

Full Length Research Paper

Evaluation of reduction approaches on frost damages of grapes grown in moderate cold climate

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Accepted 3 September, 2011

This study was done to investigate passive and active protective methods on reduction of losses from frost stresses in grapes cultivated in Shahrekord (Iran) during 2006 to 2008. Data analysis was done based on randomized block design, 3 replicates and five treatments including control without operate, Bordeaux mixture, increasing garden soil moisture up to field capacity, sprinkler irrigation upper grapes canopy, treatment by using heater between grapes canopy. Four measurements were done such as decrease of photosynthesis, increase of respiration, decrease of grapes yield, while cold stress in 2006 to 2007 and decrease of photosynthesis, increase of respiration, decrease of grape yield and tissue damages while freezing stress in 2007 to 2008 occurred at harvest time. Results showed that treatments using heaters were significant ($P<0.001$), the effective method in comparison to other applied methods during the first year because of no frozen stresses. Nevertheless, treatment of Bordeaux mixture was the best method ($P<0.001$) for reduction of damages on grapes within 2007 to 2008, since there was freezing stresses on growth of grapes.

Key words: Frost damages, grapes, cold climate, cold stress and freezing stress.

INTRODUCTION

Plants need appropriate resources such as air, light, temperature and soil in order to grow normally. When any undesirable environmental condition occur, plants could either adapt to these conditions to some extent (Amolkumar et al., 2008) or are subjected to damage by environmental stressors that is cold, drought and salinity (Beck et al., 2007). Cold and frost stresses, significant abiotic stresses, are comprised of chilling injury (0 to 20°C) and frost injury (<0°C) which has an impact on plant development, net photosynthesis and yield (Kasuga et al., 1999; Lang et al., 2005) respiration rate (Asgworth, 1986; Palta et al., 1980). Most plants like rice, maize, tomato, soybean, banana, papaya, mango, grapes and orange are sensitive to low temperature and lose their quality and productivity (Larcher, 1995). For instance a 30 to 40% yield reduction of rice was reported in temperate climate (Andaya et al., 2003). Another study revealed that metabolic rates of plants reduced at reproduction in cold climate which resulted in low yields (Thakur et al., 2010). Cold stress is affected by duration and extent of stress. Some major signs of cold stress are

as follows, surface lesion, a water-soaked appearance of the tissue, discoloration, and desiccation, tissue breaks down, accelerated senescence, ethylene production, shortened shelf life and faster decay (Sharma et al., 2005).

In addition, Frost stress also affects sensitive plant organs (Beck et al., 2007). Thus, membrane damage occurs from ice formation inside the plant tissue, cellular dehydration and increased concentration of intracellular salts in frost stresses. Lipid peroxidation caused by accelerated reactive oxygen species resulting from freezing produce cold and frost injury in plants (Amolkumar et al., 2008; Liang et al., 2008). Prolong decrease in temperature and change of cold stress into freezing stress affects plants growth to come into a critical stage. This causes visibility of damages on plants surface in some cases. Temperatures below 0°C even for short time can be fatal for plant disease and death is end of cell viability (Raghavendera et al., 1992). Despite vast number of studies on cold and frost stresses, there is fairly limited number of research on plant protection from

chilling/freezing injury to help farmers. Farmers normally know which areas of their land are suitable for planting by their own experiences and there is also some information available for them to grow suitable plants preventing from cold/frost damages (Snyder et al. 2005). Furthermore, using nitrogen and phosphorous may lead to increase of cold damage, conversely, application of silicon fertilization acts as a cold resistance (He et al., 2009). Before cold event, plant could be irrigated to field capacity in order to protect them as a result of increasing heat absorption and storage (Rosenberg et al., 1983).

There are two protective methods against frost damage including passive and active methods. The former is a good substitute for active ones which applies before a frost night. Some of these methods include appropriate site selection for planting trees and crops, managing cold air drainage, plant selection, canopy trees, plant nutritional management, proper pruning, plant covers, avoiding soil cultivation, irrigation, removing cover crops, soil covers, trunk painting and wraps, bacteria control, planting date for annual crops and copper compounds (Cartechini, 1991; Snyder et al., 2005). It should be noted that among different copper compounds such as copper hydroxide, copper chloride oxide, the Bordeaux mixture contains copper sulfate and hydrated lime and very effective on plants protection but there was no evidence of beneficial uses in frost injuries (Association, 1974; Evans, 2000).

