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Full Length Article

Post-Bloom Applied Moringa Leaf Extract Improves Growth, Productivity and Quality of Early-Season Maturing Grapes (*Vitis vinifera*)

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Abstract

Moringa (*Moringa oleifera* Lam.) is known to be a rich source of endogenous plant growth regulators and mineral elements with potential to improve plant growth. This study was performed in a commercial vineyard to evaluate the effect of exogenously applied moringa leaf extract (MLE) (3% MLE) at different phenological stages (*i.e.* flowering, berry setting and premature stages of grapevine) on growth, productivity and physico-chemical berry quality of important early-season maturing commercial table grapes cultivars (*viz.* 'Cardinal', 'Flame Seedless', 'King's Ruby', 'NARC Black' and 'Perlette'). Untreated vines were kept as control. Data were collected regardring leaf nutrient contents, vine vegetative and reproductive growth, organoleptic and physico-chemical qualitative characters of berries. Irrespective to the cultivars, foliar applied MLE showed a significant improvement in leaf potassium, iron and zinc contents, cane length, leaf size, berry set, berries per cluster, rachis length, cluster size, cluster weight, berry size and berry weight. The highest increase of yield was observed in grapes cv. 'Perlette' (32.1%) followed by 'NARC Black' (30.3%), 'Cardinal' (28.8%), 'Flame Seedless' (27.3%) and 'King's Ruby' (11.9%). MLE application also reflected a significant improvement in total soluble solids (TSS), TSS: titratable acidity ratio, total phenolic contents, vitamin C as well as reducing, non-reducing and total sugar percentages in berry juice of all studied grapes cultivars. In conclusions, 3% MLE can be efficiently applied on grapevines at post-bloom stages (flowering, berry setting and premature growth phases) to improve their vegetative and reproductive growth as well as physico-chemical berry quality. Grapes cvs. 'Perlette', 'NARC Black' and 'Cardinal' showed better response to 3% MLE sprays, as compared to 'King's Ruby' and 'Flame Seedless'.

Keywords: Berry quality; Grapes; Growth; Moringa; Physico-chemical quality; Yield

Introduction

Grapes (*Vitis vinifera* L.) have been cultivated since long for consumption as fresh, dried, and also as wine. Presently, about 47.3% of grape production is used for wine production, 35% is consumed as fresh fruit; while, 8% is used as dried fruit in the world (OIV 2017). The grapes are important source of antioxidants and biologically active dietary compounds. The most important biochemically active component of grapes is resveratrol which has certain health-promoting benefits (Shrikhande 2000; Yadav *et al.* 2009). Grapes are also a decent source of minerals such as cobalt (Co), iron (Fe), potassium (K), magnesium (Mg), and phosphorus (P) as well as vitamins like K, B1, B2, B6, and C (Anonymous 2018).

Grapes is an important fruit occupying an area of 7.5 million ha with 75.8 million tones production across the world (FAO 2016). In Pakistan, the production of grapes has been observed to be 0.065 million tonnes from an area of 0.0162 million ha with 3.98 t ha^{-1} average yield (FAO 2016). Presently, only European table grapes are being cultivated at commercial scale throughout temperate regions in the country including Gilgit-Baltistan, Balochistan and some area of Khyber Pakhtunkhwa (GOB 2016; Khan *et al.* 2011). The cultivars found in the country include 'Haita', 'Kishmishi', 'Shundokhani', 'Sahibi', 'Shekhali', 'King's Early', 'Cardinal', 'Anab-e-Shahi', 'Black Prince', 'Gold', 'Dehkani', 'Shamas Guru', 'Thompson', 'Perlette', 'Red Globe', 'Cardinal', 'King's Ruby', 'NARC Black', 'Crimson', 'Flame', 'Sultana' and 'Muscatil' (Aujla *et al.*

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2011; Khan *et al.* 2011; Anonymous 2017). Earlier, the cultivation of grapes at commercial scale was restricted only to Balochistan and some area of Khyber Pakhtunkhwa because during summer season monsoon rains in Punjab result in attack of fungal diseases and rottening of berries. Researchers have evaluated different indigenous and exotic germplasm to identify early-season maturing grapes cultivars which could escape these rains and mature before the onset of rainy season for commercial production in the monsoon regions of central Punjab such as 'King's Ruby', 'NARC Black' and 'Perlette' (Khan *et al.* 2011; PARC 2018).

Growth and development of grapevine and grape berries quality are influenced by many internal and external factors such as cultivar, exposure to sun light, temperature, humidity, soil, nutrition and hormonal regulation (Conde *et al.* 2007). Both macro and micro-nutrients affect the yield and quality related features of grapes (Usha and Singh 2002). Various physiological functions such as development of flower, berry, and seed, setting of fruit, length of cluster, compactness of bunch, physical and chemical attributes of berry are regulated by many internal plant growth substances. Such variables can also be regulated by foliar application of plant growth regulators (PGRs), though, the effects may contrast with the variation in concentration, application time, growth stage and vine age (Farooq and Hulmani 2000; Korkutal *et al.* 2008; Shah *et al.* 2015).

Moringa oleifera known as 'Drumstick Tree' exhibits outstanding health promoting benefits (Mishra *et al.* 2011) and rich source of various phytochemicals (Leone *et al.* 2015), plant growth regulator cytokinin (zeatin), macro and micro-nutrients, phenolics, antioxidants, carotenoid, vitamins and ascorbates (Foidl *et al.* 2001; Aslam *et al.* 2005; Yasmeen *et al.* 2014). It has been found that on dry weight basis its leaves contained 27.5 mg kg^{-1} cytokinins, 15.9 mg kg^{-1} auxins, 16.8 mg kg^{-1} gibberellins and 10.5 mg kg^{-1} abscisic acid (Abusuwar and Abohassan 2017). Due to presence of the above-mentioned compounds, the foliar application of MLE has been reported to promote growth and development of various agronomic and horticultural crops (Foidl *et al.* 2001; Nasir *et al.* 2016, 2018, 2020). Exogenous application of MLE significantly improved growth, quality, and yield characters of plum (Thanaa *et al.* 2017), pear (El-Hamied and El-Amary 2015) and orange (El-Enien *et al.* 2015) fruit. Similarly, 'Kinnow' mandarin trees treated with 0.3% MLE in combination with, K and Zn at fruit set stage showed significantly higher productivity and with better fruit quality (Nasir *et al.* 2016, 2018, 2020).

Presently, the excessive and frequent use of chemical inputs such as fertilizers and PGRs has increased the consumer awareness for their hazardous effects (Helmy *et al* 2015). There is now a growing demand for availability of relatively eco-friendly, affordable, easily accessible, and naturally available resources for improving crop growth, productivity and quality (Rashid *et al.* 2018). To the best of our knowledge, at present no information is available about the influence of exogenous application of MLE on growth, productivity, and quality of grapes. Therefore, present study investigated the effect of foliar application of MLE at various phenological stages on grapevine growth, productivity, and berry quality of five early-season maturing commercial table grapes cultivars grown under the subtropical climatic conditions.

Materials and Methods

Plant material

Four years old, five commercial grapes (*Vitis vinifera* L.) cultivars *viz.* 'Cardinal', 'Flame Seedless', 'King's Ruby', 'NARC Black' and 'Perlette' propagated asexually (through cuttings) and grown in uniform condition at Rana Abdul Qayyum's Commercial Grape Vineyard, 41 R.B., Rohrhi-Wala (31°38'59.8"N; 73°25'28.4"E), Tehsil Sangla, District Nankana Sahib, located about 55 km east to Faisalabad were selected for this experiment. These vines trained on permanent support as Four Arm Kniffin System (with 3.4 m × 3.4 m row and 2.1 × 2.1 m plant distance) were treated with uniform standard commercial cultural practices such as pruning, fertilizer application, irrigation, weeding, insect-pest and disease management.

