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Improving fruit quality of "Anna cv." apple trees grown on Malus rootstock by some bio compounds

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Abstract

This study was conducted during 2013 and 2014 season's atfisha village, Mansoura Governorate to improve fruit quality of Anna apple trees. Treatments included that $mixture_1$ content (Biolab) at 1 ppm {low concentration}; $mixture_1$ with high concentration at 2 L/ 600 liters water; $mixture_1$(Low+ high) concentrations; $mixture_2$content (Anti-stress) at 1 ppm; $mixture_2$with high concentration at 2 L/ 600 liters water; $mixture_2$ (low+ high) concentrations; Pepton at 1 ppm; Pepton with high concentration at 2 g/ liter water and control. All treatments sprayed three times at the mid of March, April and May. Results indicated that, $mixture_2$ (low+ high) concentrations and Pepton treatments gave the best significant fruit number per tree. $Mixture_2$ (low+ high) concentrations; pepton and $mixture_1$ indicated the highest significant yield. $Mixture_1$ gave the highest significant fruit weight. Acidity percentage decrease significantly with bio-compounds treatments than the control. $Mixture_2$ (low+ high) concentrations; $mixture_1$ with high concentration and $mixture_2$ had more pronounced positive affect on fruit color. The highest significant total phenols with control treatment. Total indoles were the highest with pepton. Total protein were the highest $mixture_{2\text{-}Leaves}$ SDS-PAGE revealed total of 24 common bands (monomorphic bands) were detected while, the remaining twenty bands were polymorphic with 45.45% polymorphism. This variation was found between present or absent bands with different treatments may be due to the differences between improving fruit quality of Anna apple cultivar. Isozymes bands with poly phenol oxidase and peroxidase and were varied between treatments under study. It can be recommended that spraying with bio compound are improving fruit quality of Anna apple cultivar.$Mixture_2$ {low and high} with concentration {1.0 ppm+ 2 L /600 liter water}; Mixture1 with concentration 1 ppm and pepton with concentration 1000ppm are improving yield and fruit quality.

Key words: bio-compounds;Biolab; Anti-stress; Pepton; SDS-PAGE; Isozymes; poly phenol oxidase and peroxidase.

Introduction

Apple (*Malusdomestica*,Brokh)is considered the most important deciduous fruit in the world .In Egypt; ″Anna″ represents the main cultivated apple variety in Egypt **(Saeid and Kalil, 1992).** It is hybrid between ″Red Hadassiya″ and ″Golden Delicious″ apple cultivars. This variety has many advantages such as low chilling requirement, an early bearing and a high yield as compared with the local apple varieties. The cultivated total area in Egypt reach 72616 Feddans produced about 629613 tons **(Ministry of Agriculture, 2013).**

Anna apple cultivar is considering un-competitor cultivarin shape or test compared with introduce apple cultivars. Therefore, it is important to improve its fruit quality.

Polyamines are low molecular weight aliphatic amino compounds existent at every place in plants. They have been associated with growth and tissue differentiation **(Franco Mora *etal*., 2005).**

Amino acids are particularly important for stimulation cell growth. They act as buffers which the help to maintain favorable pH value within the plant cell, since they contain both acid and basic groups; they remove the ammonia from the cell. This function is associated with amide formation, so they protect the plants from ammonia toxicity. They can serve as a source of carbon and energy, as well as protect the plants against pathogens. Amino acids also function in the synthesis of other organic compounds, such as protein, amines, purines and pyrimidines, alkaloids vitamins,enzymes, terpenoids and other **{Velero*etal*., 2002}**.

Also**, Goss (1973) and Hass (1975)**, stated that the biosynthesis of cinamicacids (which are the starting materials for the synthesis of phenols) are derived from phenylalanine and tyrosine. Tyrosine is hydroxyl phenol amino acid that is used to build neurotransmitters and hormones. Moreover, **Mohamed and Khalil (1992)** indicated that promotive effect of amino acids on ornamental and medicinal plants. Treatment with amino acids was accompanied by a pronounced accumulation of other organic solutes (Saccharides, proteins and total amino acids); moreoverthey increase to some extent salt tolerance of plants through osmo-regulation **(Silvaira *etal.*, 2003)**.

Application of organic N (as pepton) caused significant increase in $N0_3$-N production in nitrification samples. The increases in No_3-N generally represented a low proportion of the added pepton-N **(Adams 1986)**.Moreover, Growth and flowering parameters were significantly promoted by

increasing the concentration of pepton **(Soad *etal*., 2010)**.Also, amino acids (in form of pepton as commercial product) had a positive effect on productivity and fruit quality of Florida Prince peach trees **(El-Razek and Saleh 2012)**.
Therefore, the aim of the present study is to improve fruit quality of Anna apple cultivar by spraying some bio compounds.

Materials and Methods

The present study was conducted during two successive seasons 2013 and 2014 on Anna apple trees budded on Malus rootstock in a private orchard at Fisha village, Mansoura Governorate. Trees were five – years – old and grown in the clay soil under flood irrigation system, spaced 2.5 * 2.5 m, vase trained and subject to cultural practices recommended by ministry of agricultural. Thirty trees were considered to conduct this study in both seasons. Three replicates for each treatment were used. The chosen trees had uniform vigor and adopted in complete randomly block design.

Bio compounds concluded that:-
*** Mixture$_1$ content (Biolab)**: Acrylet organic 15%, Protein enzyme 25%, Poly amino acid enzyme 30% and inert ingredient 30%).
***Mixture$_2$ content (Anti-stress)**: Acrylet organic 10%, Polypeptide enzymes 15%, Protein enzyme 20%, Poly-amino acid enzyme 20%, inert ingredient 29%, Vitamin A 1000 L.U., Vitamin K_3 0.15 gm/liter).

***Pepton:** (Aspartic 3.29%, Tyrosin 0.52%, Glutamic 8.18%, Glycin 2.03%, Alanine 2.26%, Valine 2.51%, Isoleucine 1.11%, leucin 1.75%, Arginine 4.64%, Histidine 0.56%, Proline 3.96%, Phenyl Alanine 0.99%, Serine 4.99%, Threonine 3.57%, organ nitrogen 12% and Potassium oxide 3.50%, typical amino acids profile (%) WW) at 1000 ppm.

Treatments concentrations were used as following:-
Mixture$_1$: It spread with low concentration at 1 ppm.
Mixture$_1$: It spread with high concentration at 2 L/600 liters water.
Mixture$_1$: It spread with **(**low+ high concentrations) at (1.0ppm + 2L / 600liters water).
Mixture$_2$: It spread with low concentration at 1.0 ppm.
Mixture$_2$: It spread with high concentration at 2 liter/600 liter water.
Mixture$_2$: It spread with **(**low+ high concentrations) at (1.0ppm + 2L / 600 liters water).
Pepton: It spread with low concentration at 1000 ppm.
1- Pepton: It spread with high concentration with 2 g/L concentration
2- Control (sprayed with water).

All treatments sprayed three times (in the mid of March; April and May) in both seasons. These experiment studied the efficiency of referred bio-enzymatic compounds on tree fruiting; fruit quality; chemical analysis and genetic relate on [SDS- PAGE electrophoresis and two enzyme systems {polyphenol-oxidase (PPO) and peroxidase} were applied. The following measurements were carried out:-

1) Tree fruiting:
a- fruit number/tree: It was calculated at the end of May month.
b- Fruit yield/tree: At harvest time in both seasons the yield of selected trees were determined as kg/tree for all treatments.

2) Fruit quality:
Fruit measurements were made on 15 harvested fruits and included fruit weight, size, length, diameter, firmness (fruit without rind) using pressure tester with 1/4 'inch plunger (CataltricGeneratot, Inc. Norfolk, VA. USA), T.S.S, (Total Soluble Solids) **A.O.A.C, 1975,** Acidity, T.S.S / acidity, color lightness (L*); Fruit (rind) color lightness were quantified at stimulus colormetery data (L*) using Hunter Chroma meter model DP- 9000, color was represented by (whiteness/ darkness, ranged from 0 to 100, while 100 being the lightest) **Me Guire, 1992)** and fruit color (CI): fruit color was quantified at tri stimulus colormetery (L,a,b) using Hunter Chroma meter model DP- 9000,color was represented L (lightness), a (green - red) and b (blue - yellow) scale, **(Me Guire, 1992).** The following equation was used to determine color index, **Salvador *etal*., 1989).**

$$CI = 1000X \frac{a}{Lb}$$

3) Chlorophyll content in leaves:-At the end of the growing season, chlorophyll content was recorded using a Spd 502 chlorophyll meter as chlorophyll reading **(Yadava 1986)**.

4) Chemical analysis:
It was included that leaf content of total phenols, total indols and total protein. Samples were taken for various measurements during late August in both seasons. Total phenols, total indols for all treatments Cplorimetrical analysis according to **(A.O.A.C, 1975)**.

Statistical analysis:
A1I data were analyzed as factorial completely Randomized Design in factorial arrangement with three replications as described by **Snedecor and Cochran (1990)**. The differences between means were differentiated using Duncan multiple range test **Duncan (1955)**.

5) Genetic study:
A. Protein banding pattern in leaves:
- **Protein extraction:**

Samples of leaves were taken from Anna apple cultivar and treated with different plant extracts by spread in one season .Total soluble protein were extracted by grounding 0.25gof each sample in 0.9 ml extraction buffer (10 ml 0.5 Mtris PH 6.8 , 16 ml 10 % SDS, 30 ml D.W) with shaking thoroughly. The extracts were transferred to Eppendr of tubes and centrifuged for 10 min at 1000 rpm under cooling. Supernatant were transferred by fresh tubes and used for SDS - PAGE analysis and extraction of isozymes was used as described by **Janathan and Weeden, (1990).**

- **Protein related index:**

Fractionation electrophoresis was performed under identical conditions on sodium dedocylsulphate polyacrylamide gel (SDS - PAGE) (12 % w/v) vertical slab using BIORAD Techware 1.5 mm according to the method of **Lammli (1970) as modified by Studier (1973)**. The molecular weights of proteins were estimated relative to maker, a wide range molecular weight protein (Fermentas com.).

