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Research Article
Biodegradable Mulch Film Technology and Harpin Protein for Quality Enhancement of Anna Apples During Cold Storage

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Abstract

Background and Objective: Growers of Anna apple have been facing many production problems that hinder them and represent a main obstacle towards obtaining high quality of organic fruits. Therefore, the main goal of this work was to evaluate the possibility of producing organic fruits, extend color and enhance resistance of fruits during storability. **Materials and Methods:** The present investigation was conducted during three successive seasons 2016, 2017 and 2018 (the first season was experimental) on Anna apple trees to evaluate the effectiveness of soil biodegradable mulch films (BMF) and sprinkle fruits by harpin protein to enhance color and texture of fruits. As well as extend storability and enhanced systemic resistance of fruits during 90 days of cold storage (0°C±1 and 90-95% RH) to be available for longer period. **Results:** The results indicated that, covering the soil with (BMF) mulch produced higher organic matter at the end of experiment. All biodegradable films (BMF) treatments improved ratios of nutrient contents Ca, P and K in the fruits. In this respect, harpin protein at 40% with mulching soil with biodegradable films (BMF) led to produce higher fruit yield at harvest and reduce postharvest loss weight, respiration rate, electrolyte leakage and pathogen decay. Further, it significantly affected enhanced vitamin C content, anthocyanin, phenolic, antioxidant activity and reduced the decline in titratable acidity while, reduced percent of TSS and total sugar. **Conclusion:** From above mentioned results, it concluded that, soil biodegradable mulch films (BMF) with sprinkle fruits by harpin protein at 40% appear to be a good solution for the obtaining high quality and storability of organic fruits.

Key words: Anna apple fruits, biodegradable mulch film, harpin protein, cold storage, organic fruits, crop production

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Apple "*Malus domestica* L." is one of the most important horticultural deciduous fruit crops in Egypt. The total area cultivated with apple is 72264 feddans produced 798574 t according to FAO[1]. The organic product creation has been consistently expanding lately due to the fantastic returns for cultivators. Cultivators considering changing from traditional to organic production can confront difficulties with soil nutrition managemen[2]. Effective ways to increase the production and marketing of organic apples are crucial for farmers who have to deal with pests and diseases without using the traditional tools available to inorganic farmers. Maintaining a healthy orchard floor is the key to preventing weeds and keeping soil healthy. Using organic mulch increase the soil organic matter and can control soil temperature fluctuation under mulch. In this respect, the mulch directly affect the climate around the plant by adjusting the budget for surface irradiation and reducing water loss in the soil, which leads to more soil moisture[3].The main benefits of mulching technique with organic or inorganic materials are early crop production, higher yields, better product quality, more efficient water use, reduced leaching of fertilizers, reduced soil and wind erosion, reduced herbicide applications[3]. Therefore, the use of plastic films in agriculture has had a serious drawback in producing massive amounts of waste.

Biodegradable mulch films (BMF) represents a real challenge for enhancing sustainable and environmentally friendly agricultural activities[4]. Biodegradable mulches (BMF) offer an environmentally sustainable alternative to conventional polyethylene (PE) mulch. BMF was developed as a substitute for polyethylene mulch (PE) and was designed to be decomposed into the soil after use by microorganisms[5]. Common bio-based polymers used in BMF include polylactic acid (PLA), starch, cellulose and polyhydroxy alkanoates (PHA)[3]. Polymers used in BMF contain ester bonds or are polysaccharides, which are amenable to microbial hydrolysis[6].Mulches enhanced fruit quality such as red coloration of cv. 'Gala Mondial' apples by improving flavonoids and anthocyanins. Flavonoids increased up to 52.4% in the fruit peel and anthocyanin content improved up to 66% compared to control. While, reflective mulch did not affect chlorophyll and carotenoid content in the 'Gala' fruit peel[7]. The biodegradable polymers have two different formulations, those which the chemical structure enables direct enzymatic degradation (e.g. starch or cellulose) and those that undergo photo-oxidation or thermo- oxidation upon exposure to UV light or heat[8]. Starch is an environmentally friendly material as it is a renewable resource derived from corn and other crops.

Harpin is a heat-stable, glycine-rich protein produced by *Erwinia amylovora* that is involved in its pathogenesis of apple. Morphological and cellular changes in apple fruit treated with harpin suggest that it may trigger or intensify cellular responses in apples[9]. Harpin can provoke a hypersensitive response in non-host plants. This response is characterized by a rapid localized cell death at the site of the invasion for which reason it can act as a chemical elicitor[10]. Moreover, this protein activates reactive oxygen species (ROS) burst, salicylic acid (SA) and the jasmonate (JA)/ethylene signal transduction pathways that confer systemic acquired resistance (SAR) to different plants[10]. Harpin has been applied as an effective postharvest treatment to prevent decay in apples[11]and pears[12]. In addition, field applications demonstrated its usefulness for controlling pathogen-borne diseases in pear[13]. Harpin was described as a stimulant capable of activating enzymes such as phenylalanine ammonialase (PAL) from the biosynthesis pathway of polyphenols. Examples of this have been found in several fruits; for instance, in postharvest-treated peaches with a subsequent increase in total phenols[14].

The main goal of this work was to evaluate the effective methods of soil biodegradable mulch film and sprinkle Anna apples by harpin protein to produce organic fruits, extend color and enhance systemic resistance of fruits during storability.

MATERIALS AND METHODS

The present investigation was conducted during the three successive seasons 2016, 2017 and 2018 (the first season was experimental) on Anna apple fruits trees to evaluate the effectiveness of harpin protein with or without mulching soil for keeping quality of Anna apple fruits under cold storage. Fruits were picked from trees about eight years old, healthy and as uniform as possible in vigor and shape which, grown in sandy soil (4×4 m) apart at private orchard located at Cairo-Alexandria desert road, Egypt. The trees subjected to the recommended cultural practices in completely randomized design and divided into 5 groups, three replicates for each and two trees for a replicate.

Prior to executing the experiment, Soil samples were randomly taken from depth (0-30 cm) then, the soil physical and chemical properties were analyzed as showed in Table 1-2. Also, the main properties of the soil were evaluated at the end of experiment after two seasons of study.

Table 1: Soil physical characteristics prior to initiation of the experiment

Soil depth (cm)	Sand (%)	Clay (%)	Silt (%)	Texture	pH	EC (dS m^{-1})
0-30	92.60	3.20	4.20	Sandy	8.20	0.65

Table 2: Soil chemical characteristics prior to initiation of the experiment

Soil depth (cm)	Soluble cations (meq L^{-1})				Soluble anions (meq L^{-1})			
	Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCO_3^-	Cl^-	SO_4^-	CO_3^-
0-30	1.65	0.70	2.55	0.20	2.80	2.10	0.53	0.0

In the present study, selected trees were mulching on both sides by using biodegradable (BMF) mulch films on February. Biodegradable mulch films (BMF) (starch based) Mater-Bi® film was silver with 20 µm thick.

Treatments:

- Control (without treated or mulched)
- Spraying fruits with harpin 20%
- Spraying fruits with harpin 20%+mulching soil with biodegradable films (BMF)
- Spraying fruits with harpin 40%
- Spraying fruits with harpin 40%+mulching soil with biodegradable films (BMF)

The harpin protein was applied within two applications:

- First application 15 April: 2 weeks after full bloom (WAFB)
- Second application 15 June: 7-10 days before harvest

Harpin protein (Messenger, Plant Health Care de Mexico, D.F.) dissolved in ultrapure water by reverse osmosis (RO). Tween-20 (0.1%) as a wetting agent was added at the rate of 40 cm/100 L water to the foliar solution in order to obtain best penetration results.

Mature apple fruits were picked when the red color reached over 50%, fruit firmness was about 11-12% according to Drake and Kupferman[15]. Fruits were picked at random in the early morning and packed in plastic boxes. The selected fruits were transported directly to the fruit handling laboratory and the defective fruits were almost equal in size and free from other pathogen injury.

For storage study, treated fruits were stored in perforated carton boxes in one layer about 5 kg (3 boxes for each replicate). All boxes were stored at 0°C±1 and 90-95% relative humidity (RH) for 90 days. Fruits were examined at initial time and after 30, 60 and 90 days during cold storage. Fruit quality assessment was recorded as described below for different chemical and physical properties.

Assessments of fruits at harvest

Total yield (kg^{-1} tree): The mature fruits of each tree were weighted and then the average fruit yield per each tree was estimated.

Fruit nutrient contents: Samples of 10 fruits were taken from each tree for analysis nutrient. Content of Ca, P and K in the fruits were determined by atomic absorption spectrophotometry (AAS Shimadzu 7000 AA), as shown in the instructions specified in the ISO 11047 method, after they were extracted with nitrate (HNO_3) and sulfuric acid (H_2SO_4) according to Evenhuis and Dewaard[16].

Quality assessments of fruits during cold storage

Weight loss (%): It was determined according to the following equation:

$$\text{Loss in fruit weight (\%)} = \frac{\text{Initial weight - Weight at sampling date}}{\text{Initial fruit weight}} \times 100$$

Fruit decay (%): It was determined according to the following equation:

$$\text{Decay (\%)} = \frac{\text{Weight of decayed fruits}}{\text{Initial total fruit weight}} \times 100$$

Respiration rate (mL CO_2 kg^{-1} h^{-1}): Respiration rate was measured by gas analyzer (Model 1450-Servomex 1400) according to Saquet *et al.*[17], the airtight glass jars (4 L) were used to fruit incubation under the same storage circumstances for 24 h, respiration rate was measured as mL of CO_2 kg^{-1} fruits h^{-1}.

Electrolyte leakage (%): It was determined using the method described by Liu *et al.*[18] with a slight modification. Twelve disks of the eight fruits were collected using a cork borer (8 mm diameter) and washed in distilled water three times. The disks were soaked in a glass tube containing 20 mL distilled water and incubated in a water bath shaker at 25°C for 2 h. The initial electric conductivity (C_0) was measured using a conductivity meter. Then the glass tube was boiled for 30 min, cooled in room temperature and total electric conductivity (C_1) was taken. Three biological replications were used for each treatment. Electrolyte leakage rate was calculated using the following equation:

$$\text{Electrolyte leakage (\%)} = \frac{C_0}{C_1} \times 100$$

Fruit firmness (lb inch^{-2}): It was determined on the two opposite sides of fruit using a hand Effegi- Penetrometers and the average was estimated as lb inch^{-2} according to AOAC[19].

Vitamin C (mg /100 mL juice): Vitamin C was measured by the oxidation of ascorbic acid with using 2, 6-dichlorophenolindophenol solution and 2% oxalic acid as a substrate then the results were indicated as mg ascorbic acid per 100 mL juice according to AOAC[19].

Total soluble solids (TSS%): It was measured using drops of apple juice using a Carl-Zeiss hand refractometer according to AOAC[19].

Titratable acidity (TA%): It was determined in fruit juice by titration with 0.1 N sodium hydroxide (NaOH), using phenolphthalein as an indicator and calculated as citric acid according to the method described in AOAC[19].

Total sugars (100 µg mL^{-1} of glucose): The total sugars were determined on the dried raw fruit of each treatment using 18% phenol and 96% sulphuric acid, the absorption was recorded with spectrophotometer at 490 nm, according to the method described by Sadasiyam and Manickam[20].

A standard curve was prepared by plotting the known concentrations of glucose solution (100 µg mL^{-1} of glucose) against respective optical density (OD) value of each. From the standard curve, the amount of total sugars actually present in the sample is determined.

Anthocyanin content (mg/100 g FW): The anthocyanin for the peel of the fruit was estimated by of pH differential method[21] using a UV spectrophotometer. Absorption was measured at 533 and 700 nm in buffers with a pH of 1.0 and 4.5 with a molar extinction coefficient of 29.600. The results were expressed as mg cyanidin-3-glucoside equivalents per 100 g^{-1} of fresh apple peel for triple extracts.

Total phenolic content (mg GAE 100/g FW): The total phenol content in the apple fruits was determined using the method of Slinkard and Singleton[22] and the absorbance was measured at 760 nm. Results are expressed as milligrams of gallic acid equivalents (GAE)/100 g fresh weight (FW) using gallic acid as a standard peel for triplicate extracts.

Antioxidant activity (mg AEAC g^{-1} FW): The antioxidant capacity with the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) was performed as reported by Brand-Williams *et al.*[23] with the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH).The absorbance at 517 nm was recorded to determine the concentration of the remaining DPPH. Ascorbic acid was used as a standard and DPPH radical scavenging activity were expressed in mg of ascorbic acid equivalents antioxidant activity (AEAC) per gram fresh weight for triplicate extracts.

Statistical analysis: Data of both seasons of the study were analyzed using analysis of variance (ANOVA). Differences among treatment means were statistically compared using Duncan's multiple tests at a level 0.05, using the CoStat V6.4 program.

RESULTS

The effect of biodegradable (BMF) mulch films on soil properties and the harpin protein applications on storability of Anna apple fruits were discussed as follow during three successive seasons 2016, 2017 and 2018 (the first season was experimental).

Soil properties after treatment: Data in Table 3 showed that, soil organic matter was affected by biodegradable (BMF) mulch. Since, covering the soil with (BMF) mulch produced higher organic matter (2.50%) at the end of experiment. Moreover, Soil available nitrogen, phosphorus and potassium were enhanced by biodegradable (BMF) mulch than the control at the end of experiment, respectively.

