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# POMOLOGICAL EVALUATION OF APRICOT CULTIVARS AND THE ROLES OF POSTHARVEST APPLICATION OF SALICYLIC ACID AND METHYL JASMONATE ON STRESS RESISTANCE

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# POMOLOGICAL EVALUATION OF APRICOT CULTIVARS AND THE ROLES OF POSTHARVEST APPLICATION OF SALICYLIC ACID AND METHYL JASMONATE ON STRESS RESISTANCE

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# **Abreviations**

SSC Soluble solid content

CI Chilling injuries

FD Fruit decay

SA Salicylic acid

JA Jasmonic acid

SAR Systemic acquired resistance

MeJA Methyl jasmonic acid MeSA Methyl salicylic acid

PAL Phenylalanine ammonia-lyase

SOD Superoxide dismutase

CAT Catalaze
POD Peroxidas

TA Titratable acidity
TSS Total soluble solid

PME Pectine methylesterase

AA Ascorbic acid

SPS Sucrose-phosphate synthase
SAM S-adenosyl-L-methionine
ACC S-adenosyl-L-methionine

LOX Lipoxygenase

ROS Reactive oxygen species

HSPs Heat shock proteins
AOX Alternative oxidase

PDJ N-propyl dihydrojasmonate

cv. Cultivar

#### 1- INTRODUCTION

The apricot (*Prunus armeniaca* L.) is one of the most important fruit species grown in the world, as apricot fruit is highly appreciated by consumers. Consumers cherish the flavour and aroma of high quality apricots, with the sugar content being one of the most appreciable quality characteristics (Ruiz and Egea, 2008).

Apricot fruit is usually stored in cold storage and many apricot cultivars can be stored for 4-6 weeks and these cultivars are able to keep an acceptable level of fresh firmness and other quality attributes. However, once the fruit is taken out from cold chamber the fruit start to deteriorate (Stanley *et al.*, 2010). Apricot fruits during cold storage were reported to show chilling injury symptoms and a high percentage of fruit decay, such as mealiness development, loss of juiciness and/or gel breakdown (Stanley *et al.*, 2010; Seibert *et al.*, 2010). Apricot fruit starts to lose its physical and chemical qualities directly after harvest and through the storage period. Fruits start to lose their water content reaching 12% after 28 days at 1 °C. This is accompanied with fruit softening, increased fruit acidity and reduction in soluble solid content (SSC) (Ezzat *et al.*, 2012).

Storage at low temperature to retard tissue respiration is still the most effective postharvest method for extending the shelf-life. But most of cold stored fruit present the chilling injuries (CI) symptoms, and subsequently fruit loss most of qualities parameters.

The use of chemical compounds to potentiate the natural defense of plants represents another alternative, potentially promising way to disease control (Kessmann *et al.*, 1994). Many reports have shown that induced disease resistance in plants by biotic and abiotic elicitors is a very effective method for restricting the spread of fungal infection (Droby *et al.*, 2002; Qin *et al.*, 2003).

Two signaling pathways have been described by Thaler *et al.* (1999), one involving salicylic acid (SA) and another involving jasmonic acid (JA), which participates in the expression of plant resistance to pathogens and insect herbivores. SA is thought to be a key compound in the regulation of resistance to fungal, bacterial and viral pathogens and provides a signal for expression of PR-proteins and other potential protective compounds (Ryals *et al.*, 1996). However, exogenous application of JA has been demonstrated to induce systemic acquired resistance (SAR) in plants by stimulating many of the systemic metabolites, similar to that which occurs from challenge with pathogens or insects (Kessmann *et al.*, 1994). The importance of the phytohormones SA and JA as critical signals in induced resistance response

in plants is recognized (Bostock, 1999). These signal molecules are involved in some signal transduction systems, which induce particular enzymes catalyzing biosynthetic reactions to form defense compounds such as polyphenols, alkaloids or pathogenesis-related (PR) proteins (Tamari *et al.*, 1995; Van Loon, 1995).

SA has been extensively used for quality improvement in a number of crops (Peng and Jiang, 2006). Research argued the role of phenolic compounds such as SA in physiological or biochemical processes including ion uptake, membrane permeability, enzyme activity, heat production, growth development (Arberg, 1981). SA significantly reduced the quality loss in peaches (Wang *et al.*, 2006), tomato (Ding *et al.*, 2001), sweet peppers (Fung *et al.*, 2004), and loquat fruits (Cai *et al.*, 2005). SA and its derivatives are widely used to enhance pre- and postharvest quality of fruit such as by controlling firmness of harvested peaches during storage, (Wang *et al.*, 2006; Li and Han 1999) and banana fruits during ripening (Srivastava and Dwivedi, 2000) Thus, SA has a remarkable ability to maintain the fruit quality during storage life of fruits.

Several natural volatile compounds, such as methyl jasmonate (MeJA), were reported to maintain fruit quality. For instance, MeJA reduced the development of chilling injury symptoms in mango (González-Aguilar *et al.*, 2000). Treatment of tomato fruit with low concentration of MeJA or methyl salicylic acid (MeSA) substantially enhanced their resistance to chilling injury and decreased the incidence of decay during low-temperature storage (Ding *et al.*, 2001; Wang *et al.*, 2006).

Phenolic compounds are secondary metabolites that have important contribution to plant-derived food quality as they affect fruits, appearance, flavour and health-promoting properties. Their content in foods is modulated by many factors that influence phenolic stability, biosynthesis and degradation. In their biosynthesis, the key step is catalyzed by the enzyme phenylalanine ammonia-lyase (PAL), which can be induced by various stress conditions (Dixon and Paiva, 1995; Hiratsuka *et al.*, 2001; Ju *et al.*, 1995).

Two vital antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT) play significant roles in scavenging free radicals produced as a result of metabolic processes. SOD converts OH<sup>-</sup> into H<sub>2</sub>O<sub>2</sub> which is converted into H<sub>2</sub>O and O<sub>2</sub> by catalase (CAT) (Sala, 1998; Wang *et al.*, 2008). Higher peroxidase (POD) activity resulted in lower browning incidence in treated peach fruits compared with control (Wills *et al.*, 1998).

Appearance, flavor, and freshness of a product can play a principal role in consumer's decision to purchase it and can influence perception by other senses. The overall quality

attributes that are important to packers, transporters, and retailers are often quite different than those of consumers (Watkins and Ekman, 2005). Although the trade ensures that consumers are presented with products of excellent appearance and at least acceptable texture, flavor is often ranked lower in importance by marketers (Watkins and Ekman, 2005). However, sensory quality is important to consumer satisfaction and influences further consumption. The task of maintaining and improving the sensory quality of fresh and fresh-cut products will probably be difficult. Pre- and postharvest treatments currently used to reduce or prevent pathological deterioration, or to maintain texture and color, can compromise sensory quality. So, in this study we thought that the assessment of the effect of these elicitors in apricot fruit storability and shelf life to be more effective and provide novel information has to involve studying the effect of these chemical in sensory parameter

As a consequence, a general requirement is to raise or at least to maintain the acceptable levels of qualities and chemical characters of apricot during a cold storage period and shelf-life.

The aims of this study were to investigate

- The effect of 0, 1, and 2 mmol L<sup>-1</sup> SA concentrations on 'Flavor Cot', 'Jumbo Cot', and 'Bergeron' apricot cultivars and to understand the mechanism of SA in enhancement the storability and fruit quality attributes. This experiment included the role of SA in pomological attributes of cold stored fruit, chilling injuries symptoms development, fruit decay percentage, total carotenoid content, ascorbic acid content, total soluble phenol content, antioxidant capacity, and membrane electrolyte leakage.
- The effect of 2 mmol L<sup>-1</sup> SA and/or 0.2 mmol L<sup>-1</sup> MeJA on various fruit quality limits of apricot fruit cultivar Bergarouge. This study included the effect of SA and/or MeJA on the fruit pomological attributes, sensory analysis, chilling injuries, fruit decay, chemical compound like ascorbic acid, carotenoid content, total soluble phenol, total antioxidant capacity and enzymes activities like, PAL, SOD, POD, and CAT.
- The effect of 2 mmol L<sup>-1</sup> SA and 0.4 mmol L<sup>-1</sup> MeJA on induced resistance to *Monilinia* laxa on apricot fruit cv. 'Bergarouge'. The study concerned the effects of 2 mmol L<sup>-1</sup> SA and 0.4 mmol L<sup>-1</sup> MeJA on mycelial growth, lesion diameter, disease incidence, fruit firmness, total solube phenol content, antioxidant capacity, lignin content, measuring some defense related enzymes activities like, SOD, POD, and PAL activities.

#### 2- REVIEW

# 2.1. Fruit quality and effects of SA and MeJA on fruit storability

#### 2.1.1 Apricot fruit quality and organoleptic characters

Numerous pomological traits studied apricot fruit quality charachters, such as fruit size, colour, taste, aroma and firmness (Souty *et al.*, 1991) as well as sugar and organic acid content and volatile compounds (Ruiz and Egea, 2008). Consumers interesting for fruit quality not only including attractive and tasty fruit, but they concerned for posses health promoting or disease preventing properties.

Apricot fruits contain significant levels of various phytochemicals such as vitamins, carotenoids and polyphenols, which contribute significantly to their taste, color and nutritive values. Fruit polyphenols content occupied most of scientist's intereting because of their antioxidant properties and ability to alleviate chronic diseases (Gardner *et al.*, 2000). The major phenolic compounds in apricot are chlorogenic and neochlorogenic acids, catechin, epicatechin, and rutin (or quercetin-3-rutinoside), which have a positive and highly significant relationship with the antioxidant capacity of apricots (Dragovic-Uzelac *et al.*, 2007).

SSC is one of quality characters which attract the cunsomers. Some authors reported that apricot accessions with SSC> 12 °Brix were characterised by an excellent gustative quality (Gurrieri *et al.*, 2001). Ruiz and Egea (2008) reported that SSC is a very important quality attribute, influencing notably the fruit taste. In addition, these values are generally lower than those for a group of Turkish genotypes (Asma and Ozturk, 2005; Asma *et al.*, 2007). It was reported that the differences between the apricot cultivars, SSC values were likely due to the different eco-geographical groups of apricot genotypes studied and the environmental conditions. Akin *et al.*, (2008) reported that malic acid was the predominat organic acid in apricot genotypes. The fruit maturity stage at the harvest date is the key factor affecting fruit acidity and also the SSC. Central Asian and Irano-Caucasian cultivars have lower acidity than European and Japanese cultivars (Mehlenbacher *et al.*, 1990, 1991).

Fruit harvest maturity stage affect the quality attributes, such as SSC, titratable acidity (TA), firmness, and shelf-life potential. Moreover, the relationship between SSC and TA has an important role in consumers' acceptance of some stone fruits such as apricot, peach,

nectarine, and plum cultivars. For the cultivars with TA> 0.9% and SSC <12%, the consumers' acceptance was controlled by SSC/TA ratio rather than SSC alone (Crisosto *et al.*, 2004).

Mehlenbacher *et al.*, (1990) reported that the greatest differences in the fruit chemical composition in apricot genotypes were observed with respect to the dry matter and SSC. Also, Dolenc-Šturm *et al.*, (1999) showed that the fruit taste is resulted from interaction between individual sugars and organic acid as well as their ratio.

Valdes *et al.*, (2009) reported that organic acids play an important role in fruit taste through sugar/acid ratio. Moreover, apricot quality consists of a balance of sugar and acidity as well as a strong apricot aroma (Hormaza *et al.*, 2007; Ishag *et al.*, 2009a,b). In generally, it may be concluded that the knowledge of the qualitative and quantitative compositions of acids and sugars in apricot fruits may prove to be a powerful tool in evaluating fruit maturity and quality (Dolenc-Šturm *et al.*, 1999).

#### 2.1.2 Effects of SA and/or MeJA on fruit pomological attributes

Weight loss is mainly regulated by respiration, transpiration and metabolic activities in fruits. Decreasing of weight loss of the fruit during the storage can manage by decreasing of respiration rate. It has been demonstrated that SA effectively reduces respiration rate in plants and harvested fruits in a concentration-dependent manner (Srivastava and Dwivedi, 2000). SA treatment delayed the ripening of banana fruits by reducing the metabolic activity and closed the stomata, fruit softening, pulp: peel ratio, reducing sugar content, respiration rate have been found to decrease in SA treated fruits as compared with control ones which reflexed on reducing fruit weight loss during the storage and prolong the fruit storability. Also, ability of closing the stomata during the stress was reported as method to make the fruit resistance to loss water content. SA has been reported to close stomata which results in suppressed respiration rate and minimized weight loss of 'Ponkan' mandarin fruit (Zheng and Zheng, 2004). Similarly, peach fruits cv. 'Delicia' treated with SA exhibited less weight loss than control (Abbasi *et al.*, 2010).

The role of abscisic acid ABA in inhibition of stomatal opening is thought by the increasing in the permeability of guard cells plasma memebrane to calcium ions. Ca <sup>2+</sup> entering the guard cells then acts as a second messenger to regulate the ion fluxes that lead to the loss of guard

cell turgor and therefore stomata close (MaAinsh *et al.*, 1990). SA thought to follow the same technique as caused the collapse of the transmembrane electrochemical potential of mitochondria and ATP dependent proton gradient of the tonoplast-enriched vesicles in pea (Manthe *et al.*, 1992). This report suggests that SA can induce stomatal closing by affecting ion fluxes and membrane permeability.

Tareen, *et al.*, (2012) found that SA at 2.0 mmol L<sup>-1</sup> concentration significantly exhibited less weight loss, higher flesh firmness, increased SSC, higher total acidity, higher skin luminosity and decreased (a\*) values compared other concentration and control treatments. They suggested that SA at 2.0 mmol L<sup>-1</sup> concentration could be used commercially to preserve peach fruits for up to five weeks without any spoilage.

Decrease in fruit metabolic activities leads to lower fruit water content, weight loss, carbohydrate depletion rate and consequently it delays fruit senescence effectively (Wills *et al.*, 1998). Fattahi *et al.*, (2010) studied the physicochemical properties of fruits including weight losses, skin and flesh colour, firmness, total soluble solids (TSS), titratable acidity (TA), pH and sensory properties were monitored during storage. The results showed that fruit weight loss significantly decreased in all SA treatments dipping time in comparison to control. Dipped fruits in SA solution for 5, 7.5 and 10 minutes had higher firmness and lower TSS than 3 and 5 min dipping time and control. Harvested apricot fruit start to loss the firmness and softening develop during the storage time (Ezzat *et al.*, 2012). SA has been documented to enhance flesh firmness of harvested peaches during storage (Wang *et al.*, 2006; Li and Han, 1999), and that of banana fruits during ripening (Srivastava and Dwivedi, 2000). In bananas, SA treatment has been found to delay the ripening of banana fruits (*Musa acuminate* L.) which was attributed to the decrease in the activities of major cell wall-degrading enzymes including cellulase, polygalacturonase, and xylanase (Srivastava and Dwivedi, 2000).

The mechanism by which SA and MeJA may affect the cell wall structure and thus maintain fruit firmness is not yet clear, and no research has been carried out in apricot in regard to investegate these compound modes of action.

Fruit softening is associated with cell wall disassembly, and modifications to the pectin fraction due to pectine methylesterase (PME) activity are some of the most apparent changes that take place in the cell wall during ripening (Marin-Rodriguez *et al.*, 2002). Hence, the control of PME activity has been a subject of interest because of its activity in fruits. It has

been suggested that MeJA reduces PME activity to decrease de-esterification of pectin (Meng *et al.*, 2009), and hence maintains peach fruit texture.

Aghdam, *et al.*, (2009) found the application of exogenous MeSA vapor on kiwifruits led to prevent the softening process of fruit flesh, kept ascorbic acid content and firmness during 5 months storage. they reported that the 32 μl MeSA treatment caused the highest fruit firmness and ascorbic acid content at all determination times. Fruit treated with 24 and 32 μl MeSA showed the lowest pH and SSC, respectively. Total acidity of the fruits was not significantly affected by the use of MeSA. The same results were reported by González-Aguilar *et al.*, (2003a) in papaya fruit, the exposured of papaya (*Carica papaya* L., cv. Sunrise) fruit to MeJA vapors (10<sup>-5</sup> or 10<sup>-4</sup> M) for 16h at 20 °C inhibited loss of firmness during storage for 14 / 32 days at 10 °C and 4 days shelf-life at 20 °C. MeJA-treated fruit also retained higher organic acids than the control fruit.

MeSA treatments were highly effective in reducing ethylene production, fungal decay weight loss and TSS in Hayward kiwifruit, as well as ascorbic acid (AA) and flesh firmness loss compared with that observed in control fruit (Aghdam *et al.*, 2011).

Wang et al. (2006) immersed peach in SA solution for 5 min, stored at 0°C for 28 days, then moved to 20°C for 3 days to simulate shelf-life. Firmness of fruit was measured at the end of shelf-life. The results showed that the concentration of 1 mmol L<sup>-1</sup> of SA significantly maintained higher firmness.

A small increase in SSC during storage and/or shelf-life has been reported previously for apricots (Basile *et al.*, 1999). SSC and soluble sugars may increase during fruit ripening due to the action of sucrose-phosphate synthase (SPS), a key enzyme in sucrose biosynthesis (Hubbard *et al.*, 1991). This enzyme is activated by the ripening process, ethylene, and cool storage (Langenkämper *et al.*, 1998). Recently, an increase in SPS and invertase activities and a decrease in sucrose synthase activity have been reported during ripening of some fruits (Cordenunsi and Lajolo, 1995). Aghdam et al. (2011) treatmented kiwifruits with MeSA and the results showed maintained a lower content of TSS than the control fruits at the end of cold storage.

Cell walls contain large amounts of polysaccharides, mainly pectins and cellulose, and are digested due to the activity of the cell wall degrading enzymes leading to a significant increase in SSC content. SA effectively protects cell walls by decreasing the expression of

degrading enzymes and as a consequence prevents from dramatic increase in SSC content of the cells (Asgharia and Aghdam, 2010).

Jasmonic acid, have increased the sugar content of treated fruit. Raspberry fruits treated with MeJA had higher SSC content than the control fruits. Treated fruits recorded higher levels of sucrose, fructose and glucose than the control fruits (Wang and Zheng, 2005). A combined treatment of MeJA and ethanol also increased the SSC in strawberries (Ayala-Zavala *et al.*, 2005). Low respiration may be the cause of high sugar contents in treated fruits, while high metabolism in control fruits may justify the depletion of carbohydrate contents (Wang and Zheng, 2005).

#### 2.1.3 Ethylene production and delay the fruit ripening

Apricot is a climacteric fruit characterized by a peak in ethylene production near the ripe stage. In higher plants, ethylene is synthesized from the amino acid methionine by the conversion to S-adenosyl-L-methionine (SAM). SAM is transformed to 1aminocyclopropane-1-carboxylic-acid (ACC) by the enzyme ACC synthase (ACS), and in the last step of the pathway, ACC is oxidized to ethylene by the action of the enzyme ACC oxidase (ACO) (Lin et al., 2009). During the ripening of climacteric fruit, ethylene can stimulate its own production, inducing autocatalysis through ACS and ACO activity (Lelièvre et al., 1997). Many reports postulated the inhibition role of SA on ethylene production. Huang, et al. (1993) reported that SA effectively inhibited ethylene production within 2 h of its application. It inhibited the conversion of ACC to ethylene; they reported that the inhibitory effect of SA resulted from the inhibition of both synthesis of ACC and the conversion of ACC to ethylene.

Ethylene plays a key role in fruit ripening and senescence. This hormone triggers the induction of cell wall hydrolyzing enzymes leading to increase in respiration rate, fruit softening and senescence (Wills *et al.*, 1998). It was also documented that MeSA reduced the ethylene production and delayed ripening of kiwifruit and may help to decrease SPS enzyme activity leading to lowered sucrose synthesis and SSC content (Aghdam *et al.*, 2011). SA and MeSA are known as inhibitors of ethylene biosynthesis (Leslie and Romani, 1986). Zhang *et al.*, (2003) reported, application of SA on kiwifruit increased superoxide free radical and lipoxygenase (LOX) activity. In that case, climacteric rise in ethylene production was

retarded. So, fruit ripening and senescence were delayed. SA reported to inhibit wound induced transcription of ACC synthase expression and activity in tomato fruit (Li *et al.*, 1992). SA can delay the ripening of banana fruit, probably through inhibition of ethylene biosynthesis or action (Srivastava and Dwivedi, 2000), inhibit ethylene production in cultured pear cells (Leslie and Romani, 1986).

Fan *et al.*, (1996) demonstrated the inhibitory action of SA on ACC oxidase activity in apple fruit disks. Babalar *et al.*, (2007) reported that 2 mmol l<sup>-1</sup> SA significantly reduced ethylene production and fungal decay and retained overall quality of cv. Selva strawberry fruit.

By the contrast, exogenously applied MeJA increased ethylene biosynthesis in several plants. It enhanced flower senescence through the elevation of ethylene production in Petunia and Dendrobium (Porat and Halevy, 1993). It stimulated ethylene production in immature tomato mesophyll cells (Saniewski *et al.*, 1987) and increased ACC oxidase activity in rice (Chou and Kao, 1992).

MeJA slightly stimulates ethylene production and greatly stimulates the synthesis or activity of the ethylene-forming enzyme (EFE), the authors suggested that both the EFE system and ACC synthase are activated under the influence of MeJA. MeJA stimulates ethylene production in ripe and overripe tomatoes (Saniewski *et al.*, 1987). Different results were reported for MeJA and apple fruit, Nowacki *et al.*, (1990) reported that MeJA inhibited ethylene production in postclimacteric fruits of tested cultivars. It also inhibited ethylene-forming enzyme activity in climacteric fruits of Jonathan, but had little or no such effect in cv. McIntosh fruits.

#### 2.1.4 Sensory analysis

Sensory quality attributes and chemical content of apricot (*Prunus armeniaca* L.) fruits as well as of other fruits (Hudina and Stampar, 2000) play an important role in consumer satisfaction and decision to buy the same products again or no. So, influence further consumption. The quality of fruits can be determined by chemical analyses and mainly depends on major compounds such as sucrose, citric acid and malic acid, as well as on carotenoids, polyphenols and pectic substances. So, what can sensory analyses offer for fruit producer and customers?

It identifies the presence of notable differences, identifies and quantifies important sensory characteristics in a fast way, and identifies specific problems that cannot be detected by other analytical procedures, as consumer preference, (Nakayama and Wessman, 1979). Deciding the appropriate harvest date for fruits, which is very difficult to define, is also a feature of those methods (Sturm *et al.*, 2003). Taste, aroma, texture, colour and appearance are generally considered to be among the most important sensory attributes. Taste is related to water-soluble compounds. Sweetness is mostly attributable to mono- and disaccharides rather than to other compounds. Sour tastes are reliably linked up with organic acids and pH. Aroma is elicited by compounds which exhibit some volatility (Sturm *et al.*, 2003). The amounts of sugars and organic acids and their ratios have been correlated with some of the sensory properties of fruits (Colaric et al., 2005). Obenland *et al.*, 2009 reported that sugar/acid ration is an important factor in determining consumer acceptance.

The degradation of structural polysaccharides and carbohydrates into other simple compounds was reported as a reason reduction of the fruit taste at later stage during storage (Kays, 1991) and these bioprocesses are correlated to fruit over ripening. The reduction in fruit texture might be due to degradation of pectin substance and maximum change may be attributing to minimum texture score as reported for control as documented by Wills *et al.*, (1989). It has been documented that MeJA reduces pectin-methyl-esterase (PME) activity to decrease deesterification of pectin (Meng *et al.*, 2009), and hence maintains fruit texture. SA has been documented to enhance flesh firmness of harvested peaches during storage (Wang *et al.*, 2006).

