Some horticultural and pathological studies to reduce fruit decay of "Anna" apple and increase fruit set, yield and improve fruit quality and storability

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Abstract: The present investigation was conducted during the two successive seasons of 2012 and 2013 on "Anna" apple budded on Mauls rootstock in a private orchard, Tanboal road from Cairo–Alexandria desert road at El-Monofeya governorate. The trees were 10-years old and planted at 3x3m apart. CPPU (Sitofex at (100 cm^3/20L water), Inca at (40cm^3/ 20L water) and Calbor at (40 cm^3/20L water) were sprayed three times (at full bloom, fruit set, and month before harvest), and treatment of Kemazed 50% WP at (50 g/100 L water) was sprayed twice, 30 and 15 days before harvest. in addition, Humic acid at (60 cm^3/tree) as a soil drench was applied in the same pervious times to assess their effect on yield, fruit quality, storability and disease severity. The results showed that, Sitofex effectively increased fruit Iet percentage and number of fruits/ branch. Calbor treatment increased fruit yield, number of fruits / branch and/ tree as well as fruit firmness and decreased fruit drop percentage. Hence, CPPU+ Calbor treatment induced much more fruit yield, number of fruits / tree and fruit firmness. Humic acid could increase fruit quality (fruit weight, size and diameter). Inca treatment decreased fruit juice acidity and fruit shape index. CPPU, Inca and Inca+ CPPU effectively reduced fruit weight loss and maintained fruit firmness during storage at 3°C and 90 R.H. for 12 weeks. Calbor treatment improved all studied fruit quality attributes. The highest disease severity was obtained by two pathogens *Penicillium puberulum* and *Scytalidium dimidiatum*. Kemazed 50% WP fungicide completely inhibited the growth of both *Penicillium puberulum* and *Scytalidium dimidiatum in vitro*. Chitosan decreased the mycelium growth of both them where the inhibitor effect increased with increasing chitosan concentration up to 1.0%. The highest disease severity with *P. puberulum* and *S. dimidiatum* occurred when fruit treatment with Acetic acid at 0.5% and Control without chitosan, while Chitosan + Kemazed 50% WP and Chitosan +calboro induced the least disease severity.

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1. Introduction

Apple is considered as one of the major and the most important deciduous fruit crop in the world. Many investigators recorded that yield and quality of "Anna" apple fruits depended on several factors. One of the most vital factors which affect and play an important role in this concern is using of some plant growth regulators which enhance fruit set, reduce fruit drop, consequently increase productively. Moreover, both concentration and application date are very important factors which in turn reflect their impact on increasing and improving fruit yield and fruit characteristics.

Sitofex (CPPU) {(N-(2-chloro-1-pyridinyl)-N-phenylurea)} at different concentrations enhanced cell division, increased cell size, increased fruit weight, size and fruit yield. It also improved the most fruit properties. (**Jindal and Sharma (1986)** on plum, **Nickell (1986)** on grape, **El-Barkooky (1985)**, **Greene (1989)** on apple, **Biasl et al., (1991) and Lowes and Woolley (1992)** on Kiwi, **Rizk (1998), Feng et al., (1999), Al-Ashkar (2000), Ranpise et al., (2000), Marwad (2001)** on grapes, **Kabeel (1999)** on persimmon, **Fatma et al., (2009)** on apple and **Guirguis et al., (2003)** and **Kabeel and Fawaaz (2005)** on pear trees).

Humic acid is a constituent of organic matter. It is the most active fraction of humus coupled with fulvic acid. It is active ingredient product natural organic fertilizer that contains 1, 5, 6 NPK + 20% humates. Several researchers have determined the positive impacts of humic acid on calcareous soils. For example, **Fathi et al. (2008)** indicated that soil application of humic acid effectively enhanced shoot length, number of leaves, leaf area and yield components of "Canino" apricot. Also, **El-Shall et al., (2010)** found that soil and foliar application of humic acid increased the vegetative growth of plum trees. However, soil application was superior to foliar application. Moreover, the combined amendments (soil and foliar application of humic acid) significantly increased the height and trunk diameter of the trees

besides increasing number, length and diameter of shoots.

Calcium ions perform multiple roles in plant cell physiology. They are important intracellular messengers, mediating responses to hormones, biotic and abiotic stress signals and a variety of developmental processes **(Reddy and Reddy, 2004).** They also play an essential role in the structural maintenance of membranes and cell walls. Calcium ions cross-link free carboxyl groups on adjacent polygalacturonate chains present in the middle lamella of the plant cell wall contributing to cell-cell adhesion and cohesion. Preharvest and postharvest treatments with calcium salts have been effective in controlling several physiological disorders, reducing the incidence of fungal pathogens and maintaining fruit firmness **(Bakshi, et al, 2005).** The foliar spraying of calcium on apple trees is commonly used to increase calcium content of fruits and leaves. **(Huguet, 1980; Stahly, 1986 and Saure, 2005)** emphasized the important role of calcium in prolonging shelf-life of fruits and improving growth, nutritional status, productivity, resistance to pathological disorders and quality of the fruits. Strawberries (*Fragaria xaanassa* Duch.) were coated with Chitosan combined with calcium gluconate. Following treatment, strawberries were stored at 10°C and 70 ± 5% RH for one week. No sign of fungal decay was observed during the storage period for fruit coated with 1.5% Chitosan or 1% Chitosan + 0.5% CaGlu. By contrast, 12.5% of the strawberries coated with 1% Chitosan lacking calcium salt were infected after five days of storage. Addition calcium to the Chitosan solution increased firmness and nutrients of the fruit (**Muňoz, et al., 2008). Khalifa et al., (2009)** reported that foliar spraying with calcium (as calcium chloride) on "Anna" apple trees increased fruit yield and improved fruit physical and chemical properties as well as enhancing the nutritional status of apple trees. The treatment clearly decreased the percentage of incidence and severity of blossom end rot diseases compared with the unsprayed treatment.

Boron, is thought to have a favorable influence on the absorption of cations particularly calcium. Both elements play an important role in cell wall metabolism and are required for auxin transport process **(Dela-Fuente *et al*., 1986).** It is well known that the toxic effect of B May be reduced or prevented by adding Ca to soils (**Kabata-pendias and Pendias, 1992).**

"Anna" apple trees, treated with Boron (Boric acid) caused significant effect on fruit diameter, length, volume and weight, but sprays increased acidity, total sugars and anthocyanin content **(Mostafa et al., 1999).**

Likewise, **Wojcik and Treder (2006)** recommended boron fertigation of "Jonagold" apple trees from the stage of bud burst to petal fall at a rate of 0.5g/tree at 3-4 days intervals. This treatment improved B status in flowers and leaves of current season shoot, fruit set and yield; but had no effect on fruit weight, treatable acidity or firmness.

Zinc is an essential nutrient that has particular physiological functions in all living systems. Protein synthesis and gene expression, enzymes structure, energy production, krebs cycle and has a positive impact on crops yield; therefore crops quantitative and qualitative, yield is strongly dependent on zinc (Z) in soil **(Sayed *et al*., 2013).**

Mode of action for (Zn) was explained by **Larue and Johnson (1989).** Zinc has been identified as a component of almost 60 enzymes; therefore, it has a role in many plant functions and it has a role as enzyme in producing the growth hormone IAA. It also plays an important role in seed development.