The latter method is used alone or in combination to reduce effects of frost damages in plants. These methods heaters, wind machines, helicopters, sprinklers, surface irrigation, foam insulation and combinations of the methods (Snyder et al., 2005). These protections should be used at night before frost stress to produce energy balance otherwise frost damage will be inevitable. There are difficulties to predict air temperature due to measuring turbulent sensible heat flux in the range typical of frost nights; accounting for advection; and spatial variations in surface radiation emissivity. It is suggested to obtain the rate of cooling of a column of air. Although radiative and turbulent sensible heat fluxes is related to vertical profiles of wind, humidity and temperature (Kalma et al., 1992; Rossi et al., 2002). Furthermore, the best time to use the energy balance is before frost stress (approximately few hours before) thus up to 33% of frost damage has been reduced (Reisch et al., 1986; Lyne, 2006). In years 1992, 1997 and 2000, some experiments were conducted on apple orchards using energy balance in the east of England. The results showed that quantitative yield was increased to about 70% in apple orchards and 180% in peach and pear orchards (Wampl et al., 2000). Reduction in photosynthesis is an outcome of energy imbalance in photosystem II (Amolkumar et al., 2008).

Grape is grown suitably in areas with warm summer. Therefore, any substantial changes in climate such as spring and autumn frosts have an impact on grape

production. So, it is important to obtain information of climate changes on a daily/weekly/monthly basis (Caprio et al., 2002). Grapes frost is also one of the problems of farmers in Canada which damage grapes in several stages. The most important and sensitive period for grape buds is time of swell and opening. Two methods, use of copper compounds and heaters are applied to protect crops from cold and frost stress (Lyne, 2006). In some areas of Canada and California (Fresno) helicopters were used for air mixing of layers up to 18 m of grape trees to balance energy levels, in order to prevent temperature drop to the critical limitations (Reisch et al., 1986).

Additionally, Grape plants are protected up to -3.3°C by using wind machines and heaters. Frost damage of grapes is prevented by air mixing with 18.3 m height and up to 1.5 m modifying critical temperature to 0.6 to 1.2°C . The use of heaters alone until 2.5°C of temperature drop is also beneficial (Lyne, 2006). Dereuddre et al. (1993) concluded that if used herbicides are maintained in rows level, temperature of plant canopy will be 1.5°C higher than before which prevents crops from freezing and cold stress. Moreover, technical arrangement and grape vine pruning could be used to reduce frost damage (Dereuddre and Brum, 1993). Respectively grape shrubs with additional branches in grape gardens were effective method for reduction of frost damages in Fresno and Michigan (USA) (Einset and Robinson, 1972). Using sprinkler irrigation in the U.S. has been able to protect grape shrubs from cold stress up to 3.9°C . There is also evidence that for every ha/h, 480 L of spray water should be pumped and if the temperature drops nearly 0°C or few degrees above zero with severe wind, more water should be pumped and sprayed. Sprinkler start to operate while temperature is 1.1°C (Dereuddre et al., 1993; Reisch et al., 1986).

The objective of this study was to evaluate effects of passive and active protection methods on grape during cold and frost stress in cold climate. As can be seen in Figure 1, local farmers use different coverings such as polypropylene plastics and removable straws on grapes in order to protect plant from frost injuries. This information is significant for the progress of grape production in this area and will be useful worldwide for application to farming practice in other similar areas.

MATERIALS AND METHODS

Grape variety (*Vitis vinifera*) was grown in Tange Sayaad (Central Zagros - Shahrekord, Iran). Weather forecasts were obtained from agro-climatology station near the research location. The climate was moderate cold during 2006 to 2008. Its geographical location is $32^{\circ}31' \text{N}$, $50^{\circ}53' \text{E}$ and 2100 m above sea level, T_{\min} in the coldest month (January, -8°C) and T_{\max} in the warmest month (June, 24.6°C). Data collection was done based on randomized block design, 3 replicates and five treatments including control without any operate (CO), Bordeaux mixture (BM), increasing garden soil moisture up to field capacity (FC), sprinkler irrigation upper grapes canopy (SI), treatment by using heater between grapes canopy (H).