Table 3: Evaluation of main properties of soil at the end of experiment after two seasons of study

Treatments	Organic matter (%)	Nitrogen (mg kg^{-1})	Phosphorus (mg kg^{-1})	Potassium (mg kg^{-1})
Control	1.47	18.20	6.90	62.50
Biodegradable films (BMF)	2.50	24.50	11.50	96.20

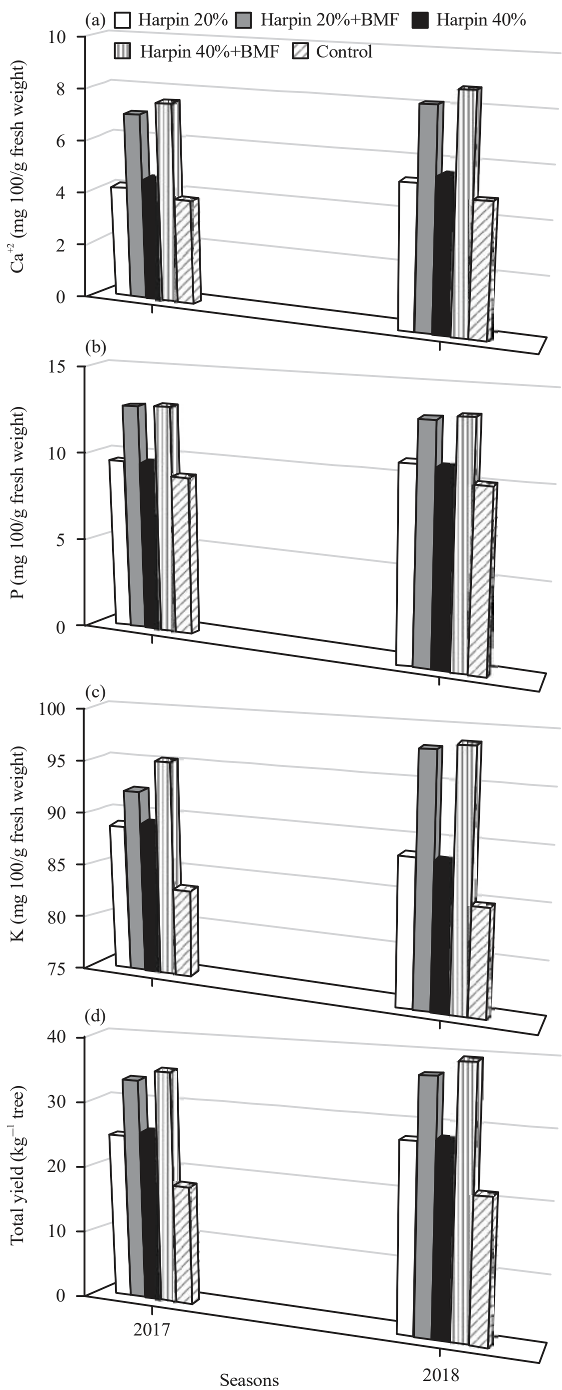

Fig. 1(a-d): (a) Total fruit calcium content, (b) Phosphorus content, (c) Potassium content and (d) Total yield (kg^{-1} tree) in "Anna" apple at harvest during 2017and 2018 seasons

Fruit nutrient contents: Nutrient contents in fruits and their ratios varied between treatments during the two years as showed (Fig. 1a-c). The Ca, P and K content in fruits varied significantly between treatments, in the two years of assessment. In this respect, all biodegradable films (BMF) treatments improved ratios of nutrient contents in fruits than the other treatments under the study. The highest contents of calcium, phosphorus and potassium were found in fruits treated with harpin 40%+mulching soil with biodegradable films (BMF) during the both seasons, respectively.

While, control produced the lowest contents of calcium, phosphorus and potassium as compare to all treatments under the study during the both seasons, respectively.

Total yield (kg^{-1} tree): Data in Fig. 1d obviously reveal that all treatments applied significantly improved fruit yield per tree rather than the control. Since, harpin at 40%+mulching soil with biodegradable films (BMF) were significantly very effective in enhancing fruit yield, which gave the highest values during both seasons, respectively. Untreated ones produced the minimum yield during both seasons, respectively.

Storability assessments

Weight loss of Anna apple fruits: Data in Table 4 presented the effect of the different conducted treatments on weight loss percentage of Anna apple fruits in 2017 and 2018 seasons. Weight loss percentage increased gradually after 90 days of cold storage. Moreover, all treatments showed lower significant weight loss percent compared with the control treatments which achieved the higher significant percent of weight loss (12.09 and 11.30%) after 90 days of cold in both seasons, respectively. On the other hand, spraying fruits with harpin 40%+mulching soil with biodegradable films (BMF) showed the lowest significant weight loss values (5.19 and 4.70%) during cold storage in the both seasons, respectively.

Decay of Anna apple fruits (%): From Table 4 data presented the influence of the different applied treatments on decay percentage of Anna apple fruits in both seasons. Decay percentage increased gradually with the prolongation of cold storage period. Moreover, all treatments showed lower significant percent of decayed fruits compared with the untreated. In this respect, spraying fruits with harpin 40%+mulching soil with biodegradable films (BMF) showed the lowest significant decay percentage (4.13 and 4.00%) after 90 days of cold storage compare with all treatments or

Table 4: Weight loss (%) and decay (%) of Anna apple fruits after 90 days of cold storage in 2017 and 2018 seasons

	Days in cold storage							
	Weight loss (%)				Decay (%)			
Treatments	Initial	30	60	90	Initial	30	60	90
Season 2017								
Harpin 20%	0.00^{p}	2.17^{l}	4.93^{g}	8.00^{b}	0.00^{k}	0.00^{k}	3.19^{g}	6.26^{c}
Harpin 20%+BMF	0.00^{p}	1.76^{n}	3.10^{j}	5.50^{e}	0.00^{k}	0.00^{k}	3.04^{h}	5.90^{d}
Harpin 40%	0.00^{p}	1.96^{m}	3.20^{i}	7.85^{c}	0.00^{k}	0.00^{k}	2.08^{i}	4.59^{e}
Harpin 40%+BMF	0.00^{p}	1.65^{o}	2.89^{k}	5.19^{f}	0.00^{k}	0.00^{k}	1.79^{j}	4.13^{f}
Control	0.00^{p}	4.73^{h}	6.14^{d}	12.09^{a}	0.00^{k}	5.91^{d}	16.84^{b}	27.94^{a}
Season 2018								
Harpin 20%	0.00^{h}	2.15f^{gh}	4.85^{cde}	7.80^{b}	0.00^{j}	0.00^{j}	3.02^{g}	6.10^{c}
Harpin 20%+BMF	0.00^{h}	1.53^{gh}	2.73^{efg}	4.90^{cde}	0.00^{j}	0.00^{j}	3.00^{g}	5.85^{d}
Harpin 40%	0.00^{h}	5.13^{cde}	3.08^{efg}	7.22^{bc}	0.00^{j}	0.00^{j}	2.05^{h}	4.23^{e}
Harpin 40%+BMF	0.00^{h}	1.26^{gh}	2.16^{fgh}	4.70^{de}	0.00^{j}	0.00^{j}	1.70^{i}	4.00^{f}
Control	0.00^{h}	4.10^{def}	5.65^{bcd}	11.30^{a}	0.00^{j}	5.88^{d}	14.43^{b}	26.06^{a}

Means followed by the same letters are not significantly different by Duncan multiple range test at 0.05 levels

