IJPS INTERNATIONAL JOURNAL OF PLANT SCIENCES
Volume 8 | Issue 2 | July, 2013 | 253-257

RESEARCH ARTICLE

Eco-friendly management of grapevine downy mildew with wanis

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SUMMARY

Anti-fungal efficacy of a botanical formulation "wanis"containing predominantly monoterpene derivatives was evaluated in vitro against sporangial germination of downy mildew pathogen Plasmopara viticola. Wanis at 0.5 per cent completely inhibited the sporangial germination of *P. viticola*. Post inoculation spraying of wanis (0.5%) controlled grapevine downy mildew incidence to an extent of 67.95 per cent in the green house. In field trials wanis spray at 0.5 per cent reduced the downy mildew incidence by 66.61 per cent. Post inoculation spraying of wanis significantly increased the peroxidase (PO) and phenylalanine ammonia lyase (PAL) activity in grapevine plants after three days of inoculation.

Key Words : Grapevine, Downy mildew, Botanical formulation, Wanis, Management

How to cite this article : Rajeswari, E. (2013). Eco-friendly management of grapevine downy mildew with wanis. *Internat. J. Plant Sci.*, **8** (2) : 253-257.

Article chronicle : Received : 04.06.2012; **Revised :** 06.02.2013; **Accepted :** 12.03.2013

Downy mildew is one of the most destructive diseases of grapevine in the world. Symptoms appear as yellowish oily lesions on the upper surface and the corresponding lower surface whitish downy growth formed consisting of sporangia and sporangiophores of *P viticola*. The disease causes severe damage on clusters and reduction in yield (Agrios, 1997; Magarey *et al.*,1990; Pearson and Goheen, 1988). The yield loss inflicted by this disease was as high as 100 per cent when the conditions favour the downy mildew on flower or young berries (Emmet *et al.*, 1992 and Rawal and Saxena., 2004). Viticulture specialists commonly use fungicides to manage the disease. The inappropriate and indiscriminate use of fungicides in grapevine has led to serious environmental threat to human life. Hence, the use of botanical fungicide is the bio rational and best alternative to manage the disease. In the present investigation a botanical formulation "wanis" which contains predominantly monoterpene derivative was tested for its efficacy against downy mildew. Wanis exhibits disease suppressing activity against root rot , leaf blight, rice blast and sheath blight pathogens (Lakshminarayanan, 2001; Narasimhan *et al.*, 1998). The effect of application of wanis on peroxidase and phenylalanine ammonia lyses activity of grapevine was also investigated.

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MATERIAL AND METHODS

Source and maintenance of *P . viticola* inoculum :

Grapevine leaves (cv. Thompson Seedless) showing typical symptoms of downy mildew were collected and the sporangia on the sporulating lesions were dispensed in sterile distilled water using camel hair brush. The sporangia were washed by centrifuging the sporangial suspension at 3500 rpm for five min. The concentration of sporangia was adjusted to 5×10^4 sporangia by using haemocytometer. This sporangial suspension was sprayed on 120 days old green house grown grape vine plants (cv. THOMPSON SEEDLESS). Sufficient water congestion was given 24 hours prior to and after inoculation to maintain high humidity. The sporangia collected from these potted plants were used for further studies.

In vitro efficacy of wanis against sporangial germination of *P. viticola* :

Wanis was prepared at the concentrations of 0.125, 0.25 and 0.5 per cent with the sterile distilled water. The above mentioned concentrations of wanis were tested against

sporangial germination of *P. viticola* by cavity slide technique. One drop of the sporangial suspension of respective concentration of wanis was placed in a sterile cavity slide and allowed to air dry. One drop of the sporangial suspension ($5x10^4$sporangia/ml) of *P. viticola* was added and mixed thoroughly. Sporangial suspension in sterile distilled water served as a control. Five replications were maintained for each treatment. The slides were kept in moist growth chamber and incubated at 20°C. After six hrs of incubation observation was recorded on sporangial germination by microscopic examination.