However, apple cultivation in Egypt is faced by several challenges such as attacks by pests and diseases. Notably among them is the ceaseless attack by a host of many air-borne pathogens such as Penicillium fungi are common contaminants, known as an apple blue mold agents worldwide. They cause a soft rot of transit, marketed and storage apples, resulting in destruction of the whole fruit in 5-7 days at ambient temperature **(Larous, et al, 2007)**. Penicillium species often infects through wounds, fruit calyx or core **(Snowdon, 1990)**. *Penicillium puberulum* Bainer. is postharvest pathogen of apple during fruit storage **(Jones and Aldwinkle, 1991; Moslem et al, 2011)** and has an interesting history with respect to its metabolic products and role as a frequent contaminant of foodstuffs **(Benjamin, et al., 1967)**. Noteworthy metabolites include mycotoxi such as Citrinin, Patulin and Penicillin acid **(Moslem, et al., 2013).** *Scytalidum dimidiatum was recorded* in many researches as pathogen on plants, for example brown spot on *Hylocereus undatus* **(Lan et al , 2012)** and fruit rot on mango **(Marques et al, 2013).** The fruit rots disease control relies heavily on synthetic fungicides **(Wedgea et al., 2007).** Recently, there has been an increased interest in the development of fungicides derived from natural compounds as an alternative to synthetic fungicides for the control of postharvest microorganisms **(El-Ghaouth et al., 2002).** Natural plant-animal-compounds have shown a great potential to control postharvest pathogens of various horticultural commodities **(Wilson et al., 1994).** Among these, Chitosan has become an important source of bio-fungicides, **(Roller and Covill 1999).** Chitosan-based coating was concerned in recent years owing to its non-toxic, biodegradable, and biocompatible properties. In these procedures, Chitosan has been reported to maintain the quality of fruits and vegetables by avoiding moisture loss and aromas loss, and reducing respiration rates, ethylene production, and transpiration. In addition, it inhibits the oxygen

penetration to the plant tissue or microbial growth **(El Ghaouth et al., 1992a, b; Li and Yu, 2000; DU, et al., 1998 and Jianglian and Shaoying, 2013)**. Moreover, edible coating is convenient and conforms to food safety **(Mantilla et al, 2013).** Chitosan has strong **antimicrobial** and antifungal activities that could effectively control fruit decay **(Aider, 2010).** Growth of important postharvest fungi such as *Alternaria alternate, Fusarium oxysporum, Rhizopus stolonifer,* and *Penicillium* spp. is inhibited by using a nutrient media ammended with various concentrations of Chitosan **(Hirano and Nagao, 1989; Benhamou, 1992; Bhaskara Reddy et al., 1997; Bautista et al., 1999).** In some studies, **El Ghaouth et al. (1991; 1992a),** reported a fungicidal effect **of** Chitosan on strawberries against *Botrytis cinerea* and R. *stolonifer*. **Luna et al. (2001)** also reported less postharvest rots when papaya fruits were dipped in chitosan solutions compared with other postharvest treatments such as heat and Thiabendazole applications. **Bautista-Banos et al., (2004)** reported that apples treated with Chitosan at 1.0% for the control of *Penicillium expansum* had less infection at both maturity stages and storage temperatures. **Bautista, et al., (2003)** evaluated the in vitro fungicidal effect of Chitosan and aqueous extracts and the combination of Chitosan and plant extracts on the development of *Colletotrichum gloeosporioides* which causes anthracnose on papaya. They found that Chitosan at 2.0% and 3.0% had a fungicidal effect on C. *gloeosporioides*. Extracts alone did not show any fungicidal effect while the combination of 2.5% Chitosan with all the tested extracts had a fungistatic rather than fungicidal effect. Control of anthracnose disease achievements were obtained with 1.5% Chitosan applied before *C. gloeosporioides* inoculation. **Yu, et al., (2007)** indicated that application of Chitosan alone was effective in inhibiting the blue mold rot in apple fruit wounds, especially with the high concentrations and low viscosities. Yet, its efficacy was declining with the incubation time so that Chitosan alone could not provide enduring protection of apple fruit from *P. expansum* infections. They found that combination of Chitosan and yeast *(Cryptococcus laureate)* resulted in a synergistic inhibition of the blue mold rot but there was a problem when applied at the concentration range from 0.001 to 0.1% (wt/vol).

The aim of the research is to overcome the phenomenon of "Tatela" in addition to improving fruit set, yield, storage quality and reduce number of fruit decay at harvest and pre-harvest. In addition, to evaluate the effect of chitosan, as a commercial fungicide on apple fruit rot caused by *Penicillium puberulum* and *Scytalidium dimidatum.*

2. Materials and Methods

The present investigation was conducted during 2012 and 2013 seasons on "Anna" apple trees budded on Malus rootstock grown at a private orchard from Tanbol road at El-Monofia governorate. Thirty three trees 10-years old, planted at 3x3m apart were chosen to carry out 11 treatments with 3 replicates each as follows:

1- Control (Sprayed with tap water).

2- Sitofex CPPU (N-(2-chloro-1-pyridinyl)-N_ phenylurea)) is a plant growth regulator which has like cytokine activity, sprayed at 10 ppm two times (at full bloom and 3 weeks after full bloom).

3- Inca (6% CaO+1% Zn) at 40 cm^3 / 20L, sprayed 3 times at full bloom, fruit set and at 1 month pre-harvest.

4- Calbor (9% Ca+1% B+ 5% N as Hepta glouconic acid) at 40 cm^3/ 20L, sprayed 3 times as Inca compound.

5- Humic acid (6% potash + 20% humates chelated) at 60 cm^3/tree soil application 3 times as Inca compound. Humic acid was used as a direct soil application through drip irrigation system by the rate of 60cm/tree during the following times: at full bloom, two weeks or three weeks of full bloom.

6- CPPU + Inca.

7- CPPU + Calbor.

8- CPPU + Humic acid.

9- Inca+ Calbor.

10- Inca+ Humic acid.

11- Calbor + Humic acid.

Eight main branches per tree were chosen at random and labeled to determine: % fruit set, % fruit drop, number of fruits / branch and/ tree as well as fruit yield as kg/tree. At picking date, samples of thirty fruits / replicate were picked to assess fruit quality characters (fruit weight as g, size as cm^3, length as cm, diameter as cm, shape index and firmness as g/cm^2). Skin color measurement was determined by using Hunter Colorimeter Type (DR– 9000) for estimation of a, b and h° hue angles where O° = red – purple, 90°= yellow, 180°= bluish green and 270° = blue as **McGuire (1992)**. Also, % total soluble solids, % total acidity and TSS/ acidity ratio were estimated in fresh juice according to **A.O.A.C (1990)**. Forty fruits / replicate were picked and stored at 3°C and 90% R.H for O, 3, 6, 9, 12 weeks to determine the effect of treatments on skin firmness, % fruit weight loss, TSS and acidity as mentioned by **A.O.A.C. (1990)**. All obtained data were statistically analyzed at random complete plots design as stated by **Steel and Torrie (1980)**. Duncan test at 5% level was used for comparison between treatments.

***1*-Fungicides Materials:**

Natural infection of flowers, fruitlets mature fruit and postharvest fruits of cv. "Anna" apple were collected during 2012-2013 from different apple

orchards and various markets in governorates: i.e. Tanbol road, (El Noubaryia), Giza markets and Cairo markets in Egypt. Chitosan (Synonyms: Poly (beta-(1,4)-2-amino-2-deoxy-D-glucose) Poly(beta-(1,4)-D-glucosamine). The product is made from the shell of shrimps "Pandalus borealis", molecular weight 100,000-300,000 from Acros Organics (New Jersey, USA). Five fungicides: Bellies 38% WG; Kemazed 50% WP; Mancozeb 80% WP; Saprol 19% EC and Teldor 50% SC as show in Table (8).

2. **Isolation, purification and identification of isolated fungi**

"Anna", apple flowers, fruitlets and mature and ripening fruits were infected. Then, they were brought to the laboratory of Fruit and Woody Trees Diseases Department, for the isolation and identification the causal pathogens. The samples were prepared according to **Logrieco et al. (1990)** procedure. Count of fungal colonies was recorded. Purified fungi were transferred to PDA, using hyphal tip method **(Tsuneo, 2002).** Genera of isolates were then identified on the basis of morphological characteristics according to **Ellis, 1971; Nelson et al. 1983; Barnett and Hunter, 1987; and Farr et al. 1989.** A stock culture from each fungal isolate was kept in a refrigerator at 5°C for further experiment. The identification was confirmed by the Mycology Department, Plant Pathology Research Institute, Agriculture Research Centre Giza, Egypt.

3- **Pathogenicity test**

"Anna" apple fruits were harvested 2 to 3 weeks before their normal harvest date. Fruits were selected for uniform size, color, and maturity, and free from visible wounds, defects and decay. Before inoculation, fruits were surface sterilized for 30 sec in a 1 % NaOCI solution. The fruits were air-dried in a laminar flow cabinet. Then were prepared for inoculation according to **Sutton and Boyne, (1983).** The inoculated and control fruits were placed on cardboard box so that they did not touch each other, and the cardboard boxes were enclosed in polyethylene bags to maintain high humidity. Fruits were incubated for 1 week at room temperature and assessed for disease, symptoms by using scale described by **Biggs and Miller (2001).** Three replicates of each fungus, three fruits were used for each replicate.