Table 5: Respiration rate (mg CO_2 kg^{-1} h^{-1}) and electrolyte leakage (%) of Anna apple fruits after 90 days of cold storage in 2017 and 2018 seasons

	Days in cold storage							
	Respiration rate (mg CO_2 kg^{-1} h^{-1})				Electrolyte leakage (%)			
Treatments	Initial	30	60	90	Initial	30	60	90
Season 2017								
Harpin 20%	13.30^{g}	12.90^{j}	13.79^{e}	14.72^{b}	9.50^{a}	18.30^{l}	24.53^{e}	26.90^{c}
Harpin 20%+BMF	13.30^{g}	13.10^{hi}	13.54^{f}	14.67^{b}	9.50^{a}	18.00^{m}	24.14^{g}	26.66^{d}
Harpin 40%	13.30^{g}	13.13^{h}	13.27^{g}	14.22^{c}	9.50^{a}	17.72^{n}	22.93^{i}	24.304^{f}
Harpin 40%+BMF	13.30^{g}	13.10^{hi}	13.21^{gh}	14.03^{d}	9.50^{a}	17.70^{n}	20.35^{j}	23.40^{h}
Control	13.30^{g}	13.00i^{j}	13.73^{e}	16.84^{a}	9.50^{a}	18.75^{k}	29.75^{b}	32.97^{a}
Season 2018								
Harpin 20%	13.01^{f}	12.26^{g}	13.10^{f}	14.31^{c}	8.93^{p}	17.93^{j}	23.86^{c}	21.89^{e}
Harpin 20%+BMF	13.01^{f}	12.19^{g}	13.10^{f}	14.24^{c}	8.93^{p}	17.23^{k}	23.14^{d}	21.21^{g}
Harpin 40%	13.01^{f}	12.21^{g}	13.70^{e}	13.80^{de}	8.93^{p}	16.30^{o}	21.83^{f}	19.82^{h}
Harpin 40%+BMF	13.01^{f}	12.20^{g}	13.83^{d}	13.72^{e}	8.93^{p}	16.53^{n}	16.65^{m}	18.57^{i}
Control	13.01^{f}	12.05^{h}	14.82^{b}	15.24^{a}	8.93^{p}	17.15^{l}	26.61^{b}	29.34^{a}

Means followed by the same letters are not significantly different by Duncan multiple range test at 0.05 levels

the untreated ones in the both seasons, respectively. Moreover, control showed the highest significant decay percentages (27.94 and 26.06%) after 90 days of cold storage period in both seasons, respectively.

Respiration rate (mg CO_2 kg^{-1} h^{-1}): As presented in Table 5, significant differences in respiration rates were recorded in response to storage periods and treatments investigated in this study. Regardless of storage period, all treatments in both seasons significantly inhibited respiration rate compared to control. Presented results showed that respiration rates were slowly declined after 30 days of cold storage and this declination was followed by consistently increase respiration rates for all the treatments till 90 days at the end of the investigated storage period.

The overall mean results of respiration rate showed that spraying fruits with harpin 40%+mulching soil with biodegradable films (BMF) recorded lower delay of respiration rate (14.03 and 13.72 mg CO_2 kg^{-1} h^{-1}) after 90 days of cold storage as compared with all treatments or the untreated ones in the both seasons, respectively. Moreover, control showed the highest significant of respiration rate (16.84 and 15.24 mg CO_2 kg^{-1} h^{-1}) after 90 days of cold storage in both seasons, respectively.

Electrolyte leakage (%): Another index of cell injury is ion leakage, which is a commonly used technique to assess cell damage or viability. Data from Table 5 showed clearly that, ion leakage increased during the progress of storage periods and showed higher values than initial. All applied treatments

Table 6: Fruit firmness (lb inch^{-2}) and vitamin C (mg/100 mL juice) of Anna apple fruits after 90 days of cold storage in 2017and 2018 seasons

	Days in cold storage							
	Firmness (lb inch^{-2})				Vitamin C (mg/100 mL juice)			
Treatments	Initial	30	60	90	Initial	30	60	90
Season 2017								
Harpin 20%	11.34^{a}	10.82^{c}	9.23^{h}	8.88^{j}	8.91^{a}	8.39^{c}	7.48^{f}	6.50^{i}
Harpin 20%+BMF	11.34^{a}	10.63^{d}	9.00^{i}	8.85^{j}	8.91^{a}	8.37^{c}	7.50^{f}	6.50^{i}
Harpin 40%	11.34^{a}	11.02^{b}	9.90^{e}	9.48^{g}	8.91^{a}	8.51^{b}	7.81^{e}	6.90^{h}
Harpin 40%+BMF	11.34^{a}	11.04^{b}	9.92^{e}	9.58^{f}	8.91^{a}	8.58^{b}	7.89^{e}	6.91^{h}
Control	11.34^{a}	10.00^{e}	8.90^{j}	7.13^{k}	8.91^{a}	8.18^{d}	7.14^{g}	6.05^{j}
Season 2018								
Harpin 20%	12.00^{a}	10.93^{e}	9.69^{j}	8.82^{m}	9.16^{a}	8.86^{cd}	7.98^{h}	6.90^{l}
Harpin 20%+BMF	12.00^{a}	11.10^{d}	9.84^{i}	9.00^{l}	9.16^{a}	8.81^{d}	7.98^{h}	6.90^{l}
Harpin 40%	12.00^{a}	11.50^{c}	10.14^{g}	9.94^{h}	9.16^{a}	8.98^{b}	8.14^{g}	7.23^{k}
Harpin 40%+BMF	12.00^{a}	11.64^{b}	10.31^{f}	10.01^{h}	9.16^{a}	8.90^{c}	8.32^{f}	7.49^{j}
Control	12.00^{a}	10.10^{g}	9.18^{k}	7.94^{n}	9.16^{a}	8.49^{e}	7.71^{i}	6.63^{m}

Means followed by the same letters are not significantly different by Duncan multiple range test at 0.05 levels

produced less percent of electrolyte leakage as compare to the control. In this respect, the untreated apples had the highest electrolyte leakage percentage (32.97 and 29.34%) after 90 days of cold storage in both seasons, respectively. While, harpin 40%+mulching soil with biodegradable films (BMF) presented the lowest percent of electrolyte leakage (23.40 and 18.57%) than all treatments after 90 days of cold storage in both seasons, respectively.

Firmness (lb inch^{-2}): Data from Table 6 showed clearly that, fruit firmness was reduced as storage period advanced during the both seasons. It also confirmed that, all treatments used significantly reduced the decline in fruit firmness than the control through the two seasons. In this respect, fruit firmness for the control treatment showed a lower fruit firmness (7.13 and 7.94) lb inch^{-2} after 90 days of cold storage in both seasons, respectively. Furthermore, treated fruits with harpin 40%+mulching soil with biodegradable films (BMF) presented a higher fruit firmness (9.58 and 10.01 lb inch^{-2}) after 90 days of cold storage in the two seasons, respectively.

