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# Nematicidal activity of seaweeds against *Meloidogyne javanica*

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## Abstract

Root-knot nematodes (*Meloidogyne* spp.) are serious disease causing agents and attack nearly all types of plants. In this study 32 seaweeds were evaluated to determine nematicidal activity against *Meloidogyne javanica* (egg hatching and larval mortality tests) *in vitro*. Results revealed that seaweed biochemical potential in two different solvents viz., water and methanol @ ratios 2.5, 5 and 10%. It is observed that *Sargasssum tenerrimum*, *Padina tetrastromatica* and *Melanothamnus afaqhusainii* showed maximum egg hatching (96%) and larval mortality (99%) and (100%), respectively in water and methanol extract @ 10% concentration after 72 hours exposure time. Similarities between Ward's cluster analysis of hatching egg and larval mortality in water and methanol extract of different seaweeds showed significant difference. Methanol extract was more effective as compared to water extract. In conclusion, use of seaweeds is positive sign against harmful microorganisms which are responsible for considerable losses in agriculture yield.

**Keywords:** Root-knot nematodes, *Meloidogyne javanica*, seaweeds, egg hatching and larval mortality.

In Pakistan, due to nematodes infestation up to 10-20% damage has been roughly estimated producing a loss of about hundred million rupees annually (Maqbool, 1988). Among other plant parasitic nematodes, root-knot nematode (*Meloidogyne* sp.) is one of the most important pest which produced considerable losses to crop plants in different parts of the world (Taylor *et al*., 1982). Root-knot nematodes (*Meloidogyne* spp.) infect almost all types of plants and caused considerable damage (Adekunle & Akinlua**,** 2007). In those areas where root-knot nematodes are not controlled, average crop yield losses were estimated 25% with damage in individual fields ranging as high as 60% (Sasser *et al*., 1982). The interaction of nematodes with other pathogens (fungi and bacteria) increased the loss of many field crops (Maiti, 1974; Hussain *et al*., 2013). Pesticides are generally used for the control of pests and diseases. These chemical pesticides caused human health and environmental hazards. So, there is a need to find alternative methods which are safe and environment friendly. An alternative control strategy of nematode developed which was safe and coast effective (Abid *et al*., 2005).

Karachi, the biggest city of Pakistan with a 100 km coastline of Arabian Sea offers a variety of sandy beaches. Rocky ledges, swampy wetland and few islands infested with marine algae (Ahmad *et al*., 1992). It was estimated that about 90% of the species of marine plant are algae and about 50% of the global photosynthesis contributed from algae (Dhargalkar & Pereira, 2005). Among all species of algae, Chlorophyta, Phaeophyta and Rhodophyta considered most important and major groups (Drew, 1955). These vast varieties of seaweeds found to possess useful untapped biochemical compounds such as carotenoids, dietary fibers, fatty acids which might be potential source of drug leads in future (Huang *et al*., 2005). In this study, seaweeds were evaluated for nematicidal activity.

## Materials and Methods

**Description of site collection:** Seaweeds were collected in different seasons during 2011-2012 in low tide. Thirty two seaweeds were collected from the Manora, Buliji and Hawksbay, Karachi, Pakistan. Seaweed species exposed on sand, rocks and along the waves in floating collected in sterilized plastic bags. Seaweeds were washed thoroughly with seawater as well as surface sterilized with 1% sodium hypochlorite (NaOCI) plant species were confirmed by the taxonomic character of seaeweeds and checked it. The samples were shade dried or oven dried at 40 ± 2 °C and ground in an electric mixture into powder, stored in polythene bags at room temperature until use.

**Extraction of water and methanolic bioactives:** For organic extract, seaweed biomass was homogenized with water and methanolic solvents at 28 °C. After 24 hours the mixture was separated for stock solution by filtration using Whatman filter paper no. 4. Stock solution of methanolic and water extract was prepared and further diluted as per dose requirement.

**Hatching test:** A suspension of eggs containing 30-35 eggs/ml was prepared from fresh roots of brinjal plant infected with root-knot nematodes (*Meloidogyne javanica*). For water extract, 1 ml of test extract and 1 ml eggs suspension were prepared. However, for methanolic extract, 2 ml methanolic extract (about 1ml for evaporation in vacuum chamber) were prepared in laboratory. After complete process of evaporation, 1 ml egg suspension was transferred in glass cavity block, 2.5 cm diam., and kept at room temperature. Each treatment was replicated 3 times. The glass cavity block containing 1 ml egg suspension for water extract and 2 ml methanol with egg suspension was served as control as suggested by Manilal *et al*., (2009). The number of hatched eggs was counted under a low power stereoscopic microscope (x6) after 24, 48 and 72 hour exposure time.