Efficacy of wanis against grapevine downy mildew in green house :

Highly susceptible Thompson seedless grapevine stem cuttings were planted in 30 cm pots and maintained in green house. These plants were maintained by regular, uniform and judicious watering. After 120 days of planting the plants were inoculated with the sporangial suspension of *P. viticola* ($5x10^4$ sporangia/ml). Necessary water congestion was given both 24 hrs prior to and after inoculation for maintaining high relative humidity. Wanis at concentrations of 0.125, 0.25 and 0.5 per cent were sprayed on the plants after 24 hr of inoculation. Bordeaux mixture (1%) was used as the chemical check. Pathogen inoculated control (without any treatment) was also maintained. Five replications were maintained for each treatment. Observation on disease severity was recorded 10 days after spraying using 0-5 scale (Brown *et al*., 1999) and per cent disease index (PDI) was worked out using the formula:

$$\text{PDI} = \frac{\text{Sum of all numerical ratings}}{\text{Number of observation}} \text{x} \frac{100}{\text{Maximum disease rating}}$$

Field efficacy of wanis against grapevine downy mildew :

Field trails were conducted with five treatments and five replications in Randomized Block Design in the commercial vineyard of Odaipatti and Kamlapuram, Tamil Nadu. These two regions were identified as an endemic area for downy mildew and the cultivar grown were Thompson seedless (Odaipatti) and Muscat (Kamlapuram). During the experimental period the annual rainfall of 950 mm and minimum and maximum temperature of 15.7 and 30°C and RH of 52-93 per cent was recorded in Odaipatti. The annual rainfall of 800 mm and the minimum and maximum temperature of 16°C and 31°C and RH of 50-90 per cent were recorded in Kamlapuram. A seven years old vineyard was selected in both regions. Pruning was done during October and the first treatments were administrated during November after the initial appearance of the disease. The treatments consisted of

T_1 – Wanis 0.125%
T_2 – Wanis 0.25%
T_3 – Wanis 0.5%
T_4 – Bordeaux mixture (1%)
T_5 – Control

Totally three sprays were given at fortnightly intervals. The observations on disease intensity were recorded 10 days after last spray (0-5 scale) and the PDI was worked out. The data were analysed statistically (Gomez and Gomez, 1984).

Effect of wanis on peroxidase (PO) and phenylalaline ammonia lyase (PAL) activity in grapevine plants :

Grapevine plants cv. Thompson seedless (120 days old) was inoculated with the sporangial suspension of *P.viticola* inoculum ($5x10^4$ sporangial/ml). After 24 hours of inoculation the plants were sprayed with the wanis at 0.5 per cent concentration. Pathogen inoculated control and un-inoculated control was also maintained. Five plants were maintained for each treatment. Grapevine leaves (5^{th}, 6^{th} and 7^{th} from the top of the plants) were collected on 0, 3^{rd}, 5^{th} and 7^{th} days after spraying for analyzing the PO and PAL activities.

Assay of peroxidase activity :

Grapevine leaf sample (1g) was homogenized in two ml of 0.1 ml phosphate buffer pH 7.0 at 4°C. The homogenate was centrifuged at 15000 rpm at 4°C for 15 min and the supernatant was used as the enzyme source. The reaction mixture consisted of 1.5 ml of 0.05 ml pyrogallol, 0.5 ml of the enzyme extract 0.5 ml of one per cent H_2O_2. The reaction mixture was incubated at room temperature (28 + 2°C). The changes in absorbance at 420 nm were recorded at 30 sec. intervals for three min. The enzyme activity was expressed as changes in the absorbance of the reaction mixture min^{-1} g^{-1} fresh weight (Hammerschmidt *et al*., 1982).

Assay of phenylalaline ammonia lyase activity :

One g of powdered leaf sample was extracted with 0.1 M sodium phosphate buffer (pH 7.0) at 4°C. The homogenate was centrifuged for 20 min at 1000 rpm. The supernatant was used as the enzyme source for the assay of PAL. The assay mixture containing 100 µl of the enzyme extract, 500 µl of 50 mM Tris Hcl, pH 8.8 and 600 µl of 1 mM L-phenylalanine was incubated for 60 min. The reaction was arrested by adding 2N Hcl. Later 1.5 ml of toluene was added, vortexed for 30 sec, centrifuged (1000 rpm, 5 min) and toluene fraction containing trans-cinnamic acid was separated. The toluene phase was measured at 290 nm against the blank of toluene. A standard curve was drawn with graded amounts of cinnamic acid in toluene. The enzyme activity was expressed as nmoles of cinnamic acid min^{-1} g^{-1} fresh tissue (Ross and Sederoff, 1992).