B. Isozymes electrophoresis:

Native - polacryiamide gel electropresis (Native - (PAGE) was performed in 12% (w/v) slab gel **(Davis, 1964).** The gel was stained after run according to **Tankseley and Rick (1980)** for poly phenyloxidase (ppo) isozymes and **Grahan *et al.*, (1964)** for peroxidase isozymes. The staining gel was incubated at 37 °c in dark for complete staining after adding the appropriate substrates and staining solutions

- **Gel documentation:**

Gels were photographed scanned, analyzed using Gel Doc Vilberlourmat system to capture the image and to calculate band intensities.

Results and Discussion

1- Tree fruiting:

1-a- Fruit number per tree:

The performance of the studied treatment in the Table (1) indicated that mixture $_2$ (low+ high) concentrations and pepton treatments gave the best significant fruit number per tree in the two seasons. Meanwhile, mixture $_2$ only was increased in the first season than the second season. In this respect, **Crisosto *etal.*, (1992)** recorded that the influence of polyamines in increasing fruit set has been observed in apple and pear particularly"Comice" pear. Putrescince enhanced pollen tube ovule penetration and delayed ovule senescence without affecting flower ethylene production. In addition, **Fayek *etal.*, (2011)** referred to that amino acids foliar spray applied twice during bud burst and full bloom stages at one g/L on Le-Conte pear trees induced the highest significant initial and final fruit set percentages. Parallel results were attained by **Yehia *etal.*, (2009)** illustrated that spraying anti-stress (polypeptide enzymes 15%) at 0.66% at bud swelling, full bloom and two weeks after petal fall and the first of July on "Le-Conte" pear trees induced the highest significant fruit set compared with control. Also, **Mouco *etal.*, (2009)** mentioned that increasing amino acid spraying rates enhanced the number of fruits per Mango plant.

1-b- Yield per tree:-

Data in Table (1) demonstrate that mixture$_2$ (low + high) concentration; Pepton and mixture$_1$ induced the highest significant yield per tree in the two seasons. Whereas, Mixture$_2$ only was higher significant yield in the second season than the first season. In this regard, **Yehia *etal.*, (2009)** stated that, foliar spray application of Anti-stress (polypeptide enzymes 15%) at 0.66% at bud swelling, full bloom and two weeks after petal fall and the first of July on Le-Conte pear trees induced the highest fruit number pear tree. Similarly, **Fayek *etal.*, (2011)** found that the amino acids foliar sprays applied twice during bud burst and full bloom stages at one g/L on Le-Conte pear trees produced the highest significant yield.

Table 1. Effect of treatments on fruit number and yield per tree on Anna apple cultivar in 2013 and 2014 seasons.

Treatments	Fruit number /tree		Yield(Kg) / tree	
	2013	2014	2013	2014
Mixture$_1$ *	122.67C	123.33CD	23.80A	22.48A
Mixture$_1$**	80.33E	107.33EF	10.95D	16.23BC
Mixture$_1$***	93.33D	125.67C	14.91C	18.32B
Mixture$_2$*	158.00A	151.00B	21.54AB	23.96A
Mixture$_2$**	122.33C	114.33DE	15.36C	17.25BC
Mixture$_2$***	154.00A	165.33A	22.45A	23.70A
Pepton *	159.67A	176.00A	23.09A	25.12A
Pepton**	137.33B	153.33B	18.57B	25.83A
Control	116.67C	102.00F	13.29C	14.23C

Mean in each column followed by the same letter (s) are not significantly different at 5%.
Mixture1= Biolab ; Mixture2= Anti-stress
*low concentration=1ppm **high concentration=2L/600 liter water
*** Low + high= {1ppm +2L/600 liter water} concentrations.

2 - Fruit quality:

Data of physical and chemical characteristics on Anna apple fruits as affected by bio-compounds spraying were arranged in Table (2) and (3)

2 –a- Fruit weight:

Data in table (2) evident that mixture$_1$ gave the highest significant weight in the two seasons respectively. The control treatment had the lowest significant value.

2 –b- Fruit size:

In the first season, mixture$_1$ surpassed in fruit size than the other treatments. But,in the second season mixture$_2$(low+ high) concentrations; mixture$_2$; pepton and the control treatments increased fruit size than the other treatments in Table (2).

2 –c- Fruit diameter:

Foliar spray with bio-compounds treatments produced higher fruit diameter with different arrangement than the control in the two seasons.

2 –d- Fruit length:

Significant changes were reported between all treatments arrangement under study from season to another.

Table 2. Effect of treatments on some physical and chemical properties at fruit maturity on Anna apple cultivar in 2013 and 2014 seasons.

