Novel Research in Microbiology Journal (2018), 2(6): 122-137
Research Article

(Print) (ISSN 2537-0286)
(Online) (ISSN 2537-0294)
https://nrmj.journals.ekb.eg/
DOI: 10.21608/NRMJ.2018.22705

Induction of defence enzymes activities in grape plant treated by seaweed algae against *Plasmopara viticola* and *Uncinula necator* causing downy and powdery mildews of grapes

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Received: 10 November, 2018; Accepted: 23 November, 2018; Published online: 25 December, 2018

Abstract

Plasmopara viticola and *Uncinula necator* are the causative agents of downy mildew and powdery mildew diseases, respectively, which become a major problem in grape crop (*Vitis vinifera* L.). The use of natural products such as seaweed provides a rich source of structurally diverse and biologically active secondary metabolites; and is the ultimate way of combating these diseases. In this context, six different seaweed algae such as; *Sargassum wightii*, *Padina gymospora*, *Caulerpa peltata*, *Halimeda gracilis*, *Acanthophora spicifera* and *Gracilaria opuntia*; were used to control the downy and powdery mildew diseases of grapes. Enzymatic studies were carried out during two growing seasons 1 and 2 on peroxidase; polyphenol oxidase, phenylalanine ammonia lyase and β-1,3-glucanase. During season 1; in grape plants treated with *Sargassum wightii*, the four enzymes raised the glucose concentration to 60.79µg, 3.87µg, 5.20µg and 225.5µg; respectively, and then these plants were treated with Bordeaux mixture at 1%. After seaweed application; enzymes raised the glucose level to 59.83µg, 3.57µg, 5.17µg, 221.7µg. During season 2; when plants were treated with *Sargassum wightii*, the enzymes raised the glucose concentration to 62.48µg, 5.93µg, 7.25µg and 226.2µg, and then these plants were treated with Bordeaux mixture at 1%. After application; the enzymes raised the glucose level to 61.92µg, 3.57µg, 7.21µg and 224.2µg, respectively. During both seasons; β-1,3-glucanase released more amounts of glucose in both normal plants and those treated with Bordeaux mixture. The present study was undertaken to evaluate whether various extracts of seaweeds algae such as; brown, red, and green seaweeds along with the defence enzymes studied, would increase the resistance of grapes to downy and powdery mildews.

Keywords: Grapes, Seaweeds algae, Peroxidase, Polyphenol oxidase, Phenylalanine ammonia lyase, β-1,3-glucanase

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 soils. This fruit is rich in minerals and vitamins such as; A, B1, B2, C and K. Grape was cultivated in an area of about 138 thousand hectares with an annual production of 2,967.00 thousand tons of fruits (Indian Horticulture database. 2017-2018). India ranks the 9th in grape production throughout the world (Shikamany, 2001; Gade *et al*., 2014). In India, Maharashtra is one of the largest grapes producing state in India with an annual productivity of 7,74,000 tons in 2015. Other major producing states are Karnataka, Tamil Nadu and Punjab with an annual production of 330.3, 53 and 27.6 thousand tons, respectively (Dethe, 2000).

Plasmopara viticola causes downy mildew of grapes; it mainly infects leaves and clusters of young berries, produces oil spot lesions on the adaxial leaf surface accompanied with massive sporulation on the abaxial surface (Perazzolli *et al*., 2012). Moreover, all green tissues of the grapevine are susceptible to powdery mildew disease caused by *Uncinula necator*. On the surface of the plant, this disease appears as whitish-grey powdery coatings on the leaves and fruits due to fungal mycelia and conidia. Late in the season, small black round reproductive structures (cleistothecia) begin to appear on the white powdery lesions. Severe leaf infections cause distortion, drying and premature drop. Infected berries covered with the fungus may turn dark brown; shrivel, split and may not ripen properly (Berkett and Cromwell, 2015).

Marine products cause plant induced systemic resistance (ISR) through fortifying the physical and mechanical strength of the cell wall; as well as chemical, physiological and biochemical reactions within host cells, leading to the synthesis of defense chemicals against pathogens. Defense reaction occurs due to accumulation of peroxidase, phenylalanine ammonia lyase and PR-protein like β-1,3-glucanase enzymes. Several authors reported the induction of defense enzymes in crop plants treated with organic products and challenged with the pathogen (Nakkeeran *et al*., 2006; Jayaraj *et al*., 2008; Flora and Rani, 2012).

According to Vidhyasekaran, (1997); peroxidase (PO) which is part of the PR-9 family has been implicated in many physiological activities within the plant cells such as; regulation of plant cell elongation, phenol oxidation, polysaccharide cross linking, Indole acetic acid oxidation, cross linking of extension monomers, oxidation of hydroxyl cinnamyl alcohols into free radical intermediates, and wound healing. Polyphenol oxidase (PPO) enzyme usually accumulates upon wounding in plants. Biochemical approaches to understand PPO function and regulation were difficult; because the quinoid reaction products covalently modify and cross link the enzyme (Constabel *et al*., 1995).

