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Studies on the Quality of Grapevine Berries Sprayed With Copper Fungicide

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Abstract: Studies were carried out by the grapevine berries sprayed with copper hydroxide (Kocide 3000) to assess its quality in terms of reducing sugars, non reducing sugars, total sugars and acidity. The total, reducing and non reducing sugars were higher in the grapevine berries sprayed with Copper hydroxide comparing with that of control plots. Fruit quality was improved in Copper hydroxide (Kocide 3000) treated plots when compared to control. The plots receiving 450 g a.i ha^{-1} recorded 25.43 per cent increase in total sugar over control. It attributes that copper hydroxide apart from managing downy mildew pathogen, it improves quality of grapevine berries fetching higher market rate.

Index Terms: Acidity- Copper hydroxide- Grapevine- Quality – Sugars

I. INTRODUCTION

Downy mildew (*Plasmopara viticola*) a fungal disease native to North America attacks most species of wild and cultivated grape. Today, the disease can be found on grapevines in most regions of the world that are wet during the growing season, which results in great failure of fruits yield and hence a major loss of economy to wine industries. The solution to control the devastating pathogen, *Plasmopara viticola* was found by Alexis Millardet who discovered in 1882 the prophylactic effect of lime and copper and developed the 'Bordeaux mixture', which became the first successful fungicide to be used in vineyards

Copper sprays applied to leaves exist predominantly as insoluble deposits of copper salts (Menkissoglu and Lindow, 1991). Copper is an essential cofactor of a number of enzymes involved in respiration such as oxygenases and electron transport proteins (Garcia *et al*., 1994). Copper has the ability to generate free radicals able to damage DNA and lipid membranes (Hoshino *et al*., 1999 and Muller *et al*., 2000). For years, copper containing compounds have been sprayed on vegetable and fruit crops to limit the spread of plant pathogenic bacteria and fungi. Copper sprays are protectant fungicides that must be applied evenly to the plant or fruit surface before the disease develops to prevent infection. Copper is not systemic chemical and cannot be carried internally through the plant to kill the pathogen. The solubility of these copper products increases as the pH drops, slowly dissolving to release a small and constant supply of cupric ions (Cu^{+2}) as long as the water remains. (Sandra Hardy., 2004).

Control of the pathogen is generally achieved with chemical fungicide and copper salt applications (Aziz *et al*., 2006). Currently the only fungicides approved as an organic option are those containing copper hydroxide and copper sulphate. These sprays only protect vines from new infections; they do not eliminate existing infections and are not systemic (Wilcox, W., 2007). Kocide 3000 manufactured by DuPont, a copper based fungicide/bactericide was reported to inhibit wide range of fungal and bacterial pathogens. The active ingredient is copper hydroxide (35 per cent) with 30 per cent metallic copper equivalent. In the present study, the quality of berries was studied by the sprayings of copper hydroxide (Kocide 3000) along with the standard fungicide Ridomil used for control of downy mildew disease.

II. MATERIALS AND METHODS

Quality of berries

In order to determine the quality of berries due to the fungicide sprayed (Copper hydroxide-Kocide 3000) the chemical analysis of the berries under each treatment was carried out for acidity and reducing sugars. The total sugar content was determined by hand refractometer and expressed as percentage.

Total sugars

To estimate total sugars in the fruits from the field trial, method given by Dubois, *et al.* (1956) was followed. Fruit sample (100 mg) was homogenized and extracted with 5 ml 80 per cent warm ethanol twice. The extracts were centrifuged at 2000 rpm for 10 minutes. Then 0.2 ml of the supernatant was taken in a test tube and 1 ml of 5 per cent redistilled reagent grade phenol solution and 5 ml of 96 per cent sulphuric acid were added and shaken well. After 10 minutes, it was reshaken and placed in a water bath at 25-30°C for 20 minutes. Simultaneously, glucose standard was prepared. Absorbance was measured at 490 ηm in a spectrophotometer. The OD value wee compared with the glucose standard graph and the amount of total sugars was calculated in per cent of fresh weight basis.

Reducing sugars

A known quantity of the pulped material was taken, clarified with neutral lead acetate, deleaded with sodium oxalate and made upto a desired volume. The clarified and deleaded solution was run down from a burette to Fehling's solution A and B till a brick red colour was reached. Then a few drops of methylene blue was added and titration continued till the end point (brick red colour) was reached. The quantity of reducing sugars was estimated from the titer value and expressed as the percentage of the material taken originally. Non reducing sugars were found out by substracting the reducing sugars from the total sugars.