Figure 1. Different coverings including polypropylene plastics and removable straws were used to protect grapes from frost damages by local farmers.



Figure 2. Spraying Bordeaux mixture on grape shrubs as highlighted.

Increasing garden soil moisture up to its field capacity (FC) were used as passive protection methods, while the temperature in grapes canopy dropped to 3°C and less during mid April to mid May. For applying Bordeaux mixture (BM) as copper compound of passive protection methods in this study, different ratios of the Bordeaux mixture were used for spraying on grape shrubs (Figure 2) in the following steps:

- 1) 1.5 kg copper sulphate, 3 kg live lime and 100 L water in stages a and b; and
- 2) 1 kg copper sulphate, 2 kg live lime and 100 L water in stages c and d. The stage a is the first time of spraying after leaves loss of grape, stage b is the second time before the complete opening of grape buds, c) the third time before grape flowering and d) the fourth time after flowering and formation of fine sour grapes.

For active protection, intensive energy operations were used to

create natural energy movement on the ground. For this operation, sprinkler irrigation (SI) and the use of heaters (H) were applied in grapes canopy when cold stress (temperature in grapes canopy dropped to 3°C) and when frost (temperature in grapes canopy dropped to below 0°C) happened. Time of on/off heaters depend on warning of automatic thermometer buzzer.

Four measurements were done such as decrease of photosynthesis, increase of respiration, decrease of grape yield, while cold stress in 2006 to 2007 and decrease of photosynthesis, increase of respiration, decrease of grapes yield and tissue damages while freezing stress in 2007 to 2008 accrued at harvest time after physiological maturity. A portable photosynthesis system (LI-Cor 6400, USA) was used to measure photosynthesis of grape leaves and respiration of grape leaves was measured by using the same systems (LI-Cor 6400, USA). Tissue damages in this study, were determined by scanning electron microscope (2003, Hitachi S - 5200).

Table 1. Results of ANOVA for 5 treatments H, SI, FC, BM and CO for two years.

Year	Treatments	Decrease of photosynthesis (%)	Increase of respiration (%)	Decrease of grape yield (%)	Tissue damages (%)
2006-2007	H	5.7 ^a	8.3 ^a	8.7 ^a	-
	SI	11.7 ^b	12.8 ^{ab}	11.6 ^b	-
	FC	12.6 ^b	14.0 ^b	13.8 ^c	-
	BM	20.6 ^c	23.9 ^c	26.8 ^d	-
	CO	22.1 ^c	25.0 ^c	27.5 ^d	-
2007-2008	H	47.3 ^b	51.8 ^b	53.0 ^b	34.2 ^a
	SI	59.3 ^c	70.0 ^d	75.4 ^d	48.0 ^b
	FC	56.0 ^c	60.3 ^c	63.9 ^c	39.7 ^b
	BM	22.3 ^a	25.3 ^a	27.7 ^a	33.3 ^a
	CO	97.0 ^d	99.0 ^e	99.9 ^e	98.0 ^e

At $P < 0.001$.

Statistical analysis

Analysis of variance (ANOVA) was performed on all experimental data using IBM SPSS 17. Means were subjected to Duncan test at 0.05 probability level.

RESULTS AND DISCUSSION

Table 1 showed ANOVA results using Duncan test. There were significant differences between treatments in terms of decrease of photosynthesis in the first year. Three levels of differences a, b and c were obtained. The H value, 5.7%, was significantly different from SI (11.7%) and FC (12.6%) and from BM (20.6%) and CO (22.1%). It indicates that the control had maximum value of reduction in photosynthesis compared to other treatments. Treatments using heaters had minimum value of reduction in photosynthesis (Figures 3 and 4). Using Bordeaux mixture for reducing damages of cold stress was not effective during several spraying. ANOVA showed that differences between the control treatment (CO) treated with Bordeaux mixture was not significant ($P < 0.05$). So if the temperature drops in the Shahrekord climate in spring, during the months of mid April to mid May do not reach 0°C or less for at least 1 h, the use of Bordeaux mixture within few stages (stages starts from late autumn to start souring grapes) was not successful in this region.

