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Potassium fertilization in relation to downy mildew disease incidence in grape leaves

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Summary

Influence of different sources of potassium and their method of application was studied on incidence of downy mildew infection in grape leaves of 'Sharad Seedless' (syn: 'Kishmish Chorni'). The total phenolic content and individual phenolic acids were analysed from healthy and downy mildew infected leaves of 'Sharad Seedless' and the degree of downy mildew infection was well correlated with potassium content in the petioles. Different sources and method of potassium application had significant effect on Phenylalanine ammonia-lyase (PAL) enzyme activity and preformed phenols in healthy vines. Significant increase in PAL enzyme activity, total phenols and individual phenolic acids was registered with increase in disease severity. Magnitude of percent change in PAL enzyme activity and total phenolic content was highest in vines with maximum disease infection. Among the phenolic acids, *o*-Coumaric acid, *p*-Coumaric acid have a definite role in disease resistance. We could also observe the variation in disease severity in vines which received different sources of potassium which was supplied in different quantities whether through soil and/ or through fertigation.

K e y w o r d s : potassium; downy mildew; phenols; phenolic acids and PAL enzyme.

Introduction

Grape (*Vitis vinifera* L.) is one of the most important fruit crops having agronomic and economic importance (Ruel and Walker 2006). Grape cultivation in India faces serious threats from several insect pests and diseases. Among the fungal diseases, downy mildew caused by pathogen *Plasmopara viticola* (Berl. & Curt.) is a predominant disease affecting grape vineyards worldwide (Goeker *et al.* 2003). Downy mildew has been reported from all grape growing regions of India though the intensity of attack varies from region to region. It is most devastating in peninsular India, particularly in south interior Karnataka, Andhra Pradesh and Maharashtra, while its severity is less in northern parts of India (Chadha and Shikhamany 1999). It is the chief limiting factor in grape cultivation, which causes damage to vines under moist and warm conditions and is prevalent in an epiphytic form (Rawal 2005). Nutrition alters the compatibility relationship of the host-parasite environment within the plant. Increasing evidence suggests that mineral nutrient plays a critical role in plant stress resistance (Amtmann 2008). Among the essential mineral nutrients, potassium (K) particularly plays a critical role in plant growth and metabolism and it contributes greatly to the survival of plants that are under various biotic and abiotic stresses. However, K is essentially critical in the production and transport of fungus inhibiting phenolic and flavonoid compounds and a shortage of K reduces the amount of the plant's natural antifungal compounds at the site of infection (Marschner 1995). A variety of factors contribute to the ability of grapevines resistance against attack of pests. One of the major factors for diseases resistance is increased phenolic compounds. There was increase in phenolic compound in different plants immediately after infection of downy or powdery mildew disease in grapes (Baydar *et al.* 2011, Mazid *et al.* 2011). The critical roles of K^+ in primary metabolism ensure optimum plant health and proper functioning of defence mechanisms. It has been proposed that K^+ encourages the stronger cell wall development and stimulates phenol production to prevent further infection (Wang 2013). Adequate potassium increases phenol concentrations, which play a critical role in disease resistance (Prasad 2010).

The mechanistic influences of K on plant disease resistance have been reported by several researchers. Higher K concentrations decreased the internal competition of pathogens for nutrient resources and enables plants to allocate more resources to develop stronger cell walls for preventing pathogen infection and insect attack and to obtain more nutrients to be used for plant defence and damage repair (Mengel 2001). Potassium is also essential for performance of multiple plant enzyme functions, and it regulates the metabolite pattern of higher plants, ultimately changing metabolite concentrations (Marschner 2012). Thus maintaining the optimum level of potassium in grapevines should become a part of the integrated disease management strategy for Indian vineyards. Indiscriminate use of water soluble fertilizers especially potassium is being practiced by grape growers which adds to cost of cultivation. In some grape growing pockets of India, excess use of potassium through fertigation has resulted in uneven bud break and lot of deadwood formation of primary and secondary arms of the vine. Hence an investigation was conducted to know the effect of different sources of potassium and their method of application (soil

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or fertigation) on physio-biochemical changes in relation to downy mildew disease incidence in grapes.