On the other hand; phenylalanine ammonia lyase (PAL) is a defense enzyme catalyzing the determination of L-phenylalanine to produce cinnamic acid, a substrate feeding to several biosynthetic routes to produce various classes of phenyl propanoid secondary plant products (Halbrook and Sheel, 1989).

PAL activity provides precursors for lignin biosynthesis and other phenolics that accumulate in plants in response to infection such as salicyclic acid (SA); a molecule that is essential for systemic acquired resistance (Klessig and Malamy, 1994). Later, Mauch-Mani and Slusarenko, (1996) reported that the major function of PAL is the production of SA precursors in the resistance of Arabidopsis to *Peronospora parasitica*. Several studies of Nayar, (1996); Jayaraj *et al*., (2008); Flora and Rani, (2012) reported that seed treatment and seedling root dipping with plant growth promoting rhizobacteria

(PGPR) induced early and enhanced levels of PAL in rice plants.

Evidence of β-1,3-glucanases activities in disease resistance was first reported by Kauffimann *et al*., (1987). In dicots, β-1,3-glucanase genes were considered to constitute a part of the general array of defense genes induced during pathogenesis (Mauch *et al*., 1988). β-1,3-glucanases especially in conjunction with chitinases were capable of hydrolyzing fungal cell walls *in vitro* (Mauch *et al*., 1988). Vogeli *et al*., (1988) added that both of these enzymes were co-induced in response to pathogen attack.

2. Materials and methods

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

A field survey was conducted to assess the extent of occurrence of grape powdery and downy mildews caused by *U. necator* and *P. viticola*, respectively, in Theni district. Villages where grape is traditionally grown were selected for assessing the prevalence of both diseases. During this survey, affected plants were recorded and their total numbers were counted. The percentage of disease incidence was checked out as per phytopathometry according to Sriram *et al*., (2000).

2.2. Names and sites of collections of seaweeds algae

The names and cities of collection of the various seaweeds algae tested for inducting resistance against *U. necator* and *P. viticola* are shown in Table. 1.

2.3. Preparation of seaweeds algae crude extracts

Each 1 Kg of alive, healthy and matured seaweed samples collected along the Coast of Pamban (Rameswaram (9°14`N; 79°14`E), Kanyakumari and Pondicherry were washed thoroughly in sea water followed by tap water to remove extraneous particles and epiphytes. These samples were air dried under shade in the laboratory for 3 days. The shade-dried samples were then chopped and pulverized. 50 g of each powdered sample in three replicates was separately extracted for 7 days in 500 ml of 1:1(v/v) chloroform: methanol under dark conditions. These extracts were pooled; concentrated using rotary evaporator under reduced pressure at 45°C, weighed and then stored at 0°C till use (Vallianayagam *et al*., 2009).

2.4. Fungal infestation

All grape vines variety Muscat (panneer) was cultivated under field conditions for powdery and downy mildews fungal infestation; three replicates were used for each fungal pathogen. For infestation of plants with powdery mildew pathogen; conidia of *U. necator* 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 sprayed at their upper surfaces with the conidial suspension at 2×10^5 conidia/ ml. Inoculated leaves were immediately covered with thin plastic bags for 6 h according to Wang *et al*., (1995).

The methods of Rumbolz *et al*., (2002); Boso *et al*., (2006) were used to propagate sporangial inoculum for downy mildew fungal infestation. *P. viticola* was obtained from naturally infected grape plants in the vineyards. Plants were sprayed with a water suspension of fungal sporangia (40,000 sporangia/ ml) on the abaxial leaf side; and then were covered with a polyethylene cover overnight. On the following day, the polyethylene covers were removed and plants were incubated at 25ºC for 5-6 days. Artificial infestation was carried out 20 days after pruning.

2.5. Seaweeds algae extract application

The seaweed extracts dosage were sprayed on all aerial parts of the infested vine using a high volume Knapsack sprayer. Sprayings were carried

out when the mildew diseases were first noticed and subsequently two sprays were undertaken at the 30th and 50th days after pruning. Field trials were conducted with eight treatments were applied during the first and second seasons (February-May, July-October, 2017; respectively). During field trails, the cultivation practices such as; pruning, irrigation, weeding and other cultural operations have been performed following standard procedures as and when required uniformly in all the experimental plots. Three replicates were used for each treatment.