Vitamin C (mg 100 mL^{-1} juice): The amount of ascorbic acid decreased continuously with storage time as showed in Table 6. Moreover, all treatments used significantly delayed the decrease of vitamin C than the control after 90 days of cold storage. The contents of vitamin C in control treatments declined to (6.05 and 6.63 mg/100 mL juice) after 90 days at cold storage during both seasons, respectively. The higher amounts of vitamin C contents were obtained with harpin 40%+mulching soil with biodegradable films (BMF) during the entire storage period. The contents of vitamin C in this treatment were (6.91 and 7.49 mg/100 mL juice) after 90 days of cold storage during both seasons, respectively.

Total soluble solid (TSS %): From Table 7 data presented that, TSS percentage of Anna apple fruits was significantly increased in all treatments applied with the progress of cold storage in both seasons. Moreover, all applied treatments showed decrement in TSS percentage as compare to control during cold storage in both seasons, respectively. Since, the higher significant TSS percent were obtained at control treatment (16.51 and 16.06%) after 90 days of cold storage in both seasons, respectively.

While, harpin 40%+mulching soil with biodegradable films (BMF) presented the lower significant TSS (15.75 and 15.09%) in both seasons, respectively.

Titratable acidity (%): Results in Table 7 showed that TA (%) of Anna apple fruits was significantly reduced in all treatments applied with the progress of cold storage in both seasons. Moreover, all applied treatments showed somewhat increment in titratable acidity as compare to control throughout this study after 90 days of cold storage in both seasons. In this respect, harpin 40%+mulching soil with biodegradable films (BMF) led to significant increment in TA percentage (0.49 and 0.54%) after 90 days of cold storage in the two seasons, respectively. While, control treatment gave lower significant acidity (0.46 and 0.49%) after 90 days of cold storage in the two seasons, respectively.

Anthocyanins (mg/100 g FW): In the study, statistical analyses showed that total anthocyanin content of Anna apple fruits were significantly reduced during 90 days of cold storage (Table 8). All treatments applied maintain the content of anthocyanin in fruits skin than the control under cold storage. The results showed that, the treatment of harpin 40%+mulching soil with biodegradable films (BMF)

Table 7: TSS (%) and titratable acidity (%) of Anna apple fruits after 90 days of cold storage in 2017 and 2018 seasons

	Days in cold storage							
	TSS (%)				Titratable acidity (%)			
Treatments	Initial	30	60	90	Initial	30	60	90
Season 2017								
Harpin 20%	13.41^{k}	14.44^{j}	15.18^{f}	15.92^{b}	0.73^{a}	0.69^{c}	0.57^{f}	0.48^{k}
Harpin 20%+BMF	13.41^{k}	14.41^{j}	15.20^{f}	15.80^{cd}	0.73^{a}	0.69^{c}	0.58^{e}	0.48^{k}
Harpin 40%	13.41^{k}	14.51^{i}	15.05^{g}	15.82^{c}	0.73^{a}	0.70^{b}	0.52^{i}	0.49^{j}
Harpin 40%+BMF	13.41^{k}	14.54^{i}	15.02^{g}	15.75^{d}	0.73^{a}	0.70^{b}	0.56^{g}	0.49^{j}
Control	13.41^{k}	14.62^{h}	15.35^{e}	16.51^{a}	0.73^{a}	0.67^{d}	0.53^{h}	0.46^{l}
Season 2018								
Harpin 20%	13.04^{h}	13.90^{g}	14.70^{d}	15.53^{b}	0.77^{a}	0.69^{cd}	0.61^{g}	0.50^{k}
Harpin 20%+BMF	13.04^{h}	13.84^{g}	14.60^{d}	15.50^{b}	0.77^{a}	0.70^{c}	0.61^{g}	0.50^{k}
Harpin 40%	13.04^{h}	13.88^{g}	14.10^{ef}	15.15^{c}	0.77^{a}	0.71^{b}	0.64^{f}	0.53^{j}
Harpin 40%+BMF	13.04^{h}	13.90^{g}	14.14^{e}	15.09^{c}	0.77^{a}	0.72^{b}	0.64^{e}	0.54^{i}
Control	13.04^{h}	14.02^{f}	15.50^{b}	16.06^{a}	0.77^{a}	0.69^{d}	0.58^{h}	0.49^{l}

Means followed by the same letters are not significantly different by Duncan multiple range test at 0.05 levels

Table 8: Anthocyanin (mg/100 mL juice) and total sugar (%) of Anna apple fruits after 90 days of cold storage in 2017 and 2018 seasons

	Days in cold storage							
	Anthocyanin (mg/100 mL juice)				Total sugar (%)			
Treatments	Initial	30	60	90	Initial	30	60	90
Season 2017								
Harpin 20%	18.53^{a}	18.09^{b}	17.19^{fg}	16.80^{i}	14.16^{f}	14.01^{g}	14.41^{cd}	14.61^{b}
Harpin 20%+BMF	18.53^{a}	18.52^{a}	17.52^{e}	17.04^{h}	14.16^{f}	14.06^{fg}	14.34^{de}	14.50^{c}
Harpin 40%	18.53^{a}	18.05^{b}	17.23^{f}	16.84^{i}	14.16^{f}	14.04^{g}	14.45^{c}	14.61^{b}
Harpin 40%+BMF	18.53^{a}	18.12^{b}	17.78^{d}	17.14^{g}	14.16^{f}	14.05^{fg}	14.30^{e}	14.49^{c}
Control	18.53^{a}	17.89^{c}	17.05^{h}	16.24^{j}	14.16^{f}	14.00^{g}	14.49^{c}	14.90^{a}
Season 2018								
Harpin 20%	18.89^{a}	18.30^{c}	18.00^{d}	17.01^{g}	14.68^{b}	14.24^{cd}	14.69^{b}	14.90^{ab}
Harpin 20%+BMF	18.89^{a}	18.49^{b}	18.08^{d}	17.20^{f}	14.68^{b}	13.99^{d}	14.62^{b}	14.84^{ab}
Harpin 40%	18.89^{a}	18.24^{c}	18.01^{d}	17.02^{g}	14.68^{b}	14.25^{cd}	14.66^{b}	14.88^{ab}
Harpin 40%+BMF	18.89^{a}	18.53^{b}	18.07^{d}	17.30^{e}	14.68^{b}	14.36^{c}	14.62^{b}	14.80^{ab}
Control	18.89^{a}	18.00^{d}	17.09^{g}	16.48^{h}	14.68^{b}	14.21^{cd}	14.87^{ab}	15.00^{a}

Means followed by the same letters are not significantly different by Duncan multiple range test at 0.05 levels

maintained anthocyanin in fruit skin (17.14 and 17.30 mg 100 g^{-1} FW) after 90 days under cold storage comparison with other treatments used during both seasons. However, control treatment presented lower values of anthocyanin in fruit skin (16.24 and 16.48 mg 100 g^{-1} FW) after 90 days of cold storage through the both seasons under the study.