Experimental treatments

For MLE, the fresh leaves after harvest were kept overnight at -20°C before grinding for their leaf extract (Nasir *et al.* 2016). MLE solution (3%) was prepared by mixing 300 mL of MLE with 10 L of distilled water. Foliar spray of 3% MLE was applied to all grape cultivars at three phonological growth stages (*i.e.*, at bloom, berry set and premature stages) using hand sprayer thoroughly until drops run-off from the leaves. The experiment was designed as two factors (treatments and cultivars) factorial arrangements under RCBD. Single vine was used as experimental unit, replicated thrice.

Determination of leaf nutrients

At harvest 40–50 healthy uniform leaves (along with petiole) apparently free from any symptoms of disease or insect pest were harvest from each experimental unit at random to determine the leaf nutrient status. After proper washing and drying, grinded leaf powder was used for further analysis. Leaf nitrogen (N) was estimated on micro kjeledhal apparatus, P on spectrophotometer and K on flame photometer prescribed by Chapman and Parker (1961) as per cent dry weight. Whereas, leaf Fe, manganese (Mn) and zinc (Zn) were estimated by following the protocol described in AOAC (2000) on atomic absorption spectrophotometer. Before the determination of leaf P, K, Fe, Mn and Zn wet digestion were done following the detailed procedure used earlier by Khan *et al.* (2015) and expressed as mg kg^{-1} DW.

Vegetative and reproductive growth

For the study of growth behavior, five uniform and health canes per experimental vine were selected and tagged. Data regarding cane length (cm), leaf size (cm^2), No. of leaves per cane, berry set percentage (%), berry drop percentage (%), total no. of berries per cluster, rachis length (cm), cluster weight (g), cluster size (cm^2), berry weight (g), berry size (mm^2), total clusters per vine (No.), yield per vine (kg), yield per acre (kg) were recorded by using the method described by Khan *et al.* (2012).

Berry quality parameters

For chemical analysis of fresh grapes berry (randomly selected 250 berries per experimental unit), juice was extracted manually by using muslin cloth. Berry juice pH was taken by digital pH meter (HI 98107, Hanna Instruments, Mauritius). A digital refractometer (RX-5000 Atago, Japan) was used for determining TSS as expressed in ºBrix. Method described by Khan *et al.* (2011) was used to estimate the titratable acidity (%) and different forms of sugars (total, reducing and non-reducing sugars) in grape berry juice. Calculation of TSS:TA ratio was done by dividing TSS with the TA percentage. Ascorbic acid contents (Vitamin C) in grape berry juice was estimated by adopting the process described by Khan *et al.* (2011) and were expressed as mg 100 g^{-1} FW. Assay reported by Brand-Williams *et al.* (1995) was used to estimate the total antioxidants in grapes berry juice by 2, 2-diphenyl-1picrylhydrazyl radical (DPPH) assay and were expressed as % inhibition. Total phenolics contents (TPC) in grapes juice were estimated using the Folin–Ciocalteu (FC) technique defined by Ainsworth and Gillespie (2007) and were expressed as mg GAE 100 g^{-1} FW.

Statistical analysis

Data were evaluated statistically by using software Statistix 8.1 and Least Significant Difference (LSD) test was applied to differentiate means by using the differences among different means at $P \leq 0.05$.

Results

Leaf mineral contents

Foliar application of MLE to the grapevine cultivars did not show significant effect on leaf N, P and Mn contents (Table 1). However, these mineral concentrations were higher in MLE-treated vine as compared to leaves of untreated vines. This impact of MLE on leaf N, P and Mn may be ascribed to very lower concentration present in the MLE which remain unaffected to improve their endogenous level. Exogenously applied MLE (3%) significantly increased leaf K, Fe and Zn contents as compared to untreated control.

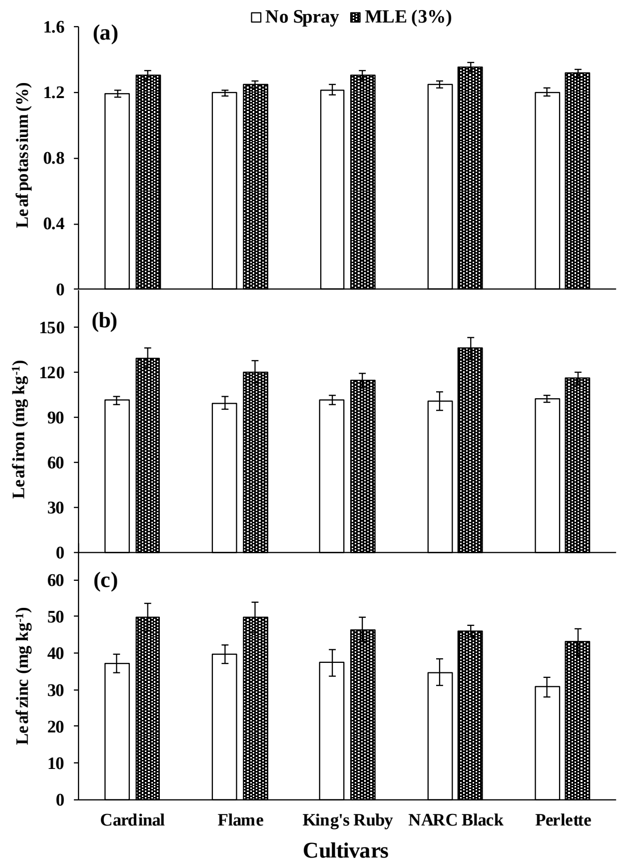

Fig. 1: Effect of foliar application of MLE (3%) on leaf K (**a**), Fe (**b**) and Zn (**c**) contents of grapes cultivars. Vertical bars indicate ± SE of means. n = 3 replicates

Application of MLE showed highest leaf K (1.36%) and Fe (136 mg kg^{-1}) contents in leaves of grapes cv. 'NARC Back' compared to untreated control (Fig. 1a–b); while, maximum increase in Zn levels (from 30.7 to 43 mg kg^{-1}) in leaves of 'Perlette' (Fig. 1c).

Vegetative and reproductive growth

The number of leaves per cane was not significantly affected by application of MLE (3%) on grapes cultivars. The exogenous spray of MLE (3%) on grapevines at various growth phases (at bloom + fruit set + premature stages) significantly improved leaf size of all cultivars. Highest leaf size was observed in 'NARC Black' (112.3 cm^2 *vs.* 79.7 cm^2 of untreated) and least increase in 'Flame' (176.9 cm^2 *vs.* 170.5 cm^2) of untreated, respectively (Table 1). Likewise, after foliar applied MLE (3%), grapevines exhibited positive significant impact on the cane length and rachis length (Table 2). Highest increase of cane length with application of MLE treatment was found in grapes cv. 'Perlette' (25.6%) followed by 'NARC Black' (19.2%), 'Flame' (18.2%), 'Cardinal' (7.9%) and 'King's Ruby' (6.8%).