# 2.2. Chilling injuries (CI) as an abiotic stress

Chilling damage to fresh produce during postharvest storage is acting dangerous for the economic importance. Storage at temperatures low enough to retard tissue respiration is still the most effective postharvest method for extending the shelf-life of produce and from the other side most of fruit which store in this way present the CI symptoms which reduce the fruit quality and is considered as big loss for the harvested fruit.

The time taken for symptoms to develop varies greatly and is influenced by a number of factors including genotype, cultivar, stage of maturity and pre-harvest growth conditions. For example, with fruit stored at 1-2°C, it takes several months for CI to develop in apples as a

brown discolouration of the cortex, several weeks for the flesh of peaches to become mealy in texture, a number of days for avocados to show areas of grey discolouration in the flesh, and only a few hours for cucumbers to display tissue breakdown in the mesocarp. CI is a type of damage and stress caused as a result of oxidative burst. The intact plants under the stress (biotic or abiotic) have to develop a broad range of defence responses to cope with that stress.

#### 2.2.1 Oxidative damage and chilling injuries

Exposure of fruit to chilling temperature results in excessive generation of reactive oxygen species (ROS) in the cell compartments (Maruthasalam *et al.*, 2010). Increase of ROS concentration bring a real threat to cells by causing lipid peroxidation, protein oxidation, enzyme inhibition and damage to DNA resulting in cell death (Mittler, 2002; Yang et al., 2010; Sharma *et al.*, 2012). This process is known as oxidative damage. Studies by Mittler *et al.*, (2004) and Karuppanapandian *et al.*, (2011) showed that ROS can cause irreversible membrane damage resulting in cell death and CI symptoms detection. However, the extent to which chilling conditions may lead to oxidative damage will depend on the degree of severity and the duration of exposure (Maruthasalam *et al.*, 2010).

#### 2.2.2 Effects of SA and/or MeJA in alleviating CI

Antioxidant capacity play vital roles in protecting the plant tissue from the stresses but it may not be sufficient to mitigate oxidative damage caused by ROS under chilling conditions (Huang *et al.*, 2008). If the chilling duration is extended, the defence system may be easily failed by excess ROS, and the fruit failed to keep away from the CI symptoms, and sever damage can be exhibited (Zhang *et al.*, 2008) leading to CI. Most of post-harvest treatments have direct or indirect role in alter and enhance the fruit antioxidant system and saving fruit quality during storage and activate other metabolic processes enabling fruit to withstand chilling conditions (Huang *et al.*, 2008).

Although, there are many methods to reduce CI in various horticultural crops. SA and MeSA treatments are inexpensive, easy to set up and applicable to various horticultural crops (Ding *et al.*, 2001). In response to abiotic stresses like cold storage, plant cells start to synthesis new proteins, as plants respond to high temperatures with the synthesis of a group of proteins known as heat shock proteins (HSPs). Often the accumulation of HSPs not only confers

protection against the stress that causes their biosynthesis but also against any other subsequent stress situation like pathogen infection or fruit wounds (Tian *et al.*, 2007).

As a hormone which plays a role in plants stress, SA has been associated with chilling tolerance in horticultural crops. Treatment with SA reduced CI in tomato (Ding et al., 2001), pomegranate (Sayyari *et al.*, 2009), cucumber (Cao *et al.*, 2009), pineapple (Lu *et al.*, 2010).

MeJA could enhance resistance to CI depends on the concentration of the treatment(González-Aguilar *et al.*, 2003). When applied at postharvest, MEJA significantly reduced CI in tomato (Ding et al., 2002), peach (Feng *et al.*, 2003), and guava (González-Aguilar *et al.*, 2000). Similarly results were reported by Wang and Buta (1994) on zucchini squash fruit treated with MeJA prior to cold storage at 5°C.

Ding *et al.*, (2001) in them studies on tomatoes, they treated the fruit with methyl jasmonate (MeJA) and methyl salicylic acid (MeSA) prior to low-temperature storage they found that these treatments induce HSPs biosynthesis and, at the same time, CI tolerance in tomatoes. Also, they reported the same results for peaches (Ding *et al.*, 2002). Accumulation of the HSPs in chilling-sensitive horticultural products with SA and MeJA treatments would allow their storage at low temperatures without CI development.

Fung et al., (2004) reported that the expression patterns of alternative oxidase (AOX) and seven other genes involved in defense against oxidative stress before and during the early chilling period suggested that pre-treatment of pepper fruit with MeSA or MeJA vapors increased preferentially the transcript levels of AOX. Overnight treatment with MeSA or MeJA vapors increased transcript levels of AOX (1.5 kb) even at room temperature of 25 °C, whereas no change was observed with untreated control. Banana fruit stored for 1 week at 5°C were protected from chilling injury by 1 mM n-propyl dihydrojasmonate (PDJ) (Chaiprasart et al., 2002). MeJA reduced decay in three strawberry cultivars stored at 5°C or 10°C (Moline et al., 1997) and delayed chilling injury in zucchini squash (Cucurbita pepo) and cucumber fruit (Wang and Buta, 1994; 1999). Droby et al., (1999) found that postharvest application of JA reduced fruit decay after and also, effectively reduced chilling injury incidence after cold storage. González- Aguilar et al., 2000 aimed to reducing the chilling injury, they tested 10<sup>-4</sup> and 10<sup>-5</sup>M of MeJA treatment before the storage of red and white cultivars of guava fruits at 5 °C for up to 15 days plus two days at 20 °C. they found that methyl jasmonate treatments reduce the chilling injury index and the ion leakage percentage. Jin et al., (2006) demonstrated that peach fruit treated with volatile MeJA showed slower decay and higher quality than untreated fruit 8 days after treatment. Strawberries stored for 12 days at 7.5°C had higher quality and were protected from decay by 100 mmol MeJA vapour (Ayala-Zavala *et al.*, 2005).

### 2.3. Pathogen infection as a biotic stress

Many reports have shown that induced disease resistance in plants by biotic and abiotic elicitors is a very effective method for restricting the spread of fungal infection (Droby *et al.*, 2002). Resistance of plants to pathogens is based on both constitutive defense mechanisms such as pre-existing antimicrobial compounds and inducible defense mechanisms. Induced disease resistance in plants by biotic or abiotic treatments is a very attractive strategy for controlling diseases (Droby *et al.*, 2002; Qin *et al.*, 2003).

### 2.3.1 Effects of SA and/or MeJA on reducing the pathogen infection

Many reports have shown that induced disease resistance in plants by biotic and abiotic elicitors is a very effective method for restricting the spread of fungal infection. The signal molecules salicylic acid (SA), jasmonic acid (JA) and methyl jasmonate (MeJA) are endogenous plant growth substances that play key roles in plant growth and development, and responses to environmental stresses. These signal molecules are involved in some signal transduction systems, which induce particular enzymes catalyzing biosynthetic reactions to form defense compounds such as polyphenols, alkaloids or pathogenesis-related (PR) proteins (Tamari *et al.*, 1995; Van Loon, 1995).

This can result in induction of defense responses and provide protection for plants from pathogen-attack (Delaney *et al.*, 1994; Kozlowski *et al.*, 1999). Such signalling molecules, when exogenously applied, have been shown to move systemically through plants, resulting in the expression of a set of defense genes that are activated by pathogen infection, thus inducing resistance against pathogens (Epple *et al.*, 1997; Kozlowski *et al.*, 1999).

Recently, MeJA has shown promise in preventing postharvest disease and disorders in horticultural crops. Application of MeJA effectively suppressed gray mold rot caused by *Botrytis cinerea* in strawberry, cut freesia and rose flowers. Darras *et al.*, (2005) treated the cut freesia flowers with methyl jasmonate vapour and they reported that the treatment

suppressed petal specking caused by *Botrytis cinerea* infection. MeJA efficacy was concentration and incubation temperature dependent. Disease severity, lesion numbers and lesion diameters decreased with increasing MeJA concentration. It was demonstrated that Jasmonate activates genes involved in pathogen and insect resistance, and genes encoding vegetative storage proteins (Creelman *et al.*, 1997). Postharvest green mold decay of grapefruit caused by Penicillium digitatum was reduced by exposure of the fruit to MeJA vapor (Droby *et al.*, 1999) MeJA dipping of grapefruit seems to provide systemic protection against the green mold rot, *P. digitatum*, by eliciting resistance responses in the treated fruit.

It has been reported that MeJA treatment could effectively suppress post-harvest diseases of various fruits including sweet cherry (Yao and Tain, 2005a), loquat (Cao *et al.*, 2008), peach (Yao and Tain, 2005b), and grapefruit (Droby *et al.*, 1999).

Yao and Tain (2005a) reported pre-harvest treatments with 2mM SA and 0.2mM MeJA significantly reduced lesion diameters on sweet cherry fruit caused by Monilinia fructicola compared with control post-harvest treatments.

Cao et al. (2008) reported treatment of loquat fruit with MeJA resulted in significantly lower disease incidence and smaller lesion diameters than in control fruit and in them in vitro experiment they postulated that MeJA significantly inhibited spore germination, germ tube elongation and mycelial growth of *C. acutatum*. These results suggest that MeJA treatment can effectively inhibit anthracnose rot caused by *C. acutatum* in postharvest loquat fruit. It is postulated that the control of the disease is directly because of the inhibitory effect of MeJA on pathogen growth, and indirectly because of the induced disease resistance triggered by +

#### 2.4. Mechanisms of stress control

Plants have the ability to create and/or develop elaborate defense mechanisms for protect themselves against different stresses like attack of pathogen and /or in appropriate environmental conditions. one of suggested mechanism include production of reactive oxygen species (ROS) such as singlet oxygen  $(O_2^*)$ , superoxide  $(O_2^-)$ , hydrogen peroxide  $(H_2O_2)$  and hydroxyl radical (OH) and in the same time the high concentration of these molecules have negative effects on the normal metabolism by oxidizing nucleic acids, lipids, proteins, and carbohydrates resulted in losing the cell membrane integrity and alleviating most of cellular function (Campo *et al.*, 2004). Other suggested mechanism to prevent injuries, plant cell

synthesizes some secondary metabolic compound (lignans, carotenoids, ascorbate, glutathione, among others) and some enzymes (SOD, CAT, POD, and PPO). These enzymes convert ROS into less toxic products. For that it is believed that producing ROS is important as it works as signal molecules in the cell which activate the production of antioxidant molecules. Therefore, the importance of cellular equilibrium between the antioxidant system and the levels of ROS must be highlighted. (Reference is missing here)

#### 2.4.1 The roles of some elicitors in alleviating the stress

Originally elicitors, in biology, are compounds that when introduced into living organisms signal the activation or synthesis of another compound. Effectors differ from hormones, compounds produced in one part of an organism to cause a change in another part of that organism, in that they do not have to be produced within the organism that they are eliciting a response in, and are usually not naturally occurring in the organism (Ebel and Cosio, 1994, Hahn, 1996, Nürnberger, 2009 and Boller,199). Eventually, the induction of defense responses may lead to enhanced resistance. This broader definition of elicitors includes both substances of pathogen origin (exogenous elicitors) and compounds released from plants by the action of the pathogen (endogenous elicitors). Elicitors are classified as physical or chemical, biotic or abiotic, and complex or defined depending on their origin and molecular structure (Table 1).

Some natural compounds treatments, which have been proved as elicitors to activate the defense system in fruit and postharvest stress-type treatments like cold storage, have been developed to preserve fruits (Fig. 1). These treatments can activate some enzymatic and/or non-enzymatic antioxidant systems of the fresh produce, therefore contributing to an adaptation process to stressful conditions and subsequently the maintenance of fruit quality and better antioxidant potential (Lim, and Tee, 2007).

Table 1 List of some elicitors' applications and their effects on different plant species.

No.	Plant	Type of elicitor used	Effects	References
1	Brassica napus	Methyl jasmonate	Accumulation of indolyl glucosinolates in the leaves. The predominant components of the response were 3-indolylmethyl- and 1-methoxy-3-indolylmethylglucosinolates, which together comprised 90% of the total glucosinolates in treated leaves.	[Doughty <i>et al.</i> , 1995]
2	Citrus sinensis	β-amino butyric acid	Inhibited Penicillium italicum spore germination and germ tube elongation in vitro. Involved in the induced resistance against <i>Penicillium italicum</i> .	[Tavallali <i>et al.</i> , 2008]
3	Solanum melongena	Salicylic acid, chitosan, methyl salicylate, and methyl jasmonate	Increased lignin deposition in cell walls of roots, accumulation of phenolics, increase in the activity of enzymes PAL, POD, polyphenol oxidase, cinnamyl alcohol dehydrogenase, and catalase. Provided resistance against Ralstonia solanacearum.	[Mandal, 2010]
4	Phaseolus vulgaris	Salicylic acid and methyl jasmonate	Controlled spider mite infestation, improved plant growth and bean yield.	[Farouk and Osman, 2011]
5	Brassica spp	Salicylic acid	Recovery from heat stress, increased seedling length, reduced electrolyte leakage, and enhanced membrane protection. Increased level of total soluble sugars, fresh/dry weight, and enzymatic activities of invertase, catalase, and peroxidase conferred thermotolerance. Enhanced expression of some new proteins including heat shock proteins (HSPs) was also observed.	[Kaur <i>et al.</i> , 2009]

Source: Thakur and Singh (2013)

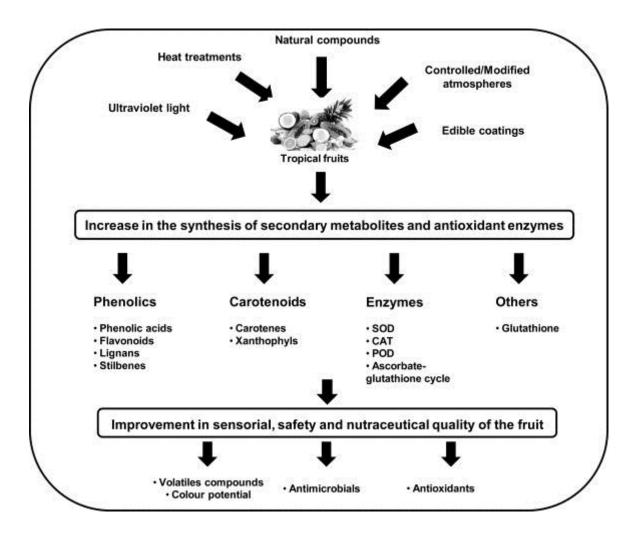


Fig. 1 Model of control postharvest stress after applied ultraviolet light, heat treatment, natural compound, modified atmosphere packages and edible coatings activate some enzymatic and/or non-enzymatic antioxidant systems of the fresh produce. Source: Gonzalez-Aguilar et al. (2010).

#### 2.4.2 Antioxidant defence mechanism induced by SA and/or MeJA

It has been proven that free radicals play an important role in many diseases, such as cardiovascular diseases, cancer, neurodegenerative diseases, diabetes and ageing (Riboli and Norat, 2003). Antioxidants have attracted more and more attention as potential agents for preventing and treating oxidative stress-related diseases. Antioxidants are compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. There are two basic categories of antioxidants, namely, synthetic and natural. In general, synthetic antioxidants are compounds with phenolic structures of various degrees of alkyl substitution, whereas natural antioxidants can be phenolic compounds (tocopherols, flavonoids, and phenolic acids), nitrogen compounds (alkaloids,

chlorophyll derivatives, amino acids, and amines), or carotenoids as well as ascorbic acid (Larson, 1988; Hudson, 1990; Hall and Cuppett, 1997). Owing to consumer preferences, internal fruit quality is becoming a priority goal, including nutritional quality, the content of health-related compounds (Zhebentyayeva *et al.*, 2012). Apricot fruit contains three major types of antioxidant molecules: water-soluble vitamin C, lipid-soluble carotenoids and polyphenolics comprising both hydro- and lipophilic components (Hegedűs *et al.*, 2011; Ruiz *et al.*, 2005).

#### 2.4.2.1 Non- enzymatic defence mechanisms

# 2.4.2.1.1 Phenolic compounds

Soleckaand and Kacperska (2003), reported that phenolic compounds are naturally occurring substances in plants, many of which are thought to play physiological roles such as antibacterial, antiviral, anticancer agents and scavengers of most types of oxidizing molecules. Phenolic compounds are ubiquitous in all plant organs and are, therefore, an integral part of the human diet. Interest in food phenolics has recently increased greatly because of the antioxidant and free radical scavenging abilities, associated with some phenolic compounds and their potential effects on human health (Bravo, 1998). Wang et al., 2009 reported that fruit treated with 10 µmol L<sup>-1</sup> MeJA exhibited significantly higher levels of total phenolics, flavonoids, and anthocyanins as well as individual phenolic compounds than control. These fruits also maintained significantly higher antioxidant activity as measured by scavenging capacity against 1,1-diphenyl-2-picrylhydrazyl, superoxide, and hydroxyl radicals and by the reducing power test compared to the control. These results indicate that MeJA can effectively reduce fruit decay and improve antioxidant capacity of Chinese bayberry fruit. In addition, it has been reported that a postharvest MeJA treatment maintained higher levels of bioactive compounds and enhanced antioxidant capacity in berry fruits including blackberries, raspberries, and strawberries (Chanjirakul et al., 2008; Wang et al., 2008)

Chanjirakul *et al.* (2008) demonstrated that MeJA might increase the resistance of tissues against decay through enhancing their antioxidant and their free radical scavenging capacities. Because MeJA is already classified by the U.S. Food and Drug Administration as a Generally Recognized as Safe (GRAS) substance, it may have potential commercial Applications in postharvest treatments for quality maintenance by reducing decay and enhancing antioxidant activity.

Exogenous application of SA at nontoxic concentrations to susceptible fruits and vegetables could enhance resistance to pathogens and control postharvest decay (Asgharia and Aghdam, 2010). Wang *et al.*, (2006) reported that treatment with SA was effective in alleviating chilling injury of peach fruit. These authors suggested that the effect of SA on alleviating chilling injury of peaches during cold storage may be attributed to its ability to induce antioxidant systems and heat shock proteins (HSPs).

MeJA has been shown to induce the synthesis of some stress-related proteins including heat-shock and pathogenesis-related proteins, which leads to an increased resistance level and results in decreased incidence of the decay (Ding *et al.*, 2001; 2002). SA also exhibits direct antifungal effects against pathogens. SA in a concentration of 2 mmol L<sup>-1</sup> showed direct toxicity on Monilinia fructicola and significantly inhibited the growth of mycelia and spore germination of the pathogen in vitro (Yao and Tian, 2005a).

#### 2.4.2.1.2 Carotenoids content

Carotenoids play a vital role in acquisition of oxidative stress tolerance (Karuppanapandian *et al.*, 2011). As antioxidants, carotenoids have been reported to detoxify ROS (Young, 1991) and serve as precursors to signalling molecules that influence environmental stress responses (Li *et al.*, 2008).

MeJA plays key roles in regulating a great diversity of physiological and biochemical processes in plants including stimulating the biosynthesis of secondary metabolites (Creeman and Mullet, 1997). MeJA has been shown to induce stilbene accumulation in leaves and berries of grapevine plants (Larrondo *et al.*, 2003) and increase the accumulation of carotenoids contents and phenolics in apples (Rudell and Mattheis 2002), raspberries (Wang and Zheng, 2005), strawberries (Ayala-Zavala *et al.*, 2005), and blackberries (Wang *et al.*, 2008).

Hayat *et al.*, (2005) reported SA applied may lower the level of oxidative stress in plants, which acts as a hardening process, improving the antioxidative capacity of the plants and helping to induce the synthesis of protective compounds (such as carotenoids) also, they reported that SA enhance the level of carotenoid is these are the role assigned to SA.

#### 2.4.2.1.3 Ascorbic acid

Ascorbic acid is one of the most abundant and potent antioxidants in plants (Davey *et al.*, 2000; Ioannidi *et al.*, 2009). Beside its role in protecting the plant tissues against oxidative damage caused by ROS, also ascorbic acid serves as an enzyme co-factor for induced

resistance (Badejo *et al.*, 2009). It is also involved in plant stress resistance by acting as a signalling compound and scavenging ROS *via* the APX reaction (Ioannidi *et al.*, 2009; Badejo *et al.*, 2009). Understanding the role of ascorbic acid in fruit physiology can provide chances to alter its concentration in fruit and more and thereby potentially minimise postharvest losses (Ioannidi *et al.*, 2009).

It is reported that the vitamin C content of apricot gradually increased through the ripening stages (Hegedüs *et al.*, 2011). Aghdam *et al.*, (2009) application of exogenous methyl salicylic acid (MeSA) vapor on kiwifruits led to prevent the softening process of fruit flesh, kept ascorbic acid content and firmness during 5 months storage. The use of MeSA also prevented the softening of fruit flesh and degrees of ascorbic acid content of fruit during the storage and kept their firmness and ascorbic acid content, so that the 32 µl MeSA treatment caused the highest fruit firmness and ascorbic acid content at all determination times.

#### 2.4.2.1.4 Lignin content

Lignin is a complex polymer of phenylpropanoid mainly deposited in cell walls (Whetten and Sederoff, 1995), and synthesis is induced by both mechanical wounding and microorganisms (Uritani and Oba, 1978; Vance and Krik, 1980). The final step in lignin biosynthesis requires oxygen for the oxidation of manomeric lignin precursors such as p-courmaryl, coniferyl and sinapyl alcohols to form polymers through the action of POD (Whetten and Sederoff, 1995).

ROS and specially H<sub>2</sub>O<sub>2</sub> can prevent micro-organism penetration in plant tissues because it contributes to wall stiffening by activate POD reactions catalysing molecular crosslinks between structural components of cell walls and lignin polymerisation. The consequent increase in mechanical barriers also slows down pathogen penetration allowing plant cells to arrange defences that require more time to be activated (De Gara *et al.*, 2003)

Peroxidases are ionically bound to the cell walls and are involved in the polymerization of phenylpropanoid lignin precursors (Cvikrová *et al.*, 2006). Oxidation of peroxidases makes the cell wall more mechanically rigid by cross-linking matrix polysaccharide and glycoprotein molecules, thus modifying the mechanical properties of the cell wall. In general, cross linking of matrix polysaccharides in cell walls are also likely to inhibit cell wall degrading enzymes of the pathogen. Some researchers were suggesting that SA or/MeJA prevent fruit from softening by affecting on lignin's biosynthesis enzymes such as PAL and POD (Yang *et al.*, 2011). Su *et al.*, (2003) reported that MeJA treatment significantly increased PAL and POD activities and lignin content, which might account for higher disease resistance and lower decay index in MeJA treated vegetable soybean pods.

#### 2.4.2.2 Enzymatic defence mechanisms

Superoxide dismutase (SOD) is the first line of defence from potential damages that may be caused by ROS. It is present in all aerobic organisms and in cellular compartments that generate ROS (Mittler, 2002). The SOD enzyme has important roles in detoxifying ROS and alleviating chilling injury (Sala 1998; Wang *et al.* 2008). SOD can protect cells from oxidant stress by dissimulating super oxide anion (O<sub>2•</sub> ) to oxygen and H<sub>2</sub>O<sub>2</sub> (Chittoor *et al.*, 1999). Increased activity of SOD is often correlated with increased tolerance of plants against environmental stresses and enhanced oxidative stress tolerance in crops (Sharma *et al.*, 2012). Sala (1998) observed that SOD activity was high in chilling tolerant mandarin fruit during cold storage at 2.5°C for 8 weeks. Catalase (CAT) is a common enzyme found in nearly all living organisms exposed to oxygen (such as vegetables, fruit or animals). It catalyzes the decomposition of hydrogen peroxide to water and oxygen (Chelikani *et al.*, 2004). It is a very important enzyme in protecting the cell from oxidative damage by ROS. Likewise, catalase has one of the highest turnover numbers of all enzymes; one catalase molecule can convert millions of molecules of hydrogen peroxide to water and oxygen each second (Goodsell, 2004).