Material and Methods

Treatment details: An experiment was laid out with eight treatments replicated four times in Completely Randomized Block Design at the experimental research farm in Block No. 1 of ICAR-Indian Institute of Horticultural Research, Hessaraghatta, Bengaluru. The experimental site has a mild tropical climate. It is situated at an elevation of 890 meters, 12° 68'- North latitude and 77° 38' East latitude. Twelve year old grape vines of 'Sharad Seedless' (*Vitis vinifera* L.), grafted on Dogridge rootstock with plants cultivated according to a 'Y' training system. In the tropical climate of India double pruning and single cropping system of grape cultivation is being practiced. Vines were pruned twice, once after harvesting of previous year's crop which is popularly called as foundation pruning/back pruning. After this pruning, the vines were encouraged to develop canes with fruitful buds. The other pruning was done on the developed canes to encourage bunch development which is called as forward pruning/fruit pruning (SATISHA *et al.* 2010). The other cultural operations like irrigation, plant protection sprays, canopy management practices like shoot thinning, shoot positioning etc were done as per the recommended practices. Shoot thinning was performed at 25-30 d after pruning leaving 40 shoots per vine (1 shoot/1.5 sq. ft canopy), while shoot positioning was done as and when required to either cover the bunches from direct western sunlight or to expose bunches to eastern sunlight.

The recommended dose of nutrients per hectare is 500:500:1000 kg of N_2O, P_2O_5 and K_2O. Keeping this recommended dose in mind, different treatments were imposed based on the potassium content in different sources of potassium fertilizers. The different treatment combinations were T_1- 100 % SOP (sulphate of potash) through soil, T_2- 60 % SOP through fertigation + 40 % SOP through soil, T_3- 60 % KNO_3 (potassium nitrate) through fertigation + 40 % SOP through soil, T_4- 60 % 19:19:19 through fertigation + 40 % SOP through soil, T_5- 40 % SOP through fertigation + 60 % SOP through soil, T_6- 40 % KNO_3 through fertigation + 60 % SOP through soil, T_7- 40 % 19:19:19 through fertigation + 60 % SOP through soil and T_8- 100 % SOP through fertigation. Treatments were imposed after 75 d after both back and forward prunings. Soil application was done once in 15 d and the fertigation was done once in 3 d from 75 d after both back and forward pruning till 120 d. The other nutrient elements were applied as per the recommended dose.

Leaf sample collection: After forward pruning during winter, leaf samples were collected before and after downy mildew incidence during the cropping year of 2017-18. Total phenols, phenol profiling and phenylalanine ammonia lyase enzyme activity were determined.

Per cent infection and per cent disease index: The disease scoring on the leaves was recorded by assigning numerical values based on the percentage of leaf area affected by the disease following five point (0-5) disease rating scale. Ten leaves selected at random from both directions of the periphery of leaf canopy of vine were scored. Later this was converted into percent disease index (PDI) with formula suggested by MCKINNEY (1923).

$$\text{PDI} = \frac{\text{Sum of all numerical ratings} \times 100}{\text{Total number of leaves observed} \times \text{Maximum disease grade}}$$

Number of sporangia per cm^2: The sporangia were enumerated by using a haemocytometer. The freshly infected leaves were collected from the field as per treatment from each plot. A section measuring one cm^2 of infected leaf section was taken, and made into a volume of 1 mL with distilled water. To this suspension, 1-2 drops of Tween 20 were added to keep the sporangia well dispersed. The number of sporangia was counted by haemocytometer under an electronic microscope and the average was calculated and expressed as sporangia per $cm^2 \cdot m^{-1}$ (CRISWELL *et al*. 2008).