4. **Preparation of Chitosan solutions**

Chitosan solution was prepared by dissolving 1.0 gram of Chitosan powder in 100 ml distilled water containing 0.5 ml (w/v) of acetic acid. The solution was heated and agitated constantly for 24 h using hotplate magnetic stirrer at 40°C. The solution was sterilized for 15 min and then pH was adjusted to 5.6 with 1N NaOH. This stock solution was used to obtain different concentrations of Chitosan (0.1, 0.2, 0.5 and 1.0 % w/v). Both the sterilized PDA and Chitosan solutions were mixed together in an equal quantity (1:1) under laminar air flow cabinet and then poured into the Petri dishes for further use. **(Benhamou et al., 1998).**

5. **Determination of antifungal activity of chitosan in vitro.**

The effect of Chitosan on the growth of Penicillium puberulum and Scytalidium dimidiatum was studied using poison food technique, **(Kumar and Tyagi, 2013).** A mycelia disc (5mm, diameter), grown on PDA was cut from the periphery of a 7 day old viable culture of two pathogens and transferred onto the center of a PDA dish containing chitosan solutions at concentrations ranged from 0.1 to 1.0% (w/v) introduced into the medium at 50°C before plating. Plates incorporated with sterile distilled water, and 0.5% acetic acid served as controls. Three replicates of each treatment and controls were arranged according to a completely randomized design and L.S.D. test at 5% level was used to compare between treatments. Petri dishes were incubated at 27+1°C. Measurements of growth were taken until the fungus reached the edge of the plate in the control treatment.

6- **Effect of different fungicides on fungal growth in vitro:**

Five fungicides different in their chemical group namely, Kemazed 50% WP, Saprol 19% EC, Mancozeb 80% WP, Bellis 38% WG, and Teldor 50% SC at the recommended concentrations (Table 8) were evaluated using the poisoned food technique **(Dhingra and Sinclair, 1995).** Under the conditions of sterilization the appropriate amount of each fungicide was added to calculate amount of PDA medium just before solidification and poured in sterilized Petri-dishes. Discs (5 mm) taken from 7 days-old of Penicillium puberulum and Scytalidium dimidiatum were placed on the plate center. Control (medium without fungicide). Three plates were used for each fungicide. All plates were incubated at 27±1°C until the fungal growth had almost filled the control plates, at which time the size of colonies in all treatments was measured.

7. **Effect of Chitosan as fungicide on apple fruits treated in the field with different products and stored on room temperature.**

The effect of Chitosan (1.0%), fertilizer compounds and Kemazed 50% WP on disease severity of Penicillium puberulum and Scytalidium dimidiatum was evaluated. The fruits of apples sprayed with different fertilizer compounds in the field, either singly or in a mixture. Three sprayes on different stages were used. Treatment of fungicide was sprayed twice, 30 and 15 days before harvest according to **(Effat Zaher et al., 1985).** Trees were sprayed with water to serve as a control. Treated fruits were picked at one week before their normal harvest date and brought to laboratory.

Apple fruits were surface sterilized with 70% ethanol and ambient air-dried. Then these fruits were artificially inoculated as mentioned above after immersed for 20 min. in Chitosan 1.0%. The fruits treatments were held at ambient temperature (28-30°C) for 7 days. Each treatment was applied to three replicates of 3 fruits. At the end of the trail period, disease was evaluated as disease severity as mentioned above.

3. Results and Discussions

I. Yield Components:

I.1. Fruit set (%):

Data in Table (1) indicated that all applied treatments increased fruit set (%) than control treatment in the two seasons of study expect Inca and CPPU + Humic acid treatments in 2nd season.

Spraying of CPPU gave the highest significant effect in both seasons (57.3 and 45.8%) followed in a descending order by treatments of Calbor + CPPU, Calbor + HA and Calbor + Inca. However, treatments of CPPU + Inca, CPPU + HA, Inca and HA did not significantly differ than control. Although CPPU alone gave good results, but when combined with Calbor or HA gave less effect. Also, HA, Calbor and Inca gave less effect when each one used alone. **Fathi et al., (2013)** recorded that spraying CPPU with 10 ppm at full bloom or fruit set had positive effect on fruit set than control. **Guirguis et al., (2010)** cleared that all tested Sitofex concentrations significantly increased fruit set percentage as compared to control. The above mentioned results are in line with those mentioned by **Fathi et al., (2013) & Guirguis et al., (2010).**

I.2. Average number of fruits/branch.

Data of Table (1) revealed that, foliar spray of Calbor achieved the highest significant average number of fruits/branch (11.77) followed by both of Calbor + humic acid added to soil (8.25), in the 1st season.

While CPPU only or mixed with Calbor gave the highest value (8.0 and 7.73), respectively in 2nd season. On the other hand, control and CPPU + humic acid were obtained the least retained fruits on branch (2.0 – 1.73) and (4.17 – 3.57), respectively in the 2nd season.

I.3. Fruit drop (%):

As shown in Table (1) Calbor treatment and Calbor + Inca resulted in the lower level of fruit drop % that gave the highest retained fruits (20.33 & 16.00%), in the two seasons, respectively. While untreated trees recorded the highest significant fruit drop % values (51.53-53.00%), respectively. The same trend was observed with humic acid + CPPU, humic acid alone in the two seasons. Using calcium (Calbor) played a role in increasing thickness of cell wall and cuticle layers, as it protects the plant from fungal invasion **(Mendez et al., 1994).**

I.4. Number of fruits / tree.

As shown in Table (1), it was noticed that trees tested with CPPU + Calbor (239 and 234) and Calbor alone yielded the highest number of fruits/tree than the control and other treatments in the both seasons. On the other hand, humic acid alone or combined had a lower values. Whereas, the lowest values were obtained from control in both seasons (60.0 and 55.3). Also, Inca in combination with CPPU gave significant effect in this respect. Our results are in line with those obtained by **Fathi et al., (2013)** who found that spraying CPPU (10 ppm) on "Le-Conte" pear trees at full bloom or at fruit set had positive effect on number of fruits/tree than control in the two seasons.

I.5. Yield (kg/tree)

Data in Table (1) indicated that the best result of yield from spraying with Calbor + CPPU (83.5 and 84.75 kg/tree), Calbor (77.25 and 82.25 kg/tree), CPPU + Inca (71.25 and 79.25 kg/tree) and Inca + H.A. in 2nd season (76.25 kg/tree). However, the lowest yield was obtained from control in both seasons (19.75 and 18.75 kg/tree). All treatments have significantly increased yield than control.

Table (1): Effect of treatments on fruit set %, average No. of fruit/branch, fruit drop %, No. of fruit/tree and yield (kg/tree) in 2012 and 2013 seasons.

Treatments	Fruit set (%)		Average No. of fruit/branch		Fruit drop %		Number of fruit/tree		Yield (kg/tree)	
	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
Control	15.33D	12.50E	2.00G	1.73G	51.53A	53.00A	60.0H	55.30I	19.65F	18.75F
CPPU	57.30A	45.80A	7.90BC	8.00A	31.10D	40.67DE	137.0E	141.0E	38.75D	37.00E
CPPU + Inca	25.10CD	19.30C-E	4.20F	5.33DE	36.00C	37.00EF	196.0C	199.0C	71.25B	79.25AB
CPPU + Calbor	37.80B	30.93B	6.63D	7.73AB	26.13E	25.67G	239.0A	234.0A	83.50A	84.75A
CPPU + Humic acid (HA)	15.65D	10.23E	4.17F	3.57F	54.33A	52.67A	108.0G	120.0FG	28.50E	32.50E
Inca	21.07CD	12.10E	4.83EF	4.97DE	45.67B	35.67F	122.00F	115.GH	46.00CD	45.75CD
Inca + Calbor	37.30B	29.33B	7.11CD	6.74BC	20.67F	19.20H	152.0D	184.0D	55.75BC	65.25B
Inca + H.A.	35.10B	27.00BC	6.63D	5.23DE	44.47B	47.13BC	107.7G	114.0GH	39.50D	76.25AB
Calbor	29.17BC	22.00B-D	11.77A	6.99CD	20.33F	16.00H	223.0B	222.0B	77.25B	82.25A
Calbor + H.A.	37.07B	30.00B	8.25B	6.67BC	22.30B	24.00CD	125.0F	126.0F	36.75D	41.00D
Humic acid (HA)	22.33CD	16.63DE	5.42E	4.67E	31.17A	38.67AB	124.0F	120.0FG	50.25C	49.50C

II- Fruit physical characters

II-1. Fruit weight (gm):

Data in Table (2) show that humic acid resulted in the highest values of fruit weight in the two seasons, (162.7 and 165.0g), followed by Inca, Sitofex + Inca. We can remark that Inca (Ca/Zn) treatment inclined to result in heavy fruit either alone or in combination with CPPU, HA or Calbor as well as Calbor alone in the second year. The lowest values were obtained by the control treatment and Sitofex + humic acid in the both seasons of the study.