PAL is a crucial enzyme in the phenylpropanoid pathway, catalyzing the formation of transcinnamic acid via the L-deamination of phenylalanine. Cinnamyl alcohol dehydrogenases catalyse the reversible conversion of p-hydroxycinnamaldehydes to their corresponding alcohols, leading to the biosynthesis of lignin in plants (Blanco *et al.*, 2003). POD are ionically bound to the cell walls and are involved in the polymerization of phenylpropanoid lignin precursors. Oxidation of peroxidases makes the cell wall more mechanically rigid by cross-linking matrix polysaccharide and glycoprotein molecules, thus modifying the mechanical properties of the cell wall (Cvikrová *et al.*, 2006).

When a hormetic dose of biotic or abiotic elicitors is absorbed by cell membrane, it can interact with atoms and molecules, mainly water, producing ROS by the univalent reduction of  $O_2$  in a rapid and controlled manner (Fig. 2). It is postulated that  $O_2$ • is the primary ROS formed in the cell and it works as activator or signal which triggers a cascade of reactions that results in the formation of a variety of ROS and induction of antioxidant enzymes such as SOD, CAT, POD, monodehydroascorbate reductase (MDAR), glutathione (GSH) and oxidized glutathione (GSSG) to keep the equilibrium in the redox reactions in the cell (Gonzalez-Aguilar *et al.*, 2010; Fig. 2).

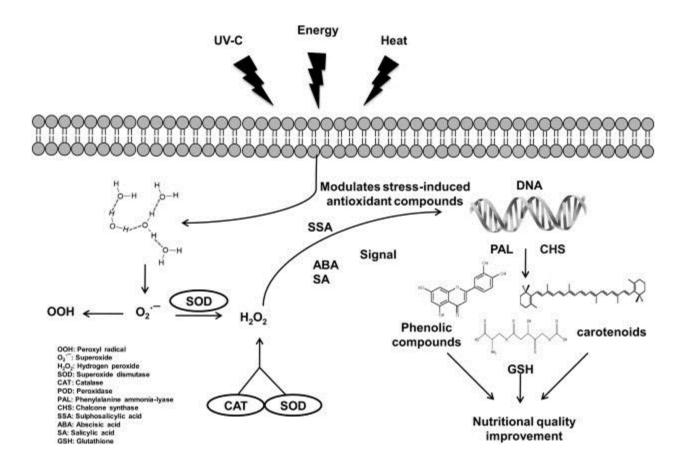


Fig. 2 Model representing possible induction of some plant hormones, secondary metabolites and antioxidant enzymes induced by stress. Source Gonzalez-Aguilar *et al.*,(2010).

SOD produces  $H_2O_2$  which is a key of ROS. It works for many cellular bioprocess like cross-tolerance, hormonal activity and gene expression (Nyathi and Baker, 2006). this supported for the theory that  $H_2O_2$  may be responsible for the improvement of antioxidant status of fruits activating gene expression of enzymes related with the synthesis and accumulation of secondary metabolites with antioxidant capacity (phenolic acids and flavonoids) (Nyathi and Baker, 2006).

Colletotrichum acutatum infection in loquat fruit was inhibited by MejA. MeJA treatment significantly inhibited activities of CAT while SOD activity was not significantly affected in early infection, thus resulting in a higher level of  $H_2O_2$  in the earlier period of incubation. The enhanced  $H_2O_2$  generation by MeJA treatment might serve as a signal to induce resistance against *C. acutatum* infection (Cao *et al.*, 2008). MeJA treatment could enhance the plant resistance to infection by highly accumulation of  $H_2O_2$  in the cells which could contribute to enhancement of disease resistance as reported by (Inze and Motagu, 1995).

Meanwhile, MeJA was also reported to decrease membrane lipid peroxidation and maintain high SOD and CAT activity in strawberry plants under water stress (Wang, 1999). Also, Peng *et al.* (2009) postulated the level of H<sub>2</sub>O<sub>2</sub> in peach fruit was significantly enhanced by MeJA and in the same time the activities of SOD and CAT were enhanced.

Chan and Tian (2006) reported that SA-treatment significantly inhibited CAT activity, but stimulated SOD and POD activities after inoculation of sweet cherry fruit with P. expansum. The same results were documented by Zeng *et al.*, (2006), found that SA treatment increased H<sub>2</sub>O<sub>2</sub> and O<sup>-2</sup> contents with low activities of CAT and enhanced resistance to anthracnose rot of mango fruit. By contrast, SA has been reported to enhance the transcription and translation of the CAT gene in treated peach fruit (Tian *et al.*, 2007). Researchers have also showed that sugar apple fruit treated with SA had increased CAT activity (Mo *et al.*, 2008).

Yao et al., (2005a), reported that pre-harvest treatment of sweet cherry with SA or MeJA induced 1,3-glucanase, PAL and POD activities during the early storage time. The SA induced defense responses are probably involved in the expression of a range of defense genes, especially those encoding PR-proteins such as PAL, chitinase, β-1, 3-glucanase and POD (Meena et al., 2001). By contrast, MeJA treatment inhibited the pathogen infection for loquat fruit and in the same time the increase in PAL, polyphenoloxidase (PPO) and POD activities and lignin content and the researchers could conclude that that lignification is not the major defense mechanism against anthracnose rot in loquat fruit (Cao et al., 2008). Cao et al. (2009) reported that SA spray applied to the trees around 30 days after full flowering enhanced accumulation of H<sub>2</sub>O<sub>2</sub> in the young fruit. Meanwhile, activities of defense enzymes, including POD, PAL, chitinase or β-1,3-glucanase in the young fruit from SA-treated trees was higher than that in the control fruit 4 days after the SA spraying. Qin et al. (2003) SA treatment induced a significant increase in PPO, PAL, and b-1,3-glucanase activity in cherry fruit, but did not alter the levels of POD. The results revealed that earlier and increased activities of PAL, was observed in salicylic acid pre-treated bhendi plants challenge inoculated with Erysiphe cichoracearum. Higher accumulation of phenolics was also noticed in plants pre-treated with salicylic acid and able to enhance the resistance against invasion of E. cichoracearum in bhendi (Vimala and Suriachandraselvan, 2003). These enzymes are considered to be the main enzymatic systems for protecting cells against oxidative damage (Tommasi et al., 2001; Wang et al., 2005). The balance between SOD and POD or CAT activities in cells is crucial for determining the steady-state level of O<sub>2</sub> and H<sub>2</sub>O<sub>2</sub>.

#### 3. MATERIALS AND METHODS

# 3.1. Effect of three SA concentration on fruit three apricot cultivars during cold storage

#### 3.1.1 Fruit sample and tested cultivars

Fruits of apricot cultivars Jumbo Cot, Flavor Cot and Bergeron were hand harvested in a commercial orchard in Boldogkőváralja, Hungary at ripe stage and selected for uniform size, colour and examined to exclude all visual defects.

#### 3.1.2 Chemical treatments

The harvested fruits of each cultivar were divided into three groups. Fruits were dipped into a solutions of 0.5, 1 and 2 mmol L<sup>-1</sup> SA for 15 minutes as well as control fruits were submerged into distilled water, for the cold storage treatment, fruit was stored at 1 °C and 90% humidity. The examinations were done on days 7, 14, 21 and 28. Each treatment was replicated three times and experiments were repeated twice.

# 3.2 Effect of SA and/or MeJA on various fruit quality parameters, of apricot fruit cultivar 'Bergarouge'

# 3.2.1 Fruit sample

Fruits of apricot cultivar 'Bergarouge' were hand harvested in a commercial orchard in Boldogkőváralja, Hungary at ripe stage and selected for uniform size, colour and examined to exclude all visual defects.

Fruit harvest was performed based on visual evaluations of the ground color of fruit skin, fruit firmness and soluble solids content (SSC) were also considered. The fruit were harvested when the skin colour was over ¾ yellow and fruit firmness was considered for each cultivar with average (4.25 N).

The fruit physical measurements including (fruit weight, fruit weight loss percentage, firmness, disease incidence) and some chemical measurements including (SSC and acidity) were performed in GYUMOLSCERT kft.

Then the fruit samples were picked up directly after harvest to the laboratory of food technology department at the Corvinus University, then freeze dried and then total polyphenols, total antioxidant capacity, and carotenoids content were performed there.3.2.2 Chemical treatments

The harvested fruits were divided into three groups. Fruits were dipped into a solution of 0.2 mmol L<sup>-1</sup> MeJA and 2 mmol L<sup>-1</sup> SA for 15 minutes as well as control fruits were submerged into distilled water. Then for each treatment, fruits were divided into 2 further groups. The first group, as the cold storage treatment, was stored at 1 °C and examinations were done on days 7, 14 and 21. The second group, as the shelf-life treatment, was stored at 1 °C and 95% humidity for 15 days then placed at room temperature (25 °C) and fruits were examined at 4 and 8 days. Each treatment was replicated three times and experiments were repeated twice.

# 3.3. Effect of SA and/or MeJA on induce resistance to *Monilinia laxa* on apricot fruit cultivar Bergarouge

# 3.3.1 Isolation of fungi

M. laxa were isolated from decayed plum fruit. The fungi were maintained on PDA at 4 °C. Spores of M. laxa were obtained from 2-week-old cultures incubated at 25 °C by flooding the cultures with sterile distilled water containing 0.05% (v/v) Tween 80. The suspensions of spores were filtered through four layers of sterilized cheese cloth. The concentrations of spores were adjusted to  $(1 \times 10^{-3} \text{ spores mL}^{-1})$  with the aid of a haemocytometer.

#### 3.3.2 Mycelial growth

The concentrations of (0.5, 2 and 5 mmol L<sup>-1</sup>) of SA and (0.1, 0.4 and 0.7 mmol L<sup>-1</sup>) of MeJA were used to study the effects on mycelial growth of monilinialaxa. The effects were assayed by the method of Yao and Tian (2005a). Each concentrate of SA and MeJA solution mixed with molten PDA-agar to give a total volume of 20 mL per petri plate (diameter: 90mm). After the agar had solidified, 5mm disks of *M. laxa* were placed in the center of each petri plate. Plates were incubated at 25 ° C. Colony diameter was determined 6 days after inoculation. Each treatment was replicated three times and the experiment was repeated twice. Mycelial growth of *M. laxa* on PDA was expressed as growth rate, which was calculated according to the following formula:

Growth rate (%) = (colony diameter after inoculation–5mm)/5mm $\times$ 100.

After the determination of the appropriate concentration the experiment was repeated as 2 mmol L<sup>-1</sup> SA and 0.4 mmol L<sup>-1</sup> MeJA solution mixed with molten PDA-agar and the growth rate was measured after 2, 4, 6 and 8 days of incubation.

### 3.3.3 Sample preparation

Fruits of cv. Bergarouge were hand harvested in the orchard of North-Cot Ltd located in Boldogkőváralja, Hungary at ripe stage and selected for uniform size, colour and examined to exclude all visual defects. primary samples were taken from the fruit directly after harvest and undergone for firmness measurements, analysis of totalphenol content, antioxidant capacity, SOD, POD, PAL activities and liginin content and the results was expressed as zero time.

# 3.3.4 Exploratory experiment

The measurement of the appropriate concentration of SA and MeJA, 30 fruits were dipped into solution of (0.5, 2 and 5 mmol L<sup>-1</sup>) of SA and (0.1, 0.4 and 0.7 mmol L<sup>-1</sup>) of MeJA for one hour as inducing treatments and water treated fruit were control. Fruit were sterilized with 2% (v/v) sodium hypochlorite for 4 minutes then washed with tap water and dried by air. Each chemical treated group was divided into two inoculation treatment. One group was wounded (a uniform hole 3mm deep and 3 mm wide) with a sterile borer. After 2 hours, the fruit were inoculated by *M. laxa* suspension. The other was not wounded, after 8 days; the fruit were examined for lesion diameter (mm) and fruit disease incidence (%). After set the tested concentrations the same experiment was repeated with 360 fruit. Divided into three chemical treatments (2 mmol L<sup>-1</sup> SA, 0.4 mmol L<sup>-1</sup> MeJA and water) then each group was divided into two inoculation treatments (inoculated and non inoculated). There were three replicates for each treatment; fruits were put in 200mmx130mmx50mm plastic boxes at humidity about (95%) at temperature 25 °C.

# 3.3.5 Effect of salicylic acid on disease severity of apricot fruit

The number of the infected fruit in non-inoculated treatment and the average lesion diameter was measured as increasing diameter over the wound of experiment after 2, 4, 6, and 8 days at 25 °C the diameter of wounds were considered as zero mm. Apricot fruits showing surface mycelial developing symptoms were considered as decayed fruit. Fruit disease incidences

were estimated by the mean proportion of fruit that showed 1 mm decay at fruit surface. Fruit firmness (N) was measured by MagnessTazlor penetrometer (model FT011, QA Supplies LLC, Italy) directly after harvested as zero time and periodically after 2, 4. 6. and 8 days of treatments.

# 3.4. Measurements of the fruit pomological characters

Measurements for weight loss, fruit firmness, SSC, acidity SSC/acidity ratio and Juice pH were performed on 30 fruits in three replicates immediately after harvest as day zero. Then 30 treated fruits per replicates were examined for each measure according to cold storage and shelf-life treatments.

The following parameters were determined:

- 3.4.1 Weight loss (%) as different between the weight at zero day and the weight at assessment days.
- 3.4.2 Fruit firmness was determined destructively using a Magness-Taylor (M-T) fruit penetrometer tester (FT Fruit-Tester, formerly known as EFFEGI) mounted in a drill press to control the movement (model FT011; Facchini Francesco Srl, Brescia, Italy). The M-T test was conducted with a 7.9-mm-diameter M-T probe (49 mm²) on whole fruit with a small area of the skin removed. The maximum penetration force to the depth of 5 mm was registered as the M-T firmness with kilograms of force (0.1 kg accuracy) converted to Newtons. The firmness of each fruit was measured on opposite sides of the equator to give two readings per fruit. Results from destructive tests were means of 30 recorded fruits
- 3.4.3 Juice SSC (degrees Brix) by portable digital refractometer (Model 53007 TR, TR-Turoni Inc Forli, Italy) at 25 oC.
- 3.4.4 Juice acidity (%) by portable digital acidity meter (Model 53101 TR, TR-Turoni Inc Forli, Italy).
- 3.4.5 SSC/acidity ratio was calculated. The prepared juice of the sample was used for chemical analysis.

3.4.6 pH measurements were performed using a (84432 HANNA instruments®, Germany) pH meter.

# 3.5. Examination of chilling injury, fruit decay, membrane electrolyte, mealiness development and lose of juiceness

Chilling injury and fruit decay were invistigated in both cold storage and shelf-life treatments by using 30 fruits per replicates.

3.5.1 The degree of chilling injury (CI) was visually investigated on the fruit surface following a double cut parallel to the axial diameter. The extent of flesh browning was divided into the following classes: 0, no browning; 1, extensive browning covering <25% of the cut surface; 2, extensive browning covering ≥25% but <50% of cut surface; 3, extensive browning covering ≥50% but <75% of cut surface; 4, extensive browning covering ≥75% of cut surface. From this, a CI index was expressed as:

CI index = [(flesh browing class) $\times$ (number of fruit at the given flesh browing class)]/(4 $\times$ total number of fruit in the treatment).

3.5.2 Fruit decay (FD) was assessed as symptomps of superficial browning on the fruit surface. The severity of the symptoms was assessed visually according to the following scale: 0, no browning; 1, browning ≥25% of the fruit surface; 2, browning ≥25% but <50% of the fruit surface; 3, browning ≥50% but <75% of the fruit surface; 4, browning ≥75% of the fruit surface. From this, FD index was expressed as:

FD index = [(superficial browning class) $\times$ (number of fruit at the given superficial browning class)]/(4 $\times$ total number of fruit in the treatment).

3.5.3 Electrolyte leakage was measured according to the method of Zeng et al., (2006). 3 mm thick of mesocarp tissue were excised from equator part of 5 fruits. Disks were put into aqueous 0.1Mmannitol under constant shaking. The conductivity of the solution (L1) was measured with a conductivity meter. Solutions were boiled for 10 min and then cooled to 20 °C. The conductivity of

- tissues (L2) was measured. The percentage of electrolyte leakage was calculated using the following formula: % Electrolyte leakage= (L1/L2) x 100.
- 3.5.4 mealiness and juiciness, each fruit was tasted three trained assessors independently and rated. Mealiness was considered to be a woolly or lumpy texture and fruit were scored on a scale from 0 (no mealiness) through to 3 (high mealiness). Juiciness was considered to take into account the amount of free fluid released from the sample during chewing and fruit were scored for juiciness on a scale from 0 (no juice) through to 3 (very juicy).

#### 3.6. Chemical measurements

# 3.6.1 Total amount of soluble phenols

Total amount of soluble phenols were determined using Folin-Ciocalteu's reagent (Singleton and Rossi, 1965). The content of soluble phenols was calculated from a standard curve obtained from different concentrations of gallic acid.

#### 3.6.2 The total antioxidant capacity

The total antioxidant capacity related to ascorbic acid was determined spectrophotometrically using the FRAP (Ferric Reducing Antioxidant Power) (Benzie and Strain, 1996). It is based on the reduction of the Fe<sup>3+</sup>-TPTZ complex to the ferrous form at low pH. This reduction is monitored by measuring the absorption change at 593nm. Results are expressed as mg equivalents of ascorbic acid (mg AA g<sup>-1</sup> frresh weight).

#### 3.6.3 Lignin content

Lignin was gravimetrically determined according the methods of Femenia *et al.*, (1998) with some modification. Samples were dispersed in 72% H<sub>2</sub>SO<sub>4</sub> at room temperature for 6 h then diluted to 1 M H<sub>2</sub>SO<sub>4</sub> and heated to 100°C for 2.5 h. Insoluble material was recovered by filtration and washed thoroughly with hot water (90°C) until acid free before drying at 105 °C

overnight. The residue weight was recorded as lignin content with the results expressed as a percentageW/W.

#### 3.6.4 Total carotenoid content

Total carotenoids were extracted according to Akin *et al.*, (2008) with some modifications. Briefly, five grams of sample was extracted with 100 mL of methanol/petroleum ether (1:9, v/v) using a high speed homogenizer, and the homogenized was transferred to a separating funnel. Petroleum ether layer was filtrated through sodium sulphate, transferred to volumetric flask and to a volume of 100 mL with petroleum ether. Finally, total carotenoid content was measured spectrophotometrically (Hitachi UV2800 spectrophotometer) at 450 nm. Carotenoid content was evaluated by using an extinction coefficient of 2500, and results were expressed as β-carotene equivalents (milligrams per 100 g of FW).

#### 3.6.5 Ascorbic acid content

Ascorbic acid content was estimated spectrophotometrically by dinitrophenylhydrazine (DNPH) method (Terada *et al.*, 1978). The ascorbic acid content was expressed as ascorbic acid on fresh weight basis, mg per 100 g fresh weight.

#### 3.7. Enzymes activities assessment

#### 3.7.1 Sampling and enzyme extraction

Fruit samples were collected at the assessment times of each experiment at 1 °C according to cold storage treatment and at 4 and 8 days after cold storage of 15 days at 1 °C according to the shelf-life treatment. Each treatment was replicated three times.

#### 3.7.2 PAL activity

Flesh (10 g) from 10 fruits was homogenized in 25 ml of 50 mmol L<sup>-1</sup> sodium borate buffer (pH 8.8, containing 5 mmolβ-mercaptoethanol) containing 0.5 g PVPP. PAL activity was then

measured according to the method of Assis *et al.*, (2001) with slight modifications. Enzyme extract (1 ml) was incubated with 2 ml of borate buffer (50 mmol L<sup>-1</sup>, pH8.8) and 1 ml of L-phenylalanine (20 mmol L<sup>-1</sup>) for 60 min at 37 °C. The reaction was stopped with 1 ml HCl (1 mol L<sup>-1</sup>). PAL activity was determined by the production of cinnamate, which was measured at 290 nm. The blank was the crude enzyme preparation mixed with L-phenylalanine with zero time incubation. PAL activity was defined as nmol cinnamic acid h<sup>-1</sup> mg<sup>-1</sup> protein.

#### 3.7.3 POD activities

0.5 ml of enzyme extract was incubated in 2 ml buffered substrate (100 mmol  $L^{-1}$  sodium phosphate, pH 6.4 and 8 mmol  $L^{-1}$  guaiacol) for 5 min at 30°C and the increasing absorbance was measured at 460 nm every 30 s for 150 s after adding 1 ml of  $H_2O_2$  (24 mmol  $L^{-1}$ ).

### **3.7.4 SOD activity**

Fruit tissue (1 g) was ground in 5 mL of 50 mmol L<sup>-1</sup> sodium phosphate buffer (PH 7.0). The extracts were then homogenized and centrifuged at 10.000 x g for 20 min at 4 °C, the supernatants were used for enzyme assay. SOD activity was then determined photochemically by the method of Rao *et al.*, (1996) The reaction mixture contained 50 mmol L<sup>-1</sup> sodium phosphate (pH 7.8), 14 mmol L<sup>-1</sup> methionine, 3 μmol L<sup>-1</sup> EDTA, 1 μmol L<sup>-1</sup> nitro-blue-tetrazolium (NBT), 60 μmol L<sup>-1</sup> riboflavin and 0.1 ml crude enzyme extract in a total volume of 3 ml. The formation of blue formazan was monitored by recording the absorbance at 560 nm. One unit of SOD activity was defined as the amount of enzyme that causes a 50% inhibition of NBT reduction under assay conditions.

### 3.7.5 CAT activity

According to the method of Abbasi *et al.*, (1998). Two buffer solutions (A and B) were used to carry out the catalase enzyme reaction. A 50  $\mu$ L enzyme extract was added to each of two cuvettes, one containing 1 mL buffer A and the other containing 1 mL buffer B. The change in optical density at 240 nm was recorded by means of a spectrophotometer after 45 s and 60 s at the time when the extract was added to the cuvettes. The difference in optical density between the 45 s and 60 s reading was used to calculate the CAT activity. CAT activity was expressed as U g<sup>-m</sup> protein.

#### 3.7.6 Protein content

In the enzyme extracts was estimated using the Bradford, (1976) method, using bovine serum albumin as a standard. Specific activity of all the enzymes was expressed as units per milligram protein.

## 3.8. Sensory analysis

Each sample was prepared on a white dish by presenting a 30 fruits and prepared short time before sensory testing, to ensure a glossy aspect and to avoid flesh browning. Each dish was marked with a 3-digit code, assigned at random. The evaluation guide provided a continuous scale for each sensory attribute, ranging from 0 to 9, and marked with two anchors (skin color: 0 = very bad, 9 = very good; flesh color: 0 = very bad, 9 = very good; Texture: 0 = very soft, 9 = very hard; Taste: 0 = low taste and 9 = good taste; Visual appearance: 0 = very bad appearance, 9 = very good appearance) and over all acceptability (0 = I dislike the sample, and 9 = I like the sample very much). Scores of 5 or above were considered acceptable for commercial purposes. These scales had been used previously to evaluate harvest maturity stage effects on apricot sensory with some modification (Infant *et al.*, 2008) and also, smillar scales was applied to study effects of storage techniques on apricot sensory (Muftuoğlu *et al.*, 2012).

# 3.9. Statistical analysis

Experiments were performed using a completely randomized design. Statistical analyses were performed with SPSS program (SPSS Inc., Chicago, IL, USA). The data were analyzed by one-way ANOVA. Means separation was performed by Duncan's multiple range tests. Differences at P<0.05 were considered as significant.

### 4. RESULTS AND DISCUSSION

# 4.1. Effect of three SA concentrations on three apricot cultivars in cold storage treatment

# 4.1.1 Effect of SA on weight loss and fruit firmness

Different concentration of SA show different effects on fruit weight loss and fruit firmness on tested cultivars. Results in (Figs. 3 and 4) showed that 0.5 mmol  $L^{-1}$  of SA had no significant effects on all the cultivars during all the cold storage dates. Treated fruit with 2 mmol  $L^{-1}$  SA of cvs. Flavor Cot and Jumbo Cot recorded significant values in comparison to water, 0.5 and 1 mmol  $L^{-1}$  SA treated fruit over 2nd and 3rd week of cold storage, respectively. While Bergeron fruit treated with 1 and 2 mmol  $L^{-1}$  SA showed better firmness and lower weigh loss over the 1st and 2nd week of storage, respectively (P<0.05). All of 2 mmol  $L^{-1}$  SA treated fruit showed the lowest values of fruit weight loss and highest fruit firmness (P≤0.05) over 3 weeks of cold storage in all the tested cultivars.

