Plant Archives Vol. 19 No. 2, 2019 pp. 2877-2882 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EFFICACY OF SEAWEED EXTRACT AGAINST DOWNY MILDEW OF GRAPES CAUSED BY *PLASMOPARA VITICOLA*

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Abstract

Grapevine downy mildew is caused by an obligate biotrophic oomycete, *Plasmopara viticola* and is a most serious problem in grapevine yards. It is potentially destructive where grape growing condition are characterized by high humidity and abundant rainfall. The mostly used technique to control this disease is the application of copper compounds that decrease the carabid and earthwarm population, enzymatic alterations, lowers the soil pH and reduce the grape wine growth and hazards to humans and environment. So the use of natural resource such as seaweed was done to compacts the disease. In this context, six different seaweeds such as *Hydroclathrus clathratus, Jania rubens, Ulva intestinalis, Ulva reticulate, Liagora ceranoides* and *Dictyota dichotoma* were used to control the downy mildew of grapes. Evaluation of marine products used against the pathogens were carried out under field trial. The disease incidence was observed on fruits, leaves and inflorescens. But the disease incidence was reduced on the spraying of *Hydroclathrus clathratus* @ 10% on 70 and 80 days on fruits (36.23 and 53.38% in downy mildew). 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.

Key words : Seaweeds, *Plasmopara viticola*, Anti-fungal compounds, Grapes.

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. The cultivated grapevine belongs to the genus *Vitis,* that probably originated in Pangea and more precisely in Laurasia, a continental area corresponding to Europe, North America and Asia, excluding India (Boso *et al.,* 2006). It is grown in a variety of soil. The fruits are rich in minerals and vitamins, *viz*., A, B_1, B_2, 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; Suthin Raj *et al.,* 2018d). 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). Fungal infections reduce mostly the yield and damage fruit and wine quality.

Downy mildew is also a severe disease of grapevine 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).

The organic control of soil borne plant pathogens is a potential alternative to the use of chemical pesticide (Rathore *et al.,* 2009; Suthin Raj *et al.,* 2016b). Seaweed provide a rich source of structurally diverse and biologically active secondary metabolites (Jeffrey Norrie *et al.,* 2014; Suthin Raj *et al.,* 2018c). The function of these secondary metabolites are defense mechanism against herbivores, fouling organisms and pathogens

(Ammirato, 1986; Suthin Raj *et al.,* 2016c). Application of seaweed extracts is proved to be better to decrease the foliar fungal diseases which ultimately increase its fertility and help the growth of plants (Jayaraj *et al.,* 2008; Stirk and Van Staden, 1997; Suthin Raj *et al.,* 2018a).

Materials and Methods

Survey on the occurrence of downy mildew of grapes in Theni district

A field survey was conducted to assess the extent of grape downy mildew occurrence in the Theni district. The villages where grapes is traditionally grown are selected for assessing the prevalence of downy mildew caused by *P. viticola*. During a survey, plants affected due to 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).

Evaluation of seaweeds against downy mildew of grapes

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

Sl. No.	Scientific name	Common name	Collected from
1.	*Dictyota dichotoma*	Brown seaweed	Rameswaram
2.	*Hydroclathrus clathratus*	Brown seaweed	Pamban
3.	*Jania rubens*	Red seaweed	Kanyakumari
4.	*Liagora ceranoides*	Red seaweed	Pamban
5.	*Ulva intestinalis*	Green seaweed	Rameshwaram
6.	*Ulva reticulate*	Green seaweed	Pondicherry

Preparation of crude seaweeds extracts (Suthin Raj *et al.,* 2016a)

Each 1 Kg of live, healthy and matured samples of each seaweed (Brown, Green and Red) 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 the laboratory for 3 days. The shade-dried samples were chopped and pulverized. Each 50 g powdered sample was separately extracted for 7 days, thrice in 500 ml of 1:1(v/v) chloroform: methanol using a 1 litre Erlenmeyer conical flask under dark condition. The extractants were pooled and concentrated by using a flask evaporator under reduced pressure at 45°C, weighed and stored at 0°C.

Artificial Inoculation

The methods of Rumbolz *et al.,* (2002) and Suthin Raj *et al.,* (2018b) 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 the incubation period lasted for five to six days at 25ºC. The trial plants were inoculated by repeating the procedure after a week.

Observation on downy mildew incidence

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

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	1 - 10% of berries diseased per bunch
2	11 - 25% of berries diseased per bunch
3	26 - 50% of berries diseased per bunch
4	51 - 75% of berries diseased per bunch
5	76 - 100% of berries diseased per bunch

$$PDF = \frac{Sum\ of\ numerical\ rating}{No\ of\ bunches\ obsoves} \times \frac{100}{Maximum\ rating}$$

Disease Incidence on leaves

Ten twigs from the treated plants 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

In the leaf areas having downy mildew, growth observation and disease incidence were recorded.