These results are in harmony with those of **Cuo et al., (2000)** who found that spraying old apple trees with liquid organic humic acid at different stages, enhanced fruit weight and improved fruit quality.

Castro et al., (1988) stated that variable results were obtained with the foliar application of HA. They increased the extra large tomato fruits, but did not affect the total yield.

II-2. Fruit size:

Table (2) indicated that application of humic acid led to larger fruit size (210.7 and 22.17cm^3) during the two seasons, also spraying CPPU + Inca recorded the highest value in the second season only (230 cm^3).

On the other side, CPPU + humic acid treatment and control induced smaller fruit size (105-106.7cm^3) and (128.3 – 118.4 cm^3) respectively in the 1st and 2nd seasons. Reults were in line with those of **Castor et al., (1988)** which recorded that Humic acid increased the yield of extra large tomato fruits. On the other hand, **Cooper (1974)** indicated that calcium treatment had no effect on fruit growth, fruit size and yield.

II-3. Fruit length (cm):

Table (2) show that spraying with Inca+ Calbor, Inca+ Humic acid and humic acid alone had the highest significant fruit length in the 1st season. The lowest fruit length was recorded with control, CPPU + Humic acid and Calbor+ Humic acid in first season. In the second season, CPPU + Inca and Calbor alone gave the highest fruit length followed by Calbor + humic acid, humic acid and CPPU+ Calbor. On the other hand, control and Inca treatment had the lowest fruit length.

II-4. Fruit diameter (cm):

As sown in Table (2) it appears that spraying with humic acid produces larger fruit diameter (7.0 and 7.03 cm) during the two seasons, followed by Inca+ Calbor, and Calbor + Humic acid in the first season, but in the second season Calbor + CPPU and Calbor + humic acid with no difference in between. Whereas, the lowest values were obtained from control and CPPU + humic acid.

II-5. Fruit shape index:

Fruit shape index (fruit length / diameter) in Table (2) revealed that, Calbor + H.A. in the 1st season (0.89) and Inca treatment (0.95) in the 2nd season effectively induced less fruit shape index (flatten fruits) than the other treatments and control, while Inca, Calbor in 2012 season as well as Inca + CPPu and Calbor in 2013 season helped apple fruits to have erect shape.

II-6. Fruit firmness:

Table (2) shows that CPPU, CPPU + Inca and + Calbor treatments significantly induced the highest firmness than control and the other treatments throughout the study. On the other hand, CPPU+H.A. and Inca+ Calbor showed less firmness in 2012 and 2013 seasons. Also, Calbor in 1st season and H.A. treatments in 2nd season showed the lowest level of fruit firmness.

Our results confirm those of **Guirguis et al., (2010)** who mentioned that CPPU 10 ppm after two weeks of full bloom on "Costata" persimmon, gave the highest yield/ kg/tree and heaviest, largest and most firmness fruit.

In this respect, **Rease and Drake (1995) and Gerasapoulos and Richardson (1997)** demonstrated the positive effect of Inca sprays in increasing pear yield and fruit firmness. In addition, calcium foliar on "Anna" apple trees increased, yield and improved fruit physical and chemical properties **Khalifa *et al*., (2009)**.

II.7. Hue angle:

As shown in Table (2), the hue angle of apple fruits was lowest with CPPU + HA treatment (69.9 and 54.4) in the two studied seasons, respectively. However, the highest hue angle was recorded with CPPU + Calbor and control treatments while the other treatments have intermediate hue angle. The decrease of hue angle means that external color developed from green to red as a result of decreasing chlorophyll pigments (**McGuire, 1992**).

III-Fruit chemical characters

III-1. Total soluble solids percentage (TSS%):

Data in Table (3) showed that, TSS values clearly higher in the 2nd season than the 1st one. This phenomenon may be as a result of accumulated effect of the present treatments. However, Calbor treatment induced higher TSS in 2012 and 2013 seasons. Also, Calbor + Inca, CPPU and CPPU + H.A. showed higher TSS in 1st season of study while, Calbor + H.A. in 2nd season was higher than the other treatments.

These results are in agreement with those reported by **Mostafa et al., (1999)** on "Anna" apple trees treated with boron (Calbor) and found that it increased total sugars, acidity and anthocyanin content.

Xie-yh et al., (1992) found that calcium treatments enhanced sugar accumulation. **Guirguis, et al., (2010)** reported that the lowest percentage of TSS and highest percentage of acidity was obtained by spraying Sitofex at (20 ppm) data were not consistent during the two

seasons TSS was high in the first season but was low in the second when spraying apple trees with CPPU.

III-2. Total acidity and TSS/acidity ratio:

Inca treatment in the 1st season as well as Inca + Calbor in 2nd season effectively could reduce apple juice acidity than the other treatments. Moreover, data of TSS/acid ratio showed that Calbor + H.A. treatment induced remark ratio in the two studied seasons and CPPU treatment in the 1st season.

Fawzia et al., (2003), indicated that K-humate foliar application on "Canino" apricot trees trees enhanced fruit acidity. The same effect was reached by **Guirguis et al., (2010)** on "Costata" persimmon they found that sprayed trees neither early nor later Sitofex total acidity and tannins content significantly increased with increased Sitofex concentration. The highest TSS and lowest acidity were achieved with control during the two seasons. These results focuses on the role of Sitofex in delaying fruit maturity.

IV- Storage ability:-

IV-1. Fruit weight loss (%):

Data in Table (4) showed that, the percentage of fruit weight loss gradually increased with increasing storage period from 10.43 to 13.43 and to 20.18% in 1st season and from 13.42 to 16.67 and to 23.12% in 2nd season after 6, 9 and 12 weeks, respectively. However, control and CPPU significantly induced the least percentage of weight loss. On the other hand, Calbor + Inca or + H.A. significantly caused the highest percentage of weight loss through the two studied seasons, while CPPU + H.A induced the highest weight loss only through 1st season. The interaction results showed that control and CPPU induced the leaset weight loss through 12 weeks of storage, Calbor and Inca + H.A. treatments induced less weight loss for 9 weeks, while H.A. and CPPU + Calbor treatment caused less fruit weight loss for 6 weeks of storage.

Table (2): Effect of treatments on fruit physical characters (fruit weight, size, dimensions, shape index, firmness and Hue angle in 2012 and 2013 seasons.

Treatments	Fruit weight (gm)	Fruit size (cm^2)	Fruit length cm	Fruit diameter cm	Fruit shape index	Fruit firmness (g/cm^2)	Hue angle
	2012						
Control	92.8H	128.3FG	6.43E	6.00EF	1.07b-d	36.33C	109.0a
CPPU	113.4F	147.3EF	6.57DE	6.23DE	1.05cd	36.33C	102.70b
CPPU + Inca	145.3CD	152.3DE	7.13C	6.48B-D	1.10a-c	45.67A	101.0bc
CPPU + Calbor	39.9DE	182.7BC	6.97C	6.23DE	1.12ab	44.67A	108.5a
CPPU + Humic acid (HA)	104.6G	105.0H	6.10F	5.87EF	1.05cd	38.67B	69.90e
Inca	151.3B	201.7AB	7.00C	6.17D-F	1.13a	35.33CD	97.90c
Inca + Calbor	146.9BC	180.0BC	7.63A	6.80AB	1.12ab	33.67D	69.00e
Inca + H.A.	147.0BC	172.7CD	7.30B	6.70A-C	1.08a-d	21.33F	90.00d
Calbor	139.0E	146.0EF	7.13C	6.40CD	1.11ab	31.67C	90.00d
Calbor + H.A.	117.9F	162.0CD	6.03F	6.70AB	0.89e	35.67C	99.00bc
Humic acid (HA)	162.7A	210.7A	7.30B	7.00A	1.04d	30.00E	110.0a
	2013						
Control	99.2D	118.4BC	6.00E	5.40F	1.10bc	32.13D-F	109.0b
CPPU	105.3D	165.8BC	6.27D	6.00E	1.05d	35.47B-D	103.0c
CPPU + Inca	159.3AB	230.0A	7.50A	6.53B-D	1.15a	44.97 AB	87.16fg
CPPU + Calbor	145.0BC	161.7BC	7.10B	6.87AB	1.03de	46.17A	115.7a
CPPU + Humic acid (HA)	78.0E	106.7E	6.70C	6.03E	1.10bc	26.80FG	54.40h
Inca	158.7AB	121.7D	5.93E	6.27DE	0.95f	33.97A-C	87.90fg
Inca + Calbor	142.0BC	173.3B	6.57C	6.33C-E	1.04d	32.63A-C	92.00e
Inca + H.A.	137.3C	144.2C	6.70C	6.40CD	1.05d	25.10F	85.00g
Calbor	148.3AC	170.0B	7.47A	6.63BC	1.13ab	31.38A-C	89.00f
Calbor + H.A.	130.3C	169.2B	7.33AB	6.83AB	1.07cd	30.87E-G	100.0d
Humic acid (HA)	165.0A	221.7A	7.17B	7.03A	1.00e	32.73A-C	102.0cd