Many papers revealed that SA has different mode of actions on the fruit cells. (Srivastava &Dwivedi, 2000) reported that SA effectively reduces respiration rate in plants and harvested fruits in a concentration-dependent manner and reducing the respiration rate subsequently reduce the water loss of harvested fruit, they reported that the 1 mmol L<sup>-1</sup> recorded the lowest respiration rate. SA has been reported to reduce fruit weight loss by closing stomata in 'Ponkan' mandarin fruit (Zheng and Zhang, 2004). Different between the cultivars responses to different SA concentrations may because the nature ability for each cultivar to keep the fruit quality over the cold storage time. Ezzat *et al.* (2012) reported that the different cultivars of apricot had different attitude toward storability.

SA has been documented to enhance flesh firmness of harvested peaches during storage (Wang et al., 2006; Li and Han, 1999) by reducing the enzyme of membrane breakdown. Banana fruit ripening was accompanied by an increase in all the three cell wall degrading enzymes namely, cellulase, polygalacturonase and xylanase. Levels of these cell wall degrading enzymes were found to be decreased, in a concentration dependent manner (Srivastava and Dwivedi, 2000). SA has a remarkable ability to maintain fruit quality during storage life of fruits and this is in accordance with our findings (Figs. 3 and 4).

## 4.1.2 Effect of SA on SSC and total acidity

Cultivars Jumbo Cot and Bergeron fruit treated with 2 mmol  $L^{-1}$  SA showed unchanged values for SSC and acidity over the 2nd week of cold storage, and then the values took to reduce slightly over the end time of storage (P<0.05). The 0.5, 1.5 mmol  $L^{-1}$  SA and water treated fruit showed high increase for SSC and acidity (P<0.05) at the 1st storage week then the values decreased dramatically over the 3rd week (Figs. 5 and 6).

Flavor Cot recorded unchanged of SSC for all the chemical treatments during two weeks of cold storage ( $P \ge 0.05$ ) then water and 0.5 mmol L<sup>-1</sup> SA treated fruit showed decrease for SSC values (P < 0.05) over the 3rd storage week. Meanwhile, 1 and 2 mmol L<sup>-1</sup> of SA treated fruit showed increase in SSC over the 4th week. Untreated, 0.5 and 1 mmol L<sup>-1</sup> treated fruit showed increase of acidity during 3 weeks of cold storage. Meanwhile, 2 mmol L<sup>-1</sup> SA treated fruit showed about stable values of acidity over 2 weeks of storage. (Figs. 5 and 6).

The increased amounts of soluble solids over the storage period could be due to weight loss and, therefore, fruit juice concentration One of the ripening symptoms is increasing of SSC and soluble sugars due to the action of sucrose-phosphate synthase (SPS), a key enzyme in sucrose biosynthesis (Hubbard *et al.*, 1991). It was also documented that MeSA reduced the ethylene production and delayed ripening of kiwifruit and may help to decrease SPS enzyme activity leading to lowered sucrose synthesis and SSC content (Aghdam *et al.*, 2011). Cell walls contain large amounts of polysaccharides, mainly pectins and cellulose, and are digested due to the activity of the cell wall degrading enzymes leading to a significant increase in SSC content. SA effectively protects cell walls by decreasing the expression of degrading enzymes and as a consequence prevents from dramatic increase in SSC content of the cells (Asgharia and Aghdam, 2010).

In the present study, firmer apricot fruits with low acidity levels were obtained by SA especially 2 mmolL<sup>-1</sup> treatments and this might be due to a decrease in pectin solubilisation which results in softening. Softening starts with the conversion of insoluble protopectin into water-soluble pectin, which results in increasing juice acidity (Pressey and Avants, 1973). Mango fruits treated with SA showed increased fruit firmness and decreased activity of cell wall degrading enzymes (Srivastava and Dwivedi, 2000),15 this can be also hypothesized in apricot based on our results as acidity was raised in control fruit (Fig. 6) with decreasing fruit firmness (Fig. 4).

### 4.1.3 Effect of SA on total carotenoids content and ascorbic acid content

All the SA concentrations treatments showed better carotenoids content that the water treated fruit. Cv. Flavor Cot fruit treated with 2 mmol L<sup>-1</sup> SA showed increase of carotenoids content over the 3rd week of cold storage (P<0.05) in comparison to water treated fruit. The 0.5 and 1 mmol L<sup>-1</sup> SA treated fruit showed intermediate values with also, significance differences than control fruit (Fig. 7). The same trend was noticed for cv. Jumbo Cot fruit (Fig. 5), except that the 0.5 SA treatments did not show any enhancement for the carotenoids content with no significance differences with control fruit. Also, the cv. Bergeron fruit (Fig. 7) treated with 1 and 2 mmol L<sup>-1</sup> SA showed high carotenoids content all over the storage times (P<0.05) in comparison to water and 0.5 mmol L<sup>-1</sup> SA treated fruit. Control fruit showed the highest increase of ascorbic acid during the 2 weeks of storage and then the values decreased dramatically over the 3 weeks (P<0.05) in all the tested cultivars.

2 mmol L<sup>-1</sup> SA Jumbo Cot treated fruit showed slightly unchanged ascorbic acid values over the 3rd week storage the slight decrease was noticed while the 1 mmol L<sup>-1</sup> SA treatment showed decrease of ascorbic acid at the 2nd week of storage. The same trend was noticed for cv. Bergeron fruit (Fig. 8).

The Flavor Cot fruit treated with 2 mmol  $L^{-1}$  exhibited about unchanged values of AS over all the assessment times with significance differences with all of other treatments. The 0.5 and 1 mmol  $L^{-1}$  SA Flavor Cot treated fruit showed increase of ascorbic acid early of storage while recorded deep reduction at the 3rd week (Fig. 8).

Our investigation showed increasing of carotenoids with SA application depends on concentration manner and this is agreed with the finding of (Hayat *et al.*, 2005). They reported SA applied may lower the level of oxidative stress in plants, which acts as a hardening process, improving the antioxidative capacity of the plants and helping to induce the synthesis of protective compounds (such as carotenoids) also, they reported that SA enhance the level of carotenoid is these are the role assigned to SA.

It is reported that, the vitamin C content of apricot gradually increased through the ripening stages (Hegedüs *et al.*, 2011). Our data showed that the high SA concentration treated fruit of all tested cultivars had the lower values of ascorbic acid at the early time of storage, and this can be supported by the role of these elicitors in delay the ripping processes as increasing of ascorbic acid is one sign of ripening process in apricot (Hegedüs *et al.*, 2011). This fact was reported by (Srivastava and Dwivedi, 2000) on delay ripening of banana. Aghdam *et al.*,

(2011) documented that MeSA reduced the ethylene production and delayed ripening of kiwifruit. Differences between the response of different cultivars may back to the genetic nature for each cultivars and water treated fruit for all the tested cultivars showed not the same trend for AS and VC content during the storage and this was documented by Hegedüs *et al.*, 2011 who reported that different apricot cultivars behave different ways in postharvest ripening.

# 4.1.4 Effect of SA on total soluble phenol, antioxidant capacity, DI, CI and membrane electrolyte leakage

Water treated fruit showed decrease in total soluble phenol content during the 3 weeks of storage followed with increase in 4th storage week with significant differences. Low concentration of SA (0.5 mmol L<sup>-1</sup>) showed non significance differences with the control fruit in all tested varieties.

1 and 2 mmol L<sup>-1</sup> SA treated Jumbo Cot and Flavor Cot fruit showed about stale total soluble phenol content during the 3 weeks of storage then the values increased (Fig. 9).

The same trend was noticed for Bergeron while, treated fruit with 2 mmol L<sup>-1</sup> SA showed increase in the total phenol content at the 1st storage week then the values took to be roughly stable over the 4th week (Fig. 9). Antioxidant capacity of SA treated fruit in all the tested cultivars, increased gradually over the 1st, 2nd and 3rd storage week then the deep depression was noticed with significant differences with control, 0.5 and 1 mmol L<sup>-1</sup> SA treated fruit all over the storage periods (Fig. 10). The data of DI and CI (Table 2) showed that all the SA concentrations had positive effect in reducing the fruit decay. Jumbo Cot and Flavor Cot fruit treated with 1 and 2 mmol L<sup>-1</sup> SA showed significant reducing of CI over the 3rd week of storage and then the values increased ( $P \ge 0.05$ ) in comparison to 0.5 SA and control fruit. Bergeron treated fruit with 2 mmol L<sup>-1</sup> SA showed the lowest values of CI at early of the storage periods and till the 4th week followed by values of 1 and 0.5 mmol L<sup>-1</sup> SA treated fruit.

The fruit decay data (Table 2) showed the same trend of CI, the values reduces with the increase of SA concentration and the differences between 1 and 2 mmol not found in all cultivars over all the storage times.

Fruit treated with 1 and/or mmol SA treatments exhibited low membrane electrolyte leakage (P<0.01) at early storage time  $(1^{st}$  and  $2^{nd}$  week) in comparison to 0.5 mmol SA and control fruit then the significance not found between the treatments in all the cultivars. 0.5 mmol L<sup>-1</sup> treated fruit showed the same behavior like untreated fruit in all the cultivars all over the storage time (Fig. 11).

In our results, fruit DI was significantly (P < 0.01; Table 2) affected by SA treatments compared to control, which was correlated with enhancing antioxidant power in treated fruits (Fig. 10). When applied exogenously at suitable concentrations, SA was found to enhance the efficiency of antioxidant system in plants (Hayat et al., 2005). Zeng et al. (2006) reported that salicylic acid treatment significantly enhanced phenylalanine (PAL, peroxidase POD and -1, 3- glucanase activity in grape berries. Increased antioxidant activity of SA treated fruits may help them to protect themselves against chilling stress during storage and this finding was matching with our results in Fig. 10 and Table 2. Exogenous application of SA at nontoxic concentrations to susceptible fruits and vegetables could enhance resistance to pathogens and control postharvest decay (Asgharia and Aghdam, 2010). SA also exhibits direct antifungal effects against pathogens. SA in a concentration of 2 mmol L<sup>-1</sup> showed direct toxicity on Monilinia fructicola and significantly inhibited the growth of mycelia and spore germination of the pathogen in vitro (Yao and Tian, 2005). Wang et al. (2006) documented that SA treatment alleviated chilling injury of peach fruits due to its capability to induce antioxidant activity. This was recorded in our study (Table 2 and Fig. 10). The same trend as noticed in peach and pomegranate fruit, (Wang et al., 2006; Sayyari et al., 2009). In peach fruit alleviation of CI was achieved at 1 mM but failed at 0.7 mM or lower SA concentrations and in pomegranate fruit SA treatments, especially at 2 mM concentration, were highly effective in reducing CI and electrolyte leakage in the husk of pomegranate.

Generally, CI occurs primarily at the cell membrane with changes in the fatty acid phospholipids composition (Lurie *et al.*, 2005) and the membrane damages initiate a cascade of secondary reactions leading to disruption of cell structures. This membrane damage measured by the electrolyte leakage, which in this study was significantly higher in the control fruit than in salicylic acid treated fruit especially high concentrations (1 and/or 2 mmol L<sup>-1</sup>) (Fig. 11).

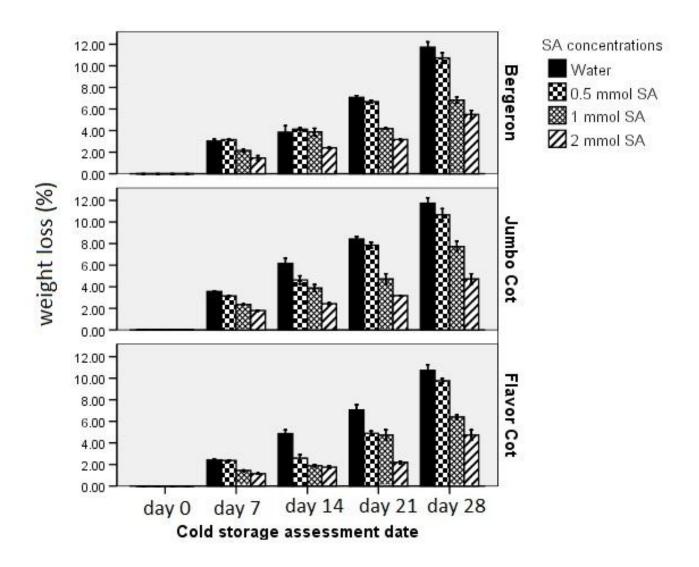
These results show a role of salicylic acid in maintaining membrane integrity, as has been reported for loquat fruit (Cai *et al.*, 2005) and pomegranate fruit (Sayyari *et al.*, 2009).

Table 2 Effect of treatments of 0, 1, and 2 mmol L<sup>-1</sup> salicylic acid (SA) on chilling injuries (CI) and decay index (DI) of cvs. 'Flavor Cot', 'Jumbo Cot', and 'Bergeron' apricot fruit

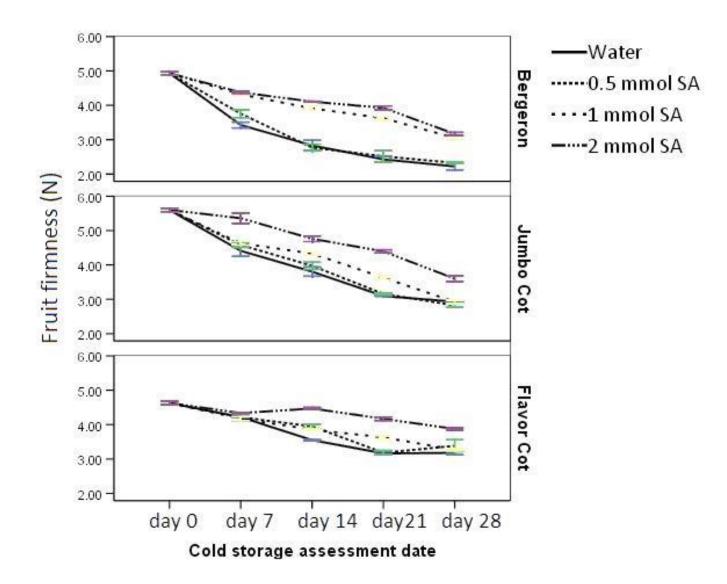
Varieties	SA conc.	Ci <sup>a</sup> Cold storage assessment date				FD (%) <sup>b</sup> Cold storage assessment date			
			water	05.26A <sup>c</sup>	18.12 A	29.12 A	38.24 A	17.25 A	22.35 A
Flavor Cot	0.5 mmol	04.21A	16.24 A	25.25 A	35.12 A	17.52 A	21.25	32.25	40.25
	1 mmol	02.01B	10.26 B	16.24 B	29.25 A	13.22 A	15.15	20.52	28.25
	2 mmol	02.51B	05.62 B	10.25 C	26.25 A	09.25 A	12.35	18.12	20.01
Jumbo Cot	water	4.23 A	17.25 A	30.25 A	39.24 A	16.58 A	25.25 A	38.25 A	55.25 A
	0.5 mmol	4.25 A	17.25 A	25.23A	36.12A	16.25	23.25	38.01	55.68
	1 mmol	3.12 B	9.15 B	10.57 B	26.15 A	10.12	12.25	22.52	40.68
	2 mmol	2.01 C	5.36 B	13.25 B	20.15 B	8.12	11.15	19.24	31.25
Bergeron	water	5.12 A	16.57 A	28.24 A	36.25 A	18.25 A	30.25 A	42.25 A	53.24 A
	0.5 mmol	4.05 B	8.25 B	18.35 B	22.54 A	17.25 A	32.25 A	34.58 A	40.25 A
	1 mmol	4.20 B	7.65 B	15.24 B	21.25 A	11.02 A	15.25 B	21.35 B	30.25 B

<sup>&</sup>lt;sup>a</sup> CI index: chilling injury index. <sup>b</sup> FD (%) index: decay index percentage of the fruit

 $<sup>^{\</sup>circ}$  Values within a column followed by different letters are significantly different at P<0.05 according to Duncan's multiple range tests. The results represent the means  $\pm$  SD of triplicate assay.



**Fig. 3** The effect of treatments of 0, 1, and 2 mmol L<sup>-1</sup> salicylic acid (SA) on on weight loss percentage of apricot fruit (cvs. 'Flavor Cot', 'Jumbo Cot', and 'Bergeron) in cold storage treatment at days 7, 14, 21 and 28 at 1 °C.



**Fig. 4** The effect of treatments of 0, 1, and 2 mmol L<sup>-1</sup> salicylic acid (SA) on on fruit firmness (N) of apricot fruit (cvs. 'Flavor Cot', 'Jumbo Cot', and 'Bergeron) in cold storage treatment at days 7, 14, 21 and 28 at 1 °C.

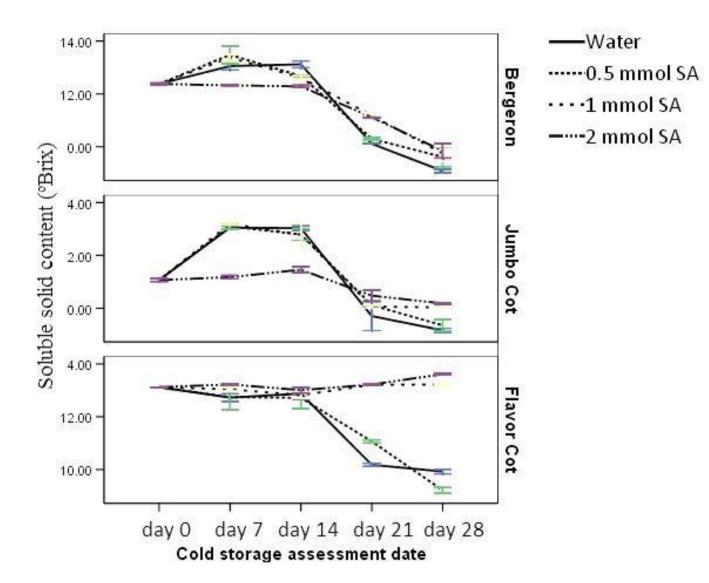


Fig. 5 The effect of treatments of 0, 1, and 2 mmol  $L^{-1}$  salicylic acid (SA) on on soluble solid content (°Brix) of apricot fruit (cvs. 'Flavor Cot', 'Jumbo Cot', and 'Bergeron) in cold storage treatment at days 7, 14, 21 and 28 at 1 °C.

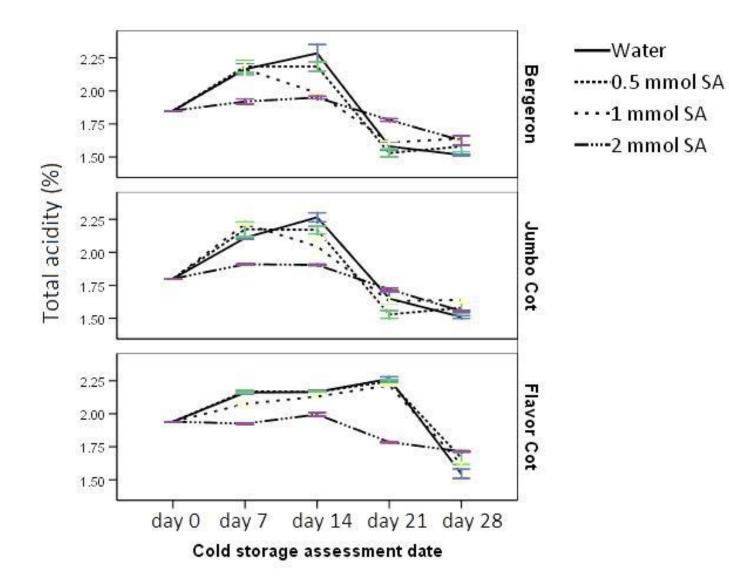


Fig. 6 The effect of treatments of 0, 1, and 2 mmol  $L^{-1}$  salicylic acid (SA) on on fruit acidity (%) of apricot fruit (cvs. 'Flavor Cot', 'Jumbo Cot', and 'Bergeron) in cold storage treatment at days 7, 14, 21 and 28 at 1  $^{\circ}$ C.

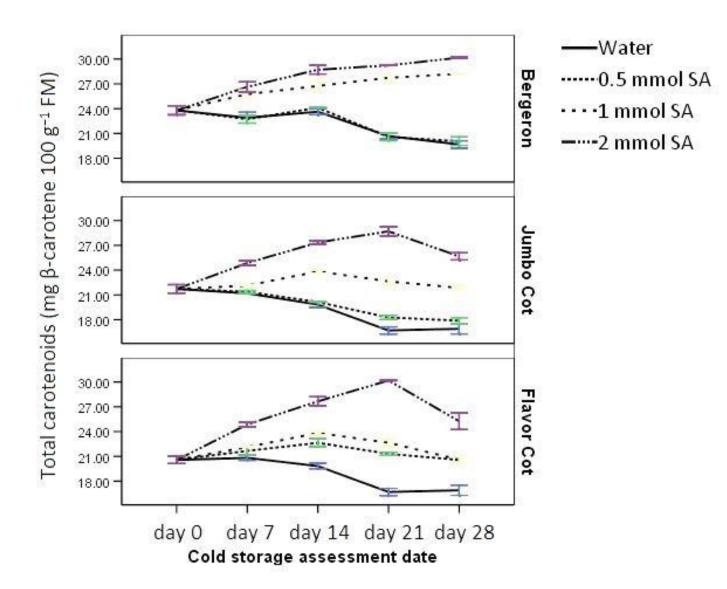


Fig. 7 The effect of treatments of 0, 1, and 2 mmol  $L^{-1}$  salicylic acid (SA) on on fruit Total carotenoids (mg β-carotene 100 g<sup>-1</sup> FM) in apricot fruit (cvs. 'Flavor Cot', 'Jumbo Cot', and 'Bergeron) in cold storage treatment at days 7, 14, 21 and 28 at 1 °C.

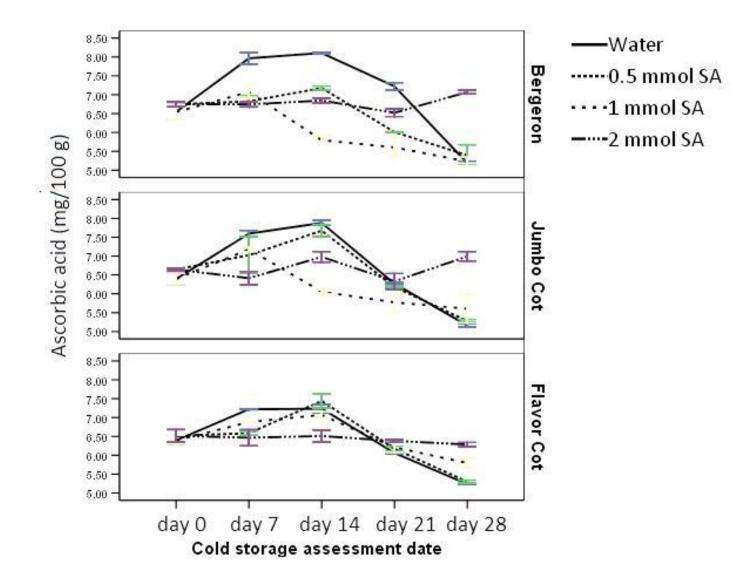


Fig. 8 The effect of treatments of 0, 1, and 2 mmol  $L^{-1}$  salicylic acid (SA) on ascorbic acid (mg/100 g) in apricot fruit (cvs. 'Flavor Cot', 'Jumbo Cot', and 'Bergeron) in cold storage treatment at days 7, 14, 21 and 28 at 1  $^{\circ}$ C.