Acidity

The berries were pulped in a blender and 50 g of the pulped material was taken which was digested with 200 ml of water for half an hour, the volume was made up to 500 ml, cooled and filtered. Twenty five milliliters of the filtrate was taken for the estimation of acidity and titrated against normal potassium hydroxide using phenolphthalein as indicator. The results are expressed as grams of acid per hundred millilitres of juice, which was approximately percentage of acid. The quality of the grapes was judged in terms of the sugars acid ratio (Krishnamurthi *et al*., 1959).

Statistical analysis

The data generated from various experiments of this study were statistically analyzed by DMRT with IRRISTAT software. The data with per cent values were subjected to arc sine transformation.

III. RESULTS

Effect of fruit quality

Since the application of Copper hydroxide (Kocide 3000) has reduced disease severity in fruits, the effect of different doses of Copper hydroxide (Kocide 3000) on fruit quality was investigated. For this purpose, total sugar, acidity, sugar acid ratio, reducing sugar and non reducing sugars were estimated. The results are presented in Tables 1 to 5

Total sugar content was higher in fruits obtained from 450 g a.i ha^{-1} of Copper hydroxide (Kocide 3000) sprayed plots by 25.43 per cent increase over control. Sugar content was found to be lower in fruits obtained from control plots (12.67) due to infection of fruits by the downy mildew pathogen (Table 1). The acidity content recorded from the fruits obtained in Copper hydroxide (Kocide 3000) treated plot (450 g a.i ha^{-1}) was 7.14 per cent increase over control plots (Table 2).

Sugar acid ratio was observed lower (16.75 per cent increase over control) in fruits taken from Copper hydroxide (Kocide 3000) treated plot (150 g a.i ha^{-1}). The ratio was higher (23.01 per cent increase over control) in fruits obtained from Copper hydroxide (Kocide 3000) treated plot (450 g a.i ha^{-1}) (Table 3). Highest reducing sugar content was recorded (9.95 per cent) in fruits obtained from Copper hydroxide (Kocide 3000 @ 450 g ai ha^{-1}) treated plots followed by 375 g ai ha^{-1} treated plot (9.92 per cent) (Table 4). Non reducing sugar content was recorded (7.25 per cent) in fruits obtained from Copper hydroxide (Kocide 3000- 450 g ai ha^{-1}) treated plots followed by 375 g ai ha^{-1} treated plot (6.94 per cent). There was no marked difference in non reducing sugar content in fungicide treated plots (Table 5).

Discussion:

The fruit quality was improved in sprayed plots when compared to control. The dosage of Copper hydroxide (Kocide 3000 at 450 g ai ha^{-1}) recorded 25.43 per cent increase in total sugar over control. This can be attributed to the reduction in disease incidence in leaves which makes more photosynthates available for fruit development and reduced disease severity in fruits leading to their normal development.

The downy mildew infects leaf and fruit and also affects the quality of developing berries. Srinivasan and Jeyarajan (1977) reported that total, reducing sugars content of the *P. viticola* infected grapevine was significantly less than that of healthy fruits. Ghure and Shinde (1987) reported that there was 3.5, 1.6 and 21.7 per cent reduction in total soluble solids, total sugars and ascorbic acid respectively when powdery mildew intensity exceeded 75 per cent. There was also increase in per cent of non reducing sugars (69.7) and acidity (24.3). As a result, fruit taste and market value was reduced.

Cupric hydroxide sprays reduces the quality, flavour and colour of grapes (Haeseler and Petersen, 1974). But the present findings showed that the fruit quality of grapevine was improved due to spraying of Copper hydroxide (Kocide 3000). This can be attributed that the Copper hydroxide effectively controls downy mildew infection in grapevine fruits and thus increases the quality of berries to fetch higher market prices.