There were significant differences at $P < 0.01$ among these treatments 2006 to 2007. The three indices were studied including effects of cold stress on photosynthesis, respiration, and grape yield.

The extent of decreasing three indices namely photosynthesis and yield as well as increase respiration was measured as losses. For example, in a normal condition of growth, photosynthesis, respiration and grape yield in cold conditions for grapes without stress has been considered. Drop in photosynthesis at any

extent has been assessed unpleasant and harmful in plant growth. Using heater treatment (H) was different (at $P < 0.01$) with other treatments due to prevention of negative effects of cold stress on growth (Table 1). In fact, using heater treatment had the lowest negative effects of cold stress on grapes compared to other treatments. These effects were obtained on a basis of some indices: the least negative effect on the reduction of photosynthesis 5.7%, the least negative impact on respiration increased 8.3%, the lowest negative impact on the reduction of grapes yield 8.7% in comparison with other experimental treatments.

Consequently, using heaters in grape gardens is the most efficient, economical and practical method for reducing the effects of cold stress in Shahrekord climate for a critical period of mid April to mid May. The treatment increased soil moisture to field capacity in the afternoon and was recommended when there are symptoms of night frost occurrence. Comparison of three treatments H, FC and SI showed that the following order of treatments was obtained to gain lesser extent of losses during cold on cold and frost stress: $H > SI > FC$. The previous studies showed that using heaters in combination with a wind machine can be effective in grape plant to prevent cold and frost damages. Using heaters alone prevent temperatures drop up to 2.5°C (Reisch et al., 2003; Lückner et al., 2009). The results indicate that the ability of different treatments compared with the incidence of cold stress condition in 2006 to 2007 and frost stress on reduction of damage in frost and cold stress are quite different. The amount of damage of indices such as photosynthesis, respiration and grapes yield of all treatments was different (F test, LSD and Duncan test at $P < 0.001$) with each other and in comparison with control. In year 2007 to 2008, four indices were studied because of frost occurrence including effects of cold stress on photosynthesis, respiration, yield and frost damages to