Total sugars (%): Considering to the effect of total sugar, data in Table 8 revealed that, total sugars were increased gradually according to the progress of cold storage during both seasons. Since, fruits of control samples had significantly the highest level of total sugars values after 90 days of cold storage (14.90 and 15.0%) in the first and second seasons, respectively. Conversely, harpin 40%+mulching soil with biodegradable films (BMF) presented the lower significant sugar percent (14.49 and14.80%) after 90 days of cold storage under the two seasons, respectively.

Total phenols (mg GAE/100 g FW): Results presented in Table 9 showed that total phenols were increased gradually according to the progress of cold storage during both seasons. Since, fruits of control samples presented significantly the lowest values in total phenol content of Anna apple fruits compared with all treatments used after 90 days of cold storage (103.33 and 112.00 mg eq. gallic acid 100 g^{-1}) in both seasons, respectively.

The higher values of total phenol were obtained by sprinkle harpin 40%+mulching soil with biodegradable films (BMF) ranged (109.66 and 115.0 mg eq. gallic acid 100 g^{-1}) after 90 days of cold storage in both seasons, respectively.

Antioxidant activity (mg AEAC g^{-1} FW): Results presented in Table 9 showed that all investigated treatments increment antioxidant activity compared to the control after 90 days of

Table 9: Total phenols (mg eq. gallic acid 100 g^{-1}) and antioxidant activity (µg mL^{-1}) juice of Anna apple fruits after 90 days of cold storage in 2017 and 2018 seasons

	Days in cold storage							
	Total phenols (mg eq. gallic acid/100 g)				Antioxidant activity (mg AEAC g^{-1} FW)			
Treatments	Initial	30	60	90	Initial	30	60	90
Season 2017								
Harpin 20%	99.00^{de}	99.00^{e}	102.00^{cde}	105.33^{abcd}	0.55^{ab}	0.52^{bc}	0.50^{bcd}	0.48^{cd}
Harpin 20%+BMF	99.00^{de}	101.33^{cde}	101.66^{cde}	106.33^{abc}	0.55^{ab}	0.51^{bc}	0.53^{abc}	0.54^{abc}
Harpin 40%	99.00^{de}	102.33^{cde}	102.00^{cde}	108.33^{ab}	0.55^{ab}	50.00^{bcd}	0.49^{cd}	0.49^{cd}
Harpin 40%+BMF	99.00^{de}	103.33^{bcde}	103.00^{bcde}	109.66^{a}	0.55^{ab}	0.50^{bcd}	0.54^{abc}	0.58^{a}
Control	99.00^{de}	100.66^{cde}	103.00^{bcde}	103.33^{bcde}	0.55^{ab}	0.54^{abc}	0.51^{bc}	0.45^{d}
Season 2018								
Harpin 20%	105.33^{cd}	105.66^{cd}	107.33^{bcd}	112.33^{abc}	0.59^{abc}	0.55^{cd}	0.55^{cd}	0.60^{abc}
Harpin 20%+BMF	105.33^{cd}	108.33^{abcd}	108.00^{abcd}	113.00^{ab}	0.59^{abc}	0.57^{bc}	0.59^{abc}	0.63^{ab}
Harpin 40%	105.33^{cd}	108.66^{abcd}	110.33^{abcd}	114.00^{ab}	0.59^{abc}	0.58^{abc}	0.58^{abc}	0.60^{abc}
Harpin 40%+BMF	105.33^{cd}	107.33^{bcd}	109.66^{abcd}	115.00^{a}	0.59^{abc}	0.58^{abc}	0.58^{abc}	0.64^{a}
Control	105.33^{cd}	109.00^{abcd}	110.66^{abcd}	112.00^{abcd}	0.59^{abc}	0.51^{d}	0.50^{d}	0.57^{bc}

Means followed by the same letters are not significantly different by Duncan multiple range test at 0.05 levels

cold storage during both seasons. It also showed that harpin 40%+mulching soil with biodegradable films (BMF) resulted significant increment in antioxidant activity compared with all treatments used or the control after 90 days of cold storage (0.58 and 0.64 mg AEAC g^{-1} FW) in both seasons, respectively. Moreover, control treatment attained the minimum significant values of antioxidant activity in apple fruits compared with all treatments used after 90 days of cold storage (0.45 and 0.57 mg AEAC g^{-1} FW) in both seasons, respectively.

DISCUSSION

In this study, covering the soil with biodegradable (BMF) mulch enhanced its organic matter. Moreover, Soil available Nitrogen, phosphorus and potassium were improved by biodegradable (BMF) mulch at the end of experiment. Mulches of organic origin typically enter a relationship with the soil, increasing the activity of the enzymes which break down plant residues. Mulches developed from biomass are increasingly used to cover the soil around the plants which improve soil properties involved the organic matter, available phosphorus and potassium. The organic matter improves environmental conditions for soil via, preventing of water and wind erosion, inhibiting drastic variation in humidity and temperature[24]. A higher amount of available phosphorus in the soil in mulched plots was determined by Sinkeviciene *et al*.[25]. Organic mulches probably have much more potassium in their structure and regulation of soil temperature and moisture which helped increase soil available potassium[26].

Biodegradable plastic mulches (BDMs) have been developed as substitutes to PE mulch films and are designed to be tilled into soil after use where resident microorganisms degrade the plastic. Biodegradable mulch 0.35 mm thick with a surface weight of 40 g m^{-2}, consisting of 16% corn starch and 84% natural fibre, allowed about 15% of radiation in the range 450-850 mm to pass, exhibited very good water permeability and thermal stability under field conditions. Its degradation rate in soil at 10-25°C and 65% humidity was the highest compared to mulches based on natural polyvinyl alcohol and polyacrylate[27]. Physical properties of the inorganic mulch depend on the type of polymer and the manufacturing process, as well as the additives during production (stabilizers, stimulants, conveyors, dyes and fillers)[28]. Since, mulching soil with biodegradable films (BMF) with sprinkle harpin at 40% were significantly very effective in enhancing fruit yield, which gave the highest values during both seasons, respectively. Maintaining high moisture content of the soil during the cucurbit vegetation period allows advancement and prolongation of the cropping season, leading to an increase in yield[29]. All biodegradable films (BMF) treatments improved ratios of nutrient contents in fruits than the other treatments under the study. The fruits showed a decreased dry matter content, with a similar amount of soluble sugars compared to the fruit from the control site[30].