Table 1: Effect of foliar application of MLE (3%) on leaf N, P, Mn contents, no. of leaves and leaf size of grapes cultivars

Cultivars	Leaf N (%)		Leaf P (%)		Leaf Mn (mg kg^{-1})		Leaves per cane (No.)		Leaf size (cm^2)	
	No spray	MLE spray	No spray	MLE spray	No spray	MLE spray	No spray	MLE spray	No spray	MLE spray
'Cardinal'	2.68	2.80	0.34	0.36	180.00	188.00	43.76	47.60	140.01	175.71
'Flame Seedless'	2.68	3.03	0.35	0.37	187.33	196.33	102.77	106.52	170.49	176.88
'King's Ruby'	2.68	2.68	0.36	0.37	200.67	208.33	109.31	116.82	183.21	190.19
'NARC Black'	2.80	2.92	0.37	0.38	190.33	196.00	67.73	69.80	79.66	112.31
'Perlette'	2.45	2.57	0.36	0.37	204.67	209.33	76.00	84.40	181.80	216.30
LSD ($P \leq 0.05$)										
Treatments	NS		NS		NS		NS		13.100	
Cultivars	NS		NS		12.095		11.714		20.713	
Treatments x cultivars	NS		NS		NS		NS		NS	

n = 3 replicates, NS = non-significant

Table 2: Effect of foliar application of MLE (3%) on cane length, rachis length and berry components of grapes cultivars

Cultivars	Cane length (cm)		Rachis length (cm)		Berries per cluster (No.)		Berry size (mm^2)		Berry weight (g)	
	No spray	MLE spray	No spray	MLE spray	No spray	MLE spray	No spray	MLE spray	No spray	MLE spray
'Cardinal'	87.27	94.13	18.60	21.70	181.95	240.20	247.55	318.25	3.69	3.92
'Flame Seedless'	106.11	125.41	18.08	19.00	281.66	293.22	236.31	246.01	2.07	2.21
'King's Ruby'	90.75	96.94	18.81	24.10	339.35	363.11	255.70	290.51	2.27	2.42
'NARC Black'	112.20	133.77	14.00	17.57	94.07	112.60	256.55	309.58	2.45	2.88
'Perlette'	108.53	136.27	21.20	25.73	423.27	514.33	200.22	223.54	1.59	1.95
LSD ($P \leq 0.05$)										
Treatments	7.3807		1.5301		22.527		11.800		0.1680	
Cultivars	11.670		2.4193		35.618		18.657		0.2656	
Treatments x cultivars	NS		NS		NS		26.385		NS	

n = 3 replicates, NS = non-significant

The grapes reproductive characters varied with the cultivar but application of moringa exogenously on grapevines showed promising improvement in reproductive characters such as rachis length, reduced berry drop and increased berry set, berries per cluster, berry size, berry weight, cluster size, cluster weight and ultimately yield of all the studied cultivars. Exogenously applied 3% MLE significantly improved the rachis length of grapevine cultivars (Table 2). The highest increase in rachis length was observed in 'King's Ruby' (28.1%) and lowest in 'Flame Seedless' (5.1%) as compared to untreated vines. Berry set on the clusters of grapes was significantly enhanced by MLE (3%). Maximum increase of berry set percentage was found in 'Cardinal' (39.2% compared to 30.7%) and followed by 'NARC Black' (50.5% compared to 41.9) and 'Perlette' (51.8% compared to 45.8%) (Fig. 2a). On the other hand, MLE (3%) treatment at three growth phases significantly reduced the berry drop from the clusters of grapes as compared to untreated vines. 'Cardinal' revealed up to (24.9%) less berry drop than the vines without treatment followed by 'Perlette' (24.3%), 'NARC Black' (16.9%), 'King's Ruby' (6.5%) and 'Flame Seedless' (1.9%) (Fig. 2b).

There was highly significant variation in number of berries per cluster, berry size and berry weight among the cultivars (Table 2). As well as, exogenous applied MLE significantly improved berry characters as compared to control without any treatment. The berries per cluster increased from (4.1%) to (32%) in 'Flame Seedless' and 'Cardinal', respectively. Whereas, 'Perlette', 'NARC Black' and 'King's Ruby' had shown an increase of berries per cluster (21.5%), (19.7%) and (7%), respectively as

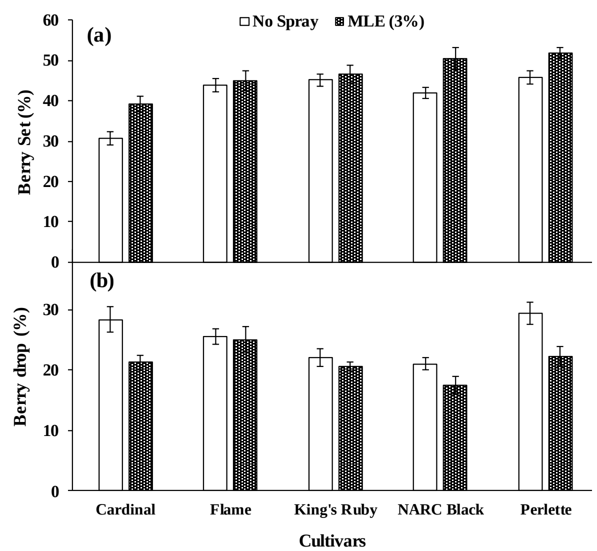

Fig. 2: Impact of foliar application of MLE (3%) on berry set (**a**) and berry drop (**b**) percentage of grapes cultivars. Vertical bars indicate ± SE of means. n = 3 replicates

compared to untreated vines. As MLE spray increased berry set and reduced berry drop percentage, it ultimately led to an increase in the number of berries which reached to harvest maturity and thereby increased yield. In response to MLE 'Cardinal' showed highest (28.6%) increase in berry size (318.3 mm^2 compared to 247.6 mm^2 of untreated vines) (Table 2). 'Perlette' had highest and 'Cardinal' had lowest significant increase in berry weight upon exogenous

Table 3: Effect of foliar application of MLE (3%) on yield components of grapes cultivars

Cultivars	Clusters per vine (No.)		Cluster size (cm^2)		Average cluster weight (g)		Yield (kg $vine^{-1}$)		Yield (tones ha^{-1})	
	No spray	MLE spray	No spray	MLE spray	No spray	MLE spray	No spray	MLE spray	No spray	MLE spray
'Cardinal'	31.00	31.33	170.50	217.23	267.33	341.73	8.33	10.72	11.6	15.0
'Flame Seedless'	43.67	48.33	147.90	158.79	339.44	389.77	14.88	18.94	20.8	26.4
'King's Ruby'	43.33	42.67	160.62	172.10	353.58	404.00	15.55	17.41	21.7	24.3
'NARC Black'	63.33	65.00	101.55	126.20	117.87	149.93	7.48	9.74	10.4	13.6
'Perlette'	61.33	64.00	225.25	275.42	423.07	538.67	26.01	34.35	36.3	47.9
LSD ($P \leq 0.05$)										
Treatments	NS		14.466		28.620		2.5056		1415.7	
Cultivars	7.1318		22.872		45.253		3.9617		2238.4	
Treatments x cultivars		NS	NS		NS		NS		NS	

n = 3 replicates, NS = non-significant

application of MLE (3%) as compared to its corresponding untreated vines.

Exogenous application of MLE did not show any significant effect on number of clusters per vine (Table 3). However, MLE applied at flowering, berry setting and premature stages significantly improved cluster size, cluster weight and ultimately yield. Amon cultivars, minimum increase of cluster size was evident in 'King's Ruby' (7.15%) followed 'Flame Seedless' (7.4%) and maximum increase was in 'Cardinal', 'NARC Black' and 'Perlette' (27.4, 24.3 and 22.3%, respectively). Maximum increase in cluster weight was observed in cv. 'Cardinal' followed by 'Perlette', 'NARC Black', 'Flame Seedless' and 'King's Ruby' cvs., as compared to untreated vines. MLE (3%) had also a remarkable significant impact on the yield of grapes per vine. The highest increase of yield per vine was observed in 'Perlette' (32.1%) followed by 'NARC Black' (30.3%), 'Cardinal' (28.8%), 'Flame Seedless' (27.3%) and 'King's Ruby' (11.9%) as compared to their corresponding untreated vines (Table 3).