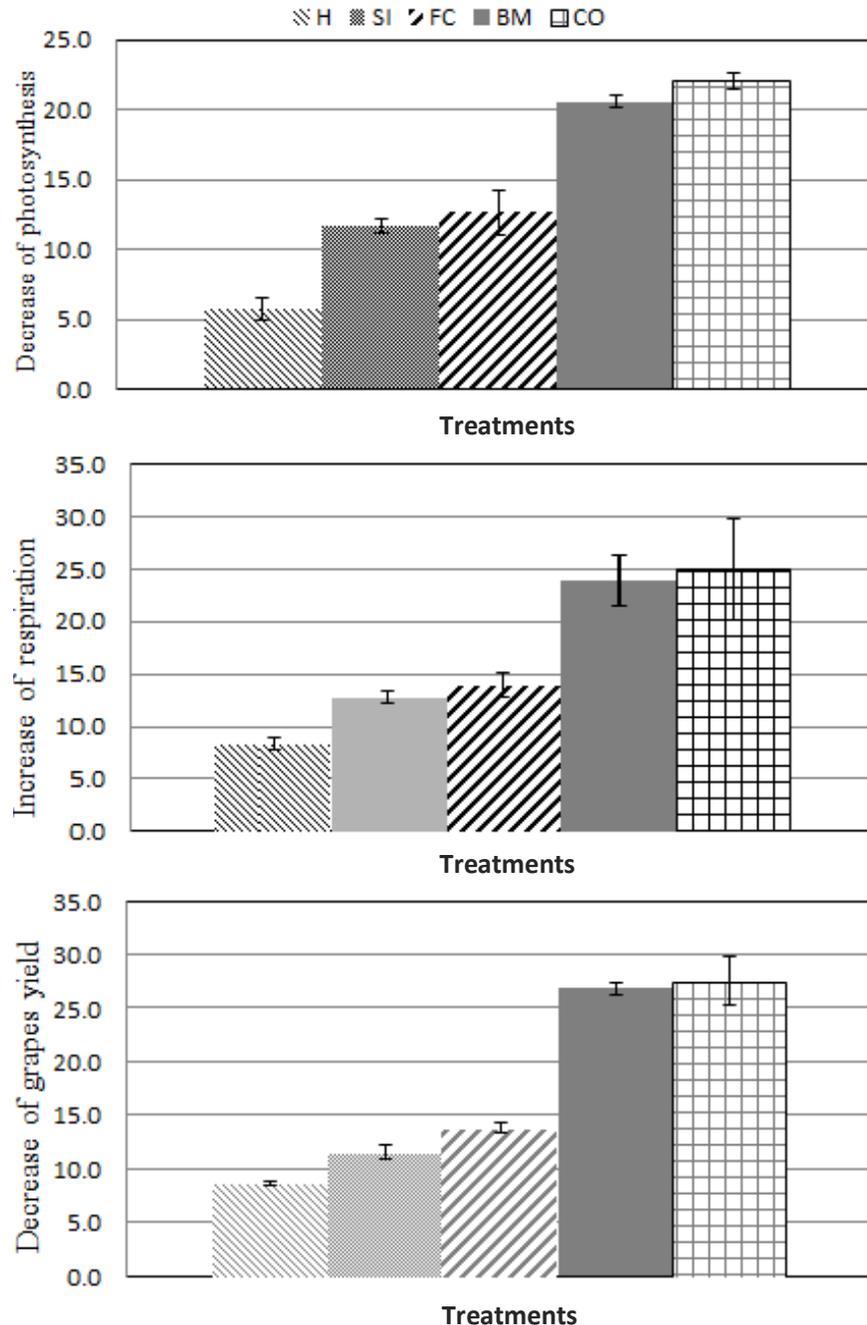


Figure 3. Values of Decrease of photosynthesis, Increase of respiration and Decrease of grapes yield versus treatments H, SI, FC, BM and CO are obtained in year 2006-7. Standard deviations are given as error bars for three replications.

plant tissues.

Additionally, the effects of frost damage caused the collapse of plant tissues in year 2007 to 2008. In the first year, the control treatment (CO) had 22.1% reduction in photosynthesis, 25% increase in respiration and 27.5% reduction in grapes yield. However, the drop in temperature and frost incidence in the second year

caused some losses in control treatment (CO) as follows: 97% photosynthesis, 99% increase of respiration, 99% grape yield reduction, and 98% tissues destruction. This indicates significant adverse effects of the temperature drop. This drop below zero can cause irreversible damage on plant tissues and negative effects on grapes yield and finally create an economic disaster for grape