In this study, harpin at 40% and mulching soil with biodegradable films (BMF) presented the lowest percent of electrolyte leakage after 90 days of cold storage. Harpin is a protein isolated from *Erwinia amylovora* bacteria which cause fire blight on fruit trees. It is thought that this protein has several cellular impacts on various processes or factors such as plant's resistance[31]activation of reactive oxygen types[32] transportation of effector proteins in plant cytoplasm[33] and depolarization of cell membrane. The product commercially called Messenger Gold which consists of 1% harpin and stimulates growth is generally used on fruits in order to increase the resistance against diseases. Previous studies have shown that postharvest treatment with harpin reduced disease in many harvested crops, such as apple[11]. Spraying fruits by harpin 40% and mulching soil with biodegradable films (BMF) showed the lowest significant decay percentage and recorded lower delay of respiration rate after 90 days of cold storage. The treatment with harpin on pear fruits can induce activities of POD, PAL, 4CL and GLU and content of TPC, all of which play important roles in disease resistance[12]. The lignin accumulations were enhanced by harpin treatment irrespective of the side considered. Lignin is extremely resistant to microbial degradation and thus constitutes one of the most effective barriers against pathogenic invasion[34]. The results showed that, the treatment of harpin at 40% with mulching soil by biodegradable films (BMF) presented a higher fruit firmness in fruit skin and maintained anthocyanin after 90 days of cold storage. The harpin treatment induced lignification of cell walls, promoting the combination of lignin with the cell wall and consequently enhancing protection of the cell wall[35]. The treatment of harpin spraying four times could reduce postharvest disease in fruits. The control for postharvest disease was clearly correlated with the activation of defense enzymes and metabolites as well as enhancement of lignin accumulation. In this study, harpin at 40% with mulching soil by biodegradable films (BMF) presented the lower significant TSS percentage while, showed somewhat increment in titratable acidity as compare to control treatment after cold storage. Conversely, harpin at 40% with mulching soil by biodegradable films (BMF) presented the lower significant sugar percent while, showed higher values of total phenol and significant increment in antioxidant activity.

Harpin are affected slightly fruit quality by enhancing vitamin C content and the ratio between soluble solids and titratable acidity. Harpin treatment increased flesh firmness and vitamin C content of fruit 10 days after storage. However, it significantly decreased titratable acidity (TA) content of fruit after storage. Moreover, it enhanced the TSS/TA ratio of fruit after storage. Therefore, scores of tastes in fruit treated with harpin were improved compared with control[36].

Harpin protein, originally isolated from *Erwinia amylovora*, is one of the plant activators that have become common in biological agriculture practices. When applied, harpin protein binds to plant receptors that activate several biochemical pathways related to growth and resistance enhancement by way of a systemic acquired resistance pathway (SAR)[37]. It is reported that an improved accumulation of phenolics may be one of the mechanisms that help control pathogen growth[38]. Harpin has been reported to stimulate the activity of phenylalanine ammonia lyase enzyme, a key to the biosynthesis of polyphenolic compounds such as gallic, caffeic, chlorogenic and some other acids[39]. The accumulation of phenolic compounds is associated with a hypersensitive response stimulated by genes involved in the synthesis of secondary metabolites such as phenolic compounds that may have antimicrobial activity. Phenylpropanoid metabolism has been reported to be involved in fruit-pathogen interactions[40]. The biosynthesis and accumulation of anthocyanins in the skin cells start from ripening to harvest and are mainly subject to genetic control however, climatic conditions and cultural practices, including the use of external plant growth regulators, often influence gene expression and activation of the biosynthetic enzymes.

Harpin application on grapes during the growing period, they trigger the expression of hundreds of genes related to the plant disease resistance, among which those involved in the biosynthesis of bioactive compounds such as anthocyanins[41]. Applying harpin protein (HrP) to grapes improved berry skin color by stimulating the biosynthesis of anthocyanins and enhancing their content in grape skin.

Harpin has been reported to stimulate the activity of the enzyme phenylalanine ammonia lyase, which is the key to the biosynthesis of polyphenols[41].

High correlations between phenolic content and antioxidant capacity (DPPH and FRAP) so, phenolic compounds may be the most important phytochemicals responsible of induce accumulation antioxidant capacity[38].

CONCLUSION

The biodegradable mulch film technology appears to be a good solution to replace the traditional polyethylene mulch and get a high-quality organic fruit. Sprinkle harpin protein 40% with mulching soil with biodegradable films (BMF) led to obtain high quality of organic fruits with the desired coloration and enhance systemic resistance of fruits during storability.

SIGNIFICANCE STATEMENT

This study discovered the possible effect of the soil biodegradable mulch films (BMF) and sprinkle fruits by harpin protein that can be beneficial to produce organic fruits, extend color and enhance systemic resistance of fruits during storability of Anna apples. This study will help the researchers to uncover the critical areas of maintaining quality of organic fruits and enhance systemic resistance accompanied by changes in various metabolic and physiological processes during storability that many researchers were not able to explore. Thus, a new theory of soil (BMF) with harpin protein are promising examples that are beginning to be adopted on a commercial scale which may be arrived at retain quality of apple fruits during storability.