IV.2. Fruit Firmness:

Data tabulated in Table (5) cleared that, the firmness of apple fruits skin decreased gradually and significantly as storage period increase up to 12 weeks (negative relation) throughout the two seasons of study. Meanwhile, CPPU, Inca and CPPU + Inca treatments significantly supported "Anna" apple fruits to maintain the highest firmness during storage in 1st season (98.6, 87.43 and 85.5 g/cm^2 respectively). While, in 2nd season Calbor, Inca and control treatments showed the highest firmness (79.86, 77.52 and 72.95, respectively). Moreover, interaction results showed that, Inca, CPPU and H.A. maintain the fruits firmness to 6 weeks then significantly decreased. However, Calbor treatment

supported the firmness till the end of storage period (12 weeks) while Inca treatment maintained firmness till 9 weeks then breakdown (20.33g/cm^2). Cytokinin natural plant growth hormones promote the fruit cell division, **(Looney, 1993).** Moreover, an obvious firmness in treated fruits was evident.

Table (3): Effect of treatments on TSS (%), acidity (%) and TSS/acid ratio in 2012 and 2013 seasons.

Treatments	TSS (%)		Acidity (%)		TSS/acid ratio	
	2012	2013	2012	2013	2012	2013
Control	11.53a	12.87ef	0.890ab	0.667bc	12.96c	19.30e
CPPU	11.50a	13.10de	0.757c	0.570de	15.19a	22.98cd
CPPU + Inca	11.27a-c	12.43g	0.790bc	0.687b	14.27ab	18.09ef
CPPU + Calbor	9.90d	13.83c	0.967a	0.600cd	10.24e	23.05cd
CPPU + Humic acid (HA)	11.43ab	12.83ef	0.823bc	0.537de	13.89bc	23.89bc
Inca	9.80d	13.40d	0.657d	0.783a	14.92ab	17.11f
Inca + Calbor	11.47a	12.63fg	0.813bc	0.507e	14.11ab	24.91b
Inca + H.A.	10.90c	12.70fg	0.923a	0.570de	11.81d	22.28d
Calbor	11.43ab	14.23b	0.790bc	0.563de	14.47ab	25.28b
Calbor + H.A.	11.00bc	14.83a	0.723cd	0.567de	15.21a	26.16a
Humic acid (HA)	10.90c	13.00ef	0.790bc	0.820a	13.80bc	15.85g

Table (4): Effect of treatments on weight loss % during storage at 3°C and 90% RH for 0, 6, 9 and 12 weeks.

Treatments	**Fruit weight loss %**									
	2012					**2013**				
	0	**6 weeks**	**9 weeks**	**12 weeks**	**Mean**	**0**	**6 weeks**	**9 weeks**	**12 weeks**	**Mean**
Control	0.00m	3.41l	6.58j-l	8.75j-l	6.24E	0.00n	3.22m	5.57lm	8.32j-m	5.70G
CPPU	0.00m	3.16l	4.42kl	9.88jk	5.82E	0.00n	7.39k=m	8.61j-m	11.42i-l	9.14F
CPPU + Inca	0.00m	16.70f-h	20.21c-g	21.86c-f	19.59AB	0.00n	14.52g-j	16.41e-i	21.98d-f	17.64C
CPPU + Calbor	0.00m	7.81j-l	11.65h-j	15.88g-i	11.78CD	0.00n	13.58i-k	15.86f-i	20.61d-g	16.68C
CPPU + Humic acid (HA)	0.00m	19.02d-g	23.51cd	24.85bc	22.46A	0.00n	3.12m	13.60i-k	22.51de	13.08DE
Inca	0.00m	15.28g-i	16.86e-h	18.90d-g	17.01B	0.00n	20.49d-h	22.49de	30.21b	24.39B
Inca + Calbor	0.00m	16.49f-h	17.86e-g	31.77a	22.04A	0.00n	25.97b-d	30.74b	38.40a	31.70A
Inca + H.A.	0.00m	4.29l	6.30j-l	29.27ab	13.29C	0.00n	11.34i-l	13.04i-k	23.73cd	16.04CD
Calbor	0.00m	3.59l	7.10j-l	17.25e-g	9.31D	0.00n	13.43i-k	16.70e-i	25.61b-d	18.58C
Calbor + H.A.	0.00m	18.44d-g	22.37c-e	25.51bc	22.11A	0.00n	26.36b-d	29.15bc	37.41a	30.98A
Humic acid (HA)	0.00m	6.56j-l	10.87ij	18.07d-g	11.83CD	0.00n	8.20j-m	11.19i-l	14.12h-k	11.17EF
Mean	0.00D	10.43C	13.43B	20.18A		0.00D	13.42C	16.67B	23.12A	

Table (5): Effect of treatments on fruit firmness during storage at 3°C and 90% RH for 0, 3, 6, 9 and 12 weeks.

Character	**Fruit firmness (g/cm^2)**					
Treatments	**0**	**3 weeks**	**6 weeks**	**9 weeks**	**12 weeks**	**Mean**
	1st season; 2012					
Control	109.00h	90.33mn	67.67q	51.00t	37.00wx	71.00F
CPPU	194.3a	134.0f	83.00o	45.33u	36.33w-y	98.60A
CPPU + Inca	160.7b	112.5h	64.00q	46.00u	44.33u	85.50C
CPPU + Calbor	140.00e	97.83jk	39.00vw	42.67uv	44.67u	72.83EF
CPPU + Humic acid (HA)	95.33kl	81.33o	67.33q	54.33st	38.67vw	67.40G
Inca	151.3c	120.2g	87.33n	45.00u	33.33x-z	87.43B
Inca + Calbor	95.33jk	81.33o	67.67q	50.33t	33.67x-z	66.27G
Inca + H.A.	151.7c	103.3i	55.00st	36.00w-y	21.33z	73.47E
Calbor	93.00lm	76.33p	59.67r	51.00t	31.67yz	62.33H
Calbor + H.A.	14.3d	101.8ij	57.67rs	57.00rs	35.67w-y	79.30D
Humic acid (HA)	117.3g	95.33kl	73.33p	39.67vw	30.00z	71.13F
Mean	132.3A	99.48B	65.61C	47.12D	35.15E	
	2nd season; 2013					
Control	113.60cd	93.10gh	70.73lm	54.00no	33.33s-u	72.95C
CPPU	140.0a	92.67gh	57.67n	33.33s-u	14.00z	67.54D
CPPU + Inca	117.0c	78.33k	43.33q	22.67wx	21.67wx	56.59F
CPPU + Calbor	114.3cd	83.33j	50.67op	37.33rs	29.67tu	63.06E

Character	Fruit firmness (g/cm^2)					
Treatments	**0**	**3 weeks**	**6 weeks**	**9 weeks**	**12 weeks**	**Mean**
CPPU + Humic acid (HA)	100.3ef	86.33ij	69.33m	53.67no	35.67s	69.07D
Inca	123.6b	90.00hi	57.33n	96.33fg	20.33wx	77.52B
Inca + Calbor	103.0e	89.00hi	75.33kl	58.00n	41.33qr	73.33C
Inca + H.A.	114.8cd	70.33m	34.33st	24.67vw	15.33yz	51.89G
Calbor	111.6d	93.67gh	77.33k	68.67m	48.00p	79.86A
Calbor + H.A.	112.7cd	75.67kl	40.67qr	37.00rs	18.00x-z	56.81F
Humic acid (HA)	97.53fg	75.20kl	54.67no	29.00uv	19.67w-y	55.21F
Mean	113.5A	84.33B	57.40C	46.79D	27.00E	

IV.3. Fruit juice TSS:

The present data (Table, 6) revealed that, total soluble solids significantly increased till 3 weeks of storage (12.0 and 13.67%) in the two studied seasons then gradually and significantly decreased till the end of storage period. However, in the 1st season Inca+ Calbor, control and Calbor treatments showed the highest TSS values (11.4, 11.17 and 11.14%). In the 2nd season Calbor + H.A., Calbor and Calbor + CPPU treatments showed the highest TSS (13.57, 13.24 and 12.83%), respectively. Also, interaction results showed that, Inca + Calbor treatment induced higher TSS throughout the storage period up to 12 weeks in the two studied seasons, while the same result was obtained by Calbor + H.A. treatment in 2013 season only.