RESULTS AND DISCUSSION

The results of the *in vitro* assay revealed that wanis was highly effective in inhibiting the sporangial germination of grapevine downy mildew pathogen. Wanis (0.5%) totally arrested (100% inhibition) the sporangial germination of *P. viticola*. Even at the lower concentration (0.125%) it exhibited

the inhibition of 66.40 per cent. Bordeaux mixture (1%) used for comparison also completely inhibited the sporangial germination (Table 1). The present investigation clearly demonstrated that the wanis has potential antifungal action against *P.viticola*. In this study complete inhibition of sporangial germination of *P.viticola* was found in wanis at 0.5 per cent. The earlier report showed that wanis was highly inhibitory to the rice pathogens, *Pyricularia oryzae*, *Drechslera* oryzae and *Rhizotonia solani* causing blast, brown spot and sheath blight, respectively in rice (Lakshminarayanan, 2001 ; Narasimhan *et al.,* 1998). Wanis totally arrested the mycelial growth of the soil borne pathogens *viz.*, *Fusarium solani*, *F.equiseti*, *F.oxysporum*, *Phytophthora capsici* and *Sclerotinia sclerotiorum* (Narasimhan *et al.,*1999). Wanis also observed to be completely inhibited the mycelial growth of *Alternaria solani*, *A.alternata*, *A.porri*, *Sclerotium rolfsii*, *Macrophomina phaseolina Pythium aphanidermatum* and *Fusarium oxysporum* fsp *lycopersici* and *Fusarium oxysporum* fsp *cepae (*Bharathi *et al.*, 2004).It is interesting to note that wanis has broad spectrum activity against wide range of pathogen.

Post inoculation spraying of wanis (0.5%) on green house grown grapevine plants reduced the disease incidence by 67.95 per cent while, wanis at 0.125 per cent showed the disease reduction of only 36.69 per cent. Bordeaux mixture inhibited the disease development by 74.31 per cent (Table 2).

Wanis was found to be promising in combating the disease in the field also. The trial conducted at Odaipatti during showed that spraying of wanis thrice, first spray after the initiation of the disease second and third at fortnightly intervals was significantly superior in inhibiting disease development by recording the lowest PDI of 23.45 per cent as against 81.62 per cent in the control. This accounted for the disease reduction of 71.26 per cent. Wanis at lower concentration (0.125%) recorded the maximum PDI (47.40) with the minimum disease reduction (41.92%), Bordeaux mixture (1%) and wanis (0.5%) were at par with each other in reducing the disease intensity.

In Kamlapuram vineyard also positive correlation was found between wanis concentration and its efficacy against the disease. The plants treated with wanis (0.5%) as

Table 1 : *In vitro* efficacy of wanis against sporangial germination of *P.viticola*

Sr. No.	Wanis concentrations (%)	Sporangial germination (%)*	Germination inhibition (%)
1.	0.125	30.50 (33.51)^{c}	66.40
2.	0.25	19.85 (26.46)^{b}	78.13
3.	0.5	0 (0.54)^{a}	100
4.	Bordeaux mixture (1%)	0 (0.54)^{a}	100
5.	Control	90.78 (72.37)^{d}	-

*Mean of five replications.Data in parentheses are arc sine transformed values, Within column mean followed by a common letter do not differ significantly (P=0.05) according to Duncan's Multiple Range Test

Table 2 : Downy mildew incidence on green house grown grapevine plants sprayed with wanis

Treatments No.	Wanis concentrations (%)	Per cent disease index*	Disease reduction (%)
T_1	0.125	57.53 (49.29)^{d}	36.69
T_2	0.25	49.85 (44.92)^{c}	45.10
T_3	0.5	29.10 (32.65)^{b}	67.95
T_4	Bordeaux mixture (1%)	23.33 (28.87)^{a}	74.31
T_5	Control (Pathogen inoculated)	90.87 (72.81)^{e}	-

*Mean of five replications. Data in parentheses are arc sine transformed values within column mean followed by a common letter do not differ significantly (P=0.05) according to Duncan's Multiple Range Test

Table 3 : Field efficacy of wanis against grapevine downy mildew

Treatment No.	Wanis concentration (%)	Odaipatti		Kamlapuram		Mean disease reduction (%)
		Per cent disease index*	Disease reduction (%)	Per cent disease index*	Disease reduction (%)	
T_1	0.125	47.40 (43.52)^{c}	41.92	49.20 (44.55)^{c}	28.49	35.20
T_2	0.25	40.75 (39.68)^{b}	50.07	40.35 (39.43)^{b}	41.35	45.71
T_3	0.5	23.45 (28.96)^{a}	71.26	26.17 (30.50)^{a}	61.96	66.61
T_4	Bordeaux mixture (1%)	20.47 (26.90)^{a}	74.92	24.10 (29.40)^{a}	64.97	69.9
T_5	Control	81.62 (64.62)^{d}	-	68.80 (56.04)^{d}	-	

