotia on potato tubers against *Rhizoctonia solani*. *Turkish Journal of Biology*, 35, 457-462. DOI:10. 3906/BIY-1004-98.

Haq, T., Khan, F. A., Begum, R. & Munshi, A. B. (2011). Bioconversion of drifted seaweed biomass into organic compost collected from the Karachi coast. *Pakistan Journal of Botany*, 43, 3049-3051.

Khalil, M. S. & Badawy, M. E. I. (2012). Nematicidal activity of a biopolymer chitosan at different molecular weights against root-knot nematode, *Meloidogyne incognita*. *Plant Protection Science*, 48, 170–178.

Khan, A. M., Ameen, M., Naz, S. & Noureen, S. (2012). Bioscreening of marine organisms from the coasts of Pakistan. *Journal of the Chemical Society Pakistan*, 34, 184-194.

Khan, A. M. & Hussain, M. S. (2015). Production of biofuels from marine macroalgae *Melanothamnus afaqhusainii* and *Ulva fasciata*. *Journal of the Chemical Society of Pakistan,* 37, 371-379.

Khan, A. M. (2000). *Phytochemical and structural studies on the chemical constituents of Taxus wallichiana, Tanacetum, gracile, Jolyna laminarioides and other marine algae.* Ph. D. Thesis, University of Karachi, Karachi, Pakistan. Retrieved from: http://eprints.hec.gov.pk/979/1/711.html.

Kositchaiyong, A. & Sombatsompop, N. (2012). Anti-fungal and anti-algal performances of biocides filled PVC and wood/PVC composites, *Advanced Materials Research*, 410, 75−78. DOI:10.4028/www.scientific.net/AMR.410.75

Rizvi, M. A. (2010). Comparative antibacterial activities of seaweed extracts from Karachi coast, Pakistan. *Pakistan Journal of Pharmacology*, 27, 53-57.

Rizvi, M. A. & Shameel M. (2006). *In vitro* nematicidal activities of seaweed extracts from Karachi coast. *Pakistan Journal of Botany*, 38, 1245-1248.

Shameel, M. (1999). *Melanothamnus afaqhusainii*, a new red alga from the coast of Karachi. *Pakistan Journal of Botany*, 31, 211-214.

Sultana, V., Baloch, G. N., Ara, J., Ehteshamul-Haque, S., Tariq, R.M. & Athar, M. (2011). Seaweeds as an alternative to chemical pesticides for the management of root diseases of sunflower and tomato. *Journal of Applied Botany and Food Quality*, 84, 162-168.

Sultana, V., Baloch, G. N., Tariq, S., Ehteshamul-Haque, S., Athar, M. & Ara, J. (2013). Role of seaweeds occurring at Karachi coast in suppressing the root diseases of cotton and chilli. *Journal of Applied Botany and Food Quality*, 86, 138-142.

Thirumaran, G., Arumugam, M., Arumugam, R. & Anantharaman, P. (2009). Effect of seaweed liquid fertilizer on growth and pigment concentration of *Cyamopsis tetrogonolaba* (L.), Taub. *American-Eurasian Journal of Agronomy*, 2, 50-56.

Wiratno, Taniwiryonoc, D., Van den, Bergb, H., Riksend, J. A. G., Rietjensb, I. M. C. M., Djiwantia, S. R., Kammengad, J. E. & Murk, A. J. (2009). Nematicidal activity of plant extracts against the root-knot nematode, *Meloidogyne incognita*, *The Open Natural Products Journal*, 2, 77-85. doi: 10.2174/1874848100902010077.

(Accepted: November 25, 2015)