Table (6): Effect of treatments on fruit TSS % during storage at 3°C and 90% RH for 0, 3, 6, 9 and 12 weeks.

Character	TSS (%)					
Treatments	**0**	**3 weeks**	**6 weeks**	**9 weeks**	**12 weeks**	**Mean**
	1st season; 2012					
Control	11.53e-h	12.30b	11.47f-i	10.97l-n	9.57xy	11.17B
CPPU	11.50e-i	11.30g-k	10.53p-r	10.87m-o	9.40yz	10.72E
CPPU + Inca	11.27h-k	12.37b	11.37f-j	11.10k-m	8.70	10.96C
CPPU + Calbor	9.90vw	12.27b	11.40f-i	11.33g-k	9.27z	10.83D
CPPU + Humic acid (HA)	11.37f-j	12.77a	11.57e-g	10.63o-q	8.50	10.97C
Inca	10.07uv	11.43f-i	10.43q-s	10.37r-t	9.20z	10.30G
Inca + Calbor	11.47f-i	11.73de	11.57e-g	11.50e-i	10.73n-p	11.40A
Inca + H.A.	10.90l-n	12.00c	11.43f-i	11.23i-k	9.03	10.92CD
Calbor	11.33g-k	12.23b	11.13j-l	11.23i-k	9.77wx	11.14B
Calbor + H.A.	10.13t-v	11.63ef	11.10k-m	10.27s-u	8.43	10.31G
Humic acid (HA)	10.20s-u	11.93cd	10.87m-o	10.97l-n	8.33	10.46F
Mean	10.88D	12.00A	11.17B	10.95C	9.18E	
	2nd season; 2013					
Control	12.85g-i	13.85cd	12.70i-k	11.27st	10.37z	12.21E
CPPU	12.53j-l	13.07fg	11.90op	11.03t-v	10.03	11.71H
CPPU + Inca	12.43kl	12.97f-h	12.07no	11.70pq	9.73	11.78H
CPPU + Calbor	13.63de	14.80a	13.07fg	11.97no	10.67xy	12.83C
CPPU + Humic acid (HA)	12.73h-j	13.50e	12.17mn	11.10tu	10.07	11.91G
Inca	13.07fg	13.93c	12.67i-l	11.83op	10.77wx	12.45D
Inca + Calbor	12.47j-l	13.07fg	12.40lm	12.07no	11.40rs	12.28E
Inca + H.A.	12.53j-l	13.47e	12.63i-l	11.53qr	10.17	12.07F
Calbor	14.23b	14.80a	13.17f	12.83j-i	11.17s-u	13.24B
Calbor + H.A.	14.83a	14.97a	14.00bc	13.07fg	11.00u-w	13.57A
Humic acid (HA)	11.27st	12.00no	10.83v-x	10.50yz	9.10	10.74J
Mean	12.96B	13.67A	12.51C	11.72D	10.41E	

IV.4. Fruit juice acidity:

Data in Table (7) showed that, juice acidity significantly and gradually decreased throughout storage period up to 6 weeks then increased till 12 weeks of storage in the two studied seasons. However, Inca + H.A. treatment effectively decreased juice acidity throughout the storage period up to 12 weeks at 3°C and 90 RH (0.38 and 0.34%) in 2012 and 2013 seasons, respectively. Moreover, Calbor treatment in the 1st season as well as H.A. treatment in the 2nd season obviously caused the least juice acidity (0.39 and 0.36%), respectively. On the other hand, Calbor +

H.A. in the 1st season as well as control in the 2nd season significantly induced the highest juice acidity (0.5 and 0.43%), respectively. Interaction data showed that, Calbor treatment effectively induced less juice acidity throughout the storage period up to 12 weeks in 2012 and 2013 seasons.

The present results cleared that, "Anna" apple stored fruits for 3 weeks did not lose weight but have better quality (more TSS and less acidy with adequate firmness). Moreover, CPPU treatment caused less fruit weight loss and maintained firmness. Also, Calbor treatment improved all studied fruit quality attributes (% fruit weight loss, firmness, TSS and acidity). However, Inca treatment alone or + CPPU effectively supported fruits to maintain the firmness through storage period.

Generally, many researchers dealed with the effect of Ca (Inca and Calbor) as well as K (Humic acid) treatments on fruits during storage period.

Eliwa et al. (1999) revealed that Ca has received a considerable attention in apple orchards not only due to its relationship to physiological disorders, but also due to its other desirable effects like extending storage life and increasing firmness and TSS. Also, **Ali et al. (2006)** found that K treatment reduced peach fruit weight loss, disorders and acidity but increased TSS during cold storage. Meanwhile, CPPU at 10 ppm and $CaCl_2$ at 2% effectively decreased pear fruit weight loss and acidity while increased firmness and TSS after 2 months of cold storage (**Nasr et al. 2009**). **Abdel–Haffeez et al. (2010)** stated that, Ca and K treatments had the least weight loss and the highest fruit texture after 6 weeks of storage.

Table (7): Effect of treatments on fruit acidity % during storage at 3°C and 90% RH for 0, 3, 6, 9 and 12 weeks.

Character	Acidity (%)					
Treatments	0	3 weeks	6 weeks	9 weeks	12 weeks	Mean
	1st season; 2012					
Control	0.97a	0.29l-n	0.31l-n	0.36j-l	0.36j-l	0.46B
CPPU	0.80b-d	0.36j-l	0.25m-o	0.42i-k	0.29l-n	0.43B-D
CPPU + Inca	0.92a	0.25m-o	0.22no	0.32k-n	0.59fg	0.46B
CPPU + Calbor	0.82bc	0.29l-n	0.18o	0.36j-l	0.38j-l	0.41CD
CPPU + Humic acid (HA)	0.66ef	0.32k-n	0.22no	0.36j-l	0.49hi	0.41CD
Inca	0.79cd	0.30l-n	0.29l-n	0.45h-j	0.38j-l	0.44BC
Inca + Calbor	0.78cd	0.34j-m	0.24m-o	0.34k-m	0.37j-l	0.41CD
Inca + H.A.	0.81b-d	0.25m-o	0.22no	0.24m-o	0.38j-l	0.38D
Calbor	0.79cd	0.32k-n	0.18o	0.29l-n	0.36j-l	0.39D
Calbor + H.A.	0.89ab	0.42i-k	0.25m-o	0.42i-k	0.54gh	0.50A
Humic acid (HA)	0.71de	0.36j-l	0.25m-o	0.32k-n	0.54gh	0.44BC
Mean	0.81A	0.32D	0.24E	0.35C	0.43B	
	2nd season; 2013					
Control	0.66a	0.43d-g	0.32i-n	0.37g-i	0.38g-i	0.43A
CPPU	0.57b	0.41e-h	0.27k-p	0.35g-k	0.33h-l	0.39BC
CPPU + Inca	0.68a	0.33h-m	0.23op	0.31i-o	0.55bc	0.42AB
CPPU + Calbor	0.67a	0.38g-i	0.23op	0.32i-n	0.3636g-k	0.39BC
CPPU + Humic acid (HA)	0.55bc	0.25l-p	0.19p	0.36g-k	0.50b-d	0.37CD
Inca	0.65a	0.37g-i	0.28j-p	0.41e-h	0.38g-i	0.42AB
Inca + Calbor	0.57b	0.38g-i	0.22p	0.31i-o	0.37g-i	0.37CD
Inca + H.A.	0.53bc	0.32i-n	0.24m-p	0.26l-p	0.36g-j	0.34D
Calbor	0.57b	0.40f-i	0.23n-p	0.27k-p	0.35g-k	0.37CD
Calbor + H.A.	0.50b-d	0.26l-p	0.25l-p	0.38g-i	0.48c-f	0.37CD
Humic acid (HA)	0.43d-g	0.32i-n	0.23op	0.32i-n	0.48c-f	0.36CD
Mean	0.58A	0.35C	0.24D	0.33C	0.41B	

1- Isolation purification and identification of the associated fungi to apple cv. Anna fruit rot

Isolation purification and identification of the associated fungi of infected samples obtained from different stages of apple trees (flower, fruit set, fruit mature and postharvest fruit) were carried out. The microscopic examination of the obtained cultures revealed that there were several fungi belonging to different genera. The obtained fungi were purified and identified as: Alternaria alternata, Aspergillums Niger, Fusarium subglutinans, Penicillium puberulum, Scytalidium dimidiatum, and stemphylium vesicarium (Table 8).