Table 1: Names and cities of collection of the various seaweed algae

S. no.	Scientific name	Common name	Cities of collection
1.	*Padina gymospora*	Brown seaweed	Pamban
2.	*Sargassum wightii*	Brown seaweed	Pamban
3.	*Gracilaria opuntia*	Red seaweed	Kanyakumari
4.	*Acanthophora spicifera*	Red seaweed	Pamban
5.	*Caulerpa peltata*	Green seaweed	Pondicherry
6.	*Halimeda gracilis*	Green seaweed	Pondicherry

2.6. Evaluation of seaweed algae activities for inducing resistance against powdery and downy mildews of grapes

2.6.1. Application of seaweeds algae extracts on grape plants in the field

Field trials were conducted using the Muscat (panneer) variety of grape plants with eight treatments during the first and second seasons (February-May, July- October, 2017, respectively); to assess the induction of defence enzymes by seaweed algae formulations after challenge inoculation with *U. necator* and *P. viticola* as described previously. The following eight treatments were adopted; Treatment 1: spraying of *Hydroclathrus clathratus* (brown seaweed) at 10%, T2: spraying of *Liagora ceranoides* (Red seaweed), T3: spraying of *Jania rubens* (Red seaweed) at 10%, T4: spraying of *Dictyota dichotoma* (brown seaweed) at 10%, T5: spraying of *Ulva intestinalis* (green seaweed) at 10%, T6: Sspraying of *Ulva reticulate* (green seaweed) at 10%, T7: spraying of Bordeaux mixture at 1% (positive control), and T8: as control. All treatments were applied after 30 and 50 days from pruning of grape plants. The treated plant samples were collected at different time intervals (1, 3, 5, 7 and 9 days) after powdery and downy mildews infestation. Three replications were maintained for each treatment, and fresh plant samples were used for enzymatic analysis.

2.6.2. Enzyme extraction from treated grape plants

The collected plant tissues were immediately homogenized with liquid nitrogen. One gram of each powdered sample was extracted with 2 ml of sodium phosphate buffer, 0.1 M (pH 7.0) at 4°C. The homogenate was centrifuged for 20 min. at 10,000 rpm. Plighting enzyme extracts prepared from leaves were used for the estimation of peroxidase (PO), polyphenol oxidase (PPO), L-phenylalanine ammonia lyase (PAL) and β-1,3-glucanase enzymes.

2.6.3. Evaluation of enzymes level in treated grape plants using spectrophotometric assays

2.6.3.1. Peroxidase (PO)

Peroxidase activity was assayed spectrophotometrically (Hartee, 1955). The reaction mixture consisted of 1.5 ml of 0.05 M pyrogallol; 0.5 ml of enzyme extract and 0.5 ml of 1 % H_2O_2 which was incubated at room temperature (28 ± 1°C). The change in absorbance at 420 nm was recorded at 30 sec. intervals for 3 min., the boiled enzyme preparation served as blank. Enzyme activity was expressed as change in the absorbance of the reaction mixture at 420 nm min^{-1} g^{-1} fresh weight of tissue as described by Hammerschmidt *et al*., (1982).

2.6.3.2. Polyphenol oxidase (PPO)

The method of Mayer *et al*., (1965) was adopted. The reaction mixture consisted of 1.5 ml of 0.1M sodium phosphate buffer (pH 6.5) and 200 µl of the enzyme extract. To start the reaction, 200 µl of 0.01 M catechol was added, and the activity was expressed as changes in absorbance at 495 nm min^{-1} g^{-1} fresh weight of tissue.

2.6.3.3. Phenylalanine ammonia lyase (PAL)

The assay mixture contained 100 µl of enzyme extract; 500 µl of 50 mM Tris HCl (pH 8.8) and 600 µl of 1mM L-phenylalanine were then incubated for 60 min. The reaction was arrested by adding 2 N HCl. Later, 1.5 ml of toluene was added and vortexed for 30 sec. The centrifuged (1000 rpm, 5 min.) toluene fraction containing trans-cinnamic acid was separated. Absorbance of the toluene phase was measured at 290 nm against the blank of toluene. Standard curve was drawn with graded amounts of cinnamic acid in toluene as described earlier. Enzyme activity was expressed as µmoles of cinnamic acid min^{-1} g^{-1} fresh tissue according to Ross and Sederoff, (1992).

2.6.3.4. β-1, 3-glucanase

Crude enzyme extract of 62.5 µl was added to 62.5 µl of 4 % laminarin and incubated at 40°C for 10 min. The reaction was stopped by adding 375 µl of dinitrosalicylic acid (DNS), and then heated for 5 min. on boiling water bath (DNS prepared by adding 300 ml of 4.5 % NaOH to 880 ml containing 8.8 g of DNS and 22.5g potassium sodium tartarate). The resulting colored solution was diluted with dist. water; vortexed and then absorbance was measured at 500 nm. Crude enzyme extract preparation mixed with laminarin at 0 time incubation served as blank. The enzyme activity was expressed as µg equivalents of glucose min^{-1} g^{-1} fresh weight of tissue (Pan *et al*., 1991).

3. Results and Discussion

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

The survey was taken in different places of Theni. Among the different locations of Theni surveyed for downy mildew (Dm) 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%) showed lesser disease incidences (Table 2).

Among the different locations of Theni surveyed for powdery mildew (Pm) 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 disease incidences (Table 3).