Berry quality

Foliar application of MLE 3% solution did not show any significant impact on the colour and flavor berries. However, berry texture, taste and overall acceptability exhibited significant improvement with MLE application in all cvs. (Table 4). Exogenous application of MLE significantly improved TSS and TSS:TA ration and reduced TA of all the grapevines cvs. Highest TSS (10.9%) was observed in 'NARC Black' and lowest value of TSS was recoded in 'Flame Seedless' (4.6%) compared their respective berries harvested from untreated vines (Fig. 3b). Similarly, MLE application significantly improved the TSS: TA ratio of berries harvested form treated than untreated vines (Fig. 3c). However, MLE effectively reduced the acidity in grapes at harvest. The 'Perlette' showed (21.7%) less TA than the untreated control vines followed by 'King's Ruby' (19.51%), 'NARC Black' (15.2%), 'Flame Seedless' (11.2%) and 'Cardinal' (8.6%) cvs. (Fig. 3a).

Foliar application of MLE showed significantly improved total, reducing and non-reducing sugar percentage of grape berry juice. These sugars also varied significantly among cultivars. 'NARC Black' had highest (20.1%) and

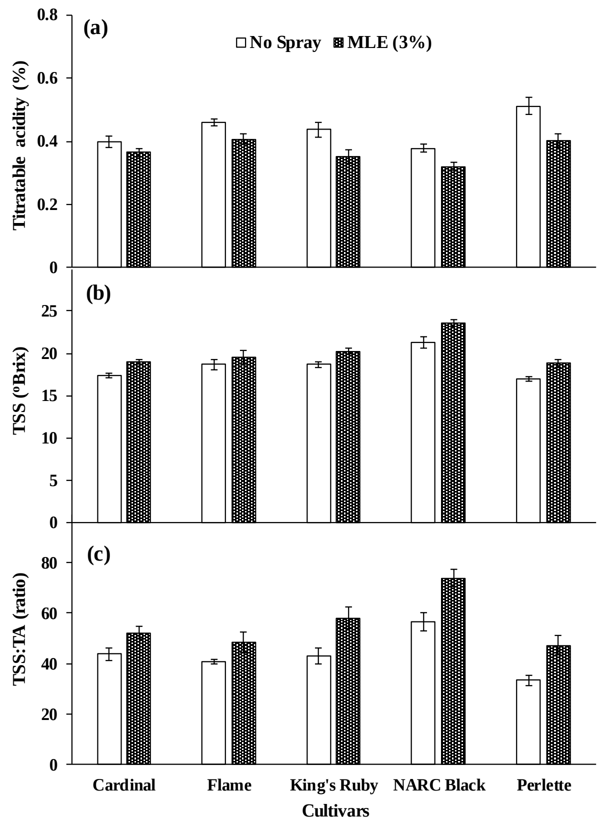

Fig. 3: Effect of foliar application of MLE (3%) on TA (**a**), TSS (**b**) and TSS:TA ratio (**c**) of berry juice of grapes cultivars. Vertical bars indicate ± SE of means. n = 3 replicates

'Flame Seedless' (14.8%) had lowest significant increase in total sugar contents in grape juice upon exogenous application of 3% MLE (Fig. 4a). However, foliar application of MLE showed non-significant results with respect to pH and total antioxidant contents of grapes berry juice (Table 5). Though, pH and total antioxidant contents of grape berry juice somewhat varied among the cultivars. 'NARC Black' had shown the highest (53.4%) inhibition and 'Perlette' (41.7%) inhibition of total antioxidant contents in juice of berries collected from untreated grapevines. On an average, irrespective of cvs. application

Table 4: Effect of foliar application of MLE (3%) on organoleptic characters of different grape cultivars

Cultivars	Colour (score)		Texture (score)		Taste (score)		Flavour (score)		Overall acceptability (score)	
	No Spray	MLE (3%)	No Spray	MLE (3%)	No Spray	MLE (3%)	No Spray	MLE (3%)	No Spray	MLE (3%)
'Cardinal'	6.53	6.67	5.80	6.53	6.00	6.60	5.87	6.20	6.47	7.07
'Flame Seedless'	5.27	5.60	5.07	5.20	5.13	5.53	5.07	5.40	5.67	6.13
'King's Ruby'	5.73	5.93	5.07	5.73	5.20	5.53	5.87	6.00	5.60	6.10
'NARC Black'	6.13	6.33	6.40	7.53	6.13	7.40	6.13	6.13	6.80	7.60
'Perlette'	6.87	7.13	5.93	6.93	6.13	7.20	5.67	6.00	6.60	7.27
LSD ($P \leq 0.05$)										
Treatments	NS		0.4089		0.2948		NS		0.2211	
Cultivars	0.3819		0.6466		0.4662		0.5447		0.3496	
Treatments x Cultivars	NS		NS		NS		NS		NS	

n = 3 replicates, NS = non-significant

Table 5: Effect of foliar application of MLE (3%) on pH and total antioxidant contents of berry juice of different grape cultivars

Cultivars	pH		Total antioxidant (% inhibition)	
	No Spray	MLE (3%)	No Spray	MLE (3%)
'Cardinal'	3.05	3.18	43.56	49.49
'Flame Seedless'	2.91	3.04	52.73	54.18
'King's Ruby'	3.03	3.07	49.66	51.04
'NARC Black'	3.24	3.32	53.39	55.01
'Perlette'	2.81	2.90	41.73	45.86
LSD ($P \leq 0.05$)				
Treatments	NS		NS	
Cultivars	0.2169		6.88	
Treatments × Cultivars	NS		NS	

n = 3 replicates, NS = non-significant

of MLE improved the level of total antioxidants of grapes berries in contrast to untreated control.

Exogenously applied MLE significantly improved berry juice Vitamin C and total phenolic contents (TPC). Highest increase in Vitamin C contents was recorded in cv. 'Cardinal' (12.82 mg 100 mL^{-1}) compared to untreated vines (10.1 mg 100 mL^{-1}) which was about 27.3% increase followed by 'Perlette' were (10.9 9.2 mg 100 mL^{-1} compared to 9.2 mg 100 mL^{-1} of untreated vines) which was (20%) increase; whereas, least 8.3% increase in Vitamin C contents was observed in cv. 'King's Ruby' (Fig. 5a). Irrespective of cvs., grapes harvested from MLE sprayed vines exhibited 1.1-fold to 1.3-fold higher TPC, as compared to untreated control. Among grapes cvs., cv. 'NARC Black' treated with MLE showed highest (302 mg GAE 100 g^{-1}) about 1.3-fold increase in the TPC as compared to control (Fig. 5b).

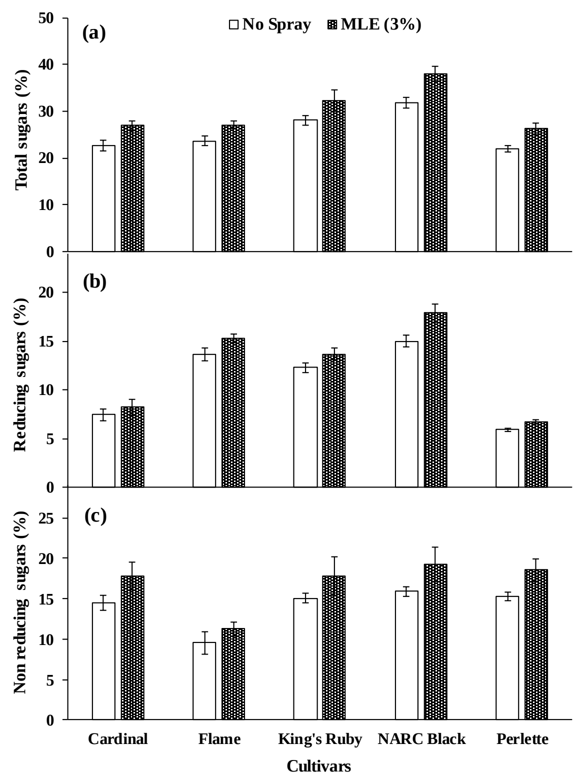

Fig. 4: Effect of foliar application of MLE (3%) on total (**a**), reducing (**b**) and non-reducing (**c**) sugar percentages of berry juice of grapes cultivars. Vertical bars indicate ±SE of means. n=3 replicates