Phenylalanine ammonia lyase: Phenylalanine ammonia lyase was estimated by the procedure by HODGINS (1971). The enzyme was extracted by homogenizing 0.25 g of leaf tissue with 5 mL of 150 mM Tris HCl buffer containing 0.1 mM EDTA. 30 µL of enzyme extract was incubated with 3 mM L-Phenylalanine solution and deionized water, increase in absorbance was measured at 270 nm up to 5 min at 1 min interval. The activity was expressed as units per mg FW.

Potassium in petioles: A known weight of petiole sample was digested in diacid mixture HNO3: HClO4 (10:4) till a colourless solution was obtained. The di-acid digested sample was fed to Atomic Absorption Spectrometer directly, with proper dilution (if required). The reading was used along with the standard curve to estimate potassium contents (PIPER 1966).

Total phenols: Total phenol content was estimated by spectrophotometric method using Folin-Ciocalteau's Phenol Reagent (SINGLETON *et al*. (1965). About 1 g leaf sample was incubated in 20 mL of methanol (80 %) for 72 h. Then it was homogenized with methanol (80 %) in a pestle and mortar 2-3 times. The extracts were pooled and volume made to 50 mL. About 0.5 mL of the extract was taken in test tubes and 2 mL of Folin-Ciocalteau's Phenol Reagent was added followed by 3.3 mL of distilled water and mixed well. After 2 min, 1 mL of 20 % sodium carbonate solution was added and mixed well. The reaction mixture was allowed to stand at room temperature for 30 min and blue color intensity was red in a spectrophotometer at 700 nm against blank. A standard curve was prepared using gallic acid as standard. Total phenol content was expressed as mg gallic acid per 100 g using the formula.

$$\text{Total phenols} = \frac{OD_{700\,nm} \times \text{Std. value (µg/OD)} \times \text{Total Vol. of extract} \times 100}{\text{Assay volume} \times \text{Wt. of tissue (g)} \times 100}$$

Phenol profiling: Individual phenolic acids were identified and quantified by the MRM method in LC-MSMS knowing their parent mass m/z and most abundant fragmented daughters as previously described by WEIDNER *et al*. (2000) and CHEN *et al*. (2001) with slight modification.

Chemicals and reagents: Phenolic acid standards namely ferulic acid, 2,4-dihydroxybenzoic acid,

caffeic acid, gallic acid, gentisic acid, o-coumaric acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, protocatechuic acid, salicylic acid, syringic acid, t-cinnamic acid, vanillic acid, chlorogenic acid, benzoic acid, 3-hydroxy benzoic acid, sinapic acid and ellagic acid were acquired from Sigma Chemical Co., USA. The standard solutions were prepared in 80 % methanol. The organic solvents used as the mobile phase for liquid chromatography were of chromatographic/ MS grade and all the other reagents were of analytical grade. Water purified in the Milli-Q (Millipore) system was used to prepare the mobile phases. All mobile phases were filtered through membranes with a pore size of 0.45 µm.

Calibration curve: The calibration curve for phenolic acids were developed by the multiple reactions monitoring (MRM) method of LC-MS or MS using the parent mass (m/z) and most abundant fragmented daughters (Figure).

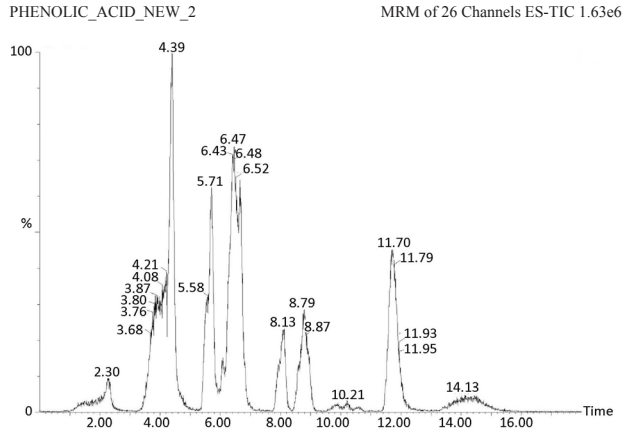

Figure: Standard chromatogram used for integration and quantification of phenolic contents.