*Mean of five replications.Data in parentheses are arc sine transformed values, Within column mean followed by a common letter do not differ significantly (P=0.05) according to Duncan's Multiple Range Test

mentioned above showed the PDI of 26.17 (61.96% disease reduction) which was at par with bordeaux mixture (24.10 PDI). The untreated control plants recorded the PDI of 68.80. Wanis (0.125%) was found to be the least effective (49.2 PDI) in reducing the disease incidence. This confirms the previous findings in rice which showed that wanis was effective against blast and brown spot disease (Lakshminarayanan, 2001). The antifungal activity of wanis might be due to the presence of plant based monoterpenes (Narasimhan *et al.*,1998; Narasimhan *et al.*, 1999). Many pathogenic wood inhabiting fungi were found to be inhibited by monoterpens of their host (Bridges, 1987; Cobb *et al.*, 1968; DeGroot, 1972 ; Ennos and Swales, 1988 ; Flodin and Fries, 1978).

Post inoculation spraying of wanis increased PAL and PO activity in grapevine plants when compared to healthy plants. The enzyme activities attained the peak, three days after treatment, after wards both the enzyme activity started declining. Increase in PO and PAL activity due to wanis treatment alone was lesser than that of post inoculation spray. Inoculation of *P.viticola* also increased these enzyme activities in grapevine plants compared to healthy plants (Fig.1 and 2).

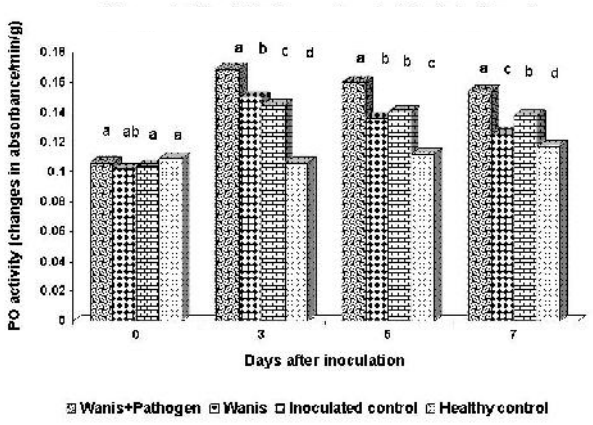

Fig. 1: Changes in PO activity of grapevine plants treated with wanis

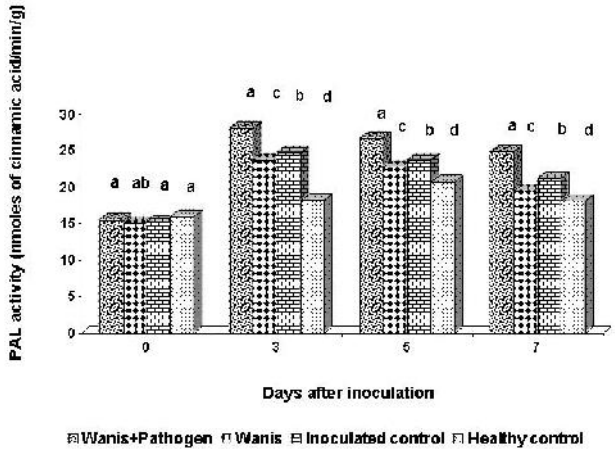

Fig. 2: Changes in PAL activity of grapevine plants treated with wanis

It was evident from the present results that the post inoculation spraying of wanis increased both PO and PAL activities in grapevine plants. Peroxidase oxidized phenols to quinines associated with disease resistance in plants (Chattopadhyay and Bera, 1980 ; Iwata *et al*., 1982 ; Mansfield, 1993). Increased PAL activity leads to the de novo synthesis of phenolics (Glazzener, 1982). Phenylalaline ammonia lyase is an enzyme of phenyl propenoid pathway and it might have a definite role in the defense mechanism of plants (Friend and Threshfall, 1976). Phenylalaline ammonia lyase activity was associated with the production of specific phenolic compounds including some antifungal isoflavonoids and phytoalexin in legumes (Friend, 1981) . The increase in PO, PAL activity due to the spraying of wanis may be attributed to reduction of downy mildew incidence in grapevine plant. Repeated application of synthetic fungicides results in development of fungicide resistant pathogens and environmental pollution (Rao and Reddy, 1988). These fungicidal risk can be minimized if botanical formulation *viz.*, wanis could be exploited for downy mildew management in grapevine.

Acknowledgement :

The authors are greatful to Southern Petro Chemicals Industries Corporation Ltd., Chennai, Tamil Nadu, India, for the financial support to carry out the research work

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