Treatments	Fruit weight (g.)	Fruit size (cm^3)	Fruit diameter (cm)	F. length (cm)	F. firmness	TSS (%)	Acidity (%)	TSS/acid ratio
				2013 season				
Mixture$_1$ *	194.1A	166.6A	6.50AB	7.37AB	14.29B	10.50C	0.50D	21.00A
Mixture$_1$ **	136.8C	125.1C	6.53A	6.87B	15.37A	10.00C	0.59D	17.05C
Mixture$_1$ ***	159.7B	147.1B	6.53A	7.73A	15.29A	10.63C	0.60D	17.82C
Mixture$_2$ *	136.8C	118.3CD	6.20B	7.10B	7.61D	10.83C	0.57D	19.10B
Mixture$_2$ **	137.2C	120.7C	6.50B	7.20B	10.08C	10.50C	0.53D	19.81B
Mixture$_2$ ***	146.1BC	115.8CD	5.90C	6.87B	15.22A	13.33A	0.80C	16.74C
Pepton *	149.0C	90.6E	6.67A	6.83B	10.41C	10.33C	0.61D	17.03C
Pepton **	134.2C	141.7B	3.37B	6.77B	9.67C	12.33B	0.98B	12.63D
Control	112.7D	102.7E	4.97C	5.00C	14.98AB	7.30D	1.20A	6.08E
				2014 season				
Mixture$_1$ *	177.1A	197.0B	6.73BC	7.17C	3.93C	10.50C	0.74CD	14.25BC
Mixture$_1$ **	152.0CD	193.0B	6.50CD	7.10C	3.97C	10.53C	0.72CD	14.63AC
Mixture$_1$ ***	145.4CD	190.0B	6.70BC	7.70AB	4.10C	10.33C	0.60D	17.22A
Mixture$_2$ *	157.7BC	201.8AB	6.80BC	7.30BC	3.33D	10.07C	0.65D	15.41AC
Mixture$_2$ **	150.6CD	175.9C	6.93AB	7.43BC	4.80B	10.90C	0.80D	13.68C
Mixture$_2$ ***	141.8D	210.8A	6.77BC	7.20C	5.00B	13.60B	0.94B	14.42C
Pepton *	141.7D	201.7AB	6.50CD	7.30C	5.20B	11.00C	0.64D	17.19A
Pepton **	167.7AB	192.6B	7.17A	7.93A	3.73D	10.03A	0.90C	16.77B
Control	139.7D	201.7B	6.23D	7.47BC	5.83A	7.67D	1.41A	5.45D

Mean in each column followed by the same letter (s) are not significantly different at 5%.
Mixture1= Biolab ; Mixture2= Anti-stress
*low concentration=1ppm **high concentration= 2L/600 liter water *** low + high= {1ppm +2L/600 liter water} concentrations

2 –E- Fruit firmness:

Mixture$_1$ (high concentration); mixture$_1$ (low + high) concentration and mixture$_2$ (low + high) concentration was significantly increased fruit firmness in the first season. Meanwhile, control treatment gave the highest value in the second season.

2 –F- Total soluble solids (T.S.S):

Mixture$_2$ (low + high) concentration and pepton high concentration were the best values of T.S.S with different arrangement from season to another. But, control had the lowest values in the two seasons.

2 –g- Acidity (%):

Acidity percentage was decrease significantly with foliar spray with bio-compounds treatments. But, control had the highest values of acidity % in both seasons.

2 –h- T.S.S / acidity ratio:

Foliar spray with bio-compounds treatments had significantly increases T.S.S / acid with different arrangement from season to another compared to the control that gave the lowest significant values of T.S.S / acid ratio.

2 –i- Fruit color:

Data in table (3) cleared that spraying with mixture$_2$ (low + high) concentration; mixture$_1$ high concentration and mixture$_2$ had more pronounced positive effect on fruit color in the two seasons.

2 –j- color index:

Pepton high concentration was the best color index in the two seasons. On the other hand, control had the lowest value in the two seasons.

These results are in harmony with **El-razek and Saeleh *etal.*,(2012)** who found that foliar and /or soil application of amino acid (in form of pept on as a commercial product) had positive effect on

productivity and fruit quality of "Florida Prince" peach. Also, In addition, a major determinate of citrus fruit quality, accumulates early in fruit development and declines towards maturation results in amino acid biosynthesis **Degu *etal.*, (2011)**. Moreover, **Yehia *etal.*, (2009)** noticed that foliar sprayed application of 0.66% Anti-stress at bud swelling, full bloom and two weeks after petal fall and first of July on Le-Conte pear trees induced the highest significant fruit weight. **Fayek *etal.*, (2011)** noticed that foliar sprayed amino acids at bud burst and full bloom stages at one g/L on Le-conte trees produced the highest significant fruit weight. **Bezold *etal.*, (2003)** declared that the plant systems that are responsible for dividing tissues exert high levels of polyamines and activities of their biosynthetic enzymes. Meanwhile, **Mouco *etal.*, (2009)** referred that amino acid sprays lightly delayed the evaluation of skin huminosity and degree of Hue of pulp, but the differences were not visually identified on Mango.