Sample Preparation and Extraction: About 1 g leaf sample was incubated in 20 mL of methanol (80 %) for 72 h. Then it was homogenized with methanol (80 %) in a pestle and mortar 2-3 times. The extracts were pooled and volume made to 50 mL. From this extract, 30 mL were evaporated to dryness under vacuum at 45 °C and the residue was dissolved in 10 mL of distilled water and extracted thrice with 40 mL petroleum ether then with 40 mL of ethyl acetate using a separating funnel. Discarding the aqueous layer, the ethyl acetate extract was evaporated to dryness under vacuum at room temperature. To the dry residue, 4 mL of 2 N NaOH was added and allowed to hydrolyze by overnight. After acidification to pH 2 using 4 mL 2N HCl, the residue was again re-extracted with 40 mL ethyl acetate using a separating funnel. The ethyl acetate layer was dried completely in a rotary evaporator and the residue dissolved in 2 mL MS grade methanol, filtered through 0.2 µm nylon filter prior to injecting in LC-MSMS for phenolic acid estimation.

Equipments: An Acquity UPLC-H class coupled with TQD-MS/MS from M/S Waters, USA with ESI source was used in the phenolic acids determination, equipped with a degasser, quaternary pump, automatic injection system (0-10 µL), with a diode array detector and a temperature control compartment for the analytical column. The detection system allowed for the simultaneous detection at various wavelengths and MRM for individual masses. The overall system was controlled by the Mass lynx software, which also administered the data collection and treatment system.

LC and MS-MS conditions: The phenolic acids were resolved on the analytical column BEH-C18 (2.1 x 50 mm, 1.7 µm) from waters India ltd., protected by a Vanguard BEH C-18 (Waters, USA) with the gradient flow of organic and aqueous phase with the flow rate of 0.3 mL·min^{-1}. The column temperature was maintained at 25 °C during analysis and the sample injection volume was 4 µL. The eluted phenolic acids were monitored by the PDA detector and the UPLC column effluent pumped directly without any split into the TQD-MS/MS (Waters, USA) system optimized for the phenolic acids and flavonoids analysis. Mobile phase used was Solvent – A as 0.1 % formic acid in water and Solvent - B: as 0.2 % formic acid in methanol.

Statistical analysis: The data is presented as arithmetic mean of four replications. The significance of given treatments were determined using one-way ANOVA statistics. Duncan's multiple range test (DMRT) was used to differentiate the mean at $p = 0.05$. Simple correlation studies were also made to understand their interaction effects. SPSS for Windows version 9.0 and Microsoft Excel 2007 were used to carry out statistical analysis and graphical data presentation.

Results and Discussion

Application of potassium through 40 % SOP through fertigation + 60 % SOP through soil application (T_5) resulted, in significant reduction of mean percentage of infected plants (32.47 %), minimum PDI (per cent disease index, 25.82), lowest no. of sporangia (6.90 cm^{-2}) and maximum potassium content in petioles of 3.60 %. Treatment T_2 (60 % SOP through fertigation + 40 % SOP through soil) was on par to T_5 treatment with mean percentage of infected plants (37.70 %), PDI (28.91), no. of sporangia (7.05) and potassium content in petioles (3.34 %). Highest mean per cent of infected plants (57.54), maximum PDI (38.89), highest no. of sporangia cm^{-2} (7.30) and least potassium content in petioles (2.03 %) had noted in treatment T_8 with 100 % SOP through fertigation (Tabs 1 and 2).

Phenylalanine ammonia-lyase activity and total phenolic content of leaves before downy mildew infection differed significantly among the treatments under investigation. Treatment T_5 with 40 % SOP through fertigation + 60 % SOP through soil registered significantly highest PAL activity and total phenols followed by T_2 treatment. Lowest PAL activity and total phenolic content was observed in T_8 treatment with 100 % SOP through fertigation. There was significant increase in PAL activity and total phenolic content of leaves due to foliar downy mildew infection. The maximum PAL activity and post inflectional accumulation of phenolics were recorded in T_5 treatment while minimum was recorded in treatment T_3 (60 % KNO_3 through fertigation + 40 % SOP through soil) and treatment T_1 (100 % SOP through soil) respectively, but magnitude of percent change in PAL activity (54.25 %) and total phenolic content (1319.40 %) was