Table (8) reveal that the frequency percentage of fungi isolated from different apple stage (flower, fruit set, fruit mature and postharvest fruit) varied from a stage to another. The same trend with regard to the yielded fungi was obtained.

Also, data show that the postharvest stage gave the highest frequency percentage of isolated fungi (30.30%), followed by the fruit mature stage (26.90 %), the fruit set stage (21.80) and the flower stage (21.00 %). This is due to the reason that the fruits contain high levels of sugars and nutrient elements and their low pH values make them particularly desirable to fungal decayed **(Singh and Sharma, 2007).** At the same time, the highest frequency percentage of Alternaria alternate that showed a high frequency, in general, the highest frequency percentage was isolated from the fruit set stage (10.1 %), followed by Penicillium puberulum the highest frequency percentage was isolated from the postharvest stage (12.6 %), Aspergillums Niger the highest frequency percentage was isolated from the postharvest stage (7.60 %), stemphylium vesicarium the highest frequency percentage was isolated from the flower stage (5.90 %), Scytalidium dimidiatum the highest frequency percentage was isolated from the fruit mature stage (5.90 %) and Fusarium subglutinans the highest frequency percentage was isolated from the flower stage (1.70 %). Similar results have been reported by many workers **(Jones and Aldwinkle, 1991; Gur, et al., 2008; Moslem et al, 2011).**

Table (8): Frequency of different fungi isolated from flower, fruit let, fruit mature and postharvest fruit stage of apple fruit cv. Anna obtained during season 2012-2013 growing season

Fungus	Frequency (%)				Total
	flower	fruit set	fruit mature	postharvest fruit	
Alternaria alternata	9.24	10.10	7.60	5.90	32.8
Aspergillus niger	4.20	4.20	2.50	7.60	18.5
Fusarium subglutinans	1.70	0.00	0.00	0.00	1.7
Penicillium puberulum	0.00	0.00	6.70	12.60	19.3
Scytalidium dimidiatum	0.00	2.50	5.90	2.50	10.9
stemphylium vesicarium	5.90	5.00	4.20	1.70	16.8
Total	21.00	21.80	26.90	30.30	

1- Fungi isolated percentage were highest with alternation alternate (32.8%) while were loest with fusarium subglutinan (1.7%) where isolated fungi increased gradually from flower to fruit set to mature fruit and to post harvest stage (21.0, 21.8, 26.9 and 30.3%). However, the highest disease severity was obtained by fungus Scytalidium dimidiatum (76.0%) while, fusarium subglutinans does not give any symptoms during the test period (0.0%).

Moreover, Kemazed 50% WP and Mancozeb 80% WP fungicides completely inhibited the growth of both Penicillium puberulum and S. dimidiatum. Also, Chitosan fungicide reduced the growth of both them where the inhibitor effect increased with increasing chitosan concentration up to 1.0%. in addition, acetic acid at 0.5% and control caused the highest disease severity with P. puberulum and S. dimidiatum while Chitosan + Calbor or + Kemazed induced the leaset disease severity.

2-Pathogenicity test:

Pathogenic capabilities of the isolated fungi obtained from the different stages, were tested to prove their responsibility for disease incidence.

Table (9) and Fig. (1) show that, in general, most of the tested fungi had the capability to cause the disease at different degrees of disease severity. At the same time Table (9) clearly reveal that the pathogenic capabilities of isolated fungi were different from fungus to another. The highest disease severity was obtained by the fungus Scytalidium dimidiatum, (76.0 %) followed by *Penicillium puberulum* (61.0 %) then stemphylium vesicarium (37.0 %), Aspergillums Niger (24.1 %) and *Alternaria alternate* (16.7%). *Fusarium subglutinan* does not give any symptoms at the fruit during the test period. **(Punithalingam et al., 1970)** reported that *Scytalidium dimidiating (synanamorph Hendersonula toruloidea)* a dematiaceous mold, is a well-recognized plant pathogen causing branch wilt, canker and dieback disease of wide range of trees and storage rot of plant tubers such as yams in tropical and subtropical regions worldwide. Also, this fungus recorded as pathogen on fruits of various hosts such as mango, banana and dragon fruit **(Sakalidis et al., 2011; Meredith, 1963; Masratul, et al., 2013).** On the other hand, obtained results also demonstrated that *Penicillium puberulum* was capable of producing typical blue mold symptoms in apple fruits. This result generally agreed with the published literature, where wound-invading Penicillium species were the most common and destructive post-harvest pathogens responsible for apple blue mold **(Jones and Aldwinkle, 1991; Moslem et al, 2011).**

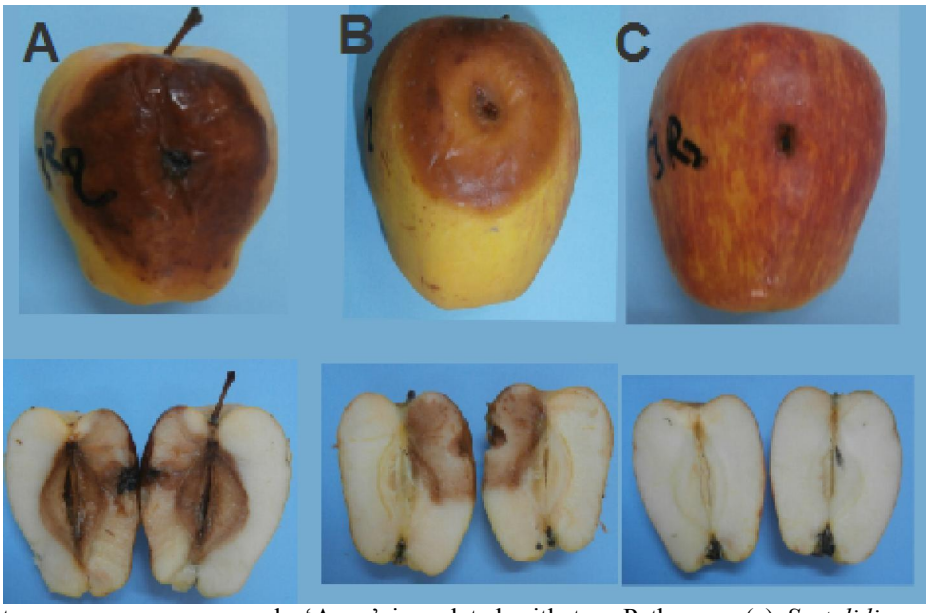

Fig. (1). Symptoms appearance on apple 'Anna' inoculated with two Pathogens (a) *Scytalidium dimidiatum* (b) *Penicillium puberulum* (C) control over a period of 7 days.