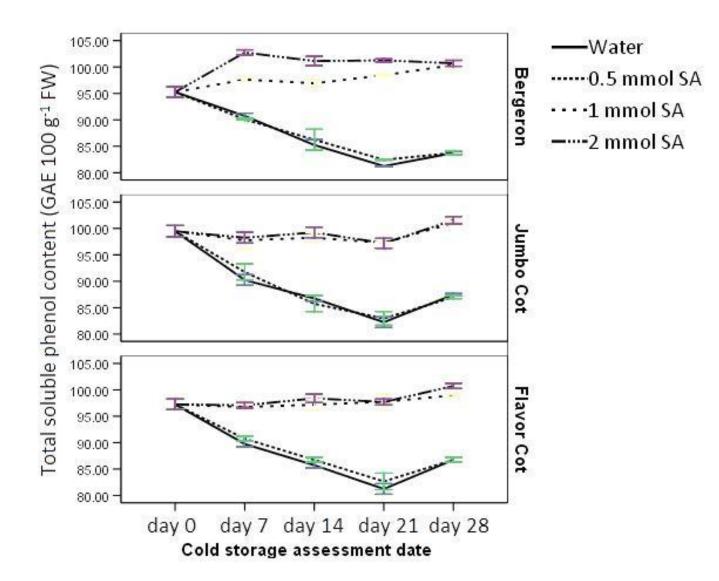


Fig. 9 The effect of treatments of 0, 1, and 2 mmol L<sup>-1</sup> salicylic acid (SA) on total soluble phenol content (GAE 100 g<sup>-1</sup> FW) in apricot fruit (cvs. 'Flavor Cot', 'Jumbo Cot', and 'Bergeron) in cold storage treatment at days 7, 14, 21 and 28 at 1 °C.

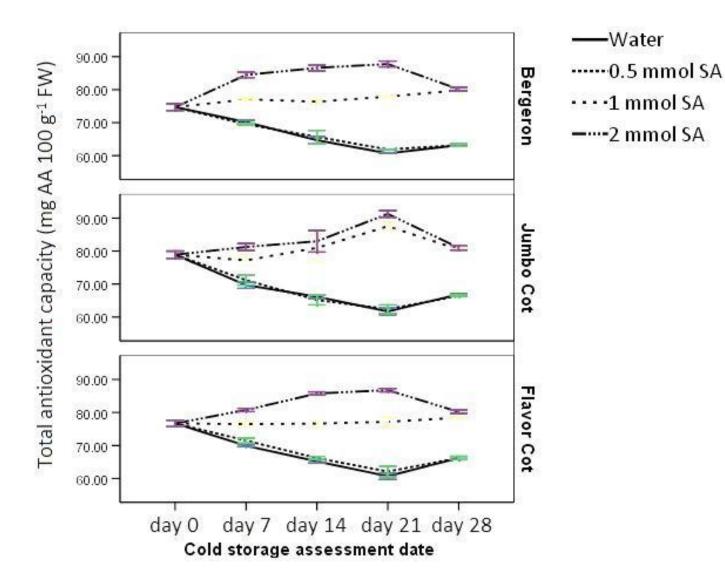
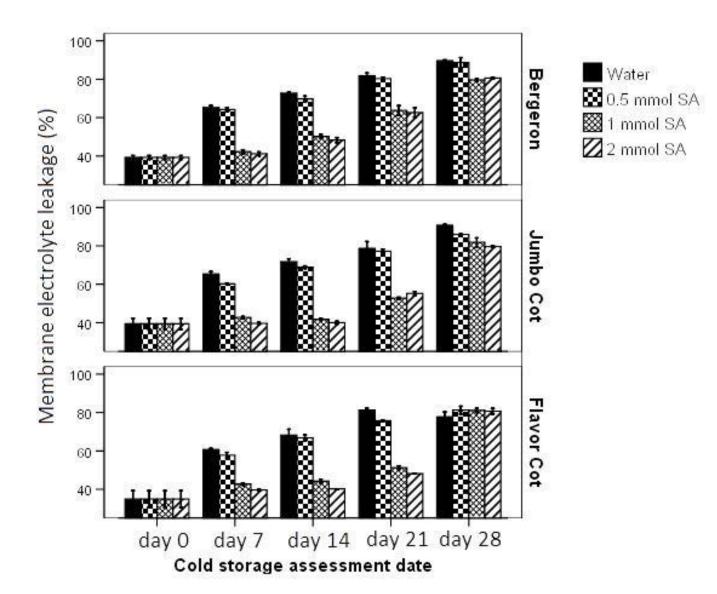


Fig. 10 The effect of treatments of 0, 1, and 2 mmol  $L^{-1}$  salicylic acid (SA) o and total antioxidant capacity (mg AA 100 g<sup>-1</sup> FW) in apricot fruit (cvs. 'Flavor Cot', 'Jumbo Cot', and 'Bergeron) in cold storage treatment at days 7, 14, 21 and 28 at 1  $^{\circ}$ C.



**Fig. 11** The effect of treatments of 0, 1, and 2 mmol L<sup>-1</sup> salicylic acid (SA) on membrane electrolyte leakage (%) of apricot fruit (cvs. 'Flavor Cot', 'Jumbo Cot', and 'Bergeron) in cold storage treatment at days 7, 14, 21 and 28 at 1 °C.

# 4.2. Effect of SA and/or MeJA on various fruit quality parameters of apricot cultivar Bergarouge

# 4. 2.1 Effect of SA and MeJA on weight loss, fruit firmness, SSC, acidity, SSC/acidity ratio and PH

Apricot fruits tended to lose weight either during cold storage or shelf-life treatment (Fig. 12). In the cold storage treatment, the weight reduction in control treatment was higher (P<0.05) than for treatments with 0.2 mmol L<sup>-1</sup> MeJA or 2 mmol L<sup>-1</sup> SA. Significant differences between water treated fruit and the either MeJA or SA treatments appeared at days 14 and 21. In the shelf-life treatment, significant differences between control and chemically treated fruit occurred from day 4 at 25 °C. The SA and MeJA treatments alleviated the weight loss of apricot fruits almost equally. It has been demonstrated that SA effectively reduces respiration rate in plants and harvested fruits in a concentration-dependent manner (Srivastava and Dwivedi, 2000). Decrease in fruit metabolic activities leads to lower fruit water content, weight loss, carbohydrate depletion rate and consequently it delays fruit senescence effectively (Wills *et al.*, 1998). SA has been reported to reduce fruit weight loss by closing stomata in cv. 'Ponkan' mandarin fruit (Zheng and Zhang, 2004).

Fruit firmness was decreased in three- week periods of the cold storage treatments and values of fruit firmness in both chemical treatments were higher (P<0.05) compared to the water treated fruits (Fig. 13a). The same trend was observed in the shelf-life treatments (Fig. 13b). The differences between SA and MeJA treatments were not significant though MeJA treatment seems to be more successful in reducing fruit softening than SA. The mechanism by which these compounds may affect the cell wall structure and thus maintain fruit firmness is not yet clear, and no research has been carried out in apricot, although it has been suggested that MeJA reduces pectin-methyl-esterase (PME) activity to decrease de-esterification of pectin (Meng *et al.*, 2009), and hence maintains fruit texture. SA has been documented to enhance flesh firmness of harvested peaches during storage (Wang *et al.*, 2006; Li and Han 1999), and that of banana fruits during ripening (Srivastava and Dwivedi, 2000). Thus, SA has a remarkable ability to maintain fruit quality during storage life of fruits and this is in accordance with our findings (Fig. 13a,b).

MeJA and SA treated fruits recorded roughly stable values of SSC at days 7 and 14 in the cold storage treatment. Meanwhile, control treatment showed SSC increasing values then sharp decreased at day 21 (Fig. 13c). Acidity decreased slightly after 7 and 14 days of cold

storage for MeJA and SA treated fruit with significant differences to the control (Fig. 13e). In the shelf-life treatments at 25 °C, the SSC and acidity values decreased with the experiment time and were higher in both chemical treatments in regard to control (P<0.05).

A small increase in SSC during storage and/or shelf-life has been reported previously for apricots (Basile *et al.*, 1999). In this study, treatment of apricot fruits with MeJA and SA maintained a higher content of SSC than control fruits at the end of cold storage (Fig. 13c,d) and this were also associated with the decreased intensity of fruit weight loss (Figs. 12). The apricot does not behave such as apple or kiwifruits. Hence, there is no conversion from starch to sugar during ripening (Kurz *et al.*, 2008).

SSC and soluble sugars may increase during fruit ripening due to the action of sucrose-phosphate synthase (SPS), a key enzyme in sucrose biosynthesis (Hubbard *et al.*, 1991). It was also documented that MeSA reduced the ethylene production and delayed ripening of kiwifruit and may help to decrease SPS enzyme activity leading to lowered sucrose synthesis and SSC content (Aghdam *et al.*, 2011). Cell walls contain large amounts of polysaccharides, mainly pectins and cellulose, and are digested due to the activity of the cell wall degrading enzymes leading to a significant increase in SSC content. SA effectively protects cell walls by decreasing the expression of degrading enzymes and as a consequence prevents from dramatic increase in SSC content of the cells (Asgharia and Aghdam, 2010).

The results of SSC and acidity reflexed on the of SSC/ acidity ratio (Fig. 14a,b) as, the SA and MeJA showed high ratio than the control treatment (P<0.05). This was agreement with the finding of Meng *et al.*, (2009), the reported that MeJA treatment and low temperature enhanced rate of SSC/TA by mainly restraining decrease of SSC in peach fruits. Also, SA treatment can enhance the SSC/acidity ratio by reducing loses of SSC during the storage as in (Fig. 14a,b).

In the present study, firmer apricot fruits with low acidity levels were obtained by SA and MeJA treatments and this might be due to a decrease in pectin solubilisation. Softening starts with the conversion of insoluble protopectin into water-soluble pectin, which results in increasing juice acidity (Pressey and Avants, 1973). This will eventually result in cell wall deterioration (Fishman *et al.*, 1993). Banana fruits treated with SA showed increased fruit firmness and decreased activity of cell wall degrading enzymes (Srivastava and Dwivedi, 2000), this can be also hypothesized in apricot based on our results as acidity was raised in control fruit (Fig. 13e,f) with decreasing fruit firmness (Figs. 13a,b). Juice pH values were

increased with the storage time, water treated fruit recorded the highest pH values (P < 0.05) at 14 and 21 day of cold storage or at room temperature (Fig.14c, d). Minimum weight loss in chemically treated fruits resulted in high firmness and maintained lower pH levels may because SA treated fruit might have reduced respiration rates this phenomenon has also been reported by some other researchers (Srivastava and Dwivedi, 2000; Wolucka *et al.*, 2005).

# 4. 2.2 Effect of SA and MeJA on chilling injury, fruit decay, mealiness developement and lose of juiceness

Degree of CI index increased with storage time in the control, MeJA and SA treatments (Table 3). Meanwhile, fruit pre-treated with MeJA and SA showed significantly (*P*<0.05) lower CI indices than water treated control fruit throughout the whole storage period. After a three-week storage at 1 °C, fruit treated with MeJA had 53.7 and 74.3% lower CI index compared to those of the SA-treated and control fruit, respectively (*P*<0.05). After 8 days of 25 °C, MeJA significantly (*P*<0.05) enhanced the resistance of apricot fruits to chilling injuries compared to either SA or water control treatment. SA treated fruit showed better and lower result for CI index in comparison to control fruit (*P*<0.05)

Non-treated apricot fruit showed a great increase in FD index at 1 °C as it reached to 96.25±2.3% after 21 days (Table 4). In contrast, MeJA and treatments (P<0.05) reduced the development of superficial browning symptoms. In addition, both MeJA and SA significantly decreased FD index in the shelf-life treatment at after 8 days of 25 °C.

Similar results were reported for mealiness development, as water treated fruit developed mealiness faster and with higher (P<0.05) values than SA and MeJA treatments (Fig.15a,b).

Juiciness measurements (Fig.15c,d) showed the priority of chemical treatment in comparison to control treatment to keep the juiciness. SA and MeJA treatments had positive effects in keeping juiciness with cold storage or shelf treatment.

Previous research papers reported that apricots also developed chilling injury symptoms when stored below 7 °C, such as mealiness, loss of juiciness or gel breakdown. Mealiness and woolliness in fruit have both been associated with textural changes and loss of juiciness (Seibert *et al.*, 2013; Stanley *et al.*, 2010).

The CI symptoms and mealiness development were generally influenced and triggered by a combination of storage temperature and period (Lurie and Crisosto, 2005). Our results

confirmed that using MeJA and SA played an important role in alleviating CI symptoms, development of mealiness, lose of juiciness and FD of cold-stored apricot. However, MeJA kept CI at lower level in comparison with SA treatment after 21 days at 1 °C and after 8 days at 25 °C.

Ding et al., (2002) reported that MeSA and MeJA treatments significantly reduced CI, and decay in tomato fruit and this beneficial effect took place by induction of pathogenesis related protein gene expression. Wang et al., (2006) reported that postharvest application of SA significantly alleviated CI and decay in peach fruit and was associated with maintainance of fruit firmness. Peng et al. (2009) indicated that MeJA vapor treatment could significantly decrease internal browning and flesh mealiness symptoms of peach fruit, the authors reported high levels of extractable juice in the MeJA treatment corresponded with low levels of mealiness, whereas low levels of extractable juice was associated with high percentage of mealiness. The same results for SA treatment were reported for fresh-cut Chinese water chestnut showed delayed discoloration with increased SA concentrations (Peng and Jiang 2006).

# 4. 2.3 Effect of SA and MeJA on total soluble phenol content, antioxidant capacity, caroteniods content and ascorbic acid content

Total soluble phenol content of apricot fruits was higher (P<0.05) in the MeJA and SA treatments compared to the control treatment in both cold storage and shelf-life treatments (Fig. 16 a,b). In the cold storage treatments, apricot fruit treated with MeJA showed increased phenol contents after 7 days of storage at 1 °C with significant differences in regard to SA treatment (Fig. 16a).

The antioxidant capacity levels increased significantly in fruit treated with MeJA and SA after 2 weeks and 4 days of cold storage and shelf-life treatments, respectively, and then it started to decrease. While the antioxidant capacity of control fruits decreased continuously in both cold storage and shelf-life treatments (Fig. 16c,d).

The carotenoids content started to increase with the time in all treatments. The SA had the pronounced values over MeJA treated fruit (P<0.05). The same line was observed for shelf-life treatment (Fig. 17a, b).

Results showed that apricot fruits treated with SA had the lowest ascorbic acid then MeJA treated fruit. Untreated fruit showed higher content of ascorbic acid during the early time of storage or shelf-life treatment (P<0.05). The difference between SA or MeJA treatments was not significant (Fig. 17c,d).

Antioxidants are predominant health-promoting phytochemical compounds in horticultural crops and have many biological activities on human health. In our results, fruit decay was significantly (P<0.01; Table 4) affected by MeJA and SA treatments at the end of both cold storage or shelf-life period compared to control, which was correlated with enhancing antioxidant power in treated fruits (Fig. 16c,d). Chanjirakul *et al.*, (2008) demonstrated that MeJA might increase the resistance of tissues against decay through enhancing their antioxidant and their free radical scavenging capacities. Exogenous application of SA at nontoxic concentrations to susceptible fruits and vegetables could enhance resistance to pathogens and control postharvest decay (Asgharia and Aghdam, 2010).

MeJA has been shown to induce the synthesis of some stress-related proteins including heatshock and pathogenesis-related proteins, which leads to an increased resistance level and results in decreased incidence of the decay (Ding et al., 2001, 2002). SA also exhibits direct antifungal effects against pathogens. SA in a concentration of 2 mmol L-1 showed direct toxicity on Monilinia fructicola and significantly inhibited the growth of mycelia and spore germination of the pathogen in vitro (Yao and Tian, 2005b). Wang et al., (2006) documented that SA treatment alleviated chilling injury of peach fruits due to its capability to induce antioxidant activity. This was recorded in our study (Table 3 and Fig 16 c,d) As an endogenous phytohormone, MeJA plays key roles in regulating a great diversity of physiological and biochemical processes in plants including stimulating the biosynthesis of secondary metabolites (Creeman and Mullet, 1997). MeJA has been shown to induce stilbene accumulation in leaves and berries of grapevine plants (Larrondo et al., 2003), increase the accumulation of carotenoids contents and phenolics in apples (Rudell and Mattheis, 2002), strawberries (Ayala-Zavala et al., 2005), and blackberries (Wang et al., 2008) and our data supported these findings as MeJA increased total phenolic contents, antioxidant capacity and carotenoids content in apricot during storage (Figs. 16, 17a,b).

Our investigation showed increasing of carotenoids with SA application and this is agreed with the finding of Hayat *et al.*. (2005). They reported SA applied may lower the level of oxidative stress in plants, which acts as a hardening process, improving the antioxidative

capacity of the plants and helping to induce the synthesis of protective compounds (such as carotenoids) also, they reported that SA enhance the level of carotenoid is these are the role assigned to SA.

It is reported that, the vitamin C content of apricot gradually increased through the ripening stages (Hegedüs *et al.*, 2011). Our data showed that SA or/ and MeJA treated fruit had the lower values of ascorbic acid at the early time of storage, and this can be supported by the role of these elicitors in delay the ripping processes as increasing of ascorbic acid is one sign of ripening process in apricot (Hegedüs *et al.*, 2011). This fact was reported by (Srivastava and Dwivedi, 2000) on delay ripening of banana. Aghdam *et al.*, (2011) documented that MeSA reduced the ethylene production and delayed ripening of kiwifruit. Our results are consistent with the finding of Gonzalez-Aguilar *et al.*, (2004) who reported that ascorbic acid was not affected by the MeJA treatment.

### 4. 2.4 Effect of SA and MeJA on enzyme activity

PAL activity in MeJA and SA treated fruits increased with storage time and was significantly higher in all dates of cold storage and shelf-life treatments compared towater treated fruits (Fig. 18a, b). PAL activity showed a significantly higher increase in MeJA treatments compared to SA treatments by day 21 and by day 8 in the cold storage and shelf-life treatments, respectively.

SA and MeJA treatment recorded increasing in POD activity for cold storage or shelf-life treatment, while control treatment resulted in fluctuated values between decreasing and increasing during the experiment time (Fig. 18 c,d).

SOD activity increased gradually after the onset of cold storage treatment in MeJA and SA treated fruit (P<0.05), while failed to show any increase in control fruits (Fig. 19a,b). The effect of MeJA was more pronounced than that of SA (P<0.05).

CAT activity increased for all the treatments, the SA and MeJA treated fruit showed higher activity than control treatment (P<0.05). The effect of SA was superior over MeJA at 14 and 21 days of cold storage or at 8 days of shelf-life treatments (Fig. 19c,d).

PAL as a key enzyme in the first step of the phenylpropanoid pathway is directly involved in the biosynthesis of phenolic compounds, including phenols, stilbenes, and flavonoids (Dixon and Paiva, 1995). It has been reported that the accumulation of phenols and anthocyanins

paralleled the increase in PAL activity in apple and grape fruits (Ju *et al.*, 1995; Hiratsuka *et al.*, 2001). Therefore, the activity of PAL was examined in this study to investigate the possible role of PAL in phenolic metabolism of apricot fruit in response to MeJA or SA treatments. We found that fruit treated with MeJA exhibited significantly higher levels of PAL activity (Fig. 18a, b), total phenolic content (Fig. 16a, b) and total antioxidant capacity (Fig. 16c, d) compared to the control fruit. These results suggest that MeJA may improve the antioxidant status of apricot fruit by inducing PAL activity and thus positively affecting phenolic metabolism. Some papers demonstrated that SA plays a vital role in the induction of systematic acquired resistance in plant cells by its ability to induce defense and antioxidant enzymes such as (POD), (PAL), polyphenoloxidase and  $\beta$ -1, 3-glucanase (Qin *et al.*, 2003) and also, Yao and Tian (2005) reported that pre-harvest treatment of sweet cherries with SA has induced PAL and POD activities during the short time storage period.

SA and MeJA treated fruit were observed with increased POD activity throughout the storage period. Numerous researchers have documented increased POD activity in fruits when treated with SA such as sugar apple fruits (Mo et al. 2008) and the same was reported or sweet cherry fruit when treated with SA or MeJA (Yao and Tian, 2005a).

The SOD enzyme has important roles in detoxifying ROS and alleviating chilling injury (Sala, 1998; Wang et al., 2008). Our results showed that MeJA and SA treatments increased SOD activity. Therefore, protection from oxidative injury is crucial to cell survival under chilling stress and is thought to be a major mechanism of resistance to chilling. It has been reported that the improvement of chilling tolerance in harvested horticultural crops is related to the enhancement of antioxidant enzyme activities. Sala (1998) found that chilling-tolerant mandarins have higher antioxidant enzyme activity than chilling sensitive cultivars. This may interpret why MeJA and SA is more beneficial during cold storage than subsequently (Table 3). Chilling as a stress factor induces the expression of some important antioxidant genes. Fung et al., (2004) demonstrated that the expression of alternative oxidase and seven other genes involved in defense against oxidative stress in pepper fruit treated with MeSA or MeJA vapor was increased even at 25 °C, whereas no changes were observed for untreated fruits. Higher expression induced prevention from chilling injury. MeJA was also reported to decrease membrane lipid peroxidation and maintain high superoxide dismutase (SOD) activity in strawberry plants under water stress (Wang, 1999) and MeJA treatment resulted in similar consequences in our study (Fig. 19a, b).

Peng *et al.* (2009) reported the level of  $H_2O_2$  in peach fruit was significantly enhanced by MeJA, also found that MeJA treatment affected reactive oxygen species (ROS)-metabolising enzymes such as SOD, which catalyses the dismutation of  $O_{-2}$  to  $H_2O_2$ , and CAT, which scavenge  $H_2O_2$  to oxygen and water. In addition, MeJA increased the activity of SOD and decreased the activity of CAT, resulting in accumulation of  $H_2O_2$ . In plants, ROS such as  $H_2O_2$  and superoxide radicals could contribute to enhancement of disease resistance (Inze and Motagu, 1995). In our study, MeJA increased the activity of SOD and decreased the activity of CAT, which may result in accumulation of  $H_2O_2$ . Thus enhancement of ROS generation may also be another part of the mechanism of MeJA in inducing stress resistance in apricot fruit.

It has been reported that there is a close relationship between accumulation of ROS and decreased fruit susceptibility to decay after harvest (Chanjirakul *et al.*, 2008). Torres *et al.*, (2003) showed that higher levels of H2O2 were correlated with lower susceptibility of early harvested apple fruit to *Penicillium expansum* infection.

In the present investigation, SA increased CAT activity in apricot fruit as compared with the control fruit (P<0.05). This might be due to SA which has been reported to enhance the transcription and translation of the CAT gene in treated peach fruit (Tian *et al.*, 2007). Researchers have also showed that sugar apple fruit treated with SA had increased CAT activity (Mo *et al.*, 2008). By contrast Zeng *et al.* (2006) found that SA treatment increased  $H_2O_2$  and  $O_{-2}$  contents and enhanced resistance to anthracnose rot of mango fruit. So the different two elicitors have different mode of action on enhancing the storability of apricot also, they can play different mechanisms in different species.

Thus our results may imply that various functions exerted by these different defense enzymes in the defense system may be collectively and coordinately induced by MeJA and SA in apricot fruit. The induced activities of defense enzymes may be one part of the mechanism of MeJA and SA in improvement storability of apricot fruit by reducing the stress of cold storage or maintenance the membrane rigidity.