**Mortality test:** Eggs of *M. javanica* were placed in distilled water and incubated at room temperature (25 ± 2 °C) for 24 hours. A suspension of freshly hatched juveniles in distilled water containing (30-35 juveniles/ml) was prepared. For water extracts 1 ml of test extract and 1 ml juveniles suspension while for methanolic extract: 2 ml methanolic extract (about 1ml for evaporation in vacuum chamber) after complete evaporation 1ml juveniles suspension was transferred in glass cavity block, diam., 2.5 cm and kept at room temperature. Each treatment was replicated 3 times and the glass cavity blocks containing 1 ml juvenile's suspension for water extract and 2 ml methanol (after evaporation) with juvenile's suspension was served as control. After 24, 48 and 72 hour exposure, the number of killed juveniles was counted under a low power stereoscopic microscope (x6) (Cayrol *et al*., 1989).

## Results and Discussion

**Eggs hatching test:** In water extract treatment, the juveniles were exposed for each seaweed extract for 72 hours in lab conditions at room temperature (26 ± 2 ºC). The result showed that egg hatching was decreased with increase in seaweeds concentration and exposure time. Maximum 96% reduction in egg hatching was recorded in three seaweeds *S. tenerrimum*, *P. tetrastromatica* and *M. afaqhusainii* (Table 1). In water extract, result indicated that extract of *P. tetrastromatica* was more effective in the reducing of egg hatching. However, methanolic extract almost all seaweeds were inhibited egg hatching significantly. No egg hatching was observed in *S. tenerrimum*, *P. tetrastromatica* and *M. afaqhusainii* (Table 1).

**Agglomerative cluster analysis of stands (Ward's method) for hatching eggs:** The dendrogram resulted from cluster analysis for hatching reduction in egg data using Ward's method is shown in Fig. 1. The dendrogram of water extract discloses two main groups at a squared Euclidean distance. Group I comprising

of 31 stands were characterized by the predominance of *S. tenerrimum*, *P. tetrastromatica* and *M. afaqhusainii* (96%). Group II included only 1 stand. It is dominated by *C. floresii* (91%). It has low abundance in this group. However, in methanolic extract, the dendrogram discloses two main groups. Group I comprising of 19 stands, is characterized by the predominance of *M. afaqhusainii*, *S. tenerrimum* and *P. tetrastromatica* (100%). Group II included 13 stands. It is dominated by *C. indica* (83%) and *Udotia* sp. (79%).

**Larval mortality test:** The freshly hatched root-knot larvae were exposed in water extract of different seaweeds separately for 72 hours in laboratory at room temperature (26 ± 2 ºC). The maximum larval morality (99%) was recorded in *S. tenerrimum*, *P. tetrastromatica* and *M. afaqhusainii* seaweeds as compared to other seaweeds (Table 2). However, in methanol extract at 10% concentration, *S. tenerrimum*, *M. afaqhusainii* and *P. tetrastromatica* seaweeds exhibited (100%) mortality (Table 2). Larval mortality was decreased with increase in dilution of all seaweed extracts. While, junvenile mortality was increased corresponding to an increase of time exposure.

**Agglomerative cluster analysis of stands (Ward's method) for larval mortality:** The dendrogram resulting from cluster analysis for larval mortality data using Ward's method is shown in Fig. 2. The dendrogram of water extract disclosed two main groups at a squared Euclidean distance. Group I comprising of 30 stands which were characterized by the predominance of *P. tetrastromatica*, *S. tenerrimum* and *M. afaqhusainii* (99%). Group II included 2 stands. It is dominated by *Valaniopsis* sp., (50%) than *Udotia* sp., (44%) which has low abundance in this group. However, dendrogram disclosed two main groups in methanolic extract. Group I comprising of 30 stands which were characterized by the predominance of *S. tenerrimum*, *P. tetrastromatica* and *M. afaqhusainii* (100%). Group II included 2 stands. It is dominated by *Udotia* sp., (64%).

The nematicidal activities of seaweeds would be a great help to completely contrast or at least reduced the nematode disease in economically important plants, which caused heavy losses to crop plants and adversely affect economy of our country (Rizvi & Shameel, 2006), used as a fertilizer for many years and widely used biostimulant in agriculture (Hattori, 1999). Different seaweeds exhibited very significant nematicidal activities (Ara *et al*., 1996; Sultana *et al*., 2000; Noreen *et al*., 2002; Whapham *et al*., 1994; Zaki *et al*., 2005). Seaweeds contained elaborate secondary metabolites that play a significant role in the defense of the host against predators and parasites which offer a potential novel approach to control population of plant parasitic nematodes (Paracer *et al*., 1987; Jacobs *et al*., 2003).

Seaweed extracts have been reported to increase plant resistance to pests and diseases, plant growth, quantity and yield (Jolivet *et al*., 1991; Pardee *et al*., 2004; Verkleji, 1992). Application of seaweed to plants resulted in decreased levels of nematode attack (Ara *et al*., 1997; Wu *et al*., 1997; 1998). Sultana *et al*., (2011) reported that seaweeds like *Spatoglossum variabile*, *Halimeda tuna* and *Melanothamnus afaqhusainii* showed more or less similar suppressive effect on root rotting fungi and root-knot nematode to chemical fungicides (Topsin-M) and nematicide (carbofuran). In a large number of marine algae, antimicrobial activities are attributed to the presence of acrylic acid. Seaweeds contain 1-aminocyclopropane-1-carboxylic acid (ACC), which has antimicrobial activity (Nelson & Van-Standen, 1985). Our present study supported by the results of Paracer *et al*., (1987); Ara *et al*., (1996; 1997); Zaki *et al*., (2005); Abid *et al*., (2005) and Rizvi & Shameel (2006).

**Table1. Effect of different concentrations of water and methanol extracts of seaweeds on egg hatching of root-knot nematode after 72 hours.**

| Name of Algae | Egg hatchability at different concentrations of seaweeds after 72 hours exposure | | | | | |
|---|---|---|---|---|---|---|
| | Water extract (%) | | | Methanol extract | | |
| | 10 | 5 | 2.5 | 10 | 5 | 2.5 |
| *Sargasssum tenerrimum* | 96±2.84 | 75±2.72 | 48±1.45 | 100±0.66 | 89±2.64 | 65±1.33 |
| *Sargassum bindarri* | 80±2.51 | 49±0.33 | 48±1.33 | 93±1.45 | 88±1.76 | 70±3.78 |
| *Sargassums wightii* | 81±4.97 | 62±3.17 | 45±1.52 | 98±0.66 | 85±1.85 | 60±1.73 |
| *Cystoseira indica* | 71±1 | 53±1 | 44±0.57 | 83±0 | 66±1.76 | 52±0.57 |
| *Colpomenia sinuosa* | 79±2.72 | 58±2.02 | 37±0.88 | 82±2.84 | 71±1.20 | 53±3.51 |
| *Dictyota dichotoma* | 95±1 | 73±3.92 | 44±3.75 | 98±0.66 | 82±0.33 | 50±1.66 |
| *Iyengaria stellata* | 93±2.33 | 74±5.5 | 45±1.33 | 99±2.02 | 83±2.30 | 47±2.33 |
| *Jolyna laminariodes* | 95±2.18 | 77±4.16 | 57±0.66 | 96±0.88 | 87±2.03 | 76±0.88 |
| *Padina tetrastromatica* | 96±0.66 | 81±6.48 | 45±2.51 | 100±0 | 89±2.64 | 51±2.84 |
| *Steochospermum polypodioides* | 94±1 | 83±4.91 | 48±5.04 | 96±1.45 | 88±3.38 | 57±4.63 |
| *Caulerpa paltata* | 74±2.66 | 50±6.76 | 32±6.38 | 86±3.51 | 60±4.35 | 37±3.84 |
| *Caulerpa scalepiliformis* | 66±3.92 | 51±2.4 | 22±3.51 | 71±2.08 | 58±6.56 | 40±1 |
| *Caulerpa taxifolia* | 57±0.88 | 54±2.4 | 39±2.18 | 84±1.33 | 71±4.66 | 57±3.52 |
| *Caulerpa racemosa* | 70±1 | 52±2.33 | 48±0.66 | 90±1.45 | 82±3.60 | 58±1.45 |
| *Chaeto morphaantinnin* | 68±4.35 | 48±6.88 | 41±3.71 | 85±2.88 | 75±5.50 | 56±4.48 |
| *Codium iyengarii* | 75±2.4 | 68±6.33 | 43±12.12 | 86±3.17 | 70±4.05 | 48±1.76 |
| *Halimeda tuna* | 63±1.85 | 54±2.3 | 45±2 | 73±3.17 | 62±9.81 | 37±2.4 |
| *Ulva rigida* | 88±1.45 | 65±7.23 | 44±2.90 | 91±3.17 | 69±4.66 | 50±1.33 |
| *Udotia* sp. | 61±0.33 | 50±3.75 | 33±2.60 | 79±1.52 | 56±2.33 | 39±4.58 |
| *Valaniopsis* sp. | 55±4.25 | 51±4.25 | 23±3.51 | 92±3.48 | 66±7.57 | 43±1.96 |
| *Gracilaria corticata* | 86±1.73 | 61±1.2 | 40±1.20 | 90±4.04 | 75±2.64 | 46±2.64 |
| *Jania adhaerens* | 93±2 | 79±3.84 | 51±0.33 | 96±1 | 64±9.93 | 41±2.6 |
| *Laurencia pinnatifida* | 87±2.96 | 68±3.48 | 55±3.46 | 95±2.33 | 80±3.71 | 45±4.04 |
| *Scinaia huismanii* | 75±3.66 | 69±1.45 | 46±5.23 | 94±3.17 | 82±4.91 | 56±5.69 |
| *Hypnea musciformis* | 91±2.66 | 72±3.52 | 54±5.23 | 96±0.33 | 79±6.17 | 57±2.33 |
| *Halymenia porphyaeformis* | 84±2.18 | 72±1 | 44±4.04 | 97±1.66 | 74±1.52 | 49±4.35 |
| *Calliblepharis floresii* | 91±2.51 | 67±5.92 | 45±1.45 | 90±3.52 | 59±6.69 | 38±0.88 |
| *Ceramium* sp. | 87±1.45 | 53±4.04 | 43±4.66 | 92±2.96 | 73±1.52 | 47±1.52 |
| *Geladium pulchrum* | 85±2.72 | 63±2.08 | 45±2.18 | 98±1.66 | 78±4.48 | 55±3.17 |
| *Porphyra perforators* | 90±1.45 | 65±2.51 | 46±1.66 | 94±2.02 | 75±1.66 | 51±0.88 |
| *Melanothamnus afaqhusainii* | 96±1.33 | 83±6.64 | 58±1.52 | 100±1 | 90±2.08 | 72±4.35 |
| *Centroceras* sp. | 89±1 | 65±2.96 | 53±4.93 | 95±4.04 | 84±6.74 | 55±2 |

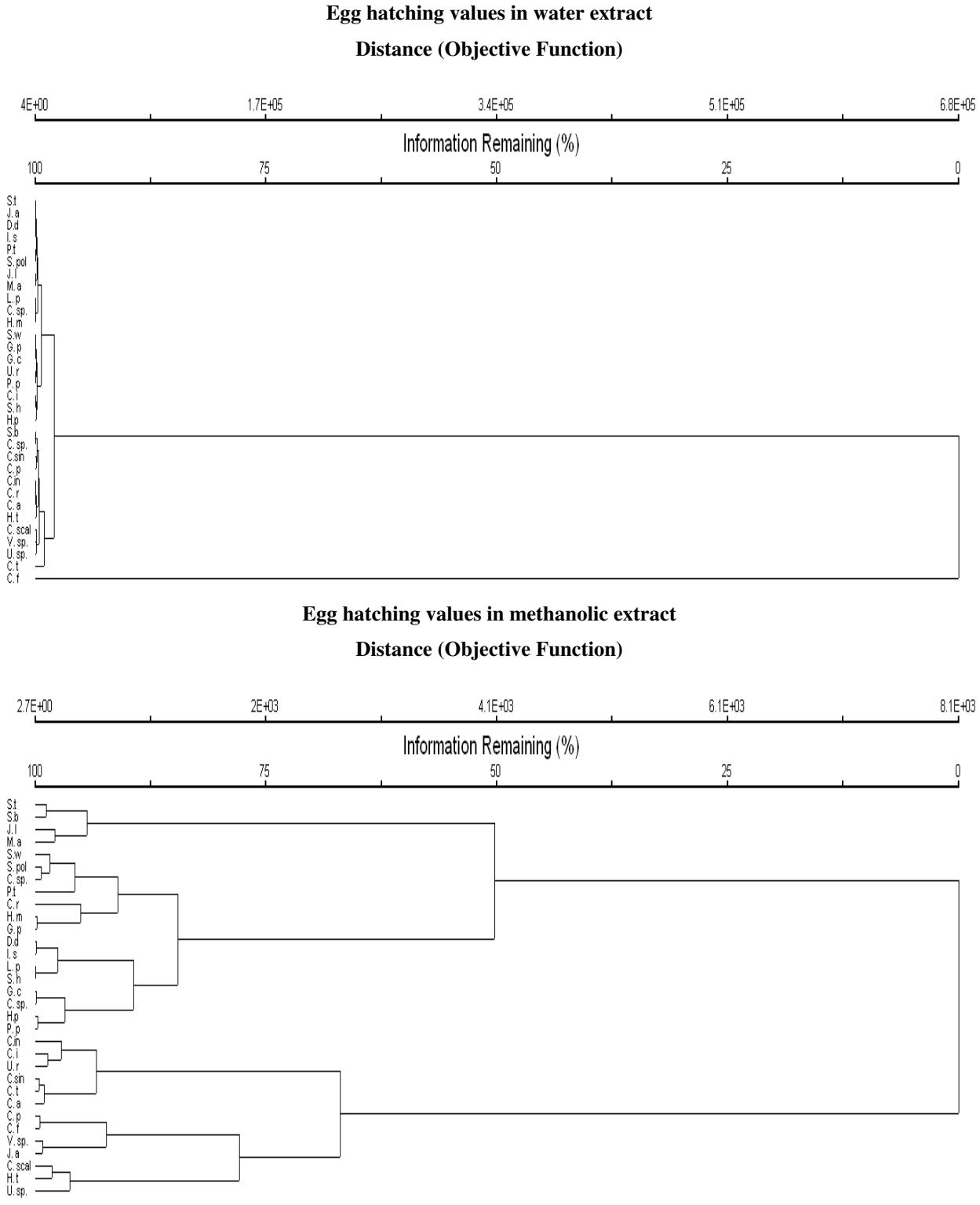


**Fig. 1.** Dendrogram obtained from Ward's agglomerative cluster analysis of egg hatching data (water and methanolic extract) of 32 stands of different seaweeds.

**Table 2. Effect of different concentrations of seaweeds extracts on larval mortality of root-knot nematode (*Meloidogyne javanica*) after 72 hours.**

| Name of Algae | Larval mortality (%) at different dilutions of seaweed extracts after 72 hours exposure | | | | | |
|---|---|---|---|---|---|---|
| | Water extract (%) | | | Methanol extract (%) | | |
| | 10 | 5 | 2.5 | 10 | 5 | 2.5 |
| *Sargasssum tenerrimum* | 99±1 | 66±4.36 | 39±5.57 | 100±0 | 82±4 | 53±4.04 |
| *Sargassum bindarri* | 98±2 | 79±4.04 | 43±0.67 | 99±1.45 | 85±1.73 | 55±2.6 |
| *Sargassums wightii* | 95±0.88 | 66±2.85 | 47±1.76 | 99±0.67 | 84±6.44 | 49±0.67 |
| *Cystoseira indica* | 97±1.67 | 72±1.15 | 54±1.67 | 98±1.20 | 83±2.91 | 57±4.67 |
| *Colpomenia sinuosa* | 88±0.33 | 71±2.31 | 55±5.24 | 91±1.76 | 73±4.1 | 54±4.26 |
| *Dictyota dichotoma* | 92±4 | 74±1.86 | 52±0.88 | 98±1.20 | 83±3.06 | 53±4.04 |
| *Iyengaria stellata* | 97±1.53 | 79±2.08 | 48±10.17 | 98±1.67 | 89±2.08 | 56±1.2 |
| *Jolyna laminariodes* | 78±7 | 75±7.93 | 50±4.16 | 98±1.45 | 86±3.33 | 60±7.21 |
| *Padina tetrastromatica* | 99±1.45 | 84±1.2 | 51±2.91 | 100±0 | 90±5.78 | 68±2.08 |
| *Steochospermum polypodioides* | 97±3 | 82±5.9 | 51±11.14 | 95±0.67 | 85±0.58 | 63±5.84 |
| *Caulerpa paltata* | 75±1.76 | 57±3.21 | 40±2.03 | 79±2.19 | 66±2.08 | 46±2.03 |
| *Caulerpa scalepiliformis* | 79±1.67 | 66±2.19 | 41±1.45 | 80±3.84 | 70±8.5 | 47±3.71 |
| *Caulerpa taxifolia* | 71±1.76 | 65±3 | 38±4.26 | 80±4.73 | 70±3.48 | 40±6.36 |
| *Caulerpa racemosa* | 81±2.67 | 75±3.48 | 46±3.18 | 83±1.53 | 79±1.53 | 49±4.93 |
| *Chaeto morphaantinnin* | 79±1.45 | 62±11.41 | 53±15.17 | 83±2.19 | 74±2.52 | 55±1.76 |
| *Codium iyengarii* | 81±6.39 | 70±7.69 | 43±3.48 | 90±3.53 | 79±3.79 | 50±3.46 |
| *Halimeda tuna* | 80±4.73 | 72±2.96 | 45±2.40 | 92±3.84 | 82±1.53 | 48±3.18 |
| *Ulva rigida* | 73±4.91 | 61±2.6 | 38±3.79 | 91±3.48 | 77±1.67 | 52±2.65 |
| *Udotia* sp. | 44±2.03 | 34±1.76 | 16±4.7 | 64±4.33 | 50±3.18 | 40±2.08 |
| *Valaniopsis* sp. | 50±0.33 | 44±2.96 | 19±1.2 | 93±3.38 | 78±4.06 | 36±6.66 |
| *Gracilaria corticata* | 86±5.24 | 61±8.95 | 40±4.41 | 97±1.33 | 80±3.48 | 51±5.84 |
| *Jania adhaerens* | 97±1.53 | 74±3.06 | 43±2.03 | 99±1 | 77±0.67 | 55±1.73 |
| *Laurencia pinnatifida* | 87±2.73 | 68±4.84 | 56±2.33 | 97±1.67 | 76±6.56 | 58±4.04 |
| *Scinaia huismanii* | 96±2.08 | 68±6.81 | 51±11.53 | 98±1 | 75±2.91 | 52±4.58 |
| *Hypnea musciformis* | 97±1.67 | 78±2.96 | 49±0.88 | 98±0.88 | 90±3.53 | 63±2.73 |
| *Halymenia porphyaeformis* | 84±2.83 | 74±2.03 | 44±3.21 | 99±1.33 | 76±6.77 | 49±2.33 |
| *Calliblepharis floresii* | 88±4.7 | 71±10.73 | 53±3.84 | 97±1.73 | 75±2.96 | 54±5.36 |
| *Ceramium* sp. | 86±4.04 | 65±2.52 | 48±2.6 | 95±2.03 | 68±1.76 | 52±7.23 |
| *Geladium pulchrum* | 95±2.08 | 79±2.19 | 47±4.26 | 99±1 | 81±4.33 | 45±2.65 |
| *Porphyra perforators* | 97±1.45 | 75±4.18 | 53±1.2 | 98±1.67 | 84±3.21 | 54±1.33 |
| *Melanothamnus afaqhusainii* | 99±0 | 90±1.67 | 63±3.06 | 100±0 | 93±1.73 | 67±6.36 |
| *Centroceras* sp. | 95±0.88 | 69±2.65 | 48±6.43 | 98±1.45 | 80±4.73 | 66±6.57 |

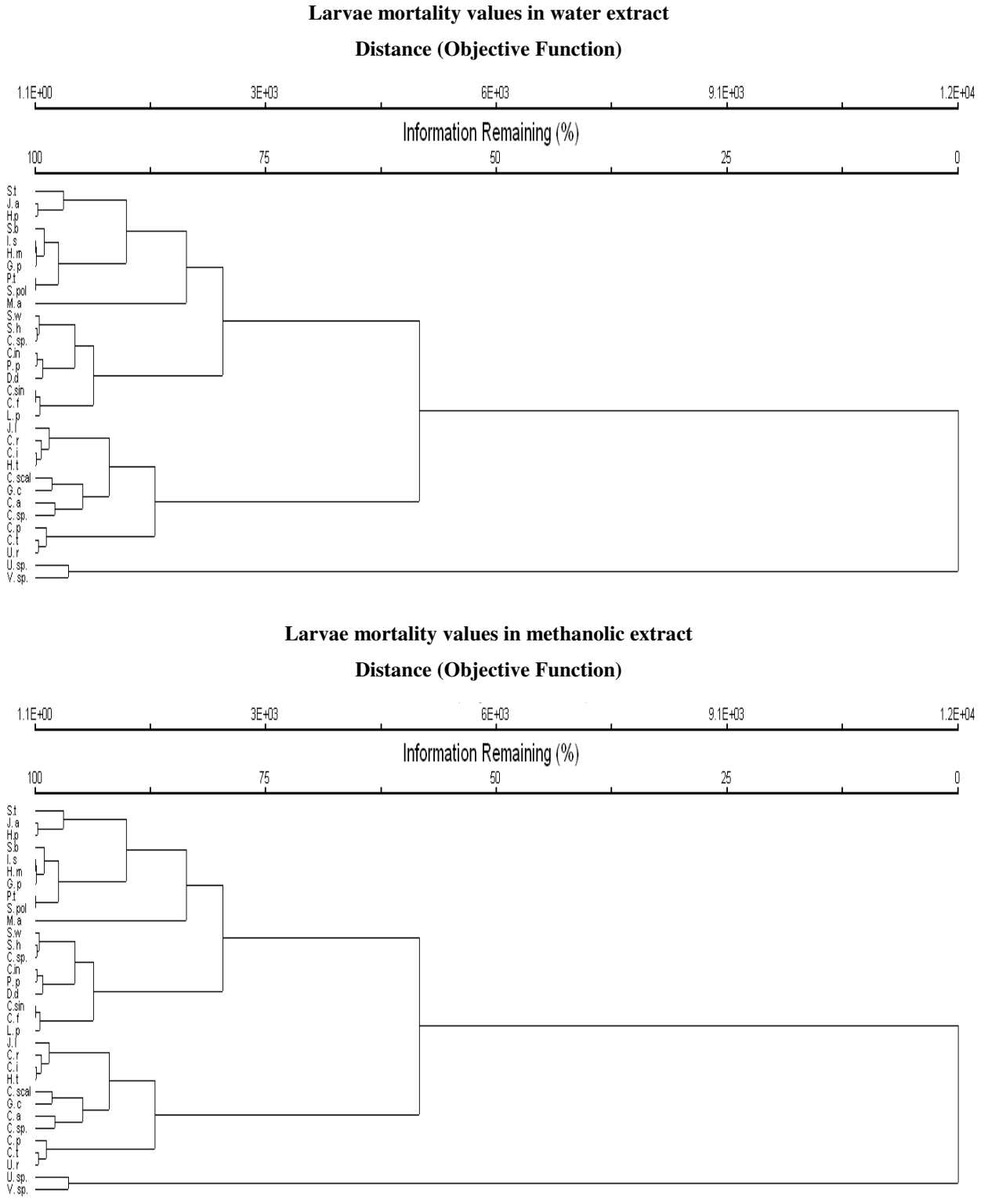


**Fig. 2.** Dendrogram obtained from Ward's agglomerative cluster analysis of larvae mortality data (water and methanolic extract) of 32 stands of different seaweeds.

## Conclusion

All extracts showed more than 50% nematicidal activity in 10% concentration against root-knot nematodes. However, extract of *S. tenerrimum*, *M. afaqhusainii* and *P. tetrastromatica* exhibited maximum nematicidal activity as compared to other seaweeds species. Contemporary investigated furthers more showed that nematicidal activity of all seaweedsis dependent on kind of extract, exposure time and concentration of the extract. It is plainly proved in this study that activity increased in the increment of the extracts and in the concentration and exposure time. Our study proved that three species of seaweeds *S. tenerrimum*, *M. afaqhusainii* and *P. tetrastromatica* can be used for the bio-control of root-knot nematodes and this method of control is also cheap environment friendly and cheap free from hazards.

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