Discussion

In current study, vines sprayed with MLE showed higher levels of macro and micronutrients as compared to control. Moringa is found to be rich in minerals including K, Fe and Zn (Nasir *et al.* 2016). These might be used as an important mineral supplement as an alternate to chemical forms of these elements for crop improvement (Aslam *et al.* 2005). The accumulation of mineral nutrients including K and Fe in rocket plants was significantly higher in the plants treated with aqueous extracts of both leaf and twig extracts of moringa (Abdalla 2013). Similarly, MLE increased leaf K percentage contents of 'Navel' orange trees (El-Enien *et al.* 2015). Earlier exogenous application of seaweed extract was found to enhance the leaf Zn content of the grapes (Sabir *et al.* 2014). Likewise, MLE treated vines exhibited expanded

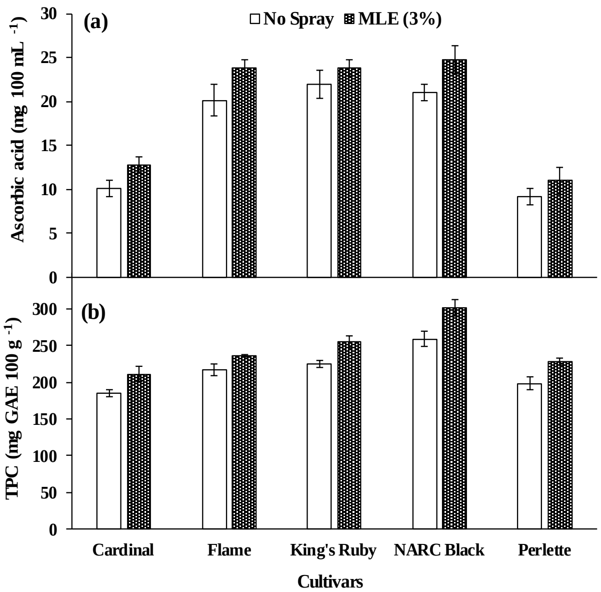

Fig. 5: Effect of foliar application of MLE (3%) on ascorbic acid (**a**) and total phenolic (**b**) contents of berry juice of grapes cultivars. Vertical bars indicate ± SE of means. n = 3 replicates

leaf size in all cultivars in contrast with non-treated vines (Table 1). Similarly, it has been reported reported that seed soaking in MLE + foliar spray of MLE on common bean (*Phaseolus vulgaris* L.) cv. Bronco significantly improved their leaf area (Rady and Mohamed 2015). The improvement in cane length and rachis length of grape cultivars under study which might be due to the availability of plant growth hormones (such as zeatin) and mineral elements found abundantly in moringa leaves, which play important role in cell division and cell elongation that lead to promote the growth of plants and can be used in agriculture industry (Ashfaq *et al.* 2012). *Phaseolus vulgaris* L. plants treated as seed soaking by salicylic acid + foliar spray with MLE were found to significantly increase shoot length (Rady and Mohamed 2015).

Reproductive growth showed significant improvement in MLE treated vines (Table 2–3). This increase can be ascribed to the improved hormonal and mineral nutrient status of treated plants (Ashfaq *et al.* 2012). Earlier, grapevines sprayed with *Ascophyllum nodosum* marine-plant extract and PGR (brassinosteroids) significantly improved their rachis length (Norrie and Keathley 2006). Such increase in fruit set may be owed to the balanced nutritional properties whereas, berry drop may be due to the imbalance of nutrients in the grapevine. As MLE is found to rich source of important macro and micro elements, growth stimulators, amino acids, carbohydrates and proteins, its foliar application might have elevated the nutritional properties of grape and consequently increased berry set and reduced berry drop. In agreement to this, in a study the application of growth promoting compounds on grapes has improved fruit set, reduced premature berry drop and improved bunch parameters (Farooq and Hulmani 2000). As *M. oleifera* leaves are high in zeatin (cytokinin) (Ashfaq *et al.* 2012) the effect of MLE supports the fact that fruit size increases with cell division and cell enlargement and may be attributed to that cytokinins (Santner *et al.* 2009). Likewise, moringa extract treatment has significantly increased fresh fruit weight 'Kinnow' mandarin (Nasir *et al.* 2016) and plum (Thanaa *et al.* 2017).

The PGRs can be used to increase yield per unit area of a plant because they are responsible for changing the patterns of growth and development as in present study (Table 3). MLE has been found to contain considerable amounts of indole-3-acetic acid, gibberellins, zeatin (natural cytokinin) and abscisic acid (Rady and Mohamed 2015). These growth hormones influence yield remarkably. Similarly, MLE has been reported to have increased the yield of crops like onion, tomato, peanut, sugar cane and corn (Foidl *et al.* 2001), navel orange (El-Enien *et al.* 2015), pear (El-Hamied and El-Amary 2015) and plum (Thanaa *et al.* 2017). Plant growth regulators are found to be effective in improving grape quality and thereby making it acceptable for the consumers. MLE having plant growth promoting properties had also improved the grape qualitative characters. Similarly, grapevine treatment with IAA, GA_3 and zeatins improved the quality variables of 'Superior Seedless' table grapes (Jalil *et al.* 2017). ABA enhanced cluster attractiveness, colour and visual liking of grapes (Reynolds *et al.* 2016).

Moringa is wonder plant with unique nutritional profile significantly affect biochemical quality of fruits and vegetables. Vines sprayed with MLE showed higher concentration of biochemical quality attributes as compared to control (Fig. 2). Application of PGRs and nutrient elements improve the TSS percentage in the plants. MLE applied on orange trees had significantly increased juice TSS (El-Enien *et al.* 2015). Accumulation of sugar and acidity levels is inversely proportional to each other. As MLE increased the TSS and sugar contents in grape berry juice it correspondingly reduced the TA percentage. Accordingly, foliar spray of MLE on the 'Hollywood' plum trees significantly reduced the TA as compared to untreated trees (Thanaa *et al.* 2017). In coincidence to our results regarding sugars (Fig. 4), MLE had significant positive influence on the total sugar contents of 'Le-Conte' pear (El-Hamied and El-Amary 2015). The reducing sugar contents of the berries harvested from the vines treated with MLE were more than control (Fig. 4b). The highest and lowest percent increase in the reducing sugar contents of berry juice were in the 'NARC Black' had (19.30%) and 'Cardinal' (10.15%), respectively in comparison with their untreated ones. MLE (3%) had a significant positive impact on non-reducing percentage of grape juice of all the cultivars (Fig. 4c).

MLE application significantly improved ascorbic acid and phenolic contents of table grapes (Fig. 5a–b). Accordingly, when common bean (*Phaseolus vulgaris*) plants, pepper (*Capsicum annuum* L.) plants and plum trees were treated with MLE, it resulted in significantly higher vitamin C contents (Thanaa *et al.* 2017). Phenolic compounds play an important role in grape organoleptic properties. MLE when applied in combination with, K and Zn applied on 'Kinnow' mandarin resulted in significantly higher total phenolic contents (Nasir *et al.* 2016). Foliar spray of MLE significantly increased TPC of spinach leaves (Aslam *et al.* 2016).

Conclusion

Grapes cvs. 'Perlette', 'NARC Black' and 'Cardinal' showed best response to MLE sprays as compared to 'King's Ruby' and 'Flame Seedless'. Thus, exogenous application of MLE can be efficiently applied on grapevines at flowering, berry setting and premature growth phases to improve its vegetative, reproductive growth as well as physico-chemical quality features.

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Author Contributions

A.S. Khan and S.M.A. Basra conceived the idea and planned the study. M. Ibrahim conducted the research trial. All authors equally contributed in providing critical feedback for analysis, editing, commenting, revising and approving the manuscript without conflict of interest.