### 4.2.5 Effect o SA and/ or MeJA on sensory characters

All the treated fruit tended to achieve low scores of most of sensory parameters with the time (Table 5). The control fruit received score below the acceptability limit (5 point) for taste and visual appearance at 7 day of cold storage and continued to decline with prolong of storage

time. While, the MeJA or/ SA treated fruit had scores over the acceptability limit up to 14 days of cold storage (P<0.05). For shelf-life treatment, the control fruit received scores below (P<0.05) the acceptability limit in flesh color, texture, taste and visual appearance attributes up to 4 days at room temperature at the same time SA and MeJA treatment saved the scores over the 5points. Overall acceptability score were above the 5 points for either SA or MeJA treatment up to 14 days of cold storage or at 4 day at room temperature

It is known that most of the sensory properties are sum of the interaction between sugars, acids, and a set of volatile compounds synthesized from a diverse set of precursors, including amino acids, lipids, and carotenoids. Some of these volatiles impart desirable qualities, while others are negatively perceived. (Beaudry, 2000). The amounts of sugars and organic acids and their ratios have been correlated with some of the sensory properties of fruits (Colaric *et al.*, 2005).

Obenland *et al.* (2009) reported that Sugar/acid ration is an important factor in determining consumer acceptance. Sugar provides sweetness and the organic acids sourness. Slightly decrease in taste score of SA and/or MeJA treatments may be because of reduce the conversion of starch/organic acid into sugars as occur in ripening stage (Arthey and Philip, 2005) and this can be the expressed as SSC/acid ratio which recorded higher values for those treatments than control treatment (Fig. 14 a,b) whereas, the degradation of structural polysaccharides and carbohydrates into other simple compounds was reported as a reason reduction of the fruit taste at later stage during storage (Kays, 1991) and this can interpret why control fruit recorded extremely low score of taste over 2 weeks of cold storage as the SSC in the same time took to decrease sharply after 2 weeks of cold storage (Fig. 13c).

The decreasing in flesh color of water treated fruit was noticed as in table 5, while the priority for control fruit was cleared for the cold stored fruit over 2 weeks an over 4 days of shelf-life storage. The breakdown of internal flesh color was expressed as fruit decay index in table 4 and the control fruit showed the highest percentage of fruit decay, this acceptance between the flesh browning as sensory parameter and fruit decay as physical parameter can be discussed as the ability of SA or MeJA to increase the tissue resistance against degradation and even fungi infection (Asgharia and Aghdam 2010; Chanjirakul *et al.*, 2008; Ding *et al.*, 2001, 2002). The skin color data is showing significance enhancement for control fruit than chemical treated fruit over 2 weeks of storage or 8 days at room temperature. Hegedüs et al. (2011) reported that The lightness factor L\*, measured on the fruit skin, increased during the

entire ripening process, a great proportion of this increase coincided with the first phase of the ripening process characterized by chlorophyll degradation in developing fruits. This can be the reason why control fruit showed this enhancement in fruit color as the fruit already continuous for ripening process while for SA or MeJA they were reported as delay or fruit ripening (Srivastava and Dwivedi, 2000; Aghdam *et al.* 2011).

Fruit texture data showed that the MeJA and SA recoded the highest score over 2 weeks of cold storage or 8 days at room temperature while the control fruit showed the extreme low texture score over 2 weeks at 1 ° C or 4 days at 25 ° C. (Table 5)

The reduction in fruit texture might be due to degradation of pectin substance and maximum change may be attributing to minimum texture score as reported for control as documented by Wills *et al.*, 1989. It has been documented that MeJA reduces pectin-methyl-esterase (PME) activity to decrease de-esterification of pectin (Meng *et al.*, 2009), and hence maintains fruit texture. SA has been documented to enhance flesh firmness of harvested peaches during storage (Wang *et al.*, 2006)

The loss in overall acceptability scores of apricot fruit might be due to degradation of different parameters during storage. Color, flavor, taste and texture are degraded due to browning, moisture losses, the breakdown of sugars, acids and volatile compounds (Ishaq *et al.*, 2009a). The ability of SA or MeJA to keep the overall acceptability better than control treatment may because them role to keep most of physical parameters unchanged over the storage. Our results were in agreement with Sartaj *et al.*, 2013 reported that use of SA was improved fresh market traits of apricot during ambient storage and may help to increase marketing options of apricot fruit.

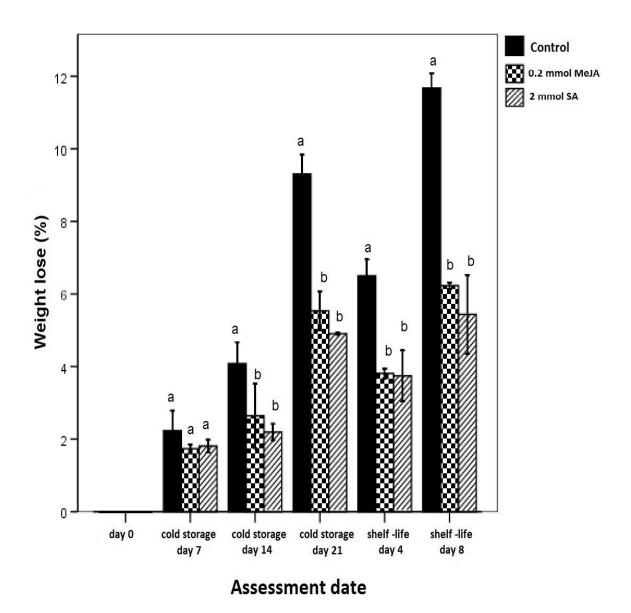


Fig. 12 Effect of 0.2 mmol  $L^{-1}$  methyl jasmonate (MeJA) and 2 mmol  $L^{-1}$  salicylic acid (SA) on weight loss percentage of apricot fruit (cv. 'Bergarouge') in cold storage treatment at days 7, 14 and 21 at 1 °C and in shelf-life treatment at days 4 and 8 at 25 °C. Values within a column for the given days followed by different letters are significantly different at P<0.05 according to Duncan's multiple range tests. Error bars represent the SD values.

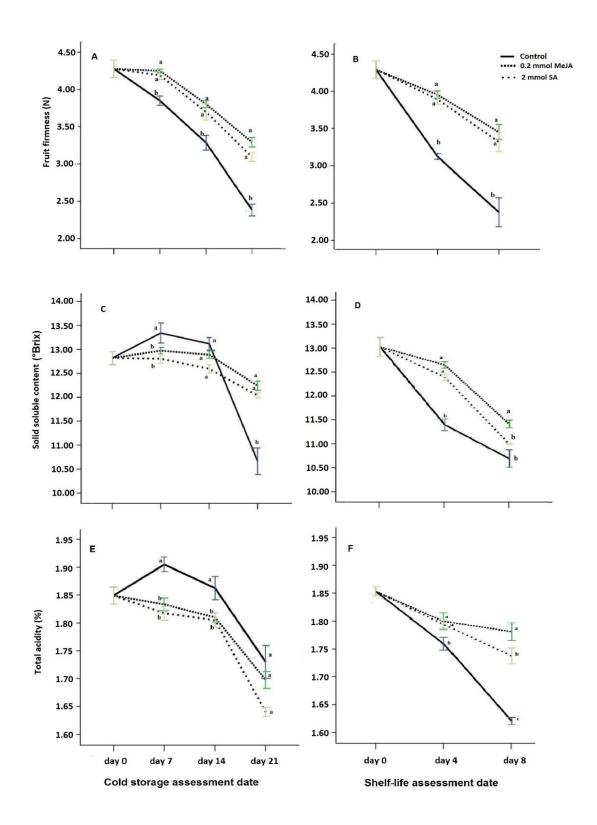


Fig.13 Effect of 0.2 mmol  $L^{-1}$  methyl jasmonate (MeJA) and 2 mmol  $L^{-1}$  salicylic acid (SA) on fruit firmness (N), soluble solid content ( $^{\circ}$ Brix) and total acidity (%) of apricot fruit (cv. 'Bergarouge') in cold storage treatment at days 7, 14 and 21 at 1  $^{\circ}$ C (A, C, and E), and in shelf-life treatment at days 4 and 8 at 25  $^{\circ}$ C (B, D and F). Values within the given days

followed by different letters are significantly different at P<0.05 according to Duncan's multiple range tests. Error bars represent the SD values.

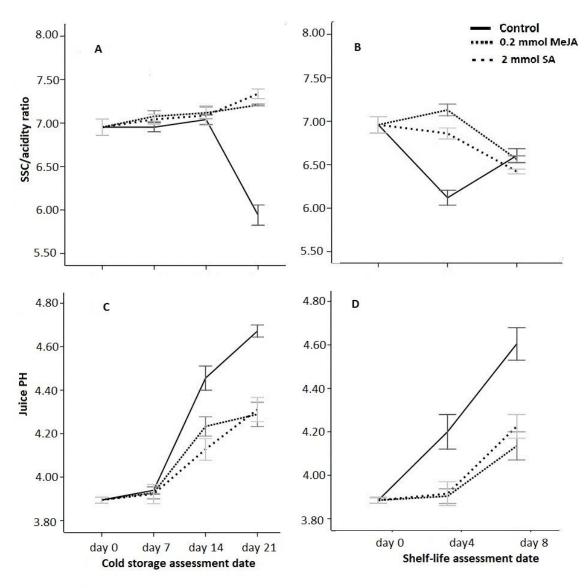


Fig. 14 Effect of 0.2 mmol L<sup>-1</sup> methyl jasmonate (MeJA) and 2 mmol L<sup>-1</sup> salicylic acid (SA) on SSC/acidity ratio, juice pH of apricot fruit (cv. 'Bergarouge') in cold storage treatment at days 7, 14 and 21 at 1 °C (A and C), and in shelf-life treatment at days 4 and 8 at 25 °C (B and D). Values within the given days followed by different letters are significantly different at P<0.05 according to Duncan's multiple range tests. Error bars represent the SD values.

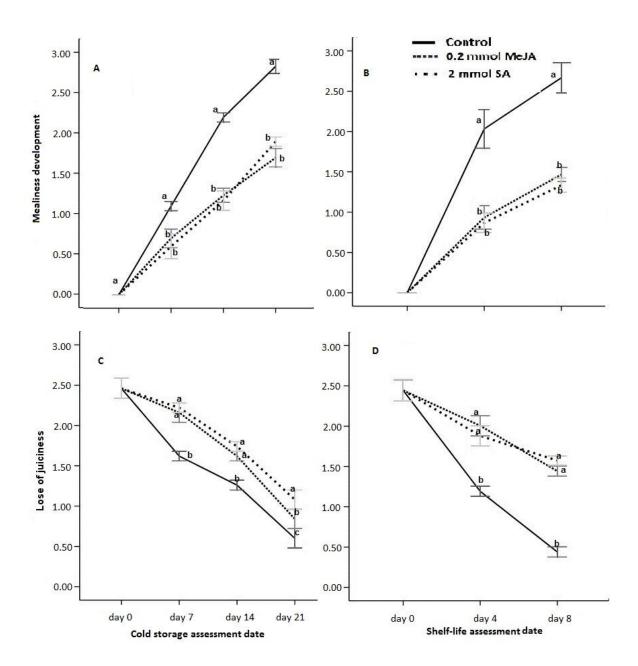


Fig. 15 Effect of 0.2 mmol L<sup>-1</sup> methyl jasmonate (MeJA) and 2 mmol L<sup>-1</sup> salicylic acid (SA) on Mealiness development and lose of juiciness of apricot fruit (cv. 'Bergarouge') in cold storage treatment at days 7, 14 and 21 at 1  $^{\circ}$ C (A and C), and in shelf-life treatment at days 4 and 8 at 25  $^{\circ}$ C (B and D). Values within the given days followed by different letters are significantly different at P<0.05 according to Duncan's multiple range tests. Error bars represent the SD values.

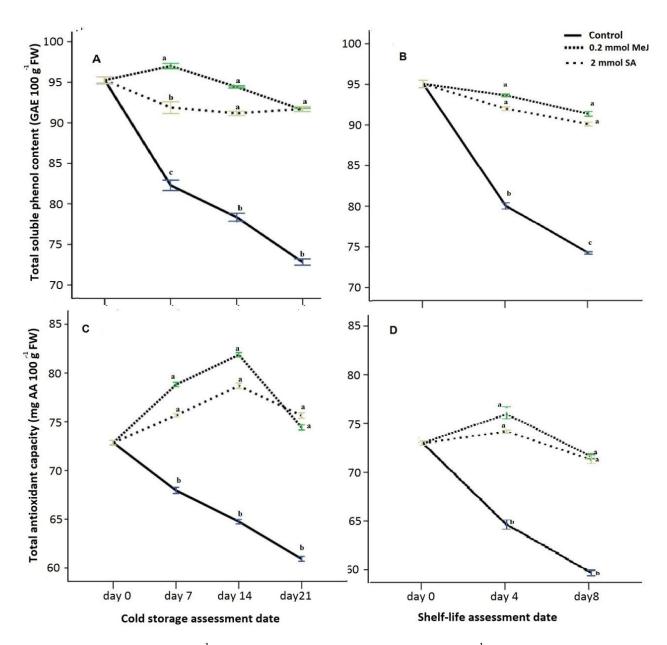
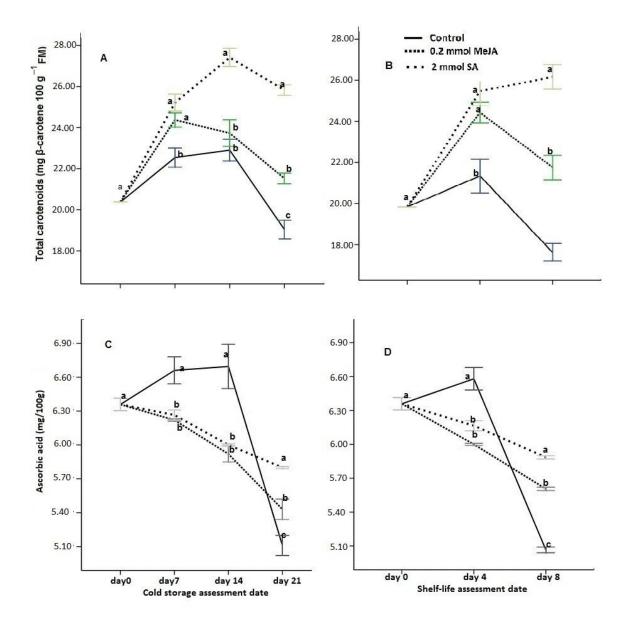
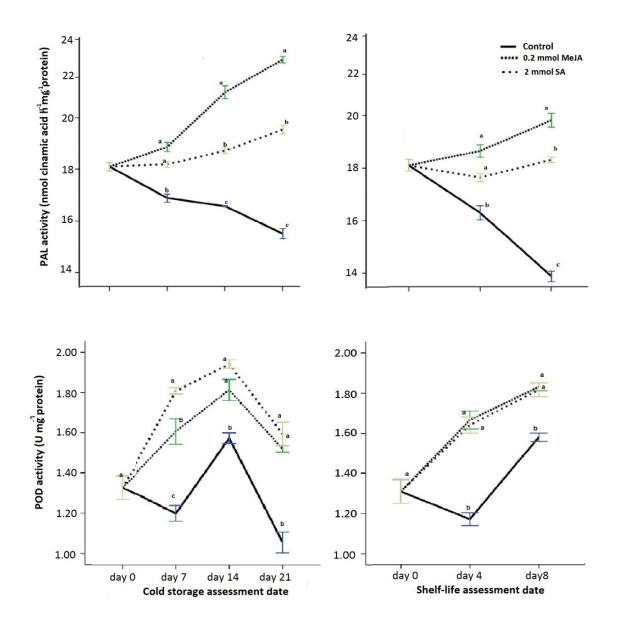


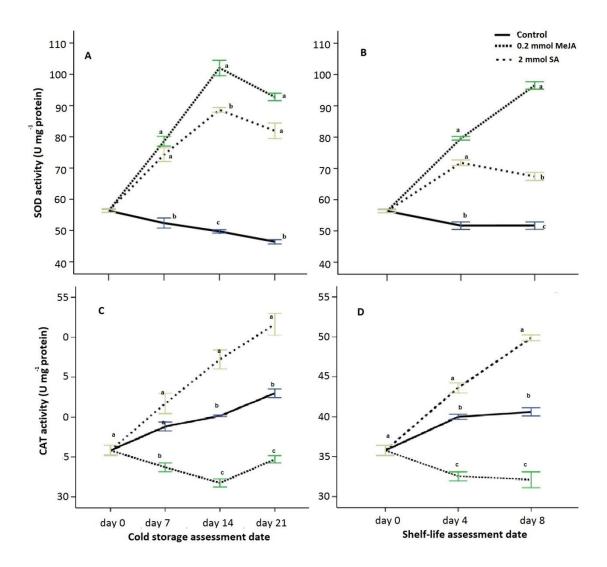
Fig. 16 Effect of 0.2 mmol L<sup>-1</sup> methyl jasmonate (MeJA) and 2 mmol L<sup>-1</sup> salicylic acid (SA)on total soluble phenol content (GAE 100 g<sup>-1</sup> FW) and total antioxidant capacity (mg AA 100 g<sup>-1</sup> FW) in apricot fruit (cv. 'Bergarouge') in cold storage treatment at days 7, 14 and 21 at 1 °C (A and C), and in shelf-life treatment at days 4 and 8 at 25 °C (B and D). Values within the given days followed by different letters are significantly different at P<0.05 according to Duncan's multiple range tests. Error bars represent the SD values.



**Fig. 17** Effect of 0.2 mmol L<sup>-1</sup> methyl jasmonate (MeJA) and 2 mmol L<sup>-1</sup> salicylic acid (SA) on Total carotenoids (mg β-carotene 100 g–1 FM) and ascorbic acid (mg/100 g)in apricot fruit (cv. 'Bergarouge') in cold storage treatment at days 7, 14 and 21 at 1  $^{\circ}$ C (A and C), and in shelf-life treatment at days 4 and 8 at 25  $^{\circ}$ C (B and D). Values within the given days followed by different letters are significantly different at P<0.05 according to Duncan's multiple range tests. Error bars represent the SD values.



**Fig. 18** Effect of 0.2 mmol L<sup>-1</sup> methyl jasmonate (MeJA) and 2 mmol L<sup>-1</sup> salicylic acid (SA) on phenylalanine ammolonia-lyase (PAL; nmol cinnamic acid h<sup>-1</sup> mg<sup>-1</sup> protein) and peroxidase (POD) activity in apricot fruit (cv. 'Bergarouge') in cold storage treatment at days 7, 14 and 21 at 1 °C (A), and in shelf-life treatment at days 4 and 8 at 25 °C (B). Values within the given days followed by different letters are significantly different at P<0.05 according to Duncan's multiple range tests. Error bars represent the SD values.



**Fig. 19** Effect of 0.2 mmol L<sup>-1</sup> methyl jasmonate (MeJA) and 2 mmol L<sup>-1</sup> salicylic acid (SA) on superoxide dismutase (SOD; U mg<sup>-1</sup> protein) and catalase (CAT; U mg<sup>-1</sup> protein) activity in apricot fruit (cv. 'Bergarouge') in cold storage treatment at days 7, 14 and 21 at 1 °C (A), and in shelf-life treatment at days 4 and 8 at 25 °C (B). Values within the given days followed by different letters are significantly different at P<0.05 according to Duncan's multiple range tests. Error bars represent the SD values.

Table 3 The effect of treatments of  $0.2 \text{ mmol } L^{-1}$  methyl jasmonate (MeJA) and  $2 \text{ mmol } L^{-1}$  salicylic acid (SA) on chilling injury of cv. 'Bergarouge' apricot fruit

	CI index (%) <sup>a</sup>								
Treatment		Cold storage at 1 °C	Shelf-life at 25 °C						
	Day 7	<b>Day 14</b>	Day 21	Day 4	Day 8				
Control	$3.12 \pm 0.87a^{b}$	$16.68 \pm 1.45a$	$37.65 \pm 4.56$ a	18.67±2.35a	30.24±3.65a				
0.2 mmol MeJA	$1.35 \pm 0.21a$	$4.52 \pm 1.98b$	$9.68 \pm 3.65c$	$8.65\pm1.09b$	$14.35 \pm 1.35c$				
2 mmol SA	$1.64 \pm 0.34a$	$5.58 \pm 0.68b$	$20.91 \pm 5.09b$	$7.68\pm2.34b$	19.26±2.65b				

<sup>&</sup>lt;sup>a</sup> CI index: chilling injury index.

<sup>&</sup>lt;sup>b</sup> Values within a column followed by different letters are significantly different at P<0.05 according to Duncan's multiple range tests. The results represent the means  $\pm$  SD of triplicate assay.

Table 4 The effect of treatments of  $0.2 \text{ mmol } L^{-1}$  methyl jasmonate (MeJA) and  $2 \text{ mmol } L^{-1}$  salicylic acid (SA) on apricot fruit decay of cv. 'Bergarouge'

Treatment	FD index (%) <sup>a</sup>							
		Shelf-life at 25 °C						
	Day 7	Day 14	Day 21	Day 4	Day 8			
Control	$16.05 \pm 1.07a^{b}$	$56.24 \pm 5.35a$	$96.25 \pm 2.3 \text{ a}$	66.36±2.65	100±3.05			
0.2 mmol MeJA	$2.11 \pm 0.98b$	$6.57 \pm 1.08b$	$16.98 \pm 6.65$ b	$16.35 \pm 3.65$	$36.65 \pm 2.03$			
2 mmol SA	$2.33 \pm 1.03b$	$7.58 \pm 2.08b$	$15.61 \pm 4.19b$	$20.36\pm4.09$	35.36±3.65			

<sup>&</sup>lt;sup>a</sup> FD index (%): fruit decay index in percentage <sup>b</sup> Values within a column followed by different letters are significantly different at *P*<0.05 according to Duncan's multiple range tests. The results represent the means  $\pm$  SD of triplicate assay.

**Table 5** The effect of treatments of 0.2 mmol L<sup>-1</sup> methyl jasmonate (MeJA) and 2 mmol L<sup>-1</sup> salicylic acid (SA) on sensory properties and overall liking of cv. 'Bergarouge' apricot fruit.

		G 11		Shelf-life			
Parameters	Chemical treatment	Cold storage assessment date				assessment date	
		Day 0	Day 7	Day 14	Day 21	Day 4	Day 8
Skin color	Control	7.20aA	7.83aA	7.95bB	7.56bB	7.10aC	7.67bB
	MeJA	7.20aA	7.40aB	6.50aA	6.10aA	6.20aB	6.30aA
	SA	7.20aA	7.90aB	6.80aA	6.20aA	6.00aB	6.40aA
	Control	8.00aA	4.20bB	3.90cB	3.10cB	2.20dB	1.90dB
Flesh color	MeJA	8.00aA	7.30aA	6.90aA	5.30bA	6.20bA	4.30cA
	SA	8.00aA	7.09aA	6.30bA	5.30bA	5.90bA	4.10cA
Texture	Control	8.90aA	5.20bB	4.30bC	2.90cB	4.30bB	3.10cC
	MeJA	8.90aA	7.10aA	6.10bA	4.30cA	6.10bA	4.10cB
	SA	8.90aA	6.90aA	5.20bB	4.70cA	6.30bA	5.10cA
Taste	Control	7.80aA	4.30bB	3.10cB	2.30cB	3.90bB	2.90cA
	MeJA	7.80aA	6.90aA	5.30bA	3.90cA	5.20bA	3.10cA
	SA	7.80aA	7.10aA	5.20bA	4.20cA	5.30bA	3.20cA
Visual appearance	Control	7.30aA	4.50bB	3.90bB	2.90cC	2.70cB	1.70dB
	MeJA	7.30aA	6.80aA	5.90bA	3.80cB	5.50bA	3.80cA
	SA	7.30aA	7.10aA	5.90bA	4.90cA	5.20bA	4.30cA
Overall acceptability	Control	8.00aA	4.10bB	3.20cB	1.80dB	2.10dC	1.20dC
	MeJA	8.00aA	6.20bA	5.30bA	3.90cA	5.30bB	4.20cB
	SA	8.00Aa	6.40bA	5.70bA	4.20cA	6.10bA	4.20bA

For each column, similar capital letters are not significantly different at  $P \le 0.05$  among Chemical treatments for each parameter. Similar small letters in rows are not significantly different at  $P \le 0.05$  during storage.