Table 1

Effect of different sources and methods of potassium fertilizers application on downy mildew disease parameters in 'Sharad Seedless' grapes

Treatment		Infected plants (%)	Percent disease index (PDI)	Log values of no. sporangia cm^{-2}
T_1	100 % SOP through soil	58.33^{ab} (49.87)	36.50^{a} (37.16)	7.11^{bc}
T_2	60 % SOP through fertigation + 40 % SOP through soil	37.50^{c} (37.70)	23.50^{bc} (28.91)	7.05^{c}
T_3	60 % KNO_3 through fertigation + 40 % SOP through soil	66.67^{a} (55.09)	34.50^{a} (35.91)	7.17^{b}
T_4	60 % 19:19:19 through fertigation + 40 % SOP through soil	62.50^{ab} (52.66)	37.00^{a} (37.40)	7.14^{bc}
T_5	40 % SOP through fertigation + 60 % SOP through soil	29.17^{c} (32.47)	19.00^{c} (25.82)	6.90^{d}
T_6	40 % KNO_3 through fertigation + 60 % SOP through soil	45.83^{bc} (42.57)	26.50^{b} (30.95)	7.07^{bc}
T_7	40 % 19:19:19 through fertigation + 60 % SOP through soil	58.33^{ab} (49.87)	27.00^{b} (31.24)	7.09^{bc}
T_8	100 % SOP through fertigation	70.83^{a} (57.54)	39.50^{a} (38.89)	7.30^{a}
	S.E.M.	3.57	1.22	0.04
	C.D. 5 %	10.49	7.31	0.12
	C.V.	15.11	3.58	1.15

Data in parentheses indicate angular transformed values.

Table 2

Effect of different sources and method of potassium fertilizers application on K content in petiole, total phenols concentration and o-Coumaric acid in 'Sharad Seedless' grapes

Treatments	Potassium content in petioles (%)	PAL (units·g^{-1} FW)			Total phenols (mg·100 g^{-1} FW)			*o*-Coumaric acid (µg·g^{-1})		
		Healthy	Infected	% Change	Healthy	Infected	% Change	Healthy	Infected	% Change
T_1	2.57^{c}	0.020^{de}	0.143^{e}	+609.58	1679.650^{cd}	2252.257^{abc}	+37.88	305.47^{c}	471.38^{e}	+56.48
T_2	3.34^{ab}	0.058^{ab}	0.191^{cd}	+231.23	2045.833^{ab}	2374.567^{ab}	+16.08	528.00^{a}	605.54^{d}	+16.80
T_3	2.49^{c}	0.028^{d}	0.166^{cde}	+533.93	1744.627^{bcd}	1864.327^{c}	+7.34	248.43^{c}	351.71^{f}	+44.46
T_4	2.13^{cd}	0.023^{d}	0.157^{de}	+645.25	1704.563^{cd}	1973.150^{bc}	+20.63	249.05^{c}	375.87^{ef}	+52.03
T_5	3.60^{a}	0.064^{a}	0.244^{a}	+282.46	2126.053^{a}	2548.350^{a}	+22.19	566.90^{a}	1562.57^{a}	+175.60
T_6	3.12^{b}	0.052^{bc}	0.228^{ab}	+353.12	2012.500^{abc}	2354.190^{ab}	+17.03	444.66^{b}	1476.11^{a}	+231.89
T_7	2.25^{cd}	0.045^{c}	0.201^{bc}	+350.36	1811.293^{abcd}	2242.547^{abc}	+23.95	421.31^{b}	1183.29^{b}	+180.91
T_8	2.03^{d}	0.013^{e}	0.189^{cd}	+1319.4	1587.257^{d}	2370.990^{ab}	+54.25	179.97^{d}	969.62^{c}	+438.26
S.E.m.±	2.57^{c}	0.003	0.012		110.82	132.429		21.06	31.96	
C.D. 5 %	3.34^{ab}	0.009	0.035		336.184	401.72		63.89	96.95	
C.V.	2.49^{c}	13.983	10.667		10.44	10.21		9.91	6.33	