Table 3. Effect of treatments on color properties at fruit maturity on Anna apple cultivar in 2013 and 2014 seasons.

Treatments	Fruit color		Color index	
	2013 season	2014 season	2013season	2014 season
$Mixture_1$ *	65.92C	64.37C	1.29BC	1.52B
$Mixture_1$ **	67.64A	65.92B	0.68CD	0.44CD
$Mixture_1$ ***	61.51D	61.03E	2.04AB	1.51B
$Mixture_2$ *	68.14A	65.90B	0.93CD	0.66CD
$Mixture_2$ **	66.40B	65.22C	1.07C	1.11BC
$Mixture_2$ ***	68.33A	68.31A	0.90CD	0.60CD
Pepton *	64.66C	61.25E	1.93B	1.46B
Pepton **	63.97C	62.22D	2.79A	2.47A
Control	62.26C	61.25E	0.14D	0.15D

Mean in each column followed by the same letter (s) are not significantly different at 5%.
Mixture1= Biolab ; Mixture2= Anti-stress
*low concentration=1ppm **high concentration= 2L/600 liter water *** low + high= {1ppm +2L/600 liter water} concentrations

3-3- chlorophyll content in leaves:-

Data in Table (4) show that there are significant differences in the values of chlorophyllcontent in leaves. $Mixture_1$ (low + high) con.and $mixture_2$ high con.had significant increases in two seasons than the other treatments which were different arrangement from season to another. Meanwhile, the lowest chlorophyll content was with the control treatment. In this respect, **El-Razek and Saieh (2012)** mentioned that chlorophyll content had a positive effect with amino acids (in form of pepton as a commercial product) foliar and/or soil application of Florida Prince peach. Also, **Refaat and Naguib (1998)** reported that application of all amino acids (alanine, cytosine, guanine and L-tyrosine) increased the total carbohydrates percentage in peppermint leaves.

Table 4. Effect of treatments on chlorophyll percentage in leaves on Anna apple cultivar in 2013 and 2014 seasons.

Treatments	**Total chlorophyll**	
	2013 season	**2014 season**
$Mixture_1$ *	45.07CD	48.00A
$Mixture_1$ **	48.90A	44.30CD
$Mixture_1$ ***	48.77A	46.03AC
$Mixture_2$ *	48.57A	45.43BC
$Mixture_2$ **	47.27AB	46.53AB
$Mixture_2$ ***	44.07D	42.90D
Pepton *	46.53BC	48.00A
Pepton **	44.43D	47.70A
Control	39.00E	38.63E

Mean in each column followed by the same letter (s) are not significantly different at 5%.
Mixture1= Biolab ; Mixture2= Anti-stress
*low concentration=1ppm **high concentration= 2L/600 liter water *** low + high= {1ppm +2L/600 liter water} concentrations

The pormotive affected of the amino acids on total carbohydrates content may be due to their important role on the biosynthesis of chlorophyll molecules which in turn affected carbohydrates content.

3-4- chemical analysis:

Data in Table (5) cleared that treatments performance on total phenols; total indoles and total protein in two seasons under study.

3-4-1- total phenols:

The highest significant total phenols with control treatment in the two seasons respectively. The lowest values with mixture$_1$; mixture$_2$; mixture$_2$ (low + high) concentrations and pepton high concentration treatments in the two seasons under study.

3-4-2- total indoles:-

Total indoles were the highest with pepton in the two seasons respectively. The lowest values with mixture$_1$(low+ high) concentrations and control treatments in the two seasons under study.

3-4-3- total protein:-

With mixture$_2$ total protein had the highest values in the two seasons of study. The lowest values were recorded with mixture$_1$ high concentration in the two seasons respectively. In this respect, **Degu *et al.*, (2011)** mentioned that treated citrus with amino acid slightly inducing its protein in fruit. Also, **Soad *et al.*, (2010)** found that total indoles levels were decreased by the increase in peptone levels.

Table 5. Effect of treatments on total phenols; total indoles and total protein of Anna apple cultivar in 2013 and 2014 seasons.

Treatments	total phenols		total indoles		total protein	
	2013	2014	2013	2014	2013	2014
Mixture$_1$ *	0.79D	0.72E	2.87C	2.81D	13.01B	13.11B
Mixture$_1$ **	0.82B	0.83AB	2.85C	2.89C	9.29F	9.22F
Mixture$_1$ ***	0.81C	0.81BC	2.57F	2.62E	10.22D	10.30D
Mixture$_2$ *	0.78D	0.77DE	2.60E	2.53F	11.07C	11.10C
Mixture$_2$ **	0.81C	0.82A-E	2.73D	2.77D	14.18A	14.22A
Mixture$_2$ ***	0.78D	0.77DE	2.72D	2.74D	8.38I	8.36I
Pepton *	0.80C	0.80C	3.25A	3.27A	8.65G	8.65G
Pepton **	0.77D	0.77DE	3.03B	3.05B	8.56H	8.51H
Control	0.84A	0.84A	2.60E	2.51F	10.16E	10.15E

Mean in each column followed by the same letter (s) are not significantly different at 5%.
Mixture1= Biolab Mixture2= Anti-stress *low concentration=1ppm
high concentration= 2L/600 liter water * low + high= {1ppm +2L/600 liter water} concentrations

3-5- Genetic study:

3-5-1-Protein banding pattern in leaves:

The electrophoretic banding pattern of proteins extracted from leaves of nine treatments on Anna Apple were show in figure (1) and their desitrometric analysis were illustrated in Table (6) , the presence and absence of bands were associated with (+) and (-) respectively.