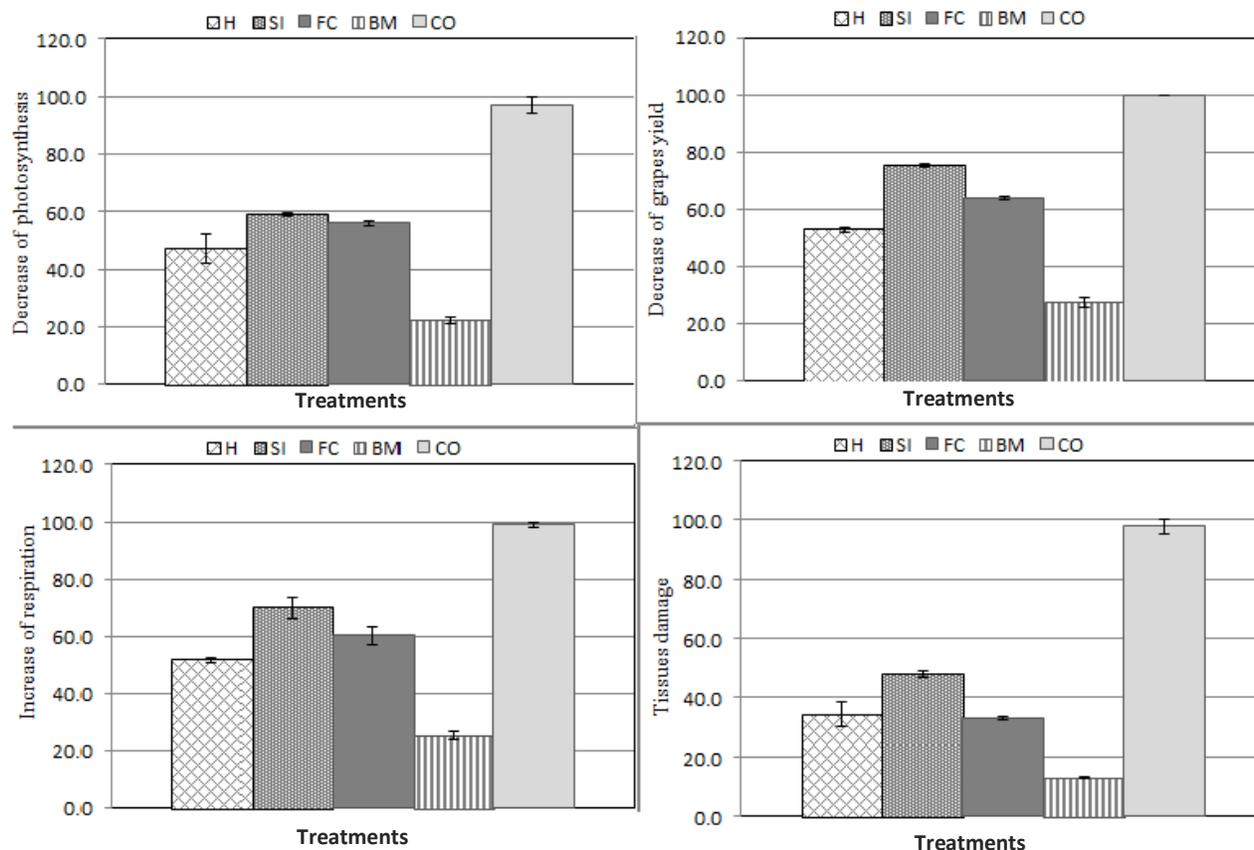


Figure 4. Values of decrease of photosynthesis, increase of respiration, decrease of grapes yield and tissues damage versus treatments H, SI, FC, BM and CO are obtained in year 2007-8. Standard deviations are given as error bars for three replications.

farmers. Using Bordeaux mixture in the reduction of cold stress damage was not successful in the first year.

Despite the second year, these treatments had the highest efficiency to reduce frost damage due to 4 measurements. This treatment was significantly different from other treatments at $P < 0.001$. These treatments had photosynthesis drop of 22.3%, respiration increase of 25.3%, reduction of quantitative yield of 27.7%, and 33.3% plant tissues destruction. Consequently, treatment of Bordeaux mixture was successful in reducing frost damage and preventing 63.3% of grapes yield which was a great achievement. During the second year, the following order of treatments was obtained to gain a lesser extent of losses during cold and frost stress: $BM > H > FC > SI$. Similar findings were obtained on grapes using copper compounds to reduce total bacteria produce, ice crystals in plants (Lücker et al., 2009).

Previous studies show plant protection from cold and frost stresses require careful considerations, as it is a very complicated subject. Several passive/active methods or a combination of these methods can be used worldwide depending on plants, environment, degree of stresses and quality properties. However, normally passive

methods are preferable and cost-effective than active methods (Amolkumar et al., 2008; Reisch et al., 2003; Raghavendera and Padmasree, 1992). The results indicate that the extent of damage of cold and frost stress in cold climate could be reduced using applied methods. Efficiency of these methods strongly depends on the extent of the temperature drop and grape varieties.

Conclusion

On the whole, using active and passive protection in cold climate (Shahrekord) could result in reduction of cold and frost stress for grape plant when grapes canopy temperature does approach to below zero. Use of heater was the most economical and practical method in grapes to control damages of cold stress when temperature drop to zero.

However, use of copper compounds- Bordeaux mixture was very effective to reduce frost damages of freezing stress, when temperature drops to below zero. It should be noted that determination of grape quality (such as sugar content) after using such protection methods is

required to carry out further research.

ACKNOWLEDGEMENTS

This research was supported by the grants from Agricultural and Food Consultant Ltd, UK. We are also grateful to the referees for their great comments on this manuscript.

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