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Cytogenetical and Physical Parameters for Using Some Food Additives and Salts to Control Anthracnose Disease on Color Sweet Pepper (*Capsicum annum* L.)

Maha S. A. Haridy[1,*]; Seham S. Ragab[2] and Thauria M. M. Abo Elwafa[2]

[1]Central Lab of Organic Agriculture, Agricultural Research Center (ARC), 9 Gamaa St., Giza 12619, **Egypt**

[2]Plant Pathol. Res. Inst., Agricultural Research Center (ARC), 9 Gamaa St., Giza 12619, **Egypt**

[*]**Corresponding author:** mahasahh@gmail.com

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Abstract: Using potassium sorbate, citric acid, sodium bicarbonate and calcium chloride as pre-harvest treatments to control Anthracnose disease which reflects the infection of Canon 58 (cv) color sweet Pepper (*Capsicum annuum*) by *Colletotrichum gloeosporioides*. The treatments with Potassium sorbate and Citric acid extending the duration of storage to four weeks at 10°C and reduced disease incidence and disease severity of artificial infection in two seasons by (33.3%) and (13.3%) respectively, compared to control which recorded (100%) for disease incidence in two seasons, and for disease severity recorded (80%) in first season and (60%) in second season. While the physical parameters or quality of sweet peppers which treated with food additives and salts was preserved better than the control. Potassium sorbate, citric acid, sodium bicarbonate and calcium chloridetreatments reduced weight loss (%), and increased total soluble solids (TSS) content and fruit firmness compared to control for natural and artificial infection in two seasons during storage periods at 10°C. Additionally, cytogenetical parameters via cells division were examined and screening the percentage of abnormal pollen mother cells (PMCs) or chromosomal aberrations in PMCs and pollen grains (n and 2n) fertile and sterile were recorded. Although the results appeared few percentage of abnormality in some treatments but must not ignore it, while fungicide which used as a monitoring control has more mutagenic effects than other treatments

Key words: *Colletotrichum gloeosporioides*, cytogenetic, chromosomal abnormality, Pollen grains.

1. Introduction

One of the most dangerous pathogenic fungi to plants in the world is the *Colletotrichum* species which cause anthracnose disease, this disease affects in fruit and plant components in a variety of commercially significant host species including legumes, fruit trees, and vegetables, is brought on by member species **(Bailey *et al.*, 1992).** In the tropics and subtropics, the production of peppers (*Capsicum* spp.) is severely

harmed by anthracnose disease**.** Fruit can become infected before or after harvest and begin to rot, which significantly reduces the amount of marketable product. In important producing nations like Thailand, losses of 50% to 100% have been documented **(Than *et al.*, 2008).** Anthracnose of pepper has been found to be associated with up to four *Colletotrichum* species: *C. truncatum* (**Damm *et al.*, