



Effect of Immunostimulatory Activity of Seaweed Algae Against *Plasmopara viticola* (Berk. and M.A. Curtis) Berl. and De Toni and *Uncinula necator* (Schwein) Burrill Causing Downy Mildew and Powdery Mildew of Grapes

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Abstract: *Plasmopara viticola* and *Uncinula necator* are the causative agent for the downy mildew and powdery mildew, which becomes a major problem in grapes. The use of natural products like seaweed provide a rich source of structurally diverse and biologically active secondary metabolite and is the ultimate way of combating this diseases. In this context, six different seaweeds such as *Chnoospora implexa*, *Dictyota dichotoma*, *Gracilaria corticata*, *Hyneea panosa*, *Enteromorpha intestinalis* and *Caulerpa racemosa* used to control the downy mildew and powdery mildew of grapes. Evaluation of marine products used against both the pathogens were carried out under field trial. The disease incidence was observed on fruits, leaves and inflorescens. But the disease incidence was reduced in fruits @ 10% on 70 and 80 days will be 51.13 and 81.27% for downy mildew. For powdery mildew, 63.76 and 78.18% on 70 and 80 days interval. For both the mildews, the growth and yield attributes @10% on 50 days interval is 69.93%. The Phytotoxicity level were analysed for both mildews. There were no symptoms exhibited on the plants but when the Bordeaux mixture applied it ranges from 1-10% damage. The antifungal compounds were identified through gas chromatography mass spectroscopy. The results revealed that 19n compounds were present in *Liagora ceranoides*. Among those compounds, d-Mannitol, 1 –decylsulfonyl shows the highest peak to reduce the mildew diseases. The present study was undertaken to evaluate whether various seaweed (brown seaweed, red seaweed and green seaweed) extracts along with commonly available fungicides are against powdery mildew and downy mildew of grapes.

Keywords: Seaweeds, powdery mildew, downy mildew, antifungal compounds, grapes.

1. INTRODUCTION

Grape (*Vitis vinifera* L.) is one of the most delicious, refreshing and nourishing sub-tropical fruit and its cultivation is one of the most remunerative farming enterprises in India. It is grown in a variety of soil. The fruit are rich in minerals and vitamins, viz., A, B₁, B₂, C and K. Grape is cultivated in an area of about 138 thousand hectares with an annual production of 2,967.00 thousand tonnes of fruit (Indian Horticulture database - 2017 to 2018). India ranks 9th in grape production (Shikamany, 2001; Gade *et al.*, 2014). In India, Maharashtra is one of the largest grapes producing state with an annual productivity of 7, 74,000 tons in 2015. Other major producing states are Karnataka, Tamil Nadu, Andhra Pradesh and Punjab with an annual production of 330.3, 53 and 27.6 thousand tons respectively (Dethe, 2000). The production of grapevine is threatened by biotic (viruses, bacteria, fungi and insects) and abiotic stresses (*i.e.* drought, winter cold).

Downy mildew is a severe disease of grapevine, one of the most cultivated plants in India. *P. viticola* is a biotrophic oomycete that causes downy mildew. This devastating disease occurs worldwide, particularly in regions with warm and wet conditions during the growing season. *P. viticola* mainly infects leaves and clusters of young berries and produces oil spot lesions on the adaxial leaf surface accompanied by massive sporulation on the abaxial surface (Michele perazzolli *et al.*, 2012).

Powdery mildew is the most widespread and destructive disease of grapevines worldwide. All green tissues of the grapevine are susceptible to powdery mildew infection. The disease appears as a whitish-grey powdery coating on the leaves or fruit caused by fungal mycelium and conidia on the surface of the plant. On leaves, initial symptoms appear as chlorotic spots on the upper leaf surface that soon become whitish lesions. Late in the season, small black round structures (chasmothecia) begin to appear on the white powdery lesions. On shoots,

infected areas have the appearance of brown/black diffuse patches; on dormant canes, these patches are reddish brown. Severe leaf infections can cause distortion, drying and premature drop. Infected berries can become covered with the fungus, may turn dark brown, shrivel and split or may not ripen properly (Lorraine Berkett *et al.*, 2015).

Downy mildew and powdery mildew are the most devastating diseases of grapes around the world especially they cause yield and quality loss in humid regions. Not only table grapes but also most of the wine grapes are damaged by both diseases.

A large number of fungicide applications are needed in order to produce high quality grapes. This situation leads to serious problems for human health. The reduction of pesticide usage is highly desirable for environmental protection, human health and food safety (Hoffmann *et al.*, 2007; Deliere *et al.*, 2010; Riaz *et al.*, 2011). Grapes might be successfully grown without spraying chemicals if naturally resistant.

2. MATERIALS AND METHODS

2.1. Survey on the occurrence of powdery mildew and downy mildew of grapes in Theni district

A field survey was conducted to assess the extent of grape powdery mildew and downy mildew occurrence in Theni district. The villages where grapes is traditionally grown are selected for assessing the prevalence of powdery mildew and downy mildew caused by *U. necator* and *P. viticola*. During a survey, plant affected due to powdery mildew and downy mildew disease were found and also the total number of plants observed were counted and recorded. The per cent disease incidence was worked out as per phytopathometry (Sriram *et al.*, 2000).

2.2. Evaluation of seaweeds against powdery mildew and downy mildew of grapes

The efficacy of the various seaweeds listed in table was tested against *U. necator* and *P. viticola*

Sl. No.	Scientific name	Common name	Collected from
1.	<i>Chnoospora implexa</i>	Brown seaweed	Pamban
2.	<i>Dictyota dichotoma</i>	Brown seaweed	Pamban
3.	<i>Gracilaria corticata</i>	Red seaweed	Kanyakumari
4.	<i>Hyneea panosa</i>	Red seaweed	Pamban
5.	<i>Enteromorpha intestinalis</i>	Green seaweed	Pondicherry
6.	<i>Caulerpa racemosa</i>	Green seaweed	Pondicherry

2.2.1. Preparation of crude seaweeds extracts (Vallianayagam et al., 2009)

Each 1 Kg of live, healthy and matured samples (Brown seaweeds, Green seaweeds and Red seaweeds) of each seaweed collected along the Coast of Pamban (Rameswaram (9°14'N; 79°14'E), Kanyakumari, Pondicherry, Velankanni, Gulf of Mannar, Tamil Nadu, India) were washed thoroughly in sea water followed by tap water to remove extraneous particles and epiphytes. Then they were air dried under shade in laboratory for 3 days. The shade-dried samples were chopped and pulverized. Each 50 g powdered sample was separately extracted for 7 days for thrice in 500 ml of 1:1(v/v) chloroform: methanol using 1 litre Erlenmeyer conical flask under dark condition. The extractants were pooled and concentrated by using flask evaporator under reduced pressure at 45°C and weighed stored at 0°C.

2.3. Artificial Inoculation

All vines were planted in pots and cultivated in the greenhouse for powdery and downy mildew inoculation. Three replicates were made while different plants were used for powdery and downy mildew artificial inoculation. For the artificial inoculation of plants with powdery mildew, the modified method of Wang et al., (1995) was used. Conidia were collected from infected leaves, washed with 78% glucose solution to imitate the osmotic pressure of powdery mildew conidia, and then suspended in sterile water. Vine leaves were

inoculated with the conidial suspension at a rate of 2×10^5 conidia/mL by spraying the upper surface of the leaves. Inoculated leaves were immediately covered with thin plastic for six hours.

The methods of Rumbolz et al., (2002) and Boso et al., (2006) were used to propagate sporangia inoculum for downy mildew inoculation. *P. viticola* was obtained from naturally infected plants in the vineyards. For the propagation of the inoculum, plants were sprayed with a suspension of sporangia (40,000 sporangia/ mL distilled water) on the abaxial leaf side and the whole plant was covered with a polyethylene cover overnight. On the following day, the polyethylene covers were removed and incubation lasted five to six days at 25°C. The trial plants were inoculated by repeating the procedure after one week.

2.4. Observation on downy and powdery mildew incidence

Observations were made on the incidence of powdery mildew and downy mildew at different stages as follows

2.4.1. Disease incidence on bunches

Observation on the development of incidence was recorded 5 days after the last spray on the randomly selected bunches for each treatment following the 0-5 grade scale (Tajinder et al., 1994). The per cent Disease Index was calculated using the formula.

Grade	Per cent berries infected per bunch		
0	No berries diseased per bunch	1	Trace to 25 % of powdery growth and downy growth on the inflorescence
1	1 - 10% of berries diseased per bunch	2	26 – 50 % of powdery growth and downy growth on the inflorescence
2	11 - 25% of berries diseased per bunch	3	51 – 75 % of powdery growth and downy growth on the inflorescence
3	26 - 50% of berries diseased per bunch	4	76 – 100% of powdery growth and downy growth on the inflorescence
4	51 - 75% of berries diseased per bunch		
5	76 - 100% of berries diseased per bunch		

$$PDI = \frac{\text{Sum of numerical ratings}}{\text{No of bunches observed}} \times \frac{100}{\text{Maximum rating}}$$

2.4.2. Disease Incidence on leaves

Ten twigs from the treated plant were randomly selected and labelled for recording the disease incidence on leaves adopting the following 0-4 grade scale as described by Rao (1991).

- 0 – No powdery growth and downy growth on the leaves
- 1 – Trace to 25 % of leaf area diseased
- 2 – 26 – 50 % of leaf area diseased
- 3 – 51 – 75 % of leaf area diseased
- 4 – 76 – 100% of leaf area diseased

Of the leaf areas having powdery growth and downy growth observation on disease incidence were recorded.

Per Cent Disease Index (PDI) was computed on the following formula given by Hoesfall and Henberger (1942).

2.4.3. Disease incidence on inflorescence

The development of powdery mildew and Downy mildew on inflorescence was recorded on 30 and 50 days after pruning, selective 10 inflorescence randomly from treatment following the procedure suggested by Rao (1991).

- 0 – No powdery growth and downy growth on the inflorescence

2.4.4. Disease incidence on fruit

The development of powdery mildew and downy mildew on fruit was recorded on 30 and 50 days after pruning, selecting 20 bunches randomly from each treatment, using 0 - 5 grade scale (Tajinder *et al.*, 1994).

- | | |
|-------|---|
| Grade | Per cent berries infected per bunch |
| 0 | No powdery and downy mildew on fruits |
| 1 | 1 – 10% of powdery and downy mildew on fruits |
| 2 | 11 – 25% of powdery and downy mildew on fruits |
| 3 | 26 – 50% of powdery and downy mildew on fruits |
| 4 | 51 – 75% of powdery and downy mildew on fruits |
| 5 | 76 – 100% of powdery and downy mildew on fruits |

2.4.5. Mean bunch weight

The number of bunches per vine and the yield in kilograms at harvest for each treatment were recorded.

2.4.6. Number of berries per bunch

In each vine ten bunches were selected randomly and the number of berries in each bunch was counted then the average number of berries per bunch was worked out and recorded.

2.4.7. Weight of berries

Fifty berries were randomly selected from the selected ten bunches at harvest and their weight was recorded in grams.

2.4.8. Shelf Life

After harvest, damaged, undesirable disease and pest affected berries removed from the bunches from treatment wrapped with polythene sheet of 100 gauge. These wrapped bunches were kept at room temperature. The observation on physiological loss in weight (PLW) (%), per cent loose berries (%) and spoiled berries were recorded on 3rd, 5th and 7th day of harvest. Separate sets of treatment boxes were used for 3rd, 5th and 7th day of harvest.

The physiological loss in weight (%), loose berries (%) and overall spoilage (%) were found out as per the method suggested by Ghure *et al.*, (2001).

Physiological loss in weight (%)

$$= \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight of sample}} \times 100$$

$$\text{Loose of berries (\%)} = \frac{\text{Loose berries}}{\text{Total berries}} \times 100$$

2.4.9. Phytotoxicity

The observations on phytotoxicity were also recorded at regular intervals. The observations were made for the following parameters:

- a. Leaf tip / surface injury
- b. Wilting
- c. Leaf injury on surface
- d. Vein clearing
- e. Necrosis
- f. Epinasty
- g. Hyponasty
- h. Fruit injury

Observation were made at regular intervals for any phytotoxicity injury using 0- 10 scale developed by Jamadar *et al.*, (1998).

Scale	Phytotoxicity
0	- No phytotoxicity
1	- 1 – 10 % leaf area/fruits affected
2	- 11 – 20 % leaf area/fruits affected
3	- 21 – 30 % leaf area/fruits affected
4	- 31 – 40 % leaf area/fruits affected
5	- 41 – 50 % leaf area/fruits affected
6	- 51 – 60 % leaf area/fruits affected
7	- 61 – 70 % leaf area/fruits affected
8	- 71 – 80 % leaf area/fruits affected
9	- 81 – 90 % leaf area/fruits affected
10	- 91 – 100 % leaf area/fruits affected

2.5. Analysis of antifungal compound through Gas Chromatography Mass Spectroscopy (GCeMS) (NIST Version. 2.0, 2005)

Based on the growth inhibition studies, Seaweed extract was selected and chemical constituents were determined with a GC Clarus 500 Perkin Elmer Gas chromatography equipped with a mass detector. Turbo mass gold containing a Elite-1 (100% Dimethyl Poly Siloxane), 30 m × 0.25 mm ID employed were the following: Carrier gas, helium (1 mL/min); oven temperature program 110°C (2 min) to 280°C (9 min); injector temperature (250°C); total GC time (36 min). The water extract was injected into the chromatograph in 2.0 ml aliquots. The major constituents were identified with the aid of a computer-driven algorithm and then by matching the mass spectrum of the analysis with that of a library (NIST Version. 2.0, year 2005). Software used for gas chromatography mass spectroscopy (GCeMS) was Turbo mass-5.1. This work was carried out in IICPT, Tanjavur.

2.6. Statistical analysis

All the experiments were of completely randomized design (CRD) and repeated twice. Data were

subjected to analyses of variance and treatment means were compared by an appropriate Duncan's multiple-range test ($P < 0.05$). The IRRISTAT package version 92-1, prepared by the IRRI Biometrics Unit, Philippines, was used for analysis.

3. RESULTS AND DISCUSSIONS

3.1. Survey of disease incidence of grapes downy mildew and powdery mildew in different localities of Theni

The survey were taken in different locations in Theni revealed the prevalence downy mildew in all the villages. Among the different locations of Theni surveyed for downy mildew disease incidence, Gandhinagar (Dm 7) registered a maximum

incidence of the disease (18%) followed by Tamarakulam (Dm 16) with 17%. The other locations *viz.*, Bodinayakanur Dm 3 (5%), Koolayanur Dm 11 (3%), Thekkampatti Dm 17 (2%) had lesser diseases incidence (Table 1).

The survey were taken in different locations in Theni revealed the prevalence powdery mildew in all the villages. Among the different locations of Theni surveyed for powdery mildew disease incidence, Odaipatti (Pm 14) and chinnamanur (Pm 4) registered a maximum incidence of the disease (22%) followed by Gopalapuram (Pm 9) with 21%. The other locations *viz.*, Kottur Pm 11 (4%), Ammapatti Pm 2 (2%), Dharmapuri Pm 6 (2%) had lesser diseases incidence (Table 2).

Table 1
Survey of disease incidence of grapes downy mildew in different localities of Theni

S. No	Locality	Crop stage	Variety	Disease incidence (%)**
1	Dm 1- Alagapuri	Vegetative	Muscat	12 ^d
2	Dm 2- Ammapatti	Vegetative	Muscat	11 ^c
3	Dm 3- Bodinayakanur	Fruiting	Medika	5 ^b
4	Dm 4- Chinnamanur	Vegetative	Muscat	15 ^b
5	Dm 5- Cumbum	Inflorescence	Red globe	10 ^c
6	Dm 6- Dharmapuri	Fruiting	Medika	7 ^g
7	Dm 7- Gandhinagar	Vegetative	Muscat	18 ^a
8	Dm 8- Ethakovil	Fruiting	A 18-3	6 ^h
9	Dm 9- Dombcherry	Vegetative	Muscat	10 ^c
10	Dm 10- Gopalapuram	Inflorescence	Red globe	8 ^g
11	Dm 11- Koolayanur	Fruiting	Medika	3 ⁱ
12	Dm 12- Kottur	Vegetative	Muscat	14 ^c
13	Dm 13- Megamalai	Fruiting	Medika	9 ^f
14	Dm 14- Odaipatti	Vegetative	Muscat	11 ^c
15	Dm 15- Surulipatti	Fruiting	A 18-3	13 ^d
16	Dm 16- Tamarakulam	Vegetative	Muscat	17 ^a
17	Dm 17- Thekkampatti	Fruiting	Medika	2 ⁱ
18	Dm 18- Vadagarai	Inflorescence	Red globe	8 ^g
19	Dm 19- Varushanadu	Inflorescence	A 18-3	11 ^c
20	Dm 20- Veerapandi	Vegetative	Muscat	16 ^b

* Values in the column followed by common letters do not differ significantly by DMRT ($P=0.05$)

** Mean of three replications

Table 2
Survey of disease incidence of grapes powdery mildew in different localities of Theni

S. No	Locality	Crop stage	Variety	Disease incidence (%)**
1	Pm 1- Alagapuri	Vegetative	Muscat	18 ^a
2	Pm 2- Ammapatti	Vegetative	Muscat	2 ⁱ
3	Pm 3- Bodinayakanur	Fruiting	Medika	7 ^g
4	Pm 4- Chinnamanur	Vegetative	Muscat	22 ^a
5	Pm 5- Cumbum	Inflorescence	Red globe	14 ^c
6	Pm 6- Dharmapuri	Fruiting	Medika	2 ⁱ
7	Pm 7- Dombcherry	Vegetative	Muscat	19 ^a
8	Pm 8- Ethakovil	Fruiting	A 18-3	8 ^g
9	Pm 9- Gopalapuram	Vegetative	Muscat	21 ^a
10	Pm 10- Gandhinagar	Inflorescence	Red globe	16 ^b
11	Pm 11- Kottur	Fruiting	Medika	4 ⁱ
12	Pm 12- Koolayanur	Vegetative	Muscat	17 ^a
13	Pm 13- Megamalai	Fruiting	Medika	9 ^f
14	Pm 14- Odaipatti	Vegetative	Muscat	22 ^a
15	Pm 15- Surulipatti	Fruiting	A 18-3	8 ^g
16	Pm 16- Tamarakulam	Vegetative	Muscat	20 ^a
17	Pm 17- Thekkampatti	Fruiting	Medika	15 ^b
18	Pm 18- Vadagarai	Inflorescence	Red globe	13 ^d
19	Pm 19- Varushanadu	Inflorescence	A 18-3	9 ^f
20	Pm 20- Veerapandi	Vegetative	Muscat	16 ^b

* Values in the column followed by common letters do not differ significantly by DMRT (P=0.05)

** Mean of three replications.

3.2. Evaluation of various seaweed extracts against grapes downy mildew incidence (%) on Leaves, inflorescence and fruits during July-October 2017

3.2.1. Downy mildew incidence on leaves

In all the treatments a reduction in the incidence level was observed after two rounds of spraying with *Dictyota dichotoma* (brown seaweed) @ 10% after 30 and 50 days after pruning. A drastic reduction in the disease incidence was observed after the first round of spray. The treatment with *Dictyota dichotoma* (brown seaweed) @ 10% after 30 and 50 days after pruning was the most effective in controlling the disease

incidence with 39.79 and 48.99 per cent decrease over control. This was followed by the spraying of Bordeaux mixture @ 1% after 30 and 50 days after pruning with 36.00 and 44.85 per cent decrease over control. A highest per cent disease index (30.00%) was recorded in the untreated control (Table 3).

3.2.2. Downy mildew incidence on inflorescence

Among all the treatments tested, *Dictyota dichotoma* (brown seaweed) @ 10% after 50 and 60 days after pruning recorded 47.25 and 54.47 per cent reduction on the disease incidence after two rounds of spraying. Generally, a drastic reduction in the disease index

was noticed after every round of spray. The treatment with spraying of Bordeaux mixture @ 1% after 50 and 60 days after pruning which recorded 44.45 and 53.61 per cent of disease index after 50th and 60th spray respectively. The unsprayed control recorded maximum per cent disease index 28.91 per cent (Table 3).

3.2.3. Downy mildew incidence on fruits

The infection of downy mildew fungus persisted throughout the cropping season in the control plot. The treatment with *Dictyota dichotoma* (brown seaweed) @ 10% after 70 and 80 days after pruning was found to be more effective than other treatments recording 51.13 and 81.27 per cent decrease on the disease index over control after 80th day after pruning. This was followed by the spraying of Bordeaux mixture @ 1% after 70 and 80 days after pruning with 46.94 and 71.71 per cent decrease on the disease incidence over control. The unsprayed control recorded a maximum per cent disease index of 26.48 per cent (Table 3).

3.3. Evaluation of various seaweed extracts against grapes powdery mildew incidence (%) on Leaves, inflorescence and fruits

3.3.1. Powdery mildew incidence on leaves

The treatment with *Dictyota dichotoma* (brown seaweed) @ 10% after 30 and 50 days after pruning was the most effective in controlling the disease incidence with 37.19 and 45.86 per cent reduction of diseases incidence over control. This was followed by the spraying of Bordeaux mixture @ 1% after 30 and 50 days after pruning 35.23 and 44.60 per cent. The highest per cent diseases index (39.40%) was recorded in the untreated control (Table 4).

3.3.2. Powdery mildew incidence on inflorescence

Among all the treatments tested, *Dictyota dichotoma* (brown seaweed) @ 10% after 50 and 60 days after

pruning recorded 43.80 and 58.30 per cent reduction of disease over control after second round of spraying. The next best result was obtained with the spraying of Bordeaux mixture @ 1% after 50 and 60 days after pruning with 43.15 and 54.50 per cent reduction of disease incidence over control. A highest per cent disease index was observed in untreated control with 36.01 per cent (Table 4).

3.3.3. Powdery mildew incidence on fruits

All the treatments tested were found to reduce the diseases incidence. However, the treatment with *Dictyota dichotoma* (brown seaweed) @ 10% after 70 and 80 days after pruning was found to be more effective than the other treatments recording, 63.76 and 78.18 per cent reduction of diseases incidence over the control. The treatment with the spraying of Bordeaux mixture @ 1% after 70 and 80 days after pruning recorded 54.16 and 76.94 per cent reduction of disease incidence over control. The unsprayed control recorded a maximum per cent disease index of 33.61 per cent (Table 4).

3.4. Effect of various seaweed extracts on growth and yield attributes under field condition

In all the treatments, a highest bunch weight was obtained with the spraying of *Dictyota dichotoma* (brown seaweed) @ 10% after 30 and 50 days after pruning with 69.93 per cent increase over control. The next best result was recorded by the spraying of Bordeaux mixture @ 1% after 30 and 50 days after pruning with 56.86 per cent increase over control. A minimum bunch weight was recorded in untreated control. Similarly, a maximum number of berries per bunch was observed in the spraying of *Dictyota dichotoma* (brown seaweed) @ 10% after 30 and 50 days after pruning with 46.27 per cent increase in the number of berries over control. This was followed by the spraying of Bordeaux mixture @ 1% after 30 and 50 days after pruning with 43.28

Table 3
Evaluation of various seaweed extracts against grapes downy mildew incidence (%) on Leaves, inflorescence and fruits

T. No	Treatments	On Leaves *				On Inflorescence *				On Fruits *			
		30 DAP		50 DAP		50DAP		60 DAP		70 DAP		80 DAP	
		PDI (18.63)**	Per cent decrease over control	PDI (16.97) ^a	Per cent decrease over control	PDI (16.01) ^a	Per cent decrease over control	PDI (14.81) ^a	Per cent decrease over control	PDI (12.70) ^a	Per cent decrease over control	PDI (5.80)	Per cent decrease over control
T ₁	Spraying of <i>Dichyota dichotoma</i> (brown seaweed) @10% after 30 and 50 days after pruning	10.21 (18.63)**	39.79	8.52 (16.97) ^a	48.99	7.61 (16.01) ^a	47.25	6.53 (14.81) ^a	54.47	4.83 (12.70) ^a	51.13	1.02 ^a (5.80)	81.27
T ₂	Spraying of <i>Hlynea panosa</i> (Red seaweed) @10% after 30 and 50 days after pruning	17.19 (24.45) ^f	20.98	15.48 (23.17) ^f	30.36	14.71 (22.55) ^e	25.70	11.48 (19.81) ^f	39.10	10.73 (19.12) ^g	26.43	8.22 ^f (16.66)	46.21
T ₃	Spraying of <i>Gracilaria corticata</i> (Red seaweed) @10% after 30 and 50 days after pruning	12.91 (21.05) ^c	31.97	10.63 (19.02) ^c	42.83	9.78 (18.22) ^b	39.67	7.89 (16.31) ^c	49.86	6.31 (14.55) ^c	44.01	4.81 ^c (12.67)	59.09
T ₄	Spraying of <i>Chnoospora implexa</i> (brown seaweed) @10% after 30 and 50 days after pruning	13.76 (21.77) ^c	29.64	11.04 (19.40) ^c	41.69	10.95 (19.32) ^c	36.34	8.48 (16.99) ^c	47.96	7.48 (15.87) ^d	38.94	5.22 ^d (13.20)	57.38
T ₅	Spraying of <i>Enteromorpha intestinalis</i> (green seaweed) @10% after 30 and 50 days after pruning	15.27 (22.99) ^d	25.70	13.71 (21.73) ^d	34.89	11.12 (19.46) ^c	35.88	9.53 (17.98) ^d	44.73	8.11 (16.55) ^e	36.32	6.78 ^e (15.09)	51.28
T ₆	Spraying of <i>Caulerpa racemosa</i> (green seaweed) @10% after 30 and 50 days after pruning	16.11 (23.64) ^e	23.60	14.22 (22.15) ^e	33.42	12.53 (20.73) ^d	31.70	10.07 (18.50) ^e	43.13	9.33 (17.79) ^f	31.55	7.92 ^f (16.34)	47.24
T ₇	Spraying of Bordeaux mixture @ 1% after 30 and 50 days after pruning	11.48 (19.20) ^b	36.00	9.91 (18.35) ^b	44.85	8.42 (16.86) ^a	44.45	6.78 (15.09) ^b	53.61	5.68 (13.79) ^b	46.94	2.32 ^b (8.76)	71.71
T ₈	Control	26.45 (30.94) ^f	-	30.00 (33.27) ^f	-	25.53 (30.35) ^e	-	28.91 (32.53) ^f	-	19.21 (25.99) ^g	-	26.48 (30.97) ^f	-

DAP – Days After Pruning PDI – Percent Disease Index

Table 4
Evaluation of various seaweed extracts against grapes powdery mildew incidence (%) on Leaves, inflorescence and fruits

T. No	Treatments	On Leaves *						On Inflorescence *						On Fruits *					
		30 DAP		50 DAP		50DAP		60 DAP		70 DAP		80 DAP		70 DAP		80 DAP			
		PDI	Per cent decrease over control	PDI	Per cent decrease over control	PDI	Per cent decrease over control	PDI	Per cent decrease over control	PDI	Per cent decrease over control	PDI	Per cent decrease over control	PDI	Per cent decrease over control	PDI	Per cent decrease over control		
T ₁	Spraying of <i>Diclytata dichotoma</i> (brown seaweed) @10% after 30 and 50 days after pruning	14.53 (22.41)**	37.19	12.91 (21.05) ^a	45.86	10.78 (19.17) ^a	43.80	7.03 (15.38) ^a	58.30	4.09 (11.67) ^a	63.76	1.83 (7.73) ^a	78.18	4.09 (11.67) ^a	63.76	1.83 (7.73) ^a	78.18		
T ₂	Spraying of <i>Hymea panosa</i> (Red seaweed) @10% after 30 and 50 days after pruning	23.15 (28.76) ^f	19.39	21.33 (27.50) ^f	29.27	20.41 (26.86) ^f	21.25	17.69 (24.87) ^g	32.57	14.48 (22.37) ^g	30.53	12.48 (20.69) ^g	41.80	14.48 (22.37) ^g	30.53	12.48 (20.69) ^g	41.80		
T ₃	Spraying of <i>Gracilaria coriicata</i> (Red seaweed) @10% after 30 and 50 days after pruning	17.33 (24.60) ^c	31.05	15.04 (22.81) ^b	41.33	13.61 (21.64) ^b	36.55	10.45 (18.86) ^c	48.86	8.51 (16.91) ^c	47.33	4.86 (12.73) ^c	64.97	8.51 (16.91) ^c	47.33	4.86 (12.73) ^c	64.97		
T ₄	Spraying of <i>chnoospora implexa</i> (brown seaweed) @10% after 30 and 50 days after pruning	19.43 (26.16) ^d	26.68	16.78 (24.18) ^c	37.81	15.00 (22.79) ^c	33.198	11.09 (19.45) ^d	47.26	10.22 (18.64) ^d	42.11	7.31 (15.68) ^d	55.74	10.22 (18.64) ^d	42.11	7.31 (15.68) ^d	55.74		
T ₅	Spraying of <i>Enteromorpha intestinalis</i> (green seaweed) @10% after 30 and 50 days after pruning	20.71 (27.07) ^e	24.13	18.19 (25.24) ^d	35.08	17.49 (24.72) ^d	27.52	14.25 (22.18) ^e	39.86	12.78 (20.95) ^e	34.94	6.45 (17.90) ^e	49.48	12.78 (20.95) ^e	34.94	6.45 (17.90) ^e	49.48		
T ₆	Spraying of <i>Caulerpa racemosa</i> (green seaweed) @10% after 30 and 50 days after pruning	22.06 (27.99) ^e	21.55	20.77 (26.67) ^e	31.40	18.22 (25.27) ^e	25.92	15.51 (23.19) ^f	37.12	13.11 (21.23) ^f	34.07	10.71 (19.10) ^f	46.09	13.11 (21.23) ^f	34.07	10.71 (19.10) ^f	46.09		
T ₇	Spraying of Bordeaux mixture @ 1% after 30 and 50 days after pruning	15.41 (23.11) ^b	35.23	13.48 (21.54) ^a	44.60	11.02 (19.39) ^a	43.15	8.33 (16.78) ^b	54.50	6.49 (14.76) ^b	54.16	2.02 (8.17) ^b	76.94	6.49 (14.76) ^b	54.16	2.02 (8.17) ^b	76.94		
T ₈	Control	34.02 (35.68) ^f	-	39.40 (38.88) ^f	-	31.45 (34.81) ^f	-	36.01 (36.88) ^g	-	28.40 (32.20) ^g	-	33.61 (35.43) ^g	-	28.40 (32.20) ^g	-	33.61 (35.43) ^g	-		

DAP – Days After Pruning PDI – Percent Disease Index

per cent increase over control. A minimum number of berries per bunch was recorded in untreated control. The spraying of *Dictyota dichotoma* (brown seaweed) @ 10% after 30 and 50 days after pruning with recorded the maximum number of berries per bunch with 54.87 per cent increase in the number of berries over control. It was followed by the spraying of Bordeaux mixture @ 1% after 30 and 50 days after pruning with 53.85 per cent of increase in the number of berries over control. A minimum number of berries per bunch (67) was recorded in the untreated control (Table 5).

3.5. Effect of various seaweed extracts on physiological changes of grape berries at room temperature:

3.5.1. Physiological loss in weight (%)

The percentage of physiological loss in weight showed a significant difference during storage consistently for the field trials. A lowest value of 11.40 per cent was recorded in the spraying of *Dictyota dichotoma* (brown seaweed) @10% after 30 and 50 days after pruning and the spraying of Bordeaux mixture @ 1% after 30 and 50 days after pruning with 11.43 respectively on the 7th day of storage. This was followed by the spraying of *Gracilaria corticata* (Red seaweed) @10% after 30 and 50 days after pruning with 11.46. The spraying of *Chnoospora implexa* (brown seaweed) @10% after 30 and 50 days after pruning with 11.47. A highest per cent physiological loss in weight was recorded in the untreated control (Table 6).

3.5.2. Loose berries (%)

There was a significant difference observed in the percentage of loose berries during storage, the lowest loose berries 14.08 was observed with the spraying of *Dictyota dichotoma* (brown seaweed) @10% after 30 and 50 days after pruning and spraying of Bordeaux mixture @ 1% after 30 and 50 days after pruning with 14.12 per cent respectively on the 7th day of storage. This was followed by the spraying of

Gracilaria corticata (Red seaweed) @10% after 30 and 50 days after pruning with 14.15. The spraying of *Chnoospora implexa* (brown seaweed) @10% after 30 and 50 days after pruning with 14.19. A highest per cent of physiological loss in weight (14.34) was recorded in the untreated control (Table 6).

3.5.3. Overall spoilage (%)

The spraying of *Dictyota dichotoma* (brown seaweed) @10% after 30 and 50 days after pruning were found significantly superior to the untreated vines which recorded a minimum spoilage of 7.22 and the spraying of Bordeaux mixture @ 1% after 30 and 50 days after pruning with 7.11 per cent respectively on the 7th day of storage. This was followed by the spraying of *Gracilaria corticata* (Red seaweed) @10% after 30 and 50 days after pruning with 7.12. The spraying of *Chnoospora implexa* (brown seaweed) @10% after 30 and 50 days after pruning with 7.14. A maximum spoilage of 7.8% was observed in the untreated control (Table 6).

3.6. Effect of various seaweed extracts on Phytotoxicity observation of grapevine-downy mildew.

Studies on the phytotoxicity of the spraying of *Dictyota dichotoma* (brown seaweed) @10% after 30 and 50 days after pruning on grapevine revealed that, there was no symptoms of phytotoxicity such as leaf injury on tops, leaf injury on surface, vein clearing, necrosis, epinasty and hyponasty. This was followed by the spraying of Bordeaux mixture @ 1% after 30 and 50 days after pruning 1-10 % Leaf area damaged. In the untreated control, 21-30 % Leaf area damaged were recorded during the field trial (Table 7).

3.7. Effect of various seaweed extracts on Phytotoxicity observation of grapevine-powdery mildew

Studies on the phytotoxicity of the spraying of *Dictyota dichotoma* (brown seaweed) @10% after 30

Table 5
Effect of various seaweed extracts on growth and yield attributes under field condition

T.No	Treatments	Bunch weight (g) *		Number of berries/ bunch (g)		Berry weight (g) *	
		Bunch weight (g)	Per cent increase over control	No. of berries/ bunch (g)	Per cent increase over control	Bunch weight (g)	Per cent increase over control
T ₁	Spraying of <i>Dicyota dichotoma</i> (brown seaweed) @10% after 30 and 50 days after pruning	260 ^a	69.93	98 ^a	46.27	3.02 ^a	54.87
T ₂	Spraying of <i>Hyne panosa</i> (Red seaweed) @10% after 30 and 50 days after pruning	167 ^b	9.15	76 ^d	13.43	2.74 ^d	40.57
T ₃	Spraying of <i>Gracilaria corticata</i> (Red seaweed) @10% after 30 and 50 days after pruning	211 ^c	37.91	93 ^a	38.81	2.97 ^b	52.31
T ₄	Spraying of <i>Chnoospora implexa</i> (brown seaweed) @10% after 30 and 50 days after pruning	195 ^d	27.45	90 ^b	34.33	2.93 ^b	50.26
T ₅	Spraying of <i>Enteromorpha intestinalis</i> (green seaweed) @10% after 30 and 50 days after pruning	181 ^e	18.30	89 ^c	32.84	2.89 ^c	48.21
T ₆	Spraying of <i>Caulerpa racemosa</i> (green seaweed) @10% after 30 and 50 days after pruning	170 ^f	11.11	82 ^c	22.39	2.78 ^d	42.56
T ₇	Spraying of Bordeaux mixture @ 1% after 30 and 50 days after pruning	240 ^b	56.86	96 ^a	43.28	3.00 ^a	53.85
T ₈	Control	153 ^g	-	67 ^d	-	1.95 ^d	-

* Mean of three replications.

Values in the column followed by common letters do not differ significantly by DMRT (P=0.05)

Table 6
Effect of various seaweed extracts on physiological changes of grape berries at room temperature

T.No	Treatments	Physiological loss in weight*			Loose berries (%)**			Overall spoilage (%)**		
		3 DAH	5 DAH	7 DAH	3 DAH	5 DAH	7 DAH	3 DAH	5 DAH	7 DAH
T ₁	Spraying of <i>Dictyota dichotoma</i> (brown seaweed) @10% after 30 and 50 days after pruning	4.38 (12.08) ^{at**}	6.58 (14.86) ^a	11.40 (19.73) ^a	3.58 (10.91) ^a	7.82 (16.24) ^a	14.08 (22.63) ^a	2.17 (8.47) ^a	4.18 (11.80) ^a	7.22 (16.22) ^a
T ₂	Spraying of <i>Hyneia pamosa</i> (Red seaweed) @10% after 30 and 50 days after pruning	4.50 (12.25) ^g	6.75 (15.06) ^e	11.54 (19.86) ^f	3.73 (11.14) ^f	7.95 (16.38) ^f	14.30 (22.22) ^e	2.36 (8.84) ^g	4.36 (12.05) ^g	7.19 (15.55) ^e
T ₃	Spraying of <i>Gracilaria corticata</i> (Red seaweed) @10% after 30 and 50 days after pruning	4.43 (12.15) ^c	6.62 (14.91) ^b	11.46 (19.79) ^c	3.65 (11.01) ^c	7.87 (16.29) ^b	14.15 (22.10) ^b	2.24 (8.61) ^c	4.25 (11.90) ^c	7.12 (15.48) ^c
T ₄	Spraying of <i>Chonospora implexa</i> (brown seaweed) @10% after 30 and 50 days after pruning	4.45 (12.18) ^d	6.65 (14.94) ^b	11.47 (19.80) ^d	3.69 (11.07) ^e	7.90 (16.32) ^c	14.19 (22.13) ^c	2.27 (8.67) ^d	4.29 (11.95) ^d	7.14 (15.50) ^c
T ₅	Spraying of <i>Enteromorpha intestinalis</i> (green seaweed) @10% after 30 and 50 days after pruning	4.46 (12.19) ^e	6.69 (14.99) ^c	11.50 (19.82) ^d	3.70 (11.09) ^d	7.92 (16.35) ^d	14.25 (22.18) ^d	2.31 (8.74) ^e	4.32 (12.00) ^e	7.17 (15.53) ^d
T ₆	Spraying of <i>Caulerpa racemosa</i> (green seaweed) @10% after 30 and 50 days after pruning	4.48 (12.22) ^f	6.73 (15.04) ^d	11.52 (19.84) ^e	3.72 (11.12) ^e	7.94 (16.37) ^e	14.27 (22.19) ^d	2.33 (8.78) ^f	4.35 (12.04) ^f	7.18 (15.54) ^d
T ₇	Spraying of Bordeaux mixture @ 1% after 30 and 50 days after pruning	4.41 (12.12) ^b	6.59 (14.87) ^a	11.43 (19.76) ^b	3.61 (10.95) ^b	7.85 (16.27) ^b	14.12 (22.07) ^b	2.20 (8.53) ^b	4.21 (11.84) ^b	7.11 (15.46) ^b
T ₈	Control	4.57 (12.34) ^g	6.78 (15.10) ^e	11.59 (19.90) ^f	3.75 (11.17) ^f	7.97 (16.40) ^f	14.34 (22.25) ^e	2.38 (8.87) ^g	4.39 (12.09) ^g	7.8 (15.59) ^e

DAH – Days After Harvest

* Mean of three replications.

**Values in parentheses are arcsine transformed value. In a column, mean followed by a common letters are not signifi? cantly differ by DMRT (P = 0.05)

and 50 days after pruning on grapevine revealed that there was no symptoms of phytotoxicity such as leaf injury on tops, leaf injury on surface, vein clearing, necrosis, epinasty and hyponasty. This was followed by the spraying of Bordeaux mixture @ 1% after 30 and 50 days after pruning 1- 1-10 % Leaf area damaged. In the untreated control, 21-30 % Leaf area damaged were recorded during the field trial (Table 8).

3.8. Gas Chromatography Mass Spectroscopy (GCeMS) analysis

On the basis of performance the nature of chemical compounds present *Liagora ceranoides* (Red seaweed) was determined. The results revealed that 19 compounds viz., l-Gala-l-ido-octose, d-Mannitol, 1-decylsulfonyl-, 2-Hexadecanol, Nonadecane, Octadecane, 6-methyl-, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Cyclobarbital, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Jetocaine, Hexadecanoic acid, ethyl ester, 2-Hexadecanol, 2-Acetoxy-1,1,10-trimethyl-6,9-epidioxydecalin, E-11-Hexadecenoic acid, ethyl ester, Octadecanoic acid, ethyl ester, Hexanoic acid, 2,7-dimethyloct-7-en-5-yn-4-yl ester, Nylidrin, Squalene, Ergosta-5,22-dien-3-ol, acetate, (3 α ,22E)-, Retinoic acid, methyl ester were present in *Liagora ceranoides*. Among these, the d-Mannitol, 1 –decylsulfonyl- which is a copper nature of the compound with highest peak area (30.12 per cent) may have been responsible for the inhibition of the growth of *U. necator* and *P. viticola*. The molecular weights, name of the compound, chemical formula, retention time and peak area percentage were given.

The results of the present study clearly indicated that, foliar spraying of *Hydroclathrus clathratus* (brown seaweed) @ 10% after 30 and 50 days after pruning reduced both downy mildew and powdery mildew diseases on leaves, inflorescence and fruits significantly during both the seasons respectively. However, encouraging results were

obtained with the spraying of *Jania rubens* (Red seaweed) @10% after 30 and 50 days after pruning and the comparison chemical spraying of Bordeaux mixture @ 1% after 30 and 50 days after pruning. A drastic reduction in the disease incidence level was observed after each spraying of the seaweed extracts and fungicide in both the seasons. On the basis of our field trial treatment with the copper-containing Bordeaux mixture provided the best crop protection against grape downy mildew which coincides with the results of Gianfranco Romanazzi *et al.*, (2016). The results of the present trial studies revealed that azoxystrobin 25 SC rapidly controlled powdery and downy mildew in grapevine plants as the findings of Ahila devi *et al.*, (2015). Certain plant extracts and fungicides are against powdery mildew of grapevine under field conditions Gamal *et al.*, (2017).

In respect to the yield parameters, the data recorded clearly showed that, *Hydroclathrus clathratus* (brown seaweed) @ 10% after 30 and 50 days after pruning not only decreased the percent disease index but also increased the yield parameters viz., bunch weight (g), berry weight number of berries per bunch and yield (g) for both the seasons. A noteworthy observation was that, the lowest yield in control plots during the first season was mainly due to inflorescence shedding during flowering stage thus causing that direct yield losses. The yield increased on fruits with increase in *Hydroclathrus clathratus* (brown seaweed) @ 10% conc. as there was a decreased PDI on leaves, inflorescence and fruits. During the second season, a similar trend was observed, but the yield decreased.

It may be inferred that, this condition may be not only due to the disease, but also it may be due to the failure of monsoon and climatic factors. Increase in yield due to seaweed-treated vines are thought to be associated with the hormonal substances present in the algal extracts. Extracts of seaweed has been reported to induce many positive changes in treated plants such as improved crop yield Stino *et al.*, (2017).

Table 7
Effect of various seaweed extracts on Phytotoxicity observation of grapevine-downy mildew

T.No	Treatments	Leaf tip/surface injury	Wilting	Veinclearing	Necrosis	Epinasty	Hyponasty	Fruit Injury	
T ₁	Spraying of <i>Dictyota dichotoma</i> (brown seaweed) @10% after 30 and 50 days after pruning	NP	NP	NP	NP	NP	NP	NP	
T ₂	Spraying of <i>Hyneia panosa</i> (Red seaweed) @10% after 30 and 50 days after pruning	NP	NP	NP	NP	NP	NP	NP	
T ₃	Spraying of <i>Gracilaria corticata</i> (Red seaweed) @10% after 30 and 50 days after pruning	NP	NP	NP	NP	NP	NP	NP	
T ₄	Spraying of <i>Chnoospora implexa</i> (brown seaweed) @10% after 30 and 50 days after pruning	NP	NP	NP	NP	NP	NP	NP	
T ₅	Spraying of <i>Enteromorpha intestinalis</i> (green seaweed) @10% after 30 and 50 days after pruning	NP	NP	NP	NP	NP	NP	NP	
T ₆	Spraying of <i>Caulerpa racemosa</i> (green seaweed) @10% after 30 and 50 days after pruning	NP	NP	NP	NP	NP	NP	NP	
T ₇	Spraying of Bordeaux mixture @ 1% after 30 and 50 days after pruning	1	NP	NP	NP	NP	NP	1	
T ₈	Control	2	2	NP	NP	NP	NP	3	
1-	1-10 % Leaf area damaged 2- 11-20 % Leaf area damaged 3- 21-30 % Leaf area damaged NP – No Phytotoxicity								

Table 8
Effect of various seaweed extracts on Phytotoxicity observation of grapevine-powdery mildew.

T.No	Treatments	Leaf tip/surface injury	Wilting	Vein clearing	Necrosis	Epinasty	Hyponasty	Fruit Injury
T1.	Spraying of Dictyota dichotoma (brown seaweed) @10% after 30 and 50 days after pruning	NP	NP	NP	NP	NP	NP	NP
T2.	Spraying of Hyncea panosa (Red seaweed) @10% after 30 and 50 days after pruning	NP	NP	NP	NP	NP	NP	NP
T3.	Spraying of Gracilaria corticata (Red seaweed) @10% after 30 and 50 days after pruning	NP	NP	NP	NP	NP	NP	NP
T4.	Spraying of Chnoospora implexa (brown seaweed) @10% after 30 and 50 days after pruning	NP	NP	NP	NP	NP	NP	NP
T5.	Spraying of Enteromorpha intestinalis (green seaweed) @10% after 30 and 50 days after pruning	NP	NP	NP	NP	NP	NP	NP
T6.	Spraying of caulerpa racemosa (green seaweed) @10% after 30 and 50 days after pruning	NP	NP	NP	NP	NP	NP	NP
T7.	Spraying of Bordeaux mixture @ 1% after 30 and 50 days after pruning	1	NP	NP	NP	NP	NP	1
T8.	Control	3	2	NP	NP	NP	NP	3

1- 1-10 % Leaf area damaged 2- 11-20 % Leaf area damaged 3- 21-30 % Leaf area damaged NP - No Phytotoxicity

In the field experiments conducted by Shamirkhan Dilavarnaik *et al.*, (2017), the different seaweed saps of various concentration and different methods of application resulted in positive and significant influence on growth and yield parameters of maize crop.

There was a significance difference observed in the physiological weight loss during storage for both the seasons. However, a lowest PLW was recorded by *Hydroclathrus clathratus* (brown seaweed) @ 10% after 30 and 50 days after pruning treated vines. It is clearly indicated that there are many factors responsible for causing weight loss during storage, but, temperature is one of the most important factor responsible for physiological weight loss. Seaweed extract increased postharvest shelf life and reduced incidence of fungal and insect attack (Stino *et al.*, 2017). Physiological loss in weight worked in grapes (Imlak *et al.*, 2017).

Loose berries per cent showed no significant difference consistently for both the seasons due to the application of various seaweed extracts. A lowest values were recorded by treatment with *Hydroclathrus clathratus* (brown seaweed) as compared to the control on the 7th day of harvest.

On the other hand, Ben-Arie *et al.*, (1997) found that the combined application of GA3 and CPPU on Thompson Seedless variety decreased the number of loose berries per box significantly as compared to the use of only GA3.

All the seaweed extracts and fungicide showed comparatively low overall spoilage than the untreated control. However, the treatment with *Hydroclathrus clathratus* (brown seaweed) @ 10% after 30 and 50 days after pruning showed significant difference on overall spoilage of grape berries at room temperature. Various fungi are responsible for the rotting of grape berries *viz.*, *Aspergillus niger*, *Penicillium* spp, *Botryodiplodia theobromae* and *Botrytis cinerea*. The activity of the pathogen was responsible for the overall spoilage of grape. The efficacy of 1 per cent garlic

extract was evaluated against three post harvest pathogens associated with Grape (Shinde *et al.*, 2016).

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