3.2. Induction of defence enzymes (Season 1)

3.2.1. Peroxidase (PO)

Plants treated with seaweed *Sargassum wightii* (10%) recorded a maximum induction of peroxidase activity (60.79 µg changes in absorbance $min^{-1}g^{-1}$ of

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

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

Table 3. Survey of incidence of powdery mildew disease of grapes in different localities of Theni

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

fresh tissue) on the 5th day after pathogen inoculation. This was followed by plants treated with Bordeaux mixture (1%); 30 and 50 days after pruning (T7), which recorded 59.83 µg changes in absorbance min^{-1} g^{-1} of fresh tissue on the 5th day after pathogen inoculation. Enzyme activity then declined slowly in all the treatments (Fig. 1).

3.2.2. Polyphenol oxidase (PPO)

Spraying of *Sargassum wightii* (10%) 30 and 50 days after pruning followed by infestation with *U. necator* and *P. viticola* (T1); recorded a maximum induction of PPO activity (3.87 µg changes in absorbance min^{-1} g^{-1} of fresh tissue) on the 5th day, and then decreased (Fig. 2).

3.2.3. Phenylalanine ammonia lyase (PAL)

PAL activity was found to increase significantly in plants treated with *Sargassum wightii* (10%) which recorded a maximum induction of PAL activity (5.20 µg changes in absorbance min^{-1} g^{-1} of fresh tissue) on the 7th day, and then it decreased. Spraying of Bordeaux mixture (1%) 30 and 50 days after pruning (T7) recorded a maximum induction on the 7th day about 5.17 µg changes in absorbance $min^{-1}g^{-1}$ of fresh tissue. The enzyme activity was then declined slowly in all the treatments (Fig. 3).

3.2.4. β -1, 3-glucanase

B -1,3-glucanase activity was observed in the leaf samples of grape plants at different days interval. Plants treated with seaweed *Sargassum wightii* (10%) 30 and 50 days after pruning followed by infestation with pathogens (T1) recorded a maximum induction of β -1,3-glucanase activity; where 225.5 µg of glucose released $min^{-1}g^{-1}$ of fresh tissue on the 5th day after pathogens inoculation. This was followed by the plants treated with of Bordeaux mixture (1%) (T7); which recorded 221.7 µg of glucose released $min^{-1}g^{-1}$ of fresh tissue (Fig. 4).

3.3. Induction of defence enzymes (season 2)

3.3.1. Peroxidase (PO)

Plants treated with seaweed *Sargassum wightii* (10%) 30 and 50 days after pruning followed by artificial infestation with *U. necator* and *P. viticola* (T1) recorded maximum induction of peroxidase activity (62.48 µg changes in absorbance $min^{-1}g^{-1}$ of fresh tissue) on the 5th day after pathogen inoculation. It was followed by the plants treated with Bordeaux mixture (1%) (Fig. 5).

3.3.2. Polyphenol oxidase (PPO)

Spraying of *Sargassum wightii* (10%) 30 and 50 days after pruning followed by artificial inoculated with pathogens (T1) recorded a maximum induction of PPO activity (5.93 µg changes in absorbance $min^{-1}g^{-1}$ of fresh tissue) on the 5th day, which then decreased further (Fig. 6).

3.3.3. Phenylalanine ammonia lyase (PAL)

PAL activity was found to increase significantly in plants treated with *Sargassum wightii* (10%) 30 and 50 days after pruning, which recorded a maximum induction of PAL activity (7.25 µg changes in absorbance $min^{-1}g^{-1}$ of fresh tissue) on the 5th day and there after it decreased. It was followed by the spraying of Bordeaux mixture (1%) which recorded a maximum induction of 7.21 µg changes in absorbance $min^{-1}g^{-1}$ of fresh tissue at the 5th day (Fig. 7).

3.3.4. β-1, 3-glucanase

Among the various treatments, plants treated with seaweed *Sargassum wightii* (10%) 30 and 50 days after pruning followed by the artificial infestation with pathogens (T1) recorded a maximum induction of β -1,3-glucanase activity (226.2 µg of glucose released $min^{-1}g^{-1}$ of fresh tissue) on the 5th day. This was followed by the plants sprayed of Bordeaux mixture (1%) under the same conditions (T7) which recorded 224.2 µg of glucose released $min^{-1}g^{-1}$ of fresh tissue on the 5th day after pathogen inoculation. The enzyme activity significantly increased at the 5th day from the pathogen inoculation, and then it declined slowly in all treatments (Fig. 8).