Table 1. Influence of total sugar in grapes by Copper hydroxide (Kocide 3000)

Treatments	Total soluble solids ^{0}brix / Days after harvesting*						Per cent increase over control
	0	1	2	3	4	Mean	
Kocide 3000@ 150 g a.i. ha^{-1}	18.16 ^{a} (25.22)	14.90 ^{d} (22.70)	15.53 ^{b} (23.21)	16.00 ^{a} (23.57)	16.56 ^{a} (24.01)	16.23 (23.74)	21.93
Kocide 3000@ 225 g a.i. ha^{-1}	17.33 ^{ab} (24.60)	16.80 ^{bc} (24.18)	16.40 ^{ab} (23.89)	16.63 ^{a} (24.07)	15.96^{a} (23.54)	16.27 (24.05)	22.13
Kocide 3000@ 300 g a.i. ha^{-1}	17.93 ^{ab} (25.05)	15.93 ^{cd} (23.52)	15.86 ^{ab} (23.47)	16.30 ^{a} (23.81)	16.46 ^{a} (23.93)	16.50 (23.96)	23.21
Kocide 3000@ 375 g a.i. ha^{-1}	17.60 ^{ab} (24.80)	17.73 ^{ab} (24.90)	15.86 ^{ab} (23.47)	15.73 ^{a} (23.36)	16.40 ^{a} (23.88)	16.67 (24.08)	23.99
Kocide 3000@ 450 g a.i. ha^{-1}	17.53 ^{ab} (24.74)	17.46 ^{ab} (24.69)	16.53 ^{ab} (23.99)	16.80 ^{a} (24.20)	16.63 ^{a} (24.06)	16.99 (24.33)	25.43
Kocide 3000@ 900 g a.i. ha^{-1}	16.40 ^{b} (23.88)	16.63 ^{bc} (24.06)	16.13 ^{ab} (23.68)	16.43 ^{a} (23.91)	15.77 ^{a} (23.39)	16.27 (23.78)	22.13
Ridomil 2.5 kg ha^{-1}	17.00 ^{ab} (24.32)	17.86 ^{ab} (24.99)	15.66 ^{ab} (23.03)	15.73 ^{a} (23.36)	16.67 ^{a} (24.09)	16.59 (24.02)	24.11
Market fruit	16.93 ^{ab} (24.28)	17.57 ^{ab} (24.77)	17.16 ^{a} (24.47)	17.23 ^{a} (24.52)	16.77 ^{a} (24.17)	17.13 (24.44)	26.04
Control	12.90 ^{c} (21.04)	13.16 ^{e} (21.30)	12.90 ^{c} (21.03)	12.23 ^{b} (20.47)	12.13 ^{b} (20.38)	12.67 (20.84)	-

*Values are mean of three replications
In a column, means followed by a common letter are not significantly different at the 5% level by DMRT

Values in parentheses are arcsine transformed values

Table 2: Influence of acidity in grape fruits by Copper hydroxide (Kocide 3000)

Treatments	Acidity (%) / Days after harvesting*						Per cent increase over control
	0	1	2	3	4	Mean	
Kocide 3000@ 150 g a.i. ha^{-1}	0.44 ^{b} (3.82)	0.44 ^{a} (3.80)	0.43 ^{bc} (3.75)	0.42 ^{a} (3.71)	0.42 ^{ab} (3.71)	0.43 (3.75)	9.30
Kocide 3000@ 225 g a.i. ha^{-1}	0.44 ^{b} (3.80)	0.41 ^{cd} (3.67)	0.43 ^{bc} (3.75)	0.42 ^{a} (3.71)	0.41 ^{b} (3.67)	0.42 (3.72)	7.14
Kocide 3000@ 300 g a.i. ha^{-1}	0.42 ^{c} (3.71)	0.42 ^{bc} (3.71)	0.44 ^{ab} (3.80)	0.42 ^{a} (3.71)	0.43 ^{a} (3.75)	0.42 (3.74)	7.14
Kocide 3000@ 375 g a.i. ha^{-1}	0.36 ^{e} (3.43)	0.39 ^{e} (3.58)	0.41 ^{de} (3.67)	0.41 ^{ab} (3.67)	0.43 ^{a} (3.75)	0.40 (3.62)	2.50
Kocide 3000@ 450 g a.i. ha^{-1}	0.40 ^{d} (3.62)	0.43 ^{ab} (3.75)	0.45 ^{a} (3.84)	0.40 ^{bc} (3.62)	0.42 ^{ab} (3.71)	0.42 (3.71)	7.14
Kocide 3000@ 900 g a.i. ha^{-1}	0.43 ^{bc} (3.75)	0.40 ^{de} (3.62)	0.40 ^{ef} (3.62)	0.40 ^{bc} (3.62)	0.42 ^{ab} (3.71)	0.41 (3.67)	4.88
Ridomil 2.5 kg ha^{-1}	0.44 ^{b} (3.83)	0.44 (3.80)a	0.45 ^{a} (3.87)	0.39 ^{c} (3.58)	0.43 ^{a} (3.75)	0.43 (3.77)	9.30
Market fruit	0.43 ^{bc} (3.75)	0.40 ^{de} (3.62)	0.40 ^{ef} (3.62)	0.40 ^{bc} (3.62)	0.42 ^{ab} (3.71)	0.41 (3.67)	4.88
Control	0.40 ^{d} (3.62)	0.39 ^{e} (3.58)	0.39 ^{f} (3.58)	0.40 ^{bc} (3.62)	0.37 ^{d} (3.50)	0.39 (3.58)	-