# 4.3. Effect of SA and/or MeJA on induce resistance to *Monilinia laxa* on apricot fruit of cv. Bergarouge

## 4.3.1 Effect of different concentrations of SA and MeJA on mycelia growth of *M. laxa in vivo*, lesion diameter (mm) and fruit disease incidence (%)

Data presented in table 6 showed that all concentrations of SA and MeJA reduced the growth rate of monilinia mycelia compared with water treated media. PDA media with 2 or 5 mmol L<sup>-1</sup> of SA produced the lowest growth rate of *M. laxa* (P<0.05). The pathogen which incubated with 0.4 or 0.7 mmol L<sup>-1</sup> MeJA also achieved lower growth rate with significant differences from the control. The same trend was found for fruit disease incidence as all the concentrations of SA and MeJA reduced the apricot fruit decay with significant differences with water treated fruit. The lesion diameter and disease incidence data revealed that the high concentration of SA and/or MeJA acheied the considered effects on M. laxa over the low concentration.

Lesion diameter increased from 0 mm to  $15.49 \pm 1.01$  mm in water treated fruits and the same was for 0.1 mmol L<sup>-1</sup>MeJA treated fruit as the lesion diameter arose to  $15.77 \pm 0.45$  mm. Meanwhile with increasing MeJA concentration the lesion diameter reduced to reach to  $11.49 \pm 0.69$  mm in 0.7 mmol MeJA treated fruit wich was significantly differentfrom control fruits (Table 6). At the same time fruit treated with 2 or 5 mmol SA recorded the lowest lesion diameter after incubation with fungi (P < 0.05) in comparison to low concentration.

# 4.3.2 Effect of 2 mmol $L^{-1}$ SA and 0.4 mmol $L^{-1}$ MeJA treatment on mycelial growth of *M. laxa in vitro* at 2, 4, 6 and 8 days of incubation

Data presented in Fig. 20, Indicated that SA and MeJA had inhibitory effects on mycelial growth of *M. laxa* in PDA in comparison to control. SA achieved the lowest growth rate of *M. laxa* with significant differences with other treatments (P<0.05) after 4 days of inoculation. In control treatment, growth rate of *M. laxa* reached more than 100 % after 6 days of incubation. It was also noticed that the effect of 0.4 mmol L<sup>-1</sup> MeJA started to disappear at 8 days of inoculation as reached to 93.16 % while 2 mmol L<sup>-1</sup> SA treted PDA media was only 68.98 %.

## 4.3.3 Effect of 2 mmol $L^{-1}$ SA and 0.4 mmol $L^{-1}$ MeJA treatment on disease incidence, lesion diameter, fruit firmness and lignin content of apricot fruit incubated with *M. laxa*

Generally, results in figs. 21 and 22 showed that the postharvest application of SA and MeJA resulted in significantly lower disease percentageand lesion development compared to control.

The decay incidence of water treated and stored fruit at 8th day was about 39.47 % with significance differences from 2 mmol L<sup>-1</sup> SA and 0.4 mmol L<sup>-1</sup> MeJA, as the decay incidence were 20.41 and 22.43 %, respectively (Fig. 21). Fruit treated with SA showed more resistance to infection than fruit treated with MeJA, as at days 4 and 6 the differences between SA and MeJA were significant.

Although all the inoculated wounds in both SA or MeJA treated and control fruit showed increase a lesion diameter during the incubation times, the lesion diameter in SA and MeJA treated fruit was still significantly lower than that in the control fruit (P<0.05), for example, at 8 days the lesion diameter were 11.84, 12.12 and 16.29 mm for treated fruit with SA, MeJA and water, respectively (Fig. 22).

For inoculated wounds with *M. laxa* in all chemical treatments, the development of fruit softening was noticed at all the incubation times, but the reduction in firmness for MeJA treated fruit was lower (P>0.05) than SA treated at 4 and 8 days of infection (Fig. 23). Meanwhile, control fruit showed sharp deterioration in fruit firmness during all the incubation assessment times. For unwound treatment, SA or MeJA treated fruit, presented approximately unchanged fruit firmness during second, 4<sup>th</sup> and 6<sup>th</sup> of storage at 25 °C, while at 8<sup>th</sup> of storage the reduction in fruit firmness was explicit (Fig. 23) but still better than control treatment (P<0.05). MeJA treatment showed priority over the SA treatment with significant differences at 4 and 8 days of storage.

#### 4.3.4 Effect of 2 mmol L-1 SA and 0.4 mmol L-1 MeJA treatment on lignin content

Lignin content of apricot fruit treated with MeJA recorded the highest value with significant differences from all other treatments (Fig. 24). Unwounded SA treated fruit did not display sensitive enhancement in lignin content in apricot fruit at 2 days of treatment while it increased bit than control fruits at 6 and 8 days of treatment. Meanwhile inoculation with *M. laxa* resulted in lignin content reduction in treated fruit with water or SA.

## 4.3.5 Effect of 2 mmol L-1 SA and 0.4 mmol L-1 MeJA treatment on levels of total phenolic and total antioxidant capacity.

Inoculated and SA or MeJA treated fruits showed slight increase in total phenol content after 2 and 4 days of incubation then it decreased gradually without explicit priority for MeJA or SA (P>0.05). While water treated fruit showed sharp decrease in phenols content (Fig. 25).

On the other hand, unwounded and SA or MeJA treated fruit recorder roughly unchanged values of total phenols content over the 6th day of treatment at 25 °C then decrease slightly at 8th day. Control fruit achieved nearly stable values at second day then the value took to decrease (P<0.05).

Fruit treated with SA or MeJA showed higher antioxidant capacity than those treated with water at all the assessment times and in both inoculated and non-inoculated treatment (P<0.05). The results showed that MeJA treatment keeps the antioxidant higher than SA (over the assessment times in both wounded or unwounded treatments (Fig. 26).

## 4.3.6 Effect of 2 mmol $L^{\text{-}1}$ SA and 0.4 mmol $L^{\text{-}1}$ MeJA treatment on changes in defense-related enzymes in apricot fruit

SOD activity in non-inoculated fruit and treated with SA, MeJA and water, increased with storage time at 25 °C, however, the SOD activity increase was higher in both SA and MeJA than control fruits (P<0.05) at all storage times. Fruit inoculated with *M. laxa* recorded increase in SOD activity at over the 6th day of treatment with SA and MeJA treatments. Meanwhile, control fruit showed a decrease in SOD activity at early inoculation time (Fig. 27). Treated fruit with MeJA recorded the highest SOD activity with significant differences with SA and control fruits at all inoculation times (P<0.05).

POD activity increased during the experiment either in inoculated or non-inoculated fruits. The differences were significant among all chemical treatments at all assessment date, except between SA and water treatments after 2 days of storage in non-inoculated fruit (p>0.05). MeJA treatment showed the highest POD activity (P<0.05) (Fig. 28).

PAL activity (Fig. 29) increased with SA or MeJA treatment at early time of storage, meanwhile the water treated fruit resulted about unchanged PAL values with significant differences from SA or MeJA treatments at all the storage times in non-inoculated fruits. Similarly, for infected fruits SA or MeJA induced PAL activity in comparison to control fruits (P<0.05).

In this study, 2 mmol L<sup>-1</sup> SA and 0.4 mmol L<sup>-1</sup> MeJA treatment significantly reduced *M. laxa* growth *in vitro* (Fig. 20). This reduction in fungal growth rate may due to direct toxicity on *M. laxa* or indirect strategy by inducing resistance in infected cells. Our results were in the line with Yao *et al.* (2005), who reported that 2 mmol L<sup>-1</sup> SA showed direct fungal toxicity on *M. fructicola* and significantly inhibited mycelial growth and spore germination of the pathogen *in vitro*. Cao *et al.* (2008) reported that the control of the disease is direct because of the inhibitory effect of MeJA on pathogen growth on loquat treated fruits, and indirect because of the induced disease resistance triggered by enhanced H<sub>2</sub>O<sub>2</sub> levels. Meanwhile, Tsao and Zhou (2000) documented that MeJA alone did not reduce brown rot on sweet cherry.

The results of this study indicated that post harvest treatments with (2 mmol L<sup>-1</sup>) SA or/and (0.4 mmol L<sup>-1</sup>) MeJA could significantly reduce disease incidence and lesion diameter of apricot fruit stored at 25 °C (Figs. 21 and 22). These results are in agreement with the findings of Yao *et al.* (2005) who reported that pre-harvest treatments with SA or/and MeJA, significantly reduced lesion diameters and disease incidence on sweet cherry fruit caused by *M. fructicola* compared with non-treated fruit. The role of SA or MeJA to reduce disease incidence or lesion diameter can be understood by direct toxicity effect (Fig. 20) or by indirect activation of some defenses enzymes which play a role in the breakdown of the fungi cell wall. Chitinase and  $\beta$ -1,3-glucanase induce activity of some enzymes which play roles either to save plant cell wall or raise the antioxidant capacity in the cells such as POD or PAL (Yao *et al.*, 2005). Our results showed that treated fruit with SA or MeJA recorded high levels of POD and PAL activities (Figs. 28 and 29)

The ability of SA and MeJA to increase the resistance of apricot fruit to *M. laxa* infection was accompanied with ability to keep fruit firmness, even after infection, much higher than the control treatment. The remaining of SA and/or MeJA treated fruit firmness higher may is due these elicitors are known as inhibitors of ethylene biosynthesis which works to increase fruit ripening, and then SA and/or MeJA are reducing fruit softening and delaying over ripening (Leslie and Romani, 1986). SA can delay the ripening of strawberry fruit, probably through inhibition of ethylene biosynthesis or action (Babalar *et al.*, 2007) and inhibits cell wall and membrane degrading enzymes such as poly galacturonase, lipoxygenase, cellulase and pectin methyl esterase, leading to decreased fruit softening rate (Asghari and Aghdam, 2010).

Lignin is a complex polymer of phenylpropanoid mainly deposited in cell walls (Whetten and Sederoff, 1995), and synthesis is induced by both mechanical wounding and microoganisms (Uritani and Oba, 1978; Vance et al., 1980). The final step in lignin biosynthesis requires oxygen for the oxidation of manomeric lignin precursors such as p-courmaryl, coniferyl and sinapyl alcohols to form polymers through the action of POD (Whetten and Sederoff, 1995). The stable fruit firmness of apricot fruit may be due to a rapid accumulation of lignin in treated fruit (Fig. 24). At the same time, the increase in phenolic content occurred (Fig. 25) concomitantly with high activities of POD and PAL (Figs. 28 and 29) which play the vital role in phenylpropaniod pathway. Some researchers were suggesting that SA or/MeJA prevent fruit from softening by affecting on lignin's biosynthesis enzymes such as PAL and POD (Yang et al., 2010).

In present study, treated fruit with 0.4 mmol L<sup>-1</sup> MeJA showed high accumulation of lignin in cell wall. Su et al. (2003) reported that MeJA treatment significantly increased PAL and POD activities and lignin content, which might account for higher disease resistance and lower decay index in MeJA treated vegetable soybean pods. These findings were in line with our results as we reported increase in activities of PAL and POD enzymes (Figs. 28 and 29.) with lower disease severity (Figs. 21 and 22) plus high accumulation of lignin in cell wall (Fig. 24) of apricot were associated with treatment of MeJA.

Total phenol content and antioxidant capacity of inoculated fruit and treated with SA or/and MeJA rose after infection with *M. laxa*. Also, it was interesting that using of SA or MeJA in non-inoculated fruit did not raise the phenol content and antioxidant capacity and the values remained approximately stable (Figs. 25 and 26). It is reported from different studies that the phenol content and antioxidant can rise in the cells as a response to infection and wound treatment. These studies showed that there are often large increases in phenolic synthesis in plants after attack by plant pathogens (De Ascensao and Dubrey 2003). Reimers and Leach, (1999) reported that the cells use high accumulation of phenolic compounds to restrict or inhibit the growth of the pathogen as

The measured enzymes are considered as key enzymes in control of plant disease in resistance systems.

Rapid generation of ROS also known as an oxidative burst, has been reported to occur upon contact between plant cell walls and fungi (Vera-Estrella et al., 1992), and has been

considered as one of the earliest events associated with plant resistance to pathogens at the site of pathogen invasion.

Plants have evolved defenses mechanisms which can be enzymatic or non-enzymatic that efficiently scavenge excess ROS (Inze and Montagu, 1995).

SOD is important enzyme in such action, SOD can protect cells from oxidant stress by dissimulating super oxide anion (O2) to oxygen and H2O2 (Chittoor *et al.*, 1999). The present study showed that SOD activity was significantly enhanced by SA and MeJA treatments in apricot fruit (Fig. 27). This can interpret how those treatments had higher resistance to infection than control by the high rate of reactive oxygen species scavenging. The same results were reported by Mittler (2002), who documented that increasing SOD activity was concomitant with superoxide radical scavenging activity increasing and decreasing of cell membrane damage and oxidative stress.

It has been shown that PAL activity can be induced by micro-organism infection (Saltveit, 2001) and this was noticed in our result as after infection PAL activity started to increase dramatically in fruit treated with SA and MeJA (Fig. 29). An increase in PAL activity is associated with biosynthesis of active metabolites, such as phytoalexins, phenols, lignins and salicylic acid in plant defense pathways (Milosevic and Slusarenko, 1996).

We postulate that may because SA and MeJA treatments could induce the cell to raise its resistance and activate its defenses system by producing high phenol content after the infection (Fig. 25) which accompanied with high activities of some phenol's biosynthesis enzymes such as POD and PAL (Figs. 28 and 29). POD has very important role to reinforcement of cell walls against fungal infection as it produces the oxidative power for cross linking proteins and phenylpropaniod radicals. (Huckelhoven *et al.*, 1999; Kristensen *et al.*, 1999). Our results indicated that postharvest traetment with SA and MeJA induced higher activities POD in apricot fruit than the controls during early storage (Fig. 28) and this increase was in line with a reduction in disease incidence and lesion diameter (Figs. 21 and 22). It was documented that SA play role in induction of some defense system enzymes like polyphenoloxidase (PPO), PAL and other enzymes like β-1,3glucanase and chitinase which hydrolyze polymers of fungal cell walls and are thought to be involved in plant defense mechanisms against fungal infection (Qin *et al.*, 2003, Collinge *et al.*, 1993; Schlumbaum *et al.*, 1986).)

Yao and Tian (2005a,b) found that treatment with MeJA induced stronger PAL and POD activities and significantly reduced lesion diameter caused by *M. fructicola* or *P. expansum* in sweet cherry and peach fruit.

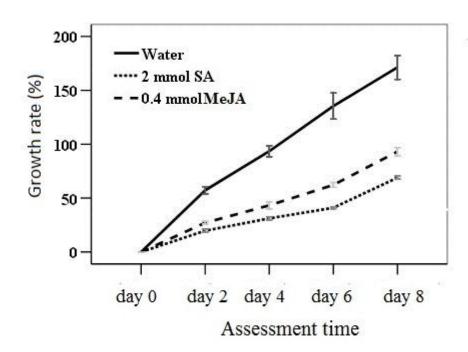
To further understanding the mechanisms which compounds like SA and MeJA inhibit brown rot caused by *M. laxa*, the inhibition was appear in two ways:

- ✓ Direct effect on mycelia growth *in vitro* and it was reported that SA and MeJA had obvious effect to reduce mycelail growth but SA had the priority than MeJA.
- ✓ Indirect effect, by inducing defenses mechanisms or induced disease resistance in infected cell. This may achieve by increasing in gene expression and high production of some defenses enzymes like PAL, POD and SOD hence, raise the lignifications of cell wall to make it rigid. It was noticed that pretreated fruit with MeJA induced high activities of these enzymes and play important role in lignifications of cell wall than SA treated fruit.

**Table 6** The effect of treatments of 0.5, 2, and 5 mmol  $L^{-1}$  salicylic acid (SA) and 0.1, 0.4, and 0.7 mmol  $L^{-1}$  methyl jasmonate (MeJA) on apricot fruit disease incidence and mycelia growth of *Monilinia laxa in vitro*.

Treatments	Control		SA			MeJA	
Concentrations	Water	Water	0.5 mmol L <sup>-1</sup>	2 mmol L <sup>-1</sup>	5 mmol L <sup>-1</sup>	0.1 mmol L <sup>-1</sup>	0.4 mmol L <sup>-1</sup>
Growth rate (%)	160.50± 12.02 a	81.98± 5.65 b	58.49± 12.01c	$55.03 \pm 5.58c$	89.91± 1.32b	74.48± 5.10b	$72.37 \pm 2.27$ b
Disease incidence (%)	58.42± 3.45 a	32.35± 2.09 b	22.50± 0.70 c	21.50± 2.12 c	36.48± 1.96 b	23.50± 0.70 c	22.84± 0.08 c`
Lesion diameter (mm)	15.49± 1.01a	12.99± 0.61b	10.31± 0.47 c	10.44± 0.61 c	15.77± 0.45 a	$12.81 \pm 0.23$ b	11.49± 0.69 b

Numbers in the rows followed by different letters showing the significance differences (P<0.05)



**Fig. 20** Effect of 0.4 mmol L<sup>-1</sup> methyl jasmonate (MeJA) and 2 mmol L<sup>-1</sup> salicylic acid (SA) on growth rate of *Monilinia laxa* in apricot fruit (cv. 'Bergarouge'). Error bars represent the SD values.

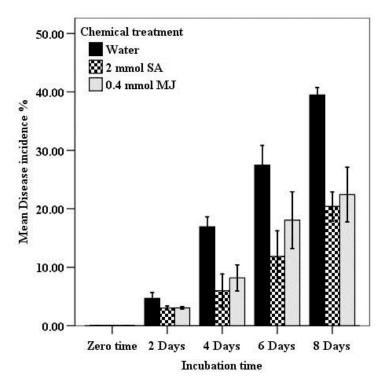
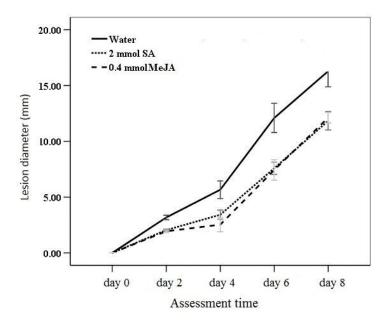
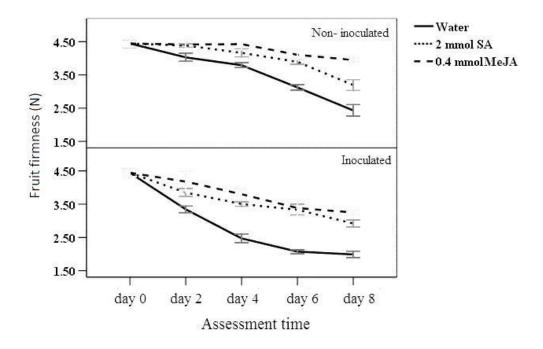


Fig. 21 Effect of 0.4 mmol L<sup>-1</sup> methyl jasmonate (MeJA) and 2 mmol L<sup>-1</sup> salicylic acid (SA) on disease incidence in apricot fruit (cv. 'Bergarouge'). Error bars represent the SD values.



**Fig. 22** Effect of 0.4 mmol L<sup>-1</sup> methyl jasmonate (MeJA) and 2 mmol L<sup>-1</sup> salicylic acid (SA) on lesion diameter in apricot fruit (cv. 'Bergarouge'). Error bars represent the SD values.



**Fig. 23** Effect of 0.4 mmol L<sup>-1</sup> methyl jasmonate (MeJA) and 2 mmol L<sup>-1</sup> salicylic acid (SA) on fruit firmness in apricot fruit (cv. 'Bergarouge'). Error bars represent the SD values.

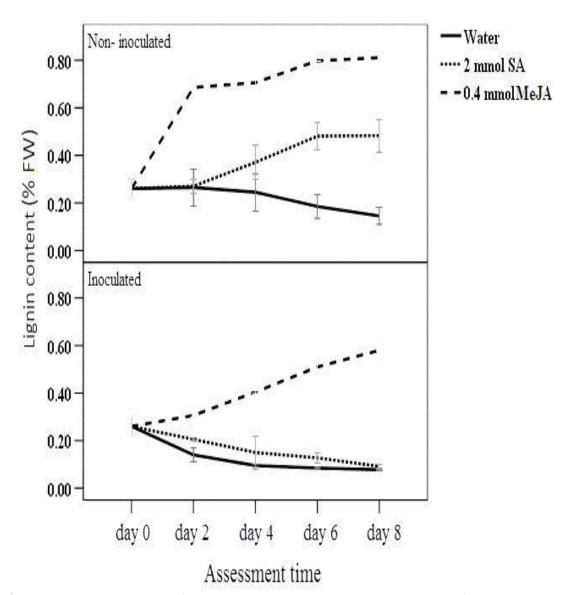
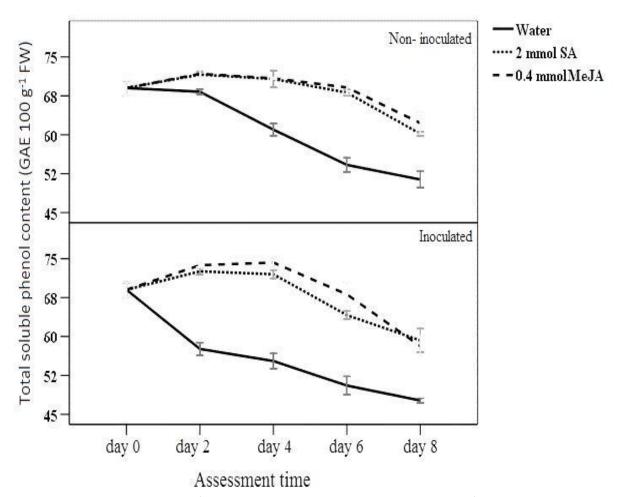
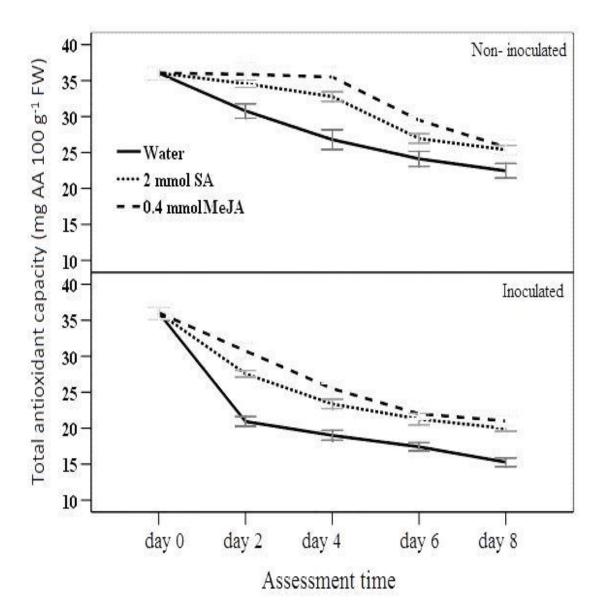


Fig. 24 Effect of 0.4 mmol L<sup>-1</sup> methyl jasmonate (MeJA) and 2 mmol L<sup>-1</sup> salicylic acid (SA) on lignin content (%) in apricot fruit (cv. 'Bergarouge'). Values within the given days followed by different letters are significantly different at P<0.05 according to Duncan's multiple range tests. Error bars represent the SD values.