REFERENCES

1. FAO., 2017. FAOSTAT database. Food and Agriculture Organization of the California. United Nation. http://www.fao.org/
2. Granatstein, D., 2002. Tree fruit production with organic farming methods. Organic Fruit Prod., 15: 1-10.
3. Kasirajan, S. and M. Ngouajio, 2012. Polyethylene and biodegradable mulches for agricultural applications: A review. Agron. Sustain. Dev., 32: 501-529.
4. Kyrikou, I. and D. Briassoulis, 2007. Biodegradation of agricultural plastic films: A critical review. J. Polym. Environ., 15: 125-150.
5. Bandopadhyay, S., L. Martin-Closas, A.M. Pelacho and J.M. DeBruyn, 2018. Biodegradable plastic mulch films: Impacts on soil microbial communities and ecosystem functions. Front. Microbiol., Vol. 9. 10.3389/fmicb.2018.00819.
6. Brodhagen, M., M. Peyron, C. Miles and D.A. Inglis, 2015. Biodegradable plastic agricultural mulches and key features of microbial degradation. Applied Microbiol. Biotechnol., 99: 1039-1056.
7. Overbeck, V., M.A. Schmitz Eiberger and M.M. Blanke, 2013. Reflective mulch enhances ripening and health compounds in apple fruit. J. Sci. Food Agric., 93: 2575-2579.
8. Sivan, A., 2011. New perspectives in plastic biodegradation. Curr. Opin. Biotechnol., 22: 422-426.
9. Capdeville, G.D., S.V. Beer and C.L. Wilson, 2008. Some cellular correlates of harpin-induced resistance to blue mold of apples. Trop. Plant Pathol., 33: 103-113.
10. Dong, H., T.P. Delaney, D.W. Bauer and S.V. Beer, 1999. Harpin induces disease resistance in *Arabidopsis* through the systemic acquired resistance pathway mediated by salicylic acid and the NIM1 gene. Plant J., 20: 207-215.
11. De Capdeville, G., S.V. Beer, C.B. Watkins, C.L. Wilson, L.O. Tedeschi and J.R. Aist, 2003. Pre-and post-harvest harpin treatments of apples induce resistance to blue mold. Plant Dis., 87: 39-44.
12. Wang, J.J., Y. Wang, Y.H. Ge and Y. Bi, 2006. Inhibiting effect of postharvest Harpin treatment on Alternaria rot and induction to resistance enzymes of *Pyrus bretschneideri* ‘Pingguo’. J. Gansu Agric. Univ., 41: 114-117.
13. Saour, G., H. Ismail and A. Hashem, 2010. Impact of kaolin particle film, spirodiclofen acaricide, harpin protein and an organic biostimulant on pear psylla *Cacopsylla pyri* (Hemiptera: Psyllidae). Int. J. Pest Manage., 56: 75-79.
14. Danner, M.A., S.A.Z. Sasso, J.G.S. Medeiros, J.A. Marchese and S.M. Mazaro, 2008. Induction of resistance to brown-rot on peaches by elicitors use in post-harvest. Pesquisa Agropecuaria Bras., 43: 793-799.
15. Drake, S.R. and E.M. Kupferman, 2000. Maturity and storage quality of 'Jonagold' apples related to starch index. J. Am. Pomol. Soc., 54: 213-218.
16. Evenhuis, B. and P.W. Dewaard, 1980. Principles and practices in plant analysis. FAO Soils Bull., 38: 152-163.
17. Saquet, A.A., J. Streif and F. Bangerth, 2000. Changes in ATP, ADP and pyridine nucleotide levels related to the incidence of physiological disorders in ‘Conference’ pears and ‘Jonagold’ apples during controlled atmosphere storage. J. Hortic. Sci. Biotechnol., 75: 243-249.
18. Liu, R., T. Lai, Y. Xu and S. Tian, 2013. Changes in physiology and quality of Laiyang pear in long time storage. Scient. Hortic., 150: 31-36.
19. AOAC., 2005. Official Methods of Analysis. 16th Edn., Association of Official Analytical Chemists, Washington, DC., USA.
20. Sadasivam, S. and A. Manickam, 1996. Biochemical Method. 2nd Edn., New Age International, India, ISBN-13: 9788122409765, Pages: 256.
21. Giust, M.M. and R.E. Wrolstad, 2001. Characterization and Measurement of Anthocyanins by UV-Visible Spectroscopy. In: Current Protocols in Food Analytical Chemistry, Wrolstad, R.E. and S.J. Schwartz (Eds.). John Wiley and Son, New York, pp: F1.2.1-F1.2.13.
22. Slinkard, K. and V.L. Singleton, 1977. Total phenol analysis: Automation and comparison with manual methods. Am. J. Enol. Vitic, 28: 49-55.
23. Brand-Williams, W., M.E. Cuvelier and C. Berset, 1995. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol., 28: 25-30.
24. Jodaugienė, D., R. Pupalienė, A. Sinkevičienė, A. Marcinkevičienė, K. Žebrauskaitė, M. Baltaduonytė and R. Čepulienė, 2010. The influence of organic mulches on soil biological properties. Zemdirbyste-Agric., 97: 33-40.
25. Sinkeviciene, A., D. Jodaugiene, R. Pupaliene and M. Urboniene, 2009. The influence of organic mulches on soil properties and crop yield. Agron. Res., 7: 485-491.
26. Kumar, V., 2014. Effect of different organic mulching materials on soil properties of NA ‘7’ aonla (*Emblica officinalis* Gaertn.) under rainfed condition of Shiwalik foothills of Himalayas India. Bioscan, 9: 561-564.
27. Tan, Z., Y. Yi, H. Wang, W. Zhou, Y. Yang and C. Wang, 2016. Physical and degradable properties of mulching films prepared from natural fibers and biodegradable polymers. Applied Sci., Vol. 6, No. 5. 10.3390/app6050147.
28. Vieira, M.G.A., M.A. da Silva, L.O. dos Santos and M.M. Beppu, 2011. Natural-based plasticizers and biopolymer films: A review. Eur. Polym. J., 47: 245-263.

29. Kuslu, Y., U. Sahin, F.M. Kiziloglu and S. Memis, 2014. Fruit yield and quality and irrigation water use efficiency of summer squash drip-irrigated with different irrigation quantities in a semi-arid agricultural area. J. Integr. Agric., 13: 2518-2526.
30. Siwek, P., I. Domagala-Swiatkiewicz and A. Kalisz, 2015. The influence of degradable polymer mulches on soil properties and cucumber yield. Agrochimica, 59: 108-123.
31. Qiao, F., X.L. Chang and P. Nick, 2010. The cytoskeleton enhances gene expression in the response to the *Harpin elicitor* in grapevine. J. Exp. Bot., 61: 4021-4031.
32. Sang, S., X. Li, R. Gao, Z. You and B., Lü *et al*., 2012. Apoplastic and cytoplasmic location of harpin protein $Hpa1_{Xoo}$ plays different roles in H_2O_2 generation and pathogen resistance in *Arabidopsis*. Plant Mol. Biol., 79: 375-391.
33. Choi, M.S., W. Kim, C. Lee and C.S. Oh, 2013. Harpins, multifunctional proteins secreted by gram-negative plant-pathogenic bacteria. Mol. Plant-Microbe Interact., 26: 1115-1122.
34. Hématy, K., C. Cherk and S. Somerville, 2009. Host-pathogen warfare at the plant cell wall. Curr. Opin. Plant Biol., 12: 406-413.
35. Zhang, Z., Y. Bi, Y. Ge, J. Wang, J. Deng, D. Xie and Y. Wang, 2011. Multiple pre-harvest treatments with acibenzolar-S-methyl reduce latent infection and induce resistance in muskmelon fruit. Scient. Hortic., 130: 126-132.
36. Wang, J., Y. Bi, Y. Wang, J. Deng, H. Zhang and Z. Zhang, 2014. Multiple preharvest treatments with harpin reduce postharvest disease and maintain quality in muskmelon fruit (cv. Huanghemi). Phytoparasitica, 42: 155-163.
37. Akbudak, N., H. Tezcan, B. Akbudak and V. Seniz, 2006. The effect of harpin protein on plant growth parameters, leaf chlorophyll, leaf colour and percentage rotten fruit of pepper plants inoculated with *Botrytis cinerea*. Scient. Hortic., 109: 107-112.
38. Fonseca, J.M., H.J. Kim, W.L. Kline, C.A. Wyenandt, M. Hoque, H. Ajwa and N. French, 2009. Effect of preharvest application of a second-generation harpin protein on microbial quality, antioxidants and shelf life of fresh-cut lettuce. J. Am. Soc. Hortic. Sci., 134: 141-147.
39. Boo, H.O., B.G. Heo, S. Gorinstein and S.U. Chon, 2011. Positive effects of temperature and growth conditions on enzymatic and antioxidant status in lettuce plants. Plant Sci., 181: 479-484.
40. Ballester, A.R., M.T. Lafuente and L. Gonzalez-Candelas, 2006. Spatial study of antioxidant enzymes, peroxidase and phenylalanine ammonia-lyase in the citrus fruit-*Penicillium digitatum* interaction. Postharvest Biol. Technol., 39: 115-124.
41. Li, M., M.L. Yu, Z.Q. Zhang, Q.N. Liu, Y.C. Wu and Z.G. Liu, 2013. Effects of Harpin protein on fruit quality and shelf life of winter jujube (*Ziziphus jujuba* Mill. cv. Dongzao). Biol. Agric. Hortic., 29: 58-68.