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Grapes Post-harvest Decaying Process,Associated Fungal Pathogens and their Ecofriendly Control by Plant Extracts and Oils

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Abstract

Grapes (*Vitis vinifera*L.) are amongst widely consumed fruit in the world. In Pakistan, about 66 thousand tons of grapes are produced annually from an area of 78.30 thousand hectares. Grapes are highly perishable commodity during transportation, storage, and marketing for general consumption and post-harvest losses are up to 16-23%. Numerous biotic and abiotic factors reduce the production and quality of grapes. In this study, samples of rotten grapes were collected from different fruit markets of Pishin (Balochistan) and Faisalabad (Punjab). The samples were subjected to isolate the fungal pathogens, responsible for the post-harvest decay of fruits. The fungal pathogen isolated was identified as *Aspergillus flavus,* and *A. carbonarius* causing post-harvest decay of grape berries. The pathogenicity of the two isolated pathogens was evaluated on different temperatures (5, 20, 30& 40 °C) on Thompson seedless variety of grapes. The temperature 40°C was found conducive for infection and proliferation of fungi. Five organic origin chemicals namely Neem, Castor and Clove oils, with concentrations of (500, 1000, 2000 ppm) for each oil and also Neem and Marigold Extracts with concentrations of (12.5, 25, 50%) of both extracts were used as antifungal agents. Among the tested organic compounds, Clove oil @ 2000 ppm was found better in delaying the decay process of both fungal pathogens. The decay was reduced up to 50% as compared to control. This study indicates that post-harvest application of essential oils can potentially enhance the storage life of grapes prior to marketing.

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Introduction

Grapes (*Vitis vinifera* L.) of *Vitaceae* family are among the most delicious fruits in world. In Pakistan the area under grapes cultivation is 78.30 thousand hectares with total production of 66 thousand tons (FAO, 2017). There are many abiotic stresses (drought, winter, cold and hot) and biotic (fungi, bacteria, viruses, MLOs and insects), which affect the quality production and cause post-harvest losses during storage and marketing. Some major grape field diseases are powdery mildew (*Uncinulanecator*), black rot (*Guignardiabidwelli*), downy mildew (*Plasmoparaviticola*), anthracnose (*Elsinoeampelina*) and crown gall (*Agrobacterium vitis*) (Odile *et al.*, 2006).Post-harvest losses due to fungal infections to grapes berries may be direct during ripening, packing, storage and transportation by a latent pathogen, thus becoming curious to grapes (Pezet*et al.*, 2003).

In Pakistan, post-harvest losses due to diseases, during packing and transport accounts to 16-23% (Aujla *et al.*, 2011). Nelson, 1979 reported that major post-harvest decay of grape are grey mold (*Botrytis cinerea*), sour rot (*Aspergillus carbonarius*), Rhizopus rot (*Rhizopus stolonifer*) and blue mold (*Penicillium expansum*). Genus *Aspergillus* is a serious threat for table grapes, wines and rasinsas its mycotoxins are also harmful to human (Perrone *et al.*, 2007; Somma *et al.,* 2012). The contamination of grapes and wine products are mostly due to Ochratoxin A (OTA) (Cabañes *et al.,* 2002). *A. carbonarius* and *A. niger* are the main to producer of OTA which results in inferior quality of grapes and its by products (Stefanaki *et al.*, 2003).

Post-harvested decay is reduced by sulfur dioxide (SO_2) treatment, bunches are usually stored in the presence of SO_2, this compound is registered as a supportive in most countries, while it has been removed from the GRAS (Generally Recognized as Safe) list and classified as a pesticide in USA (Anon, 1986). SO_2 causes phytotoxicity symptoms, such as discoloration, bleaching, sulfurous taste and browning of the rachis of grapes (Zoffoli *et al.,* 2008).

Keeping in view the notorious effects of synthetic fungicides on environment and human health, a study was designed to test the efficacy of natural products like neem, castor and clove oils along with neem and marigold extracts as antifungal agents at different concentration. The antifungal activities of essential oils are well documented being safe, easily decomposable and non-toxic (Gogoi *et al.*, 1997). Many researchers agree the effectiveness of plant extracts as fungicides, among which include cinnamon, lemongrass oils, oregano, palmarosa and clove (Marin *et al.*, 2004).

Cinamamic aldehyde present in *Cinnamon casia*oil and eugnol present in leaves of clove and cinnamon are famous for their antifungal activities (Davidson and Naidu., 2000). Neem oil solution (2-10%) controled*A. niger, A. alternate* and *F. oxysporum* (Locke, 1995). Plant extracts having antifungal and antibacterial effects are being used to control plant diseases commercially (Lee *et al.*, 2007). It has been also confirmed that different types of phenolic compounds are present in plant extracts (Al-Zoreky, 2009). *Azadirachtaindica* is commonly known as 'neem, have a strong antifungal effect (Murthy and Sirsi., 1957).

The present study was aimed to isolate and identify the pathogens associated with decay of grapes and to assess their pathogenicity on harvested grapes. Further, it was also a mandatory to work out the optimum temperature for fungal growth and to increase the shelf lives of harvested fruit using eco-friendly plant origin fungicides.

Materials and method

Isolation and identification of pathogens associated with post-harvest decaying of grapes

The samples of rotten (diseased) grapes were collected from different vineyards, local fruit stores and different markets of districts of Pishin and Faisalabad. Pathogens were isolated on potato dextrose agar. The growing pathogens were identified and purified on the basis of morphological characteristics.

Pathogenicity and temperature optimization

Pathogenicity test was carried out on 5, 20, 30 and 40°C to standardize the optimum temperature. Inoculum was prepared from fresh culture, spores were maintained up to 1×10^7 spores/ml and growth behavior of fungi was observed.

Inoculation and incubation

Thompson, a seedless variety of grapes is being widely consumed in Pakistan. 100 grams of bunches were prepared. Each bunch was immersed in 70% ethanol for two minutes to sterilize the surface, bunches washed of twice in sterilized distilled water and dried in air flow chamber. Three to four grape berries in each bunch were punctured up to 2 mm deep with the help of sterilized common pin and about 4 µl of adjusted spores solution (1×10^7 spores/ml) injected to each puncture berry with the help of micropipette and berries were placed in sterilized plastic boxes which thereafter were incubated at 5, 20, 30 and 40°C for 8 days.

In-vitro management through oils and plant extract

Essential oils

Plant essential oils neem (*Azadirachtaindica*), castor (*Ricinus communis*) and clove (*Syzygiumaromaticum*) purchased from the market were sterilized by heating at 45°C under 125 psi pressure. Solutions were prepared as 2000ppm (200mg/100ml), 1000ppm (100mg/100ml) and 500ppm (50mg/100ml) in acetone (Tripathi *et al.*, 2008).

Plant extracts

Leaves of marigold (*Tagetes*) andneem (*Azadirachtaindica*) were collected from University of Agriculture Faisalabad main campus and were sterilized in 70% ethanol. About 40 grams of leaves were blended in 100 ml of sterilized distilled water.

After blending aqueous solution were passed through muslin cloth and then filtered from whatman filter paper and kept in refrigerator until use (Riaz *et al.*, 2008). The extracts were used in three different concentrations 12.5, 25and 50%.

Evaluation of fruit decay

Infection data were collected using disease rating scale, 1-9 (0) no symptoms; (1) 1-5%, small white growth on inoculated portion; (2) 10-15%, little extent of fungal growth on inoculated portion; (3) 20-25%, spores production and little extent to adjacent berries; (4) 30-40%,the inoculated berries covered with fungus growth almost; (5) 50-60%, infection started on all berries; (6) 70-80%, severe infection; (7) >80 all berries completely covered with fungus growth (Artés-Hernández *et al.*,2004).