*Values are mean of three replications
In a column, means followed by a common letter are not significantly different at the 5% level by DMRT
Values in parentheses are arcsine-transformed values

Table 3: Influence of Sugar acid ratio of grapes by Copper hydroxide (Kocide 3000)

Treatments	Sugar acid ratio / Days after harvesting*						Per cent increase over control
	0	1	2	3	4	Mean	
Kocide 3000@ 150 g a.i. ha^{-1}	39.33^{cd}	40.62^{bc}	34.53^{d}	38.37^{b}	38.74^{ab}	38.32	16.75
Kocide 3000@ 225 g a.i. ha	41.31^{bcd}	36.43^{de}	36.13^{cd}	38.12^{b}	40.33^{ab}	38.46	17.06
Kocide 3000@ 300 g a.i. ha^{-1}	39.41^{cd}	38.22^{bcd}	37.84^{ad}	39.86^{ab}	38.05^{ab}	38.67	17.51
Kocide 3000@ 375 g a.i. ha^{-1}	42.70^{bc}	37.96^{cd}	36.36^{bcd}	38.82^{b}	38.31^{ab}	38.83	17.85
Kocide 3000@ 450 g a.i. ha^{-1}	45.08^{a}	45.50^{a}	38.73^{abc}	38.38^{b}	38.14^{ab}	41.76	23.61
Kocide 3000@ 900 g a.i. ha^{-1}	35.30^{ef}	40.86^{bc}	40.92^{a}	43.11^{a}	41.98^{a}	40.43	20.84
Ridomil 2.5 kg ha^{-1}	43.85^{b}	39.67^{bcd}	36.75^{bcd}	42.00^{ab}	39.61^{ab}	40.37	20.98
Market fruit	38.16^{de}	41.61^{bc}	40.34^{ab}	41.09^{ab}	37.51^{b}	39.74	19.73
Control	32.79^{f}	33.88^{e}	30.31^{e}	30.58^{c}	31.96^{c}	31.90	-

*Values are mean of three replications

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT
Values in parentheses are arcsine-transformed values

Table 4. Influence of reducing sugar in grapes by Copper hydroxide (Kocide 3000)