Results of leaves SDS-PAGE revealed a total number of 44 protein bands with molecular weight (MW) ranging from about 185 .364 to 15 KDa. A total of 24 common bands (monomorphic bands) were detected while, the remaining twenty bands were polymorphic with 45.45% polymorphism. This variation was found between present or absent bands with different treatments may be due to the differences between improving fruit quality of Anna apple cultivar. In this respect, **Degu*et al.*, (2011)**mentioned that treated citrus with amino acid slightly inducing its fruit protein. **Rasmia(2013)** mentioned that treatments with either proline or phenylalanine might play an important role in protein synthesis. Also, amino acids have traditionally been considered as precursors and constituents of protein **(Sabry *etal.*, 2009)**.

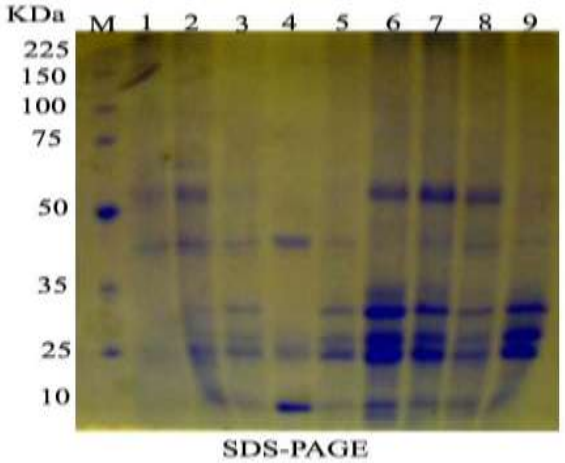

Figure (1):- SDS- Protein banding patterns of leaf proteins for the eight "Anna" apple treatments in compared with control.

1-Mixture$_1$ * 4-Mixture$_2$ * 7-Pepton *
2-Mixture$_1$ ** 5-Mixture$_2$ ** 8-Pepton **
3-Mixture$_1$ *** 6-Mixture$_2$ *** 9-Control

Mixture1= Biolab ; Mixture2= Anti-stress
* low concentration=1ppm
** high concentration= 2L/600 liter water
*** low + high= {1ppm +2L/600 liter water} concentrations

Table 6. Protein banding patterns of improving fruit qualities of Apple trees ″Anna cv.″ grown on Malus rootstock by bio-enzymatic compounds.

Band No.	M. W	Mixture$_1$*	Mixture$_1$ **	Mixture$_1$ ***	Mixture$_2$ *	Mixture$_2$**	Mixture$_2$** *	Pepton *	Pepton **	control
1	185.364	+	+	-	-	-	-	-	-	-
2	168.474	+	-	-	-	-	-	-	-	-
3	165.485	-	+	-	-	-	-	-	-	-
4	75.695	-	+	-	-	-	-	-	-	-
5	62.158	-	-	-	-	+	-	+	-	-
6	61.604	-	+	-	-	-	-	-	-	-
7	59.971	+	-	-	-	-	-	-	-	-
8	59.436	-	-	-	-	-	+	-	-	-
9	58.381	-	-	-	-	-	-	+	+	-
10	43.313	-	-	-	+	-	-	-	-	-
11	42.544	-	-	-	-	+	-	-	-	-
12	42.165	-	-	-	+	-	-	-	-	-
13	41.789	-	+	+	-	-	-	+	-	-
14	41.416	-	-	-	-	-	-	-	+	+
15	41.047	+	-	-	-	-	-	-	-	-
16	39.721	-	-	-	-	-	-	-	+	-
17	39.367	-	-	-	-	-	-	-	-	+
18	29.381	-	-	-	-	-	+	-	-	-
19	25.611	-	-	-	-	-	-	+	-	+
20	25.383	-	+	+	-	-	-	-	+	-
21	25.156	-	-	-	-	-	+	-	-	+
22	24.932	-	-	-	-	-	-	+	-	-
23	23.698	-	-	-	-	-	+	-	-	-
24	21.157	-	+	-	-	-	+	-	-	-
25	20.658	-	-	-	-	-	-	+	-	-
26	20.473	-	-	-	-	-	-	-	+	+
27	20.291	-	-	+	-	-	-	+	-	-
28	20.110	-	-	-	-	+	-	-	-	-
29	19.931	-	-	-	-	-	-	+	-	+
30	18.774	-	-	-	-	-	-	+	-	-
31	18.442	-	-	+	-	-	-	-	-	-
32	18.278	-	+	-	-	-	-	-	+	+
33	18.115	+	-	-	-	+	-	-	-	-
34	18.007	-	-	-	+	-	+	-	-	-
35	17.846	-	-	-	-	-	-	+	-	+
36	17.530	-	-	-	-	+	-	-	-	-
37	16.812	-	-	-	-	-	+	-	-	-
38	13.201	-	+	-	-	-	-	-	-	-
39	12.623	-	-	-	-	-	-	+	-	-
40	12.436	-	-	-	-	-	+	-	+	-
41	12.325	-	-	-	+	-	-	-	-	-
42	12.215	-	-	+	-	+	-	-	-	-
43	11.891	-	-	-	+	-	-	-	-	-
44	11.610	-	-	-	-	-	+	-	-	-
	Total	5	9	5	5	7	9	11	7	8