The per cent Disease Index (PDI) was computed by the following formula given by Hoesfall and Henberger (1942);

Disease incidence on inflorescence

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

0– No powdery growth and downy growth on the inflorescence

1– Trace to 25 % of downy growth on the inflorescence

2– 26 – 50 % of downy growth on the inflorescence

3– 51 – 75 % of downy growth on the inflorescence

4– 76 – 100% of downy growth on the inflorescence

Disease incidence on fruit

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

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

Mean bunch weight

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

Number of berries per bunch

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

Weight of berries

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

Shelf Life

After harvest, damaged, undesirable disease and pest affected berries were removed from the bunch of 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).

$$Physiological\ loss\ in\ weight = \frac{Initial\ weight - final\ weight}{Initial\ weight\ of\ sample} \times 100$$

$$Loose\ of\ berries = \frac{Loose\ berries}{Total\ berries} \times 100$$

Results

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

The survey were taken in different places of Theni. Among the different locations of Theni surveyed for downy mildew disease incidence, Gandhinagar (Dm 7) registered a maximum incidence of the disease (19%) followed by Tamarakulam (Dm 16) with 18%. The other

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- Ammachiapuram	Vegetative	Muscat	13[d]
2	Dm 2- Ammapatti	Vegetative	Muscat	12[e]
3	Dm 3- Bodithasanpatti	Fruiting	Medika	6[h]
4	Dm 4- Chinnamanur	Vegetative	Muscat	16[b]
5	Dm 5- Chinnaovalapuram	Inflorescence	Red globe	11[e]
6	Dm 6- Dharmapuri	Fruiting	Medika	8[g]
7	Dm 7- Gandhinagar	Vegetative	Muscat	19[a]
8	Dm 8- Ethakovil	Fruiting	A 18-3	7[h]
9	Dm 9- Dombcherry	Vegetative	Muscat	11[e]
10	Dm 10- Gopalapuram	Inflorescence	Red globe	9[g]
11	Dm 11- Kothaluthu	Fruiting	Medika	4[i]
12	Dm 12- Kamatchipuram	Vegetative	Muscat	15[c]
13	Dm 13- Megamalai	Fruiting	Medika	10[f]
14	Dm 14- Odaipatti	Vegetative	Muscat	12[e]
15	Dm 15- Surulipatti	Fruiting	A 18-3	14[d]
16	Dm 16- Tamarakulam	Vegetative	Muscat	18[a]
17	Dm 17- Thekkampatti	Fruiting	Medika	3[i]
18	Dm 18- Vadagarai	Inflorescence	Red globe	9[g]
19	Dm 19- Varushanadu	Inflorescence	A 18-3	12[e]
20	Dm 20- Veerapandi	Vegetative	Muscat	17[b]

* Values in the column followed by common letters do not differ significantly by DMRT (P=0.05). ** Mean of three replications.

Table 2: 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	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 *Hydroclathrus clathratus* (brown seaweed) @10% after 30 and 50 days after pruning	14.23(22.16)^{a**}	24.50	12.76(20.93)^{a}	31.71	11.86(20.15)^{a}	32.15	9.69(18.14)^{a}	46.82	7.38(15.77)^{a}	36.23	5.28(13.29)^{a}	53.38
T_2	Spraying of *Liagora cer anoide* (Red seaweed) @ 10% after 30 and 50 days after pruning	21.58(27.68)^{f}	5.69	19.62(26.29)^{f}	35.99	18.79(25.69)^{e}	13.50	16.41(23.90)^{f}	29.93	14.32(22.23)^{f}	10.11	12.53(20.73)^{e}	37.53
T_3	Spraying of *Jania rubens* (Red seaweed)@10% after 30 and 50 days after pruning	16.68(24.10)^{c}	17.88	14.71(22.55)^{b}	26.43	13.11(21.22)^{b}	28.55	11.51(19.83)^{b}	41.86	9.49(17.94)^{c}	27.46	7.72(16.13)^{c}	43.42
T_4	Spraying of *Dictyota dich otoma* (brown seaweed)@ 10% after 30 and 50 days after pruning	17.66(24.85)^{d}	15.33	15.22(22.96)^{c}	25.09	14.43(22.33)^{c}	24.81	12.22(20.46)^{c}	40.12	10.91(19.29)^{d}	22.00	8.42(16.87)^{c}	40.83
T_5	Spraying of *Ulva intest inalis* (green seaweed)@ 10% after 30 and 50 days after pruning	19.13(25.94)^{e}	11.62	17.17(24.48)^{d}	20.13	16.38(23.87)^{d}	19.63	14.91(22.71)^{d}	33.42	12.73(20.90)^{e}	15.49	10.91(19.29)^{d}	32.34
T_6	Spraying of *Ulva reticulata* (green seaweed) @10% after 30 and 50 days after pruning	20.60(26.99)^{f}	8.04	18.64(25.58)^{c}	16.54	17.91(25.04)^{e}	15.69	15.73(23.37)^{e}	31.49	13.41 (21.48)^{e}	13.14	11.01(19.37)^{d}	32.06
T_7	Spraying of Bordeaux mixture @ 1% after 30 and 50 days after pruning	21.21(22.41)^{b}	23.65	13.75(21.76)^{a}	29.00	12.78(20.95)^{b}	29.46	10.33(18.75)^{a}	45.03	8.21(16.65)^{b}	32.67	6.41(14.67)^{b}	48.54
T_8	Control	24.03(29.35)^{f}	-	26.00(30.65)^{f}	-	24.55(29.70)^{e}	-	31.45(34.11)^{f}	-	17.51(24.73)^{f}	-	22.78(28.51)^{e}	-