highest in T_8 treatment (Tab. 2). The phenol profile of healthy and downy mildew infected leaves exhibited significant differences among the eight treatments. In healthy leaves, significantly highest *o*-coumaric acid (566.90 µg·g^{-1}), *p*-coumaric acid (1987.77 µg·g^{-1}), caffeic acid (192.45 µg·g^{-1}), Ferulic acid (271.93 µg·g^{-1}) and salicylic acid (246.68 µg·g^{-1}) were registered in treatment T_5 and T_8 and lowest recorded content of *o*-coumaric acid (179.97 µg·g^{-1}), *p*-coumaric acid (1080.48 µg·g^{-1}), caffeic acid (85.80 µg·g^{-1}), ferulic acid (53.41 µg·g^{-1}) and salicylic acid (85.56 µg·g^{-1}; Tabs. 2 and 3). Due to downy mildew disease infection, the amount of individual phenolic acids was increased significantly over the healthy period. T_8 treatment recorded maximum increase in *o*-coumaric acid (438.26 %), ferulic acid (748.59 %), and salicylic acid (582.29 %) and T_7 treatment registered maximum increase of *p*-coumaric acid (232.97 %), caffeic acid (2252.39 %). Quantitatively, concentration of *p*-coumaric acid was highest in healthy leaves and infected leaves among all phenolic acids, whereas the highest magnitude of percent change in concentration, over the healthy period was observed in caffeic acid. Highly significant and negative correlation (-0.862) was deleted between K petiole content

Table 3

Effect of different sources and method of potassium fertilizers application on phenolic acids in 'Sharad Seedless'

Treatments	*p*-Coumaric acid (µg·g^{-1})			Caffeic acid (µg·g^{-1})			Ferulic acid (µg·g^{-1})			Salicylic acid (µg·g^{-1})		
	Healthy	Infected	% Change	Healthy	Infected	% Change	Healthy	Infected	% Change	Healthy	Infected	% Change
T_1	1513.99^{cd}	3523.62^{de}	+133.35	110.61^{c}	169.37^{f}	+52.43	114.35^{d}	417.23^{c}	+264.91	92.81^{f}	338.35^{c}	+264.62
T_2	1940.73^{a}	3862.24^{d}	+99.8	119.10^{bc}	347.44^{e}	+191.94	216.93^{b}	314.60^{d}	+46.06	107.03^{e}	174.05^{e}	+62.86
T_3	1389.37^{d}	2667.17^{f}	+92.35	116.40^{bc}	124.10^{f}	+13.14	99.55^{e}	256.63^{de}	+158.86	116.41^{e}	133.42^{e}	+15.34
T_4	1441.56^{cd}	1612.47^{g}	+11.95	90.95^{d}	149.05^{f}	+63.13	55.53^{f}	223.41^{e}	+314.93	158.02^{d}	75.81^{f}	-51.39
T_5	1987.77^{a}	6068.83^{a}	+205.31	192.45^{a}	842.30^{d}	+340.07	271.93^{a}	484.73^{bc}	+78.22	246.68^{a}	287.56^{d}	+16.35
T_6	1823.44^{ab}	4510.19^{c}	+147.35	131.70^{b}	950.90^{c}	+623.63	153.63^{c}	486.83^{b}	+216.81	210.59^{b}	300.02^{cd}	+42.68
T_7	1641.02^{bc}	5452.34^{b}	+232.97	121.05^{bc}	2846.35^{a}	+2252.39	103.93^{de}	739.78^{a}	+610.33	184.03^{c}	509.05^{b}	+176.57
T_8	1080.48^{e}	3261.96^{e}	+204.28	85.80^{d}	1673.00^{b}	+1848.95	53.41^{f}	438.38^{bc}	+748.59	88.56^{f}	604.61^{a}	+582.29
S.E.m.±	69.24	156.75		6.29	33.11		4.85	22.88		4.68	14.17	
C.D. 5 %	210.03	475.45		19.09	100.43		14.7	67.88		14.18	42.97	
C.V.	7.48	7.02		9.01	6.46		6.28	9.22		5.38	8.10	