Statistical analysis

Trial was laid out in Completely Randomized Design (CRD) and the results were subjected to analysis to differentiate treatments and concentrations turkey's (HSD) test were used for comparing different means. With three concentrations along with control having three replications each.

Results

Isolated pathogens

Isolated pathogens are (*R. stolonifer, A. carbonarius, A. flavus* and *A. niger*).*In-vitro* various plant essential oils and plant extract were evaluated against *A. flavus* and *A. carbonarius*.

Pathogenicity and temperature optimization

The pathogenicity of *A. carbonarius*and *A. flavus* was standardized. *A. carbonariu* showed maximum decay of 80.4% at 40°C after 8 days followed by *A. flavus* with decay values of 78.3% at the same temperature, while minimum decay growth was recorded up to 7.11% at 5°C on *A. carbonarius*after lapse of 8 days. It might be concluded that 30°C and 40°C temperature is more favorable for growth of fungi. It was learnt that grapes could be stored at 5°C to save precious fruit from decay (Fig.1.).

In-vitro management through oils and plant extract

Essential oils

Data regarding impact of natural oils (neem, castor and clove oil) on fungal growth (*A. flavus* and *A. carbonarius)* on grapes after 3, 5 and 8 days after picking are depicted in (Table 1.). It is evident that with the passage of time fungal infection increased but remained under control as compared to check.

Three days data showed that minimum infection (1%) was showed by clove oil @ 2000 ppm against *A. flavus* and 1.64% decay by *A. carbonarius* while maximum damage (9.22%) was recorded in control. Similarly, after five days post-harvest decay data showed that minimum infection 3.50% was marked when applied 2000 ppm clove oil on *A. flavus* while 5.63% infection was recorded in *A. carbonarius* by antifungal effects of clove oil @ 2000 ppm. Infection data analyzed after 8 days of harvesting, showed that minimum damage 11.23% was observed by applying clove oil @ 2000 ppm against *A. flavus* and *A. carbonarius*, respectively followed by castor oil. While maximum infection was observed 75.55% in control. Overall scenario showed that among the test material, Clove oil @ 2000 ppm might be the best solution to increase the shelf life of grapes. Our finding can get support from (Sukatta *et al.*, 2008; López-Malo *et al.*, 2007.

Table 1. Pathogenic effects of neem oil, castor oil and clove oil on *A. flavus* and *A. carbonarius* growth on grapes.

Fungal strains	Treatment	3 days				5 Days				8 Days			
		Control	500 ppm	1000 ppm	2000 ppm	Control	500 ppm	1000 ppm	2000 ppm	Control	500 ppm	1000 ppm	2000 ppm
A. flavus	Neem Oil	7.86	4.33	3.82	3.13	26.55	10.73	9.62	7.72	63.96	29.3	21.56	20.5
	Castor Oil	6.49	5.3	4.32	3.32	31.59	14.0	9.37	8.51	66.78	37.9	28.6	25.5
	Clove Oil	9.22	2.70	1.53	1.00	30.03	9.40	5.66	3.50	64.78	28.8	17.6	11.23
A. carbonarius	Neem Oil	5.67	5.21	4.41	3.86	27.80	13.66	12.26	8.27	76.25	42	34.8	18.97
	Castor Oil	6.23	4.36	3.33	2.99	28.98	11.11	9.65	8.60	66.46	36.5	23.5	14.44
	Clove Oil	8.16	3.32	2.87	1.64	30.77	9.55	7.75	5.63	75.55	26.7	22.1	11.28
CV		14.00				6.48				3.71			
Tukey's Value		1.65				2.85				3.41			

Table 2. Pathogenic effect of plant extracts on fungal growth on harvested grapes.

Fungal strains	Extracts	3 days				5 Days				8 Days			
		Control	12.5%	25%	50%	Control	12.5%	25%	50%	Control	12.5%	25%	50%
A. flavus	Neem Extract	7.99	5.64	4.99	2.4	30.14	14.5	12.3	9.61	64.9	44.33	39.33	28.76
	Marigold Extract	6.97	4.33	3.33	2.20	31.81	13.03	13.00	8.11	75.5	35.37	31.61	21.33
A. carbonarius	Neem Extract	9.35	5.10	3.32	3.12	33.88	11.16	9.46	7.33	70.5	44.76	39.43	32.9
	Marigold Extract	5.66	3.33	3.32	2.94	33.59	19.40	14.50	10.6	72.6	58.8	39.44	32.92
CV		14.48				7.62				3.26			
Tukey's Value		1.84				3.38				3.77			

Plant extracts

The data regarding pathogenic effects of neem and marigold extracts against *A. flavus* and *A. carbonarius* on grapes presented in (Table 2.).

It is depicted that with passage of time fungal infection increase but remained under control. Marigold extract 50% after three days of harvest showed a decay of 2.20% followed by 50% neem extract with a decay value of 2.4% while control showed a decay infection 7.99% against *A. flavus*.Similarly, 50% marigold extract treatment showed decay value of 2.94% after three days of harvesting while control showed a decay infection up to 9.35% against *A. carbonarius*.

With lapse of time, after 5 days of harvesting decay percentage increased to 33.88% in control while 50% Neem extract showed minimum decay of 7.33% and more decay of fruits decay were recorded under low concentration of treatment. Similarly after 8 days of harvesting, maximum decay of fruit were recorded as

75.5% against 21.33% decay in fruit flask treated with 50% marigold extract. Critical comparison showed that oils proved more effective then plant extract against decay causing organisms *i.e.A. flavus* and *A. carbonarius* thus these treatments need to be standardized to increase the shelf life of grapes. (Anjum Malik *et al.*, 2016) evaluated the efficacy of marigold and neem leaf extract against thevarious post-harvest diseases of fruits and vegetable that developed by fungal pathogen.

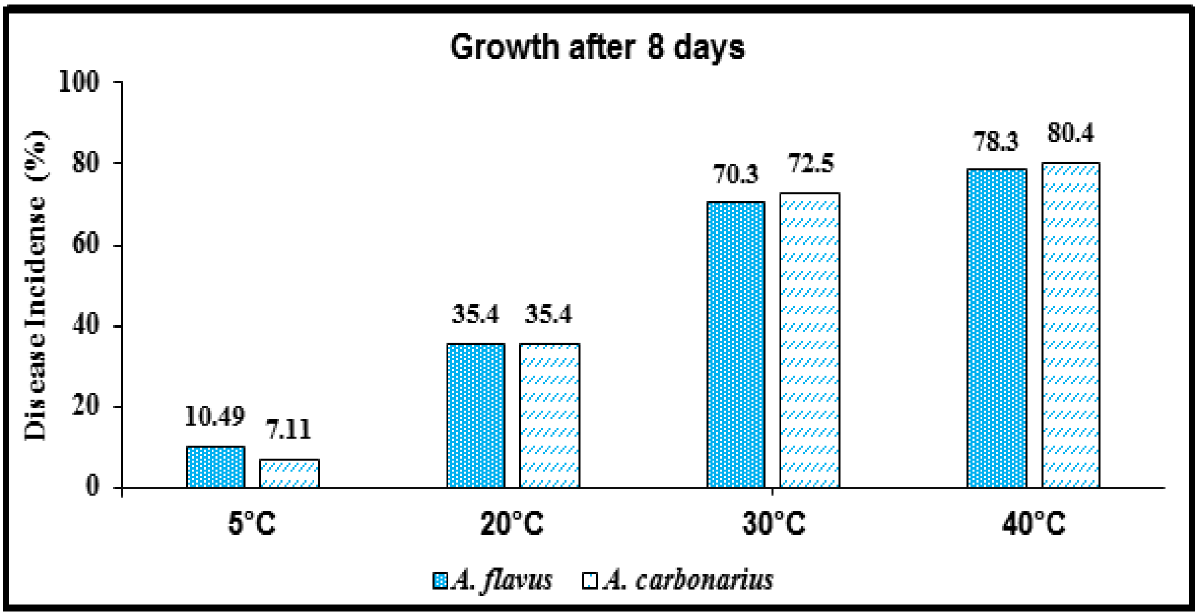

Fig. 1. Pathogenicity and temperature optimization of *A. flavus* and *A. carbonarius*.