Treatments	Reducing Sugar (%) / Days after harvesting*						Per cent increase over control
	0	1	2	3	4	Mean	
Kocide 3000@ 150 g a.i. ha^{-1}	9.68 ^{ab} (18.12)	10.21 ^{ab} (18.63)	10.07 (18.49)a	9.85 ^{ab} (18.29)	9.51 ^{b} (17.96)	9.86 (18.30)	28.80
Kocide 3000@ 225 g a.i. ha^{-1}	9.33 ^{b} (17.78)	10.33 ^{a} (18.73)	10.14 ^{a} (18.57)	9.57^{b} (18.01)	9.36 ^{b} (17.81)	9.74 (18.18)	27.93
Kocide 3000@ 300 g a.i. ha^{-1}	10.05 ^{ab} (18.47)	10.18 ^{ab} (18.60)	9.36 ^{b} (17.81)	10.53 ^{a} (18.93)	9.32 ^{b} (17.77)	9.88 (18.32)	28.95
Kocide 3000@ 375 g a.i. ha^{-1}	10.07 ^{ab} (18.50)	10.04 ^{ab} (18.47)	10.09 ^{a} (18.52)	9.93 ^{ab} (18.37)	9.48 ^{b} (17.92)	9.92 (18.360	29.23
Kocide 3000@ 450 g a.i. ha^{-1}	9.63 ^{ab} (18.07)	10.47 ^{a} (18.87)	10.20 ^{a} (18.62)	9.25 ^{b} (17.70)	10.23 ^{a} (18.65)	9.95 (18.38)	29.45
Kocide 3000@ 900 g a.i. ha^{-1}	9.64 ^{ab} (18.08)	9.50 ^{b} (17.95)	10.09 ^{a} (18.51)	9.69 ^{b} (18.13)	9.57 ^{ab} (18.01)	9.69 (18.140	27.55
Ridomil 2.5 kg ha^{-1}	9.82 ^{ab} (18.26)	9.55 ^{b} (17.99)	9.87 ^{ab} (18.30)	9.49 ^{b} (17.94)	9.53. ^{b} (17.98)	9.65 (18.09)	27.25
Market fruit	9.64 ^{ab} (18.08)	9.50 ^{b} (17.95)	10.09 ^{a} (18.51)	9.69 ^{b} (18.13)	9.57 ^{ab} (18.01)	9.69 (18.140	27.55
Control	7.5 ^{c} (15.89)	7.10 ^{c} (15.44)	6.70 ^{c} (14.99)	6.93 ^{c} (15.26)	6.90 ^{c} (15.20)	7.02 (15.36)	-

*Values are mean of three replications
In a column, means followed by a common letter are not significantly different at the 5% level by DMRT
Values in parentheses are arcsine-transformed values

Table 5: Influence of non reducing sugar in grape fruits by Copper hydroxide (Kocide 3000)

Treatments	Non reducing sugar (%) / Days after harvesting*					Mean	Per cent increase over control
	0	1	2	3	4		
Kocide 3000@ 150 g a.i. ha^{-1}	8.48 ^{a} (16.92)	4.79 ^{d} (12.63)	6.36 ^{ab} (14.53)	6.14 ^{abc} (14.34)	7.05 ^{a} (15.38)	6.56 (14.76)	17.38
Kocide 3000@ 225 g a.i. ha^{-1}	7.70 ^{a} (16.11)	6.32 ^{bcd} (14.40)	6.20 ^{ab} (14.40)	7.48 ^{ab} (15.87)	5.73 ^{ab} (13.83)	6.68 (14.92)	18.86
Kocide 3000@ 300 g a.i. ha^{-1}	7.88^{a} (16.29)	5.75^{cd} (13.86)	6.51^{ab} (14.77)	5.76^{bc} (13.88)	7.14 ^{a} (15.49)	6.61 (14.86)	18.00
Kocide 3000@ 375 g a.i. ha^{-1}	7.53 ^{a} (15.90)	7.69 ^{ab} (16.06)	5.77 ^{ab} (13.87)	6.80 ^{abc} (15.05)	6.92 ^{a} (15.24)	6.94 (15.23)	21.90
Kocide 3000@ 450 g a.i. ha^{-1}	8.22^{a} (16.64)	7.14^{abc} (15.43)	6.38^{ab} (14.62)	7.23^{ab} (15.59)	7.27 ^{a} (15.64)	7.25 (15.58)	25.24
Kocide 3000@ 900 g a.i. ha^{-1}	6.76 ^{ab} (15.06)	7.12 ^{abc} (15.47)	6.04 ^{ab} (14.22)	6.74 ^{abc} (15.04)	6.19 ^{ab} (14.41)	6.57 (14.84)	17.50
Ridomil 2.5 kg ha^{-1}	7.18^{a} (15.52)	8.32^{a} (16.74)	5.80^{ab} (13.90)	6.26^{abc} (14.44)	7.13 ^{a} (15.47)	6.93 (15.21)	21.79
Market fruit	6.73 ^{ab} (14.98)	7.67 ^{ab} (16.05)	7.13 ^{a} (15.48)	7.77 ^{a} (16.18)	7.40 ^{a} (15.78)	7.34 (15.69)	26.16
Control	5.40 ^{b} (13.43)	6.06 ^{bcd} (14.25)	5.10 ^{b} (13.03)	5.30 ^{c} (13.30)	5.23 ^{b} (13.22)	5.42 (13.45)	-

*Values are mean of three replications
In a column, means followed by a common letter are not significantly different at the 5% level by DMRT
Values in parentheses are arcsine-transformed values

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