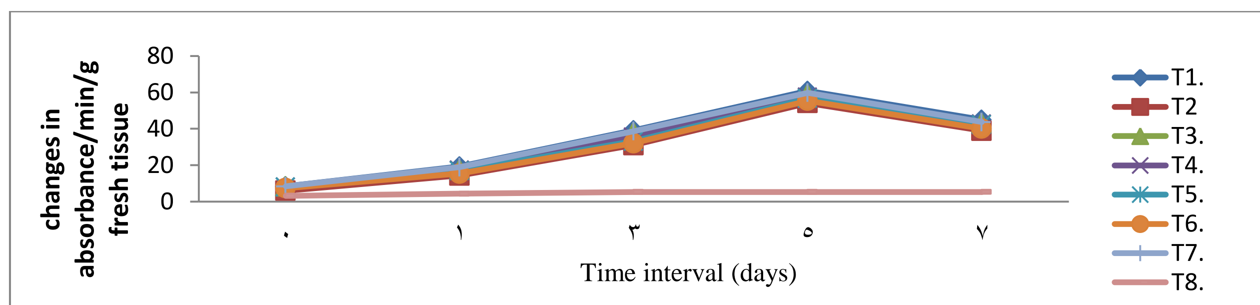

Fig. 1. Peroxidase activity* in grapes plants treated with different seaweed extracts under field conditions (season-1)

*Changes in absorbance min^{-1} g^{-1} of fresh tissue. T1, spraying of *Sargassum wightii* (10%); T2, spraying of *Acanthophora spicifera* (10%); T3, spraying of *Gracilaria opuntia* (10%); T4, spraying of *Padina gymospora* (10%); T5, spraying of *Caulerpa peltata* (10%); T6, spraying of *Halimeda gracilis* (10%); T7, spraying of Bordeaux mixture (1%); T8, control. All treatments were carried out 30 and 50 days after pruning.

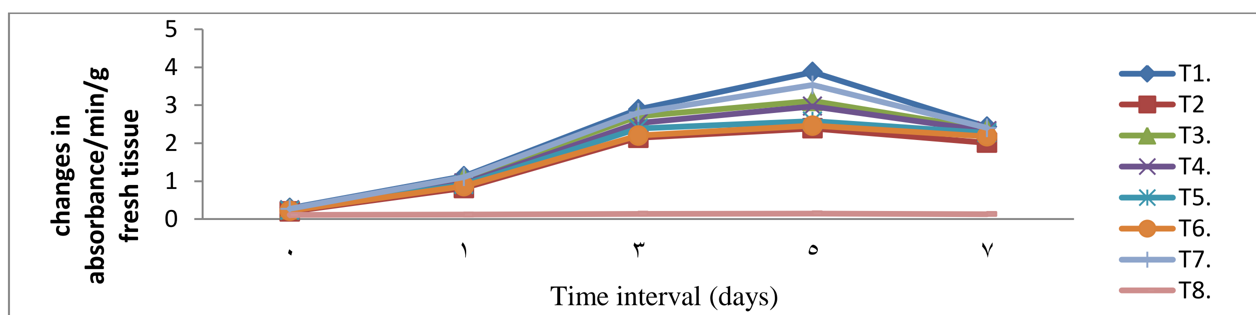

Fig. 2. Polyphenoloxidase activity* in grapes plants treated with different seaweed extracts under field conditions (season-1)

*Changes in absorbance $min^{-1}g^{-1}$ of fresh tissue. T1, spraying of *Sargassum wightii* (10%); T2, spraying of *Acanthophora spicifera* (10%); T3, spraying of *Gracilaria opuntia* (10%); T4, spraying of *Padina gymospora* (10%); T5, spraying of *Caulerpa peltata* (10%); T6, spraying of *Halimeda gracilis* (10%); T7, spraying of Bordeaux mixture (1%); T8, control. All treatments were carried out 30 and 50 days after pruning.

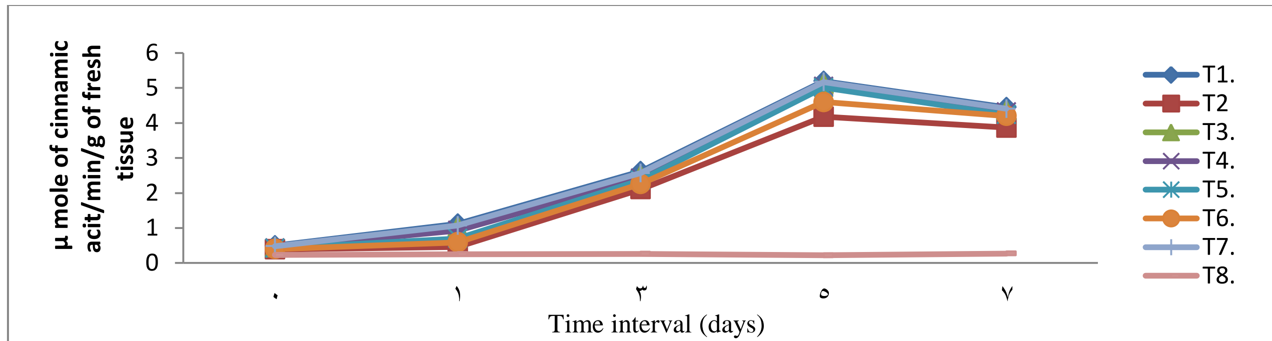

Fig. 3. Phenylalanine ammonia lyase activity* in grapes plants treated with different seaweed extracts under field conditions (season-1)

*µ mole of cinnamic acid $min^{-1}g^{-1}$ of fresh tissue. T1, spraying of *Sargassum wightii* (10%); T2, spraying of *Acanthophora spicifera* (10%); T3, spraying of *Gracilaria opuntia* (10%); T4, spraying of *Padina gymospora* (10%); T5, spraying of *Caulerpa peltata* (10%); T6; spraying of *Halimeda gracilis* (10%); T7, spraying of Bordeaux mixture (1%); T8, control. All treatments were carried out 30 and 50 days after pruning.