Discussion

Gabler *et al.* (2004) exposed spores of *A.niger* to ethanol at 25-50°C andthey revealed maximum inhibition of spore at 40°C.Esteban *et al.* (2004) reported*A. carbonarius*and*A.niger*explained that better growth at 10-40°C and produced much OTA at 20-25°C. Due to production of OTA at wide range they also declared that *A. carbonarius*and*A.niger* can mostly infect grapes.

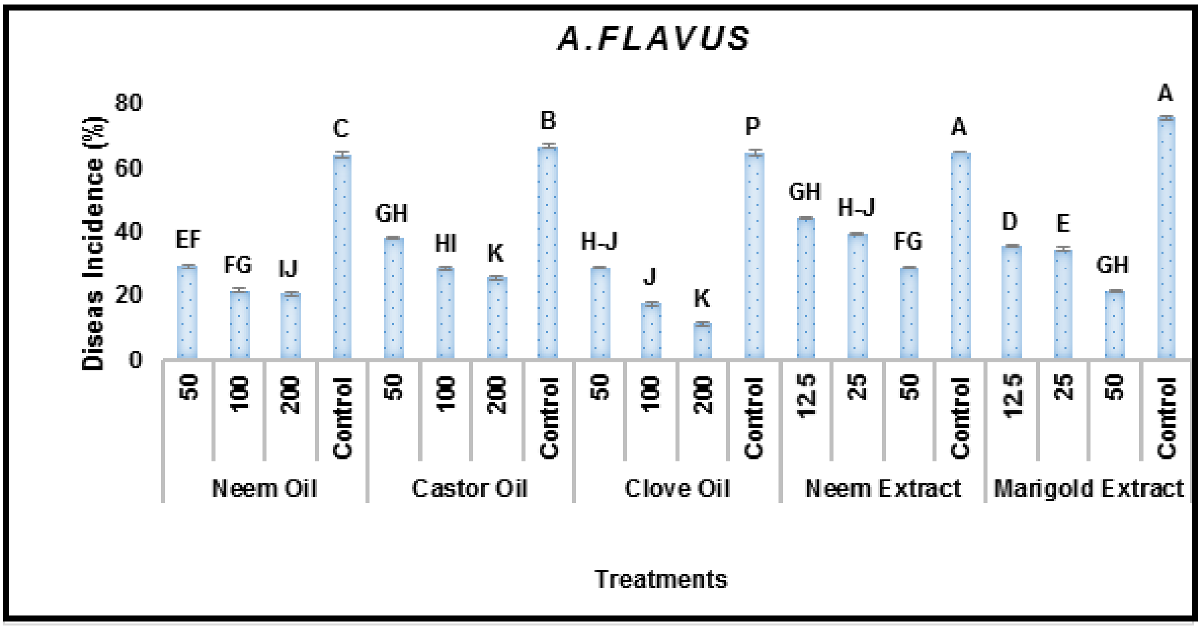

Fig. 2. Graphical representation of all treatments against *A. flavus*

In present study, the pathogenicity test of isolated fungi (*A. flavus* and *A. carbonarius* were done on 5, 20, 30 and 40°C with the aim to find out the optimum temperature for storage for grapes. After 8 days,*A. flavus* showed maximum decay expansion of 78.3% at 40°C and *A. carbonarius*gave maximum spread of 80.4% at 40°C.These studies made it clear that two pathogens showed minimum growth at 5°C and maximum proliferation of fungus at 30 and 40°C. So if grapes stored at 5-8°C, the decay will be very slow, whereas*A. carbonarius*was still able to produce infection even at 5°C.

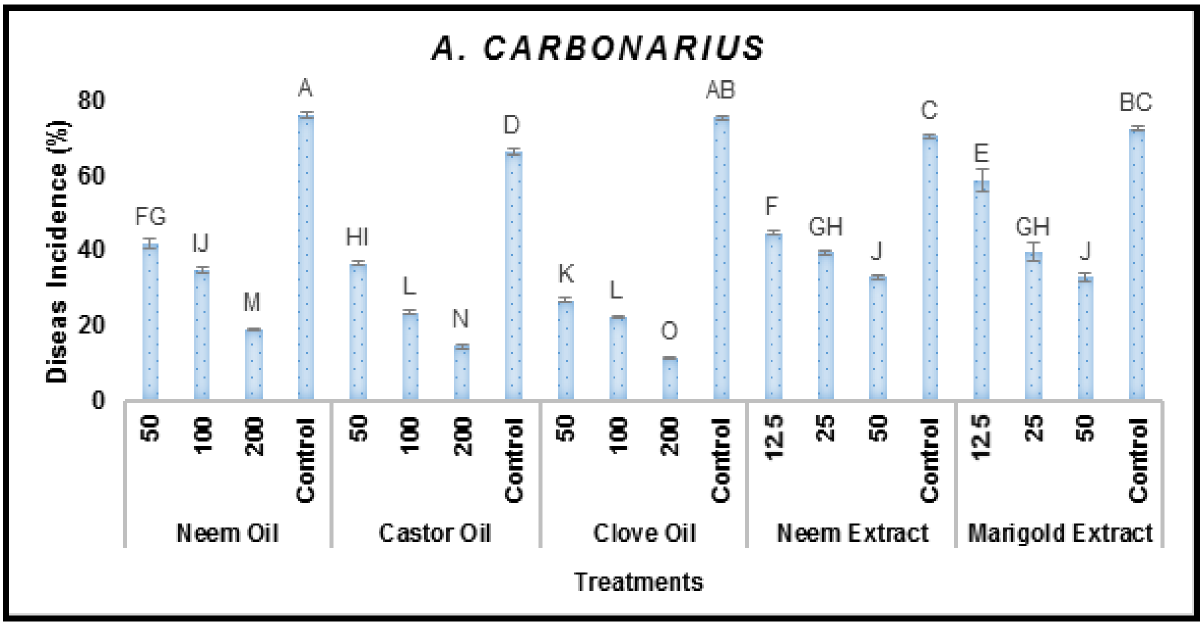

Fig. 3. Graphical representation of all treatments against *A. carbonarius.*

Xing *et al.* (2012) stated that the main constituent of clove bud oil is eugenol which is strong antioxidant, and it controlled the *A. flavus* at different concentrations with greater success but at 3% it inhibited more than 80% infection. He suggested that clove oils can be used as good agent for control of fungal pathogens. Sukatta *et al.* (2008) argued that clove oil showed better result on grapes post-harvest pathogens (*A. niger*, *R. stolonifer* and *A. alternate*) with different concentration, at higher 400 mg/ml. Amagase *et al.* (2001) stated that castor oil is a good source of monounsaturated 18-carbon fatty acid, which include ricinoleic acid. It has hydroxyl group on 12th carbon. So, it makes castor oil more polar compared to most fats, while garlic oil has allicin derivative component that's why both have more antimicrobial activity. In present study oils of neem, castor and clove were test against *A. flavus* and *A. carbonarius*at different concentration. All oils showed better result even on low concentration, but clove oil wasbetter than other oils.

Conclusion

Aspergillus species were prominent in decay as compared to other fungi. There was maximum proliferation of fungi at higher temperature, thereby maximum losses can occur. Recommendations for natural storage temperature is 5-8°C.

All oils and extracts were effective in controlling *A. carbonarius* and *A. flavus,* but clove oils proved the best as compared to other oils. Selection of solvent is an issue in the use of oils and itneeds to be addressed as the oils are soluble greatly in acetone (organic solvents). The results are based on *in-vitro* trials on fruit.

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