(%) and percent disease index (PDI). Highly significant and positive correlation for % K in petiole was observed with total phenols in healthy leaves (0.937) followed by *p*-coumaric acid (0.894), o-coumaric acid (0.880) and PAL (0.864). Total phenols showed significant and positive correlation with phenylalanine ammonia lyase activity (0.977). The reduced incidence of downy mildew in T_5 may be attributed to increased potassium content in the petioles of the same treatment. The higher concentration of potassium might be one of the responsive factors for increased production of phenolic compounds. The mechanisms of phenolic toxicity to fungal pathogens may be either substrate deprivation, membrane disruption or enzyme inhibition by the oxidized compounds which might be possibly through reactions with sulfhydryl groups or through more nonspecific interaction with the proteins (Ahmed 2016). The enhanced induction of enzymes like peroxidases, phenylalanine ammonia lyase and polyphenoloxidase might have also been contributed to induced systemic resistance triggered by potassium content in petioles as reported by several works (Tian *et al.* 2006, Barilli *et al.* 2010). These enzymes are known to have hydrolytic action and can effectively degrade the fungal cell wall (Mathivanan *et al.* 1997).

Vines which received T_5 treatment had highest potassium content in petioles and least downy mildew disease incidence and the same treatment also witnessed maximum content of constitutive (preformed) as well as post -infectional total phenols and individual phenolic acids, before and after disease infection which was principally due to its highest activity of Phenylalanine ammonia lyase (PAL; Tab. 2). This could be ascribed to the role of potassium in enzyme activation, as it acts as cofactor in phenylalanine ammonia lyase (PAL) activity. After microbial attack, the first step of the defence mechanism involves accumulation of phenols at the infection site, which restricts or slows down the growth of the pathogen (Matern and Kneusal 1988). With adequate levels of available potassium to vines, the activity of PAL increases, which is the key enzyme for metabolism of phenols, as it catalyses the deamination of L-phenylalanine to yield ammonia and *trans*-cinnamic acid from which phenolic compounds are produced. PAL has been demonstrated in metabolic activity of many higher plants and it plays a significant role in the synthesis of several defence-related secondary compounds like phenols (Benoit *et al.* 2000, Tahsili *et al*. 2014, Tsuge *et al*. 2004). The presence of phenolic compounds in plants and their synthesis in response to infection is associated with disease resistance. Phenolic compounds possess antimicrobial properties against fungi and play an important role in the host/pathogen relationship (Martini *et al.* 2009).

Results of the present investigation were in agreement with the finding of Taware *et al.* (2010), as they noted significant increase of caffeic acid and *p*-coumaric acid in powdery mildew infected leaves as compared to healthy grape leaves. Nguyen *et al*. (2010) had reported the increase in phenolics with increased application of potassium on phenolics in Basil leaves. Gao *et al*. (2018), also confirmed higher levels of ferulic and salicylic acids which were enhanced by K application and they concluded that phenolic acids can dramatically curtail soybean cyst nematode *Heterodera glycines* infection in Soybean.

Conclusion

The application of potassium sulphate more through soil (60 %) and less (40 %) through fertigation was favourable for lowest downy mildew disease incidence on grape leaves. More probably the increased potassium content in petiole increased the constitutive and post inflectional accumulated total phenols and phenolic acids with amplified phenylalanine ammonia-lyase activity in leaves. Magnitude of percent change in PAL activity and total phenolic content was highest in vines with maximum disease infection.

Among the phenolic acids, *o*-coumaric acid, *p*-coumaric acid have a definite role in disease resistance. This suggests the importance of potassium for phenol metabolism to boost the plant immune system and the importance to study the question in relation to the vineyard yield.

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