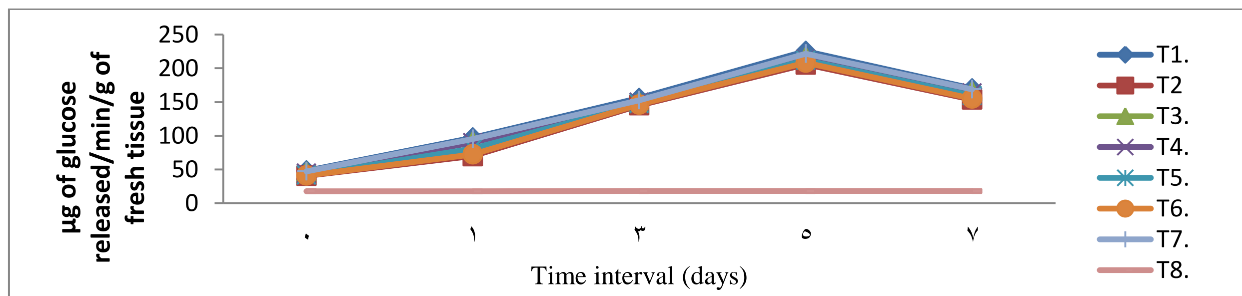

Fig. 4. β -1,3-glucanase activity* in grapes plants treated with different seaweed extracts under field conditions (season-1)

*µg of glucose released $min^{-1}g^{-1}$ of fresh tissue. T1; spraying of *Sargassum wightii* (10%), T2, spraying of *Acanthophora spicifera* (10%), T3, spraying of *Gracilaria opuntia* (10%), T4; spraying of *Padina gymospora* (10%), T5; spraying of *Caulerpa peltata* (10%), T6; spraying of *Halimeda gracilis* (10%), T7; spraying of Bordeaux mixture (1%), T8; control. All treatments were carried out 30 and 50 days after pruning.

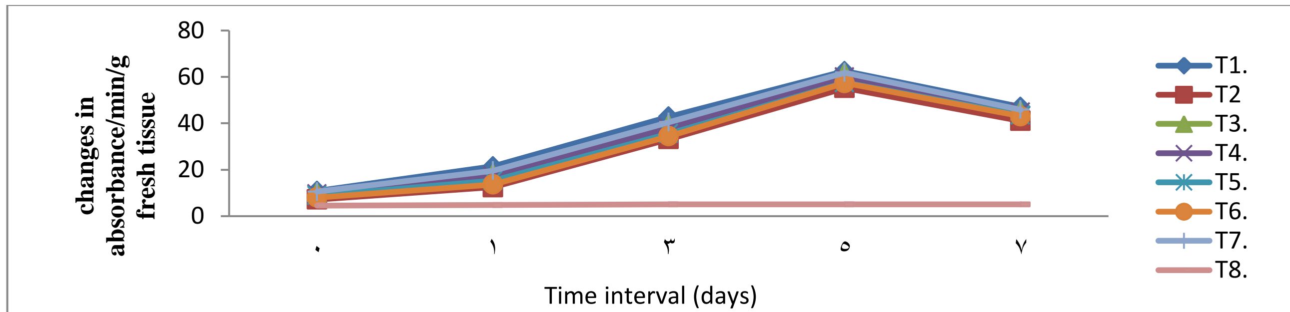

Fig. 5. Peroxidase activity* in grapes plants treated with different seaweed extracts under field conditions (season -2)

*Changes in absorbance $min^{-1}g^{-1}$ of fresh tissue. T1, spraying of *Sargassum wightii* (10%); T2, spraying of *Acanthophora spicifera* (10%); T3, spraying of *Gracilaria opuntia* (10%); T4, spraying of *Padina gymospora* (10%); T5, spraying of *Caulerpa peltata* (10%); T6; spraying of *Halimeda gracilis* (10%); T7, spraying of Bordeaux mixture (1%); T8, control. All treatments were carried out 30 and 50 days after pruning.

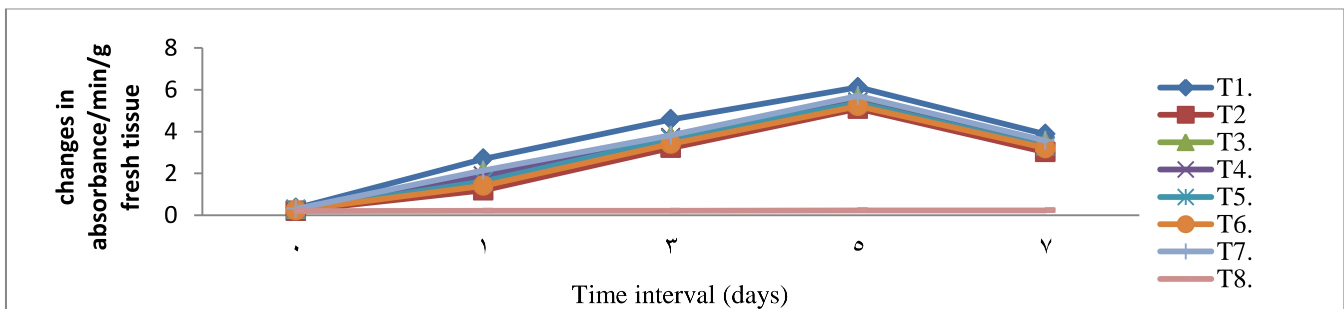

Fig. 6. Polyphenol oxidase activity* in grapes plants treated with different seaweed extracts under field condition (season-2)

*Changes in absorbance $min^{-1}g^{-1}$ of fresh tissue. T1, spraying of *Sargassum wightii* (10%); T2, spraying of *Acanthophora spicifera* (10%); T3, spraying of *Gracilaria opuntia* (10%); T4, spraying of *Padina gymospora* (10%); T5, spraying of *Caulerpa peltata* (10%); T6; spraying of *Halimeda gracilis* (10%); T7, spraying of Bordeaux mixture (1%); T8, control. All treatments were carried out 30 and 50 days after pruning.

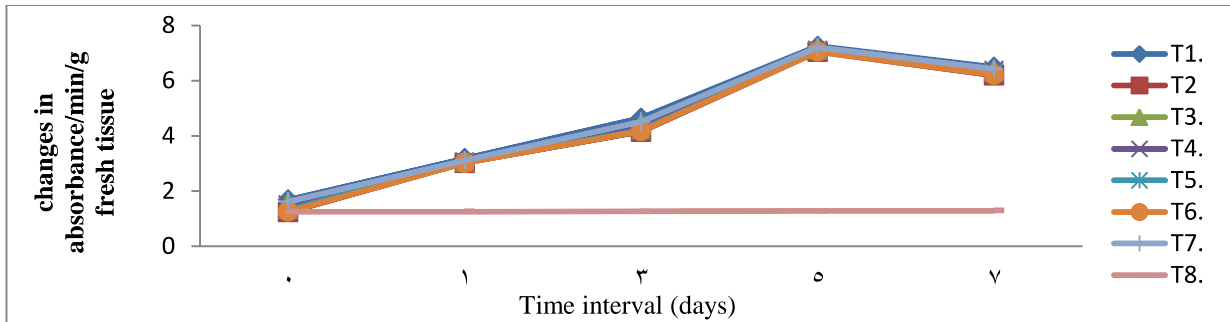

Fig. 7. Phenylalanine ammonia-lyase activity* in grapes plants treated with different seaweed extracts under field conditions (season-2)

*Changes in absorbance $min^{-1}g^{-1}$ of fresh tissue. T1, spraying of *Sargassum wightii* (10%); T2, spraying of *Acanthophora spicifera* (10%); T3, spraying of *Gracilaria opuntia* (10%); T4, spraying of *Padina gymospora* (10%); T5, spraying of *Caulerpa peltata* (10%); T6; spraying of *Halimeda gracilis* (10%); T7, spraying of Bordeaux mixture (1%); T8, control. All treatments were carried out 30 and 50 days after pruning.

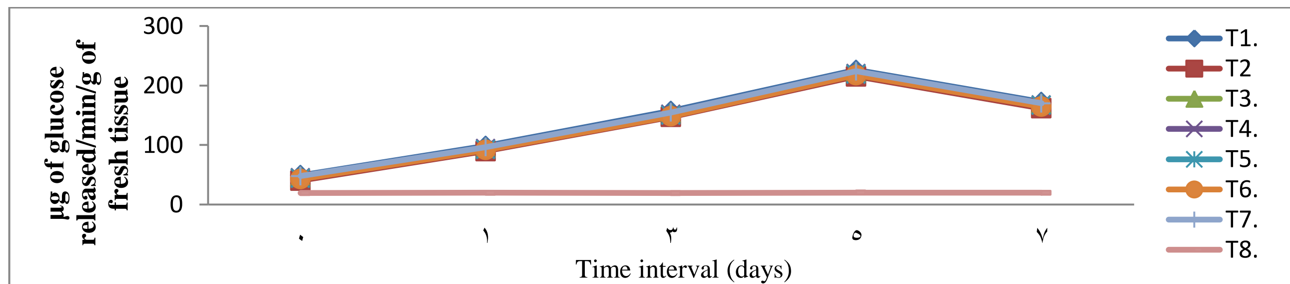

Fig. 8. β -1,3-glucanase activity* in grapes plants treated with different seaweed extracts under field conditions (season-2)

*µg of glucose released $min^{-1}g^{-1}$ of fresh tissue. T1, spraying of *Sargassum wightii* (10%); T2, spraying of *Acanthophora spicifera* (10%); T3, spraying of *Gracilaria opuntia* (10%); T4, spraying of *Padina gymospora* (10%); T5, spraying of *Caulerpa peltata* (10%); T6; spraying of *Halimeda gracilis* (10%); T7, spraying of Bordeaux mixture (1%); T8, control. All treatments were carried out 30 and 50 days after pruning.

Plants are bestowed with various defence related genes, these genes are sleeping genes and appropriate stimuli or signals were needed to activate them. Inducing a plants own defence mechanisms by prior application of a biological inducer is thought to be a novel plant protection strategy. Inductions of systemic resistance by marine products and organic amendments have been reported earlier by several workers (Jayaraj *et al*., 2008; Flora and Rani, 2012).

In the present study, grape plants pretreated with *Sargassum wightii* (brown seaweed) (at 10%) 30 and 50 days after pruning (T1) significantly induced the synthesis and accumulation of β -1,3-glucanase; PO, PPO and PAL enzymes against pathogenic *U. necator* and *P. viticola*, when compared to all the other treatments.

Enzymes activities significantly increased from the 5th day after powdery and downy mildews pathogens infestation and growth formation; and then declined in all treatments. Similar results were recorded by Jayaraj *et al*., (2008), who reported that *Ascophyllum nodosum* + chlorothalonil treatment recorded maximum induction of PO, PPO, PAL, β-1,3-glucanase enzymes activities and total phenols; more than the application of *A. nodosum* alone in carrot against Alternaria leaf spot disease.

In Lizzi *et al*., (1998) study, application of seaweeds extracts at 0.8 or 1.6 l ha^{-1} stimulated PO enzyme activity; however, two applications caused an eight-fold increase in PO activity. Moreover, treated leaves accumulated a highest capsidiol (a phytoalexin in peppers) concentration when compared to the control. Garcia-Mina *et al*., (2004) demonstrated that incorporation of *A. nodosum* extract into the planting medium caused delayed and reduced incidence of *Verticillium* wilt of pepper plants. These plants also contained higher levels and had early accumulation of phenolics. In accordance with our findings, Jayaraj *et al*., (2008); Solanki *et al*., (2012) revealed that the activity of certain defence related enzymes including; peroxidase, polyphenyl oxidase, phenylalanine ammonia lyase, chitinase and β -1,3-glucanase significantly increased in plants treated with seaweeds.

Increase in PO and PPO enzymes activities at late stage may contribute to cross linking of hydroxyproline rich glycoproteins (HRGPs) lignification's; which act as barriers against pathogen entry. Chen *et al*., (2000) added that PO generated hydrogen peroxide may function as an antifungal agent in disease resistance. Hydrogen peroxide inhibits pathogens directly or it may generate other free radicals which have antimicrobial activities. PO is a key enzyme in the biosynthesis of lignin and other oxidized phenols (Bruce and West, 1989). Meanwhile, PAL played an important role in the biosynthesis of phenolics and phytoalexins (Daayf *et al*., 1997). In the current study, an increase in PAL activity indicated the activation of the phenylpropanoid metabolism which leads to the synthesis of phenols as confirmed earlier by Massala *et al*., (1980). Klessig and Malamy, (1994) later reported that the product of PAL activity is trans-cinnamic acid; which was an immediate precursor for the biosynthesis of salicylic acid, a signal molecule in systemic acquired resistance (SAR). These enzymes act on the fungal cell wall, resulting in degradation and loss of inner contents of these pathogen cells (Benhamou *et al*., 1996). Enzymatic degradation of the fungal cell wall may release non-specific elicitors (Hammerschmidt *et al*., 1982); which in turn elicits various defence reactions in the plant cells. Ramanathan *et al*., (2000) reported that fungal cell wall elicitors have been reported to elicit various defense reactions in green gram.

Conclusion

In conclusion, prior treatment and combined application of *Sargassum wightii* (brown seaweed) (at 10%) 30 and 50 days after pruning (T1) followed

by challenge inoculation with *U. necator* and *P. viticola*, triggered plant mediated defence mechanism that in turn reduced the powdery and downy mildews incidences in grape.

Conflict of interests

The authors declare that they have no conflict of interests.

Acknowledgment

The authors would like to thank the authorities of Science and Engineering Research Board (SERB), Department of Science and Technology, India, for their financial support; and would like to acknowledge the co-operation of Department of Plant Pathology, Faculty of Agriculture, Annamalai University, India, for the successful completion of this research work.

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