Table (9). Pathogenic capability of fungi isolated from apple fruit cv. Anna collected from different growth stages

Tested fungi	Disease severity %
	Fruit rot
Alternaria alternata	16.7
Aspergillus niger	24.1
Fusarium subglutinans	00.0
Penicillium puberulum	61.1
Scytalidium dimidiatum	76.0
Stemphylium vesicarium	37.0
L.S.D. at 5 % level:	3.22

3-Effect of the fungicides tested on fungal growth

The data of Table (10) show that the fungicides differed in their ability to inhibit the mycelia growth of the two pathogens. Kemazed 50% WP gave the highest reduction in linear growth of two pathogens *Penicillium puberulum* and *Scytalidium dimidiatum* compared control treatment and other fungicides treatments. Kemazed 50% WP and Mancozeb 80% WP completely inhibited the growth of both pathogens, while Kemazed 50% WP was more inhibitory to *Scytalidium dimidiatum* than Mancozeb 80% WP. In general the pathogen *Penicillium puberulum* was more effect with fungicides than *Scytalidium dimidiatum.*

Table (10) *In vitro* effect of five fungicides on growth of *Penicillium puberulum* and *Scytalidium dimidiatum*

Trade name	Common name	Recommended dose	Mean diameter of growing colonies (cm)	
			Penicillium puberulum	*Scytalidium dimidiatum*
Kemazed 50% WP	Carbendazim	50 g/100 L water	0.0	1.3
Saprol 19% EC	Triforine	150 cm^3/100L water	2.6	4.0
Mancozeb 80% WP	Mancozeb	250 g/100 L water	0.0	3.1
Bellis 38% WG	25.2% Boscalid + 12.8% Pyrachlorstrobin	30g/100L water	5.1	7.1
Teldor 50% SC	Fenhexamid	50 cm^3/100L water	6.6	8.0
Control	-	-	9.0	9.0

4- Effect of Chitosan fungicide in vitro:

Results in Table (11) indicated that all chitosan concentration reduced linear growth of two pathogens. The inhibitor effect increased with increased chitosan concentration. The most effective concentration was 1.0% with highest inhibition of *Penicillium puberulum* and *Scytalidium dimidiatum,* 85.6 % and 77.8 %, respectively.

Theses results are in harmony with those obtained by **Palma-Guerrero, et al., (2009)** who reported that different cell types (conidia, germ tubes and vegetative hyphae) exhibited differential sensitivity to chitosan with ungerminated conidia being the most sensitive. Also, they reported that chitosan killed conidia in less than four minutes, conidial germlings within 35-45 min, and vegetative hyphae within 40 min. and they added that the lower sensitivity to chitosan of

germlings and hyphae than conidia may relate to differences in the composition of their plasma membranes. For example, different amounts of ergosterol have been found in different fungal cell types **(Alvarez et al., 2007; Martin and Konopka, 2004; Van Leeuwen et al., 2008).**

Table (11). Effects of chitosan concentration on mycelial growth of *Penicillium puberulum* and *Scytalidium dimidiatum* 7 days after incubation at 25 ◦C.

Chitosan concentration (%)	*Penicillium puberulum*		*Scytalidium dimidiatum*	
	Mycelial growth (cm)	Reduction (%)	Mycelial growth (cm)	Reduction (%)
0.1	6.6	26.7	7.1	21.1
0.2	5.9	34.4	6.4	28.9
0.5	3.8	57.8	4.1	54.4
1.0	1.3	85.6	2.0	77.8
Acetic acid (0.5%)	8.2	8.9	7.2	20.0
Sterile distilled water	9.0	00.0	9.0	00.0
L.S.D. at 5 % level:	Fungi 0.16; concen. 0.25; F&C 0.35			

5-Effect Chitosan and combination

Data in Table (12) and fig. (2) clearly demonstrated that the effect was high in reduction disease severity on *Penicillium* than *Scytalidium.* Also, acetic acid, and control (without chitosan) treatments had same trend in case *Penicillium.* Chitosan alone was their effect equal with treatments (chitosan + citofax + Enka), (chitosan+Enka) and (chitosan+citofax) on *Penicillium,* while in case Scytalidium was their effect equal in both treatment (chitosan+Enka) and (chitosan+citofax) in reduction disease severity, however was low in their effect than other treatments in reduction disease severity, while it was their effect high in reduction disease severity compared with acetic acid, and control treatments. Also, observed that non-significantly different between (chitosan + kemazd) or (chitosan+calboro) and (chitosan + calboro + humic acid) in reduction disease severity, it was clearly in two pathogen under study. In addition, it was found that in all combinations that subscribe calboro with chitosan gives high effect in reduction disease severity, than other compounds that incorporate with chitosan. These results may explain as noted by **Chardonnet et al., (2003)** pre-harvest calcium treatment used to increase the calcium content of the cell walls of fruit tissue after harvest. Moreover, it is effective in delaying senescence, resulting in firmer, higher quality fruit. Also, **Khalifa et al., (2009)** founded that the fruit firmness of Anna apple was significantly affected by foliar spraying with calcium on Anna apple trees. And they added that the incidence of the disease decreased with increase concentration of calcium and disease severity took the same trend of controlling the disease incidence results. **Effat Zaher et al., (1985)** carried out that in vivo studies for chemical control of apple fruit rot using five fungicides for pre-harvest treatment followed by storage at 3-4 °C for 3 months. They founded that all the fungicides tested gave complete inhibition of fruit rot for one month. After 2, 3 month the fungicides differed in their effect. Topsin M-70% showed the best effect followed Bavistin. These results are in agreement with, those of **Zhu et al., 2008 and Abd-A11A and Wafaa Haggag (2010)** was reported that, disease progress in the mango fruits inoculated with Colletotrichum gloeosporioides was significantly inhibited by the treatment with chitosan coating. Chitosan treated fruit inhibited the growth of a wide variety of bacteria and fungi as compared to the control treatments. Various defense responses in several fruit have been induced, including the elicitation of phenylalanine ammonia lyase (PAL) activity in grape berries **(Romanazzi et al., 2002),** and chitinase and B. -1,3- glucanase in oranges, strawberries and raspberries **(Fajardo et al., 1998; Zhang and Quantick, 1998). Jiang et al. (2005)** observed an increase in anthocyanin levels in chitosan-coated fruit already after 6 hours of storage. **Jiang and Li (2001)** found that, 1%, 2% and 3% chitosan coating on the activity of polyphenol oxidase on the third and sixth day of storage of lychee fruit, **Jiang et al. (2005)** confirmed chitosan's inhibitory effect when analyzing the influence of 2% chitosan coating on the activity of polyphenol oxidase in lychee fruit stored at 25 °C. **Pen and Jiang (2003)** also noted that chitosan's inhibitory effect on polyphenol oxidase increases at higher concentrations of the chitosan solution which is applied to coat food products. **Du, et al., (1998)** studied the effects of chitosan coating on respiration, ethylene production, and storage of Jonagold' apples *(Malus pumila Mill. var. domestica Schneid.)* and found that when coating the fruit with chitosan significantly reduced the respiration rate and ethylene production in storage. Postharvest coating increased the internal CO2, and decreased the internal O_2 levels of the fruits markedly. Firmness of the treated fruits were considerably retained during

storage. Observation by SEM revealed that the chitosan films covered overall surface of the treated fruits. A plenty of deep cracks were observed on the pericarp of uncoated fruits, but much less on the surface of coated fruits. Growing hyphae, which was resulted from an inoculation of conidia of apple gray mold caused by Botrytis cinerea, were recognized on the pericarp of uncoated'fruits, whereas many deformed spores were observed on the surface of the coated fruits. These observations support the view that chitosan coating could not only suppress the ethylene production and respiration, but also inhibit conidial germination and fungal development resulting in preserving the quality of 'Jonagold' apples.

Table (12): Effect compounds, fungicide and chitosan on two pathogens at room temperature

treatments	Disease severity %	
	Penicillium puberulum	*Scytalidium dimidiatum*
Chitosan + kemazd	5.6	24.1
Chitosan + calboro	11.1	24.1
Chitosan+calboro+humic acid	11.1	25.9
Chitosan + calboro + citofax	16.7	31.5
chitosan + calboro + inka	16.7	33.3
citofax + humic	22.2	37.0
Humic	31.5	44.4
chitosan + citofax + inka	33.3	44.4
Humic + inka	31.5	46.3
Chitosan + inka	33.3	53.7
Chitosan + citofax	33.3	53.7
Acetic acid (0.5%)	63.0	70.4
Control only chitosan	33.3	53.7
Control without chitosan	63.0	79.6
L.S.D. at 5 % level:	Teat: 6.56; F: 2.48; Treat & F N.S.	

Fig (2): Effect compounds, fungicide and chitosan on two pathogens at room temperature (A) *Penicillium puberulum* (B) *Scytalidium dimidiatum*: Treatment (1) Chitosan + kemazd; (2) Chitosan + calboro; (3) Chitosan + calboro + humic acid (4) Control without chitosan

Over all, the present study was performed on "Anna" apple trees to tackle the phenomenon of "Tatiela" improve fruit set, yield storage and reduce number of fruit decay. It is clearly obvious that Sitofex sprays achieved the highest fruit set percentage, whereas Calbor and Calbor plus Inca attained the lowest percent of fruit drop. Yet, the highest yield/tree was accomplished by Calbor plus

Sitofex treatment as well as humic acid improved fruit quality (weight, size and diameter). Chitosan treatment reduced the growth of Pathogens attacking the fruits.

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