Mixture1= Biolab ; Mixture2= Anti-stress

*low concentration=1ppm **high concentration= 2L/600 liter water

*** low + high= {1ppm +2L/600 liter water} concentrations Polymorphic = 44 bands

20 ×100 = 45.45 % (+) present of bands , (-) Absent of bands

44

3-5-1-Isozyme electrophoresis:

Table (7) and figure (2) demonstrated poly phenol oxidase (PPO) banding patterns among examined fresh leaves of the eight treatments with some bio-enzymatic compounds from (t_1 to t_8) and untreated of Anna apple cultivar.

Data presented in table (7) and figure (2) explains that, the total of four poly phenol oxidase (PPO) bands were present with differences in density of bands. In PPO1 was present with mixture$_1$; mixture$_2$ high con.; mixture$_2$ (low +high) con.; pepton; pepton high con. and control. In PPO_2 was present with mixture$_2$ high con.; pepton ;pepton high con. and control. In PPO $_3$ was present with all treatments under study.

Table 7. Endeogram analysis for leaf polyphenol oxidase isozyme banding patterns leaf for the eight Anna apple treatments in compared with control.

Mixture$_1$ *	Mixture$_1$ **	Mixture$_1$ ***	Mixture$_2$ *	Mixture$_2$ **	Mixture$_2$ ***	Pepton *	Pepton **	control
▬				▬	▬	▬	▬	▬
				▬		▬	▬	
▬	▬		▬	▬	▬	▬		
▬	▬	▬	▬	▬	▬	▬	▬	▬

*low concentration **high concentration *** low + high concentrations

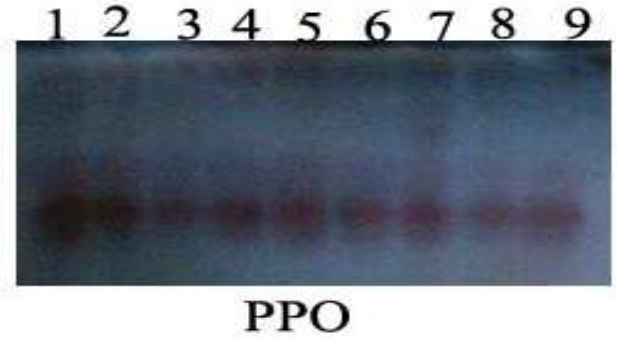

Figure (2):- Poly Phenol oxidase (PPO) isozyme banding patterns of leaf proteins for the eight "Anna" apple treatments in compared with control.

1-Mixture$_1$ *
2-Mixture$_1$ **
3-Mixture$_1$ ***
4-Mixture$_2$ *
5-Mixture$_2$ **
6-Mixture$_2$ ***
7-Pepton *
8-Pepton **
9-Control

Mixture1= Biolab ; Mixture2= Anti-stress
*low concentration=1ppm
**high concentration= 2L/600 liter water
*** low + high= {1ppm +2L/600 liter water} concentrations

Table (8) and figure (3) demonstrated peroxidase banding patterns among examined fresh leaves of the eight treatments with some bio-enzymatic compounds from ($t_1$to t_8) and untreated of Anna apple cultivar.

Data presented in table (8) and figure (3) explains that, the total of seven peroxidase (PX) bands were present with differences in density of bands. In PX_1; PX_2; PX_3 and PX_4 were present in all treatments under study. However, PX_5 was present in all treatments except mixture 1 (low + high) con.; Mixture$_2$ (low +high) con. And pipton high con. ; PX_6 and PX_7 were present in pepton and control only.

Table 8. Endeogram analysis for leaf peroxidase isozyme banding patterns leaf for the eightAnna apple treatments in compared with control.

Mixture$_1$ *	Mixture$_1$ **	Mixture$_1$ ***	Mixture$_2$ *	Mixture$_2$**	Mixture$_2$ ***	Pepton *	Pepton **	control
▬	▬	▬	▬	▬	▬	▬	▬	▬
▬	▬	▬	▬	▬	▬	▬	▬	▬
▬	▬	▬	▬	▬	▬	▬	▬	▬
▬	▬	▬	▬	▬	▬	▬	▬	▬
▬	▬		▬	▬		▬		▬
						▬		▬
						▬		▬

Mixture1= Biolab ; Mixture2= Anti-stress
*low concentration=1ppm **high concentration= 2L/600 liter water *** low + high= {1ppm +2L/600 liter water} concentrations

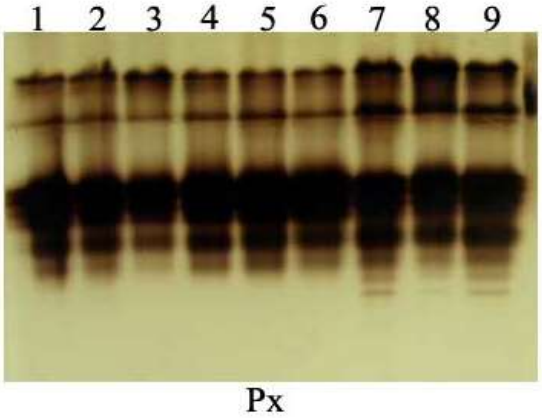

Figure (3):- Peroxidase (PX) isozyme banding patterns leaf for eight treatments of Anna apple cultivar in compared with control.

1-Mixture$_1$ * 4-Mixture$_2$ * 7-Pepton *
2-Mixture$_1$ ** 5-Mixture$_2$ ** 8-Pepton **
3-Mixture$_1$ *** 6-Mixture$_2$ *** 9-Control
Mixture1= Biolab ; Mixture2= Anti-stress
*low concentration=1ppm
**high concentration= 2L/600 liter water
*** low + high= {1ppm +2L/600 liter water} concentrations.

Conclusion

It can be recommended that spraying with bio compound are improving fruit quality of Anna apple cultivar.Mixture$_2$ {low and high} with concentration {1.0 ppm+ 2 L /600 liter water}; Mixture1 with concentration 1 ppm and pepton with concentration 1000ppm are improving yield and fruit quality.

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تحسين جودة ثمار أشجار التفاح " صنف أنا " النامي على أصل مالص ببعض المركبات الحيوية

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أجريت التجربة خلال موسمي ٢٠١٣ ، ٢٠١٤ لتحسين جودة ثمار أشجار التفاح الأنا ٠ وأشتملت المعاملات على

-محتوى خليط ١ (بيولاب)المنخفض التركيز بتركيز (١) جزء فى المليون ٠

-خليط ١العالي التركيز وذلك بتركيز ٢ لتر/ ٦٠٠لتر ماء.

- خليط ١ (منخفض + عالي) التركيزوذلك{بتركيز(١) جزء فى المليون+٢ لتر/ ٦٠٠لتر} ماء.

- خليط ٢ (أنتي – ستريس)المنخفض التركيزوذلك بتركيز (١) جزء فى المليون.

-خليط ٢العالي التركيز وذلك بتركيز ٢ لتر/ ٦٠٠لتر ماء.

- خليط ٢ (منخفض + عالي) التركيزوذلك { بتركيز(١) جزء فى المليون+٢ لتر/ ٦٠٠لتر} ماء.

- ببتون المنخفض التركيز وذلك بتركيز (١) جزء فى المليون .

- ببتون العالي التركيز وذلك بتركيز ٢ جرام/ لتر ماء.

- والكنترول {الرش بالماء}.

أشارت النتائج أن معاملات خليط ٢ (منخفض + عالي) التركيز والببتون أعطى أعلى عدد معنوي من الثمار بالنسبة للشجرة ٠ خليط ٢ (منخفض + عالي) التركيز والببتونخليط ١منخفض التركيز أعطى أعلى محصول معنوي ٠ خليط١ أعطى أعلى وزن معنوي للثمرة ٠ نسبة الحموضة منخفضة معنوياً مع معاملات المركبات الحيوية عن الكنترول. خليط٢(منخفض+عالى)التركيز، وخليط١عالى التركيز،خليط٢ منخفض التركيز كان تأثيرهماإيجابى على لون الثمار. الفينولات الكليه كانت أعلى معنوياً مع الكونترول. البروتين الكلى كان أعلى معنوياً مع خليط٢. SDS–PAGEللاوراق.وأوضحت أن ٢٤ حزمه أحاديه المظهر تم تحديدها. بينما ٢٠ حرمه كانت متنوعه بنسبة ٤٥و٤٥% تعدد مظهرى . هذاالتباين وجد بين الحزم الموجوده والغائبه مع مختلف المعاملات. ربما يرجعهذاالخلاف بين التحسين لجودة ثمار التفاح صنف أنا٠ حزم الآنزيمات مع أنزيم البولى فينول أوكسيديز (PPO) والبيوكسيديز (PX) تباينت بين المعاملات تحت الدراسه٠

ويمكن التوصيه بالإتى:-

الرش بالمركبات الحيويه حسنت جودة الثمار لصنف التفاح الانا٠ كما أن خليط ٢(منخفض +عالى) التركيز وخليط ١ المنخفض التركيزوكذلك الببتون حسن المحصول وجودة الثمار.