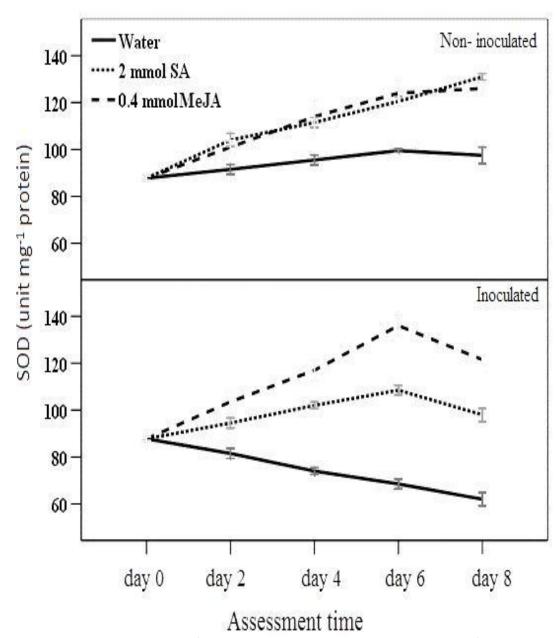


**Fig. 25** Effect of 0.4 mmol L<sup>-1</sup> methyl jasmonate (MeJA) and 2 mmol L<sup>-1</sup> salicylic acid (SA) on total soluble phenol content (GAE 100 g<sup>-1</sup> FW) in apricot fruit (cv. 'Bergarouge'). Error bars represent the SD values.

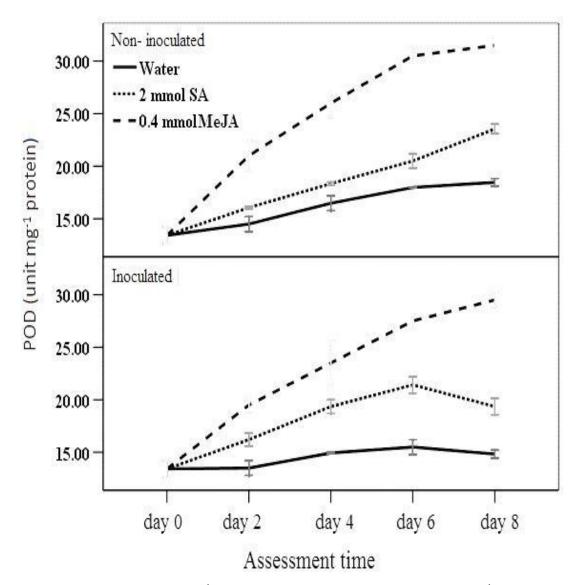


**Fig. 26** Effect of 0.4 mmol L<sup>-1</sup> methyl jasmonate (MeJA) and 2 mmol L<sup>-1</sup> salicylic acid (SA) on total antioxidant capacity (mg AA 100 g<sup>-1</sup> FW) in apricot fruit (cv. 'Bergarouge'). Error bars represent the SD values.

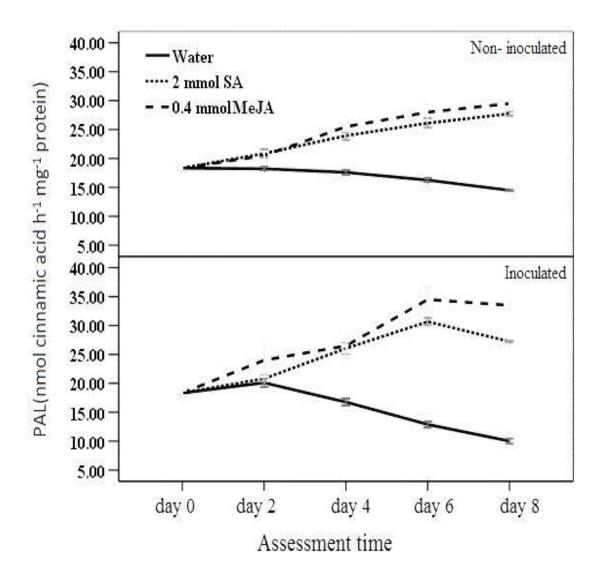
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**Fig. 27** Effect of 0.4 mmol L<sup>-1</sup> methyl jasmonate (MeJA) and 2 mmol L<sup>-1</sup> salicylic acid (SA) on supeoxide dismutase (SOD; unit mg<sup>-1</sup> protein) activity in apricot fruit (cv. 'Bergarouge'). Error bars represent the SD values.



**Fig. 28** Effect of 0.4 mmol L<sup>-1</sup> methyl jasmonate (MeJA) and 2 mmol L<sup>-1</sup> salicylic acid (SA) on peroxidase ( POD; unit mg<sup>-1</sup> protein) activity in apricot fruit (cv. 'Bergarouge'). Error bars represent the SD values.



**Fig. 29** Effect of 0.4 mmol L<sup>-1</sup> methyl jasmonate (MeJA) and 2 mmol L<sup>-1</sup> salicylic acid (SA) on phenylalanine ammolonia-lyase (PAL; nmol cinnamic acid h<sup>-1</sup> mg<sup>-1</sup> protein) activity in apricot fruit (cv. 'Bergarouge'). Error bars represent the SD values.

#### 5. CONCLUSIONS AND RECOMMENDATIONS

The study was about of increase the apricot storability and raises the fruit resistance to biotic and abiotic stress. Different apricot cultivars showed responses to different SA concentration treatments. Basically, high SA concentration of 2 mmol L<sup>-1</sup> recorded the highest response for most of pomological parameters and chemical analysis. By contrast, it caused for the lowest values of CI and/or FD.

Generally, applications of SA and/or MeJA much reduced fruit weight loss and fruit softening and maintained soluble solid content and acidity over the storage periods.

Chilling injury and fruit decay index indicated SA and/or MeJA significantly reduced deteriorate apricot fruit. Fruits treated with SA and/or MeJA were characterized by high total polyphenolic content, antioxidant capacity and carotenoids content while these parameters significantly decreased quickly in control fruits. Treated fruit with MeJA and SA enhanced SOD and CAT activities during the early phase of storage period which may cause for increasing the resistance of apricot fruit in scavenging the ROS and then, reducing the oxidative stress.

SA and/or MeJA enhanced the POD activity which reflexed on maintaining the fruit firmness and reducing the fruit decay and this suggested being the technique which how theses elicitors can activate some defense related enzymes to face the biotic and abiotic stress.

PAL activity was enhanced with SA and/or MeJA treatments in cold storage or shelf-life treatments. The increase in polyphenols content was attributed to increase in PAL activity.

Different sensory parameter data showed that the SA and/or MeJA had positive effects and these compounds did not increase the sensory analysis score for the tested fruit but they able to maintain the most limited sensory parameter in acceptable level over 3 weeks of cold storage and, make the shelf-life longer than the control fruit. Mealiness development and loss of juiceness were decreased with SA and MeJA treatments.

We reported direct toxicity of these compounds for SA and/or MeJA on the M. laxa growth rate. The disease incidence and lesion diameter decreased with sa and /or MeJA treatments.

These elicitors showed low disease severity during 6 days of incubated fruit with M. laxa and this accompanied with increasing of some dense related enzymes activities (SOD and POD).

Generally, the effects of the elicitors apricot fruit storability enhance needs more and more investigations and determines if pre or postharvest is better application and which maturity stage of fruit maturation is the best time for it but basically as these materials are considered as health safety, and cheap application, we recommend that treatment the apricot directly after harvest with 2 mmol l<sup>-1</sup> SA and/or 0.2 mmol L<sup>-1</sup> MeJA can contribute in enhancement the apricot industry from different ways as fellow:

- ✓ This treatment can at least save most of quality attributes during cold storage or shelflife better than untreated fruit.
- ✓ Reducing the fruit loss because of chilling injuries and fruit decay.
- ✓ Reducing the fruit loss because of fungi infection.

#### 6. NEW SCIENTIFIC RESULTS

- 1. Apricot fruit showed positive responses to SA and/orMeJA and we can apply these elicitors as postharvest application for apricot fruit.
- 2. Different apricot cultivars showed different responses to different SA concentration but generally, SA enhanced the different apricot fruit storability.
- 3. SA and/or MeJA improved and kept ed up the most of sensory parameters which sound directly with the customers.
- 4. SA and/or MeJA application reduced the fruit loss because of chilling injuries in apricot industry.
- 5. SA and/or MeJA treated apricot fruit enhanced shelf-life then we can increase the potential apricot fruit marketing.
- 6. Fruit loss because of *Monilia laxa* infection can be reduce with SAand/or MeJA treatments.

#### 7. SUMMARY

Apricot fruit production occupies very important position in regard to fruit production in Hungary. The last 10 years many cultivars were introduced into Hungary and the evaluation of these cultivars acceptability to Hungary conditions is very important for enhancement this industry.

The first experiment: effect of different salicylic acid SA concentations on different apricot cultivas in regad to storability

Cultivar Flavor Cot, Jambo cot and Bergeron were used in during cold storage (1 C for 7, 14, 21 and 28 days). Applications of 1 and/or 2 mmol L<sup>-1</sup> SA significantly reduced fruit weight loss and fruit softening and maintained soluble solid content and acidity over the whole storage period. Chilling injury and fruit decay index indicated SA significantly reduced the deterioration of apricot fruit. Fruit treated 2 mmol L<sup>-1</sup> SA had priority to keep the fruit firmness and at the same time was able to reduce the chilling injury and decay development. Fruits treated with SA were characterized by high total polyphenolic content, antioxidant capacity and carotenoids content while these parameters significantly decreased quickly in control fruits. In contrast the ascorbic acid values increased in control fruit.

The second experiment: effect of SA and/or methyl jasmonate (MeJA) on fruit quality, alleviating chilling injuiries and pomological characters of apricot fruit (*Prunus armeniaca* L.) cv. 'Bergarouge'

Postharvest treatments of apricot fruit with 0.2 mmol MeJA and 2 mmol SA were studied to enhance fruit quality and decrease chilling injury during storage. MeJA and SA application helped to keep fruit quality in early phases of storage by reducing fruit weight lost, fruit softening, maintaining soluble solid content (SSC) and acidity over the whole storage period. The decay and chilling injury index indicated that MeJA and SA reduced the deterioration of apricot fruit and this trend was also clear during 4 days at 25 C. Fruit treated with MeJA and SA were characterized by high total polyphenolic content and antioxidant capacity while these parameters decreased quickly in control fruits. Treated fruit with MeJA and SA enhanced phenylalanine ammonia-lyase (PAL) and superoxide dismutase (SOD) activities during the early phase of storage period. Our results support the idea of using MeJA and SA can help to enhance and prolong the storability of apricot fruits during cold storage and also

can help to prolong its shelf-life period. These beneficial effects might be attributed to the modification of stress-related enzyme activities, which results in an increase of polyphenolic contents of fruit tissues. this study we thought that the assessment of the effect of these elicitors in apricot fruit storability and shelf life to be more effective and provide novel information has to involve studying the effect of these chemical in sensory parameter. Most of sensory parameter data showed that the MeJA and SA recoded the highest score over 2 weeks of cold storage or 8 days at room temperature while the control fruit showed the extreme low texture, and taste score over 2 weeks at 1 o C or 4 days at 25 o C. Fruit color score showed priority of control fruit at 2nd week cold storage or at 4th day a room temperature and this may because the fast over ripening of untreated fruit.

# The third experiment: effect of SA and/or MeJA on induce resistance to *Monilinia laxa* on apricot fruit (*Prunus armeniaca* L.) cv. 'Bergarouge'

This experiment showed favorable effect of 2 mmol L<sup>-1</sup> of SA and 0.4 mmol L<sup>-1</sup> of MeJA than the other concentrations in regard to reduce growth rate, disease incidence and lesion diameter of M. laxa. The 2 mmol L<sup>-1</sup> of SA and 0.4 mmol L<sup>-1</sup> concentrations were used to investigate how the SA and MeJA can improve the resistance of cultivar Bergarouge fruit to M. laxa. One group of the fruit was wounded and incubated with M. laxa and other group was not wounded. The two groups were stored at shelf at 25 -C for 8 days and the samples were taken and measured 2 days intervals. The SA and MeJA showed high direct toxicity in vivo in regard to the growth rate of the fungi than water treated media. At the same time the disease incidence and lesion diameter were low in SA and MeJA treated fruit than control fruit. For fruit firmness, control fruit showed sharp deterioration in fruit firmness while treated fruit with SA or MeJA in non inoculation fruit, present approximately unchanged fruit firmness during second, 4th and 6th of storage at 25 C. MeJA showed high accumulation of lignin than the other treatment, SA did not affect lignin content. Phenolic content was enhanced and increased significantly by SA and MeJA treatment in comparison to water treated fruit. The defense enzymes, superoxide dismutase (SOD), peroxidase (POD) and phenyl alanine ammonia layase (PAL) were measured periodically and MeJA showed high influence on these enzymes activities. the role of MeJA appear obviously in infected fruit as SOD and POD activity increased significantly than SA after infection. Fruit treated with SA or MeJA showed enhance PAL activity than control fruit. In conclusion, these elicators seems to play vital role to improve apricot fruit resistance by two ways, the first by the direct toxicity on fungi and the second way by improving the antioxidant statement of the fruit and raising the defenses enzymes.

#### 8. HUNGARIAN SUMMARY

A tudományos kutatómunka célkitűzése volt i) a szalicilsav kezelések hatásának vizsgálata 3 kajszifajta tárolhatóságára, ii) a szalicilsav és a metiljazmonát kezelések hatásának vizsgálata 'Bergarouge' fajta gyümölcsök beltartalmi paramétereire 2 különböző tárolási körülmény között; és iii) a *M. laxa*-val fertőzött kajszi gyümölcsök beltartalmi paramétereinek összehasonlítása szalicilsav és metiljazmonát kezelések mellett.

Flavor Cot, Jambo Cot and Bergeron fajtákat 1 C-on tároltuk és a 7., 14., 21. és 28. napokon felvéteztük a beltartalmi paramétereiket. 1 és 2 mmol L<sup>-1</sup> szalicilsav szignifikánsan csökkentette a gyümölcsök tömegvesztését és puhulását és javította a szárazanyagtartalmat és savtartamat a teljes tárolási időszak alatt. A szalicilsav kezelés csökkentette a gyümölcsrothadást és az alacsony tárolási hőmérséklet károsító hatását. A szalicilsav kezelés növelte a gyümölcsök total fenoltartalmát, antioxidáns kapacitását és a karotinoidtartalmát.

A betakarítást követő 0.2 mmol metiljazmonát és 2 mmol szalicilsav javította a <u>'Bergarouge'</u> fajta szinte valamennyi beltartalmi jellemzőjét, mind a hűtőtárolóban (1 C) és mind a pulton (25 C). Mind a metiljazmonát és mind a szalicilsav kezelések szignifikánsan csökkentették a gyümölcsök tömegvesztését és puhulását és javították a szárazanyagtartalmat és savtartamat a teljes tárolási időszak alatt. A két kezelés csökkentette a gyümölcsrothadást, az alacsony tárolási hőmérséklet károsító hatását valamint növelték a gyümölcsök total fenoltartalmát és antioxidáns kapacitását is. A két kezelés fokozta a PAL és SOD enzimek aktivitását is mind a két tárolási feltétel mellett.

A 2 mmol L<sup>-1</sup> szalicilsav és a 0.4 mmol L<sup>-1</sup> metiljazmonát csökkentette a *M. laxa* gomba növekedési rátáját a gyümölcsök fertőzöttségi gyakoriságát és a képződött rothadó foltok átmérőjét is. Minkét kezelés direkt és indirekt gátló hatást gyakorolt a kórokozó gombára. A beltartalmi vizsgálatok igazolták a metiljazmonát növelte a gyümölcsök lignintartalmát. A metiljazmonát növelte a SOD és POD enzimek aktivitását a fertőzött gyümölcsökben. Mindkét kezelés növelte a fertőzött gyümölcsök fenoltartalmát és a PAL enzim aktivitását.

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#### **PUBLICATIONS**

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- Ezzat, A., Nessreen, N.B., Ammar, A.K.: Study of some cooking and eating quality characters on some Egyptian rice genotype.
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- Ezzat, A., Szabó, Z., Nyéki, J., Holb, I.J.: Preliminary results on salicylic acid treatment on brown rot caused by Monilinia Iaxa on Jumbo Cot fruit, Prunus armeniaca L. Int. J. Hortic. Sci. 19 (3-4,), 013), p. 37-40, 2013. ISSN: 1585-0404.
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#### **PUBLICATIONS**

- Ezzat, A., Nyéki, J., Soltész, M., Amriskó, L., Balázs, G., Mikita, T., Szabó, Z.: Storability of some apricot varieties as affected by storage period.
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- 6 Effect of treatments of 0, 1, and 2 mmol L<sup>-1</sup> salicylic acid (SA) on on fruit 48 acidity (%) of apricot fruit (cvs. 'Flavor Cot', 'Jumbo Cot', and 'Bergeron) in cold storage treatment at days 7, 14, 21 and 28 at 1 oC.
- 7 Effect of treatments of 0, 1, and 2 mmol L-1 salicylic acid (SA) on fruit 49 total carotenoids (mg β-carotene 100 g-1 FM) in apricot fruit (cvs. 'Flavor Cot', 'Jumbo Cot', and 'Bergeron) in cold storage treatment at days 7, 14, 21 and 28 at 1 oC.
- 8 Effect of treatments of 0, 1, and 2 mmol L<sup>-1</sup> salicylic acid (SA) on on and 50 ascorbic acid (mg/100 g) in apricot fruit (cvs. 'Flavor Cot', 'Jumbo Cot', and 'Bergeron) in cold storage treatment at days 7, 14, 21 and 28 at 1 oC.
- 9 Effect of treatments of 0, 1, and 2 mmol L<sup>-1</sup> salicylic acid (SA) on total 51 soluble phenol content (GAE 100 g<sup>-1</sup> FW) in apricot fruit (cvs. 'Flavor Cot', 'Jumbo Cot', and 'Bergeron) in cold storage treatment at days 7, 14, 21 and 28 at 1 oC.
- Effect of treatments of 0, 1, and 2 mmol L<sup>-1</sup> salicylic acid (SA) o and total 52 antioxidant capacity (mg AA 100 g-1 FW) in apricot fruit (cvs. 'Flavor Cot', 'Jumbo Cot', and 'Bergeron) in cold storage treatment at days 7, 14, 21 and 28 at 1 oC.
- 11 Effect of treatments of 0, 1, and 2 mmol L<sup>-1</sup> salicylic acid (SA) on 53 membrane electrolyte leakage (%) of apricot fruit (cvs. 'Flavor Cot', 'Jumbo Cot', and 'Bergeron) in cold storage treatment at days 7, 14, 21 and 28 at 1 oC.
- Effect of 0.2 mmol L<sup>-1</sup> methyl jasmonate (MeJA) and 2 mmol L<sup>-1</sup> 64 salicylic acid (SA) on weight loss percentage of apricot fruit (cv. 'Bergarouge') in cold storage treatment at days 7, 14 and 21 at 1 oC and in shelf-life treatment at days 4 and 8 at 25 oC.
- 13 Effect of 0.2 mmol L<sup>-1</sup> methyl jasmonate (MeJA) and 2 mmol L<sup>-1</sup> 65 salicylic acid (SA) on fruit firmness (N), soluble solid content (°Brix) and total acidity (%) of apricot fruit (cv. 'Bergarouge') in cold storage treatment at days 7, 14 and 21 at 1 oC, and in shelf-life treatment at days 4 and 8 at 25 oC.
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- acid (SA) on SSC/acidity ratio, juice PH of apricot fruit (cv. 'Bergarouge') in cold storage treatment at days 7, 14 and 21 at 1 oC, and in shelf-life treatment at days 4 and 8 at 25 oC.
- 15 Effect of 0.2 mmol L<sup>-1</sup> methyl jasmonate (MeJA) and 2 mmol L<sup>-1</sup> 67 salicylic acid (SA) on Mealiness development and lose of juiciness of apricot fruit (cv. 'Bergarouge') in cold storage treatment at days 7, 14 and 21 at 1 oC, and in shelf-life treatment at days 4 and 8 at 25 oC.
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- 17 Effect of 0.2 mmol L<sup>-1</sup> methyl jasmonate (MeJA) and 2 mmol L<sup>-1</sup> 69 salicylic acid (SA) on Total carotenoids (mg β-carotene 100 g<sup>-1</sup> FM) and ascorbic acid (mg/100 g) in apricot fruit (cv. 'Bergarouge') in cold storage treatment at days 7, 14 and 21 at 1 oC, and in shelf-life treatment at days 4 and 8 at 25 oC.
- 18 Effect of 0.2 mmol L<sup>-1</sup> methyl jasmonate (MeJA) and 2 mmol L<sup>-1</sup> 70 salicylic acid (SA) on phenylalanine ammolonia-lyase (PAL; nmol cinnamic acid h<sup>-1</sup> mg<sup>-1</sup> protein) and peroxidase (POD) activity in apricot fruit (cv. 'Bergarouge') in cold storage treatment at days 7, 14 and 21 at 1 oC, and in shelf-life treatment at days 4 and 8 at 25 °C.
- Effect of 0.2 mmol L<sup>-1</sup> methyl jasmonate (MeJA) and 2 mmol L<sup>-1</sup> 71 salicylic acid (SA) on superoxide dismutase (SOD; U mg-1 protein) and catalaze (CAT; U mg-1 protein) activity in apricot fruit (cv. 'Bergarouge') in cold storage treatment at days 7, 14 and 21 at 1 oC, and in shelf-life treatment at days 4 and 8 at 25 °C.
- **20** Effect of 0.4 mmol L<sup>-1</sup> methyl jasmonate (MeJA) and 2 mmol L<sup>-1</sup> 83 salicylic acid (SA) on growth rate of Monilinia laxa in apricot fruit (cv. 'Bergarouge')
- 21 Effect of 0.4 mmol L<sup>-1</sup> methyl jasmonate (MeJA) and 2 mmol L<sup>-1</sup> 83 salicylic acid (SA) on disease incidence in apricot fruit (cv. 'Bergarouge')
- 22 Effect of 0.4 mmol L<sup>-1</sup> methyl jasmonate (MeJA) and 2 mmol L<sup>-1</sup> 84 salicylic acid (SA) on lesion diameter in apricot fruit (cv. 'Bergarouge')
- 23 Effect of 0.4 mmol L<sup>-1</sup> methyl jasmonate (MeJA) and 2 mmol L<sup>-1</sup> 84 salicylic acid (SA) on fruit firmness in apricot fruit (cv. 'Bergarouge').
- 24 Effect of 0.4 mmol L<sup>-1</sup> methyl jasmonate (MeJA) and 2 mmol L<sup>-1</sup> 85

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- 27 Effect of 0.4 mmol L<sup>-1</sup> methyl jasmonate (MeJA) and 2 mmol L<sup>-1</sup> 88 salicylic acid (SA) on peroxidase (unit mg-1 protein) activity in apricot fruit (cv. 'Bergarouge').
- **28** Effect of 0.4 mmol L<sup>-1</sup> methyl jasmonate (MeJA) and 2 mmol L<sup>-1</sup> 89 salicylic acid (SA) on peroxidase (unit mg-1 protein) activity in apricot fruit (cv. 'Bergarouge').
- 29 Effect of 0.4 mmol L<sup>-1</sup> methyl jasmonate (MeJA) and 2 mmol L-1 90 salicylic acid (SA) on phenylalanine ammolonia-lyase (PAL; nmol cinnamic acid h<sup>-1</sup> mg<sup>-1</sup> protein) activity in apricot fruit (cv. 'Bergarouge').

#### **STATEMENT**

I prepared this thesis as a student of the Kerpely Kálmán Doctoral School of the University of Debrecen in order to attain the doctoral (Ph.D.) degree of the University of Debrecen.		
Debrecen,		
Signature of the candidate		
STATEMENT		
I certify that doctoral candidate Ahmed Ezzat Gaballa Kassem performed his work between 2011-2014 within the above mentioned Doctoral School with our supervision. The candidate significantly contributed to the findings described in his thesis as a result of his independent creative activity and the thesis is the independent work of the candidate. I recommend the acceptance of the thesis.		
Debrecen,		
Signature of the advisor		

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