DAP – Days After Pruning PDI – Percent Disease Index

* Mean of three replications** In a column, values in parentheses are arcsine transformed value and followed by common letters are not differ significantly by DMRT (P = 0.05).

locations *viz.,* Bodithasanpatti Dm 3 (6%), Kothaluthu Dm 11 (4%), Thekkampatti Dm 17 (3%) had a lesser diseases incidence (Table 1).

Evaluation of various seaweed extracts against grapes downy mildew incidence (%) on leaves, inflorescence and fruits:

Downy mildew incidence on leaves

In all the treatments a reduction in the incidence level was observed after two rounds of spray with *Hydroclathrus clathratus* (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. A treatment with *Hydroclathrus clathratus* (brown seaweed) @ 10% after 30 and 50 days after pruning was most effective in controlling the disease incidence with 24.50 and 31.71 per cent decrease over control. This was followed by the spraying of Bordeaux mixture @ 1% after 30 and 50 days after pruning with 23.65 and 29.00 per cent decrease over control. A highest per cent disease index was recorded in the untreated control 26.00% (Table 2).

Downy mildew incidence on inflorescence

Among all the treatments tested, *Hydroclathrus clathratus* (brown seaweed) @ 10% after 50 and 60 days after pruning recorded 32.15 and 46.82 per cent reduction on the disease incidence after two rounds of spray. Generally a drastic reduction in the disease index was noticed after every round of spray. A treatment with spraying of Bordeaux mixture @ 1% after 50 and 60 days after pruning recorded 29.46 and 45.03 per cent of disease index recorded. A highest per cent disease index was recorded in the untreated control 31.45% (Table 2).

Downy mildew incidence on fruits

The infection of downy mildew fungus persisted throughout the cropping season in the control plot. A treatment with *Hydroclathrus clathratus* (brown seaweed) @ 10% after 70 and 80 days after pruning was found to be more effective than the other treatments recording, 36.23 and 53.38 per cent decrease on the disease index over control. This was followed by the vspraying of Bordeaux mixture @ 1% after 70 and 80 days after pruning with 22.67 and 48.54 per cent decrease on the disease incidence over control. A highest per cent disease index 22.78% was recorded in the untreated control (Table 2).

Discussion

Field surveys were conducted which indicated that, powdery mildew and downy mildew infections with highest incidence of PDI on leaves, inflorescence and fruits were observed in the Cumbum area of the Theni. According to Ahila devi *et al.,* (2013) the amount of crop and yield loss (50 %) by the disease varied from place to place because of the existence of different races and biotypes of strain of the pathogen. So, the incidence and severity of downy mildew of grape might having differed in different locations of Theni. A drastic reduction in the disease incidence level was observed after each spraying of the seaweed extract and fungicide. On the basis of our field trials, treatment with the copper-containing Bordeaux mixture provided the best crop protection against grape downy mildew which coincides with the results of Gianfranco *et al.,* (2016) and Suthin Raj *et al.,* (2018). In 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 is 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) and Hane Graff and Suthin Raj (2018).

Conclusion

In the present study, a survey on the occurrence of downy mildew and powdery mildew of grapes in twenty grapes growing areas of Tamilnadu was carried out. Evaluation of various seaweed extracts including *Hydroclathrus clathratus* (brown seaweed) prophylactic spray @ 10% after 30 and 50 DAP recorded a least incidence of downy mildew on leaves, inflorescence and fruits and also treatment with Bordeaux mixture prophylactic spray @ 1% after 30 and 50 DAP were also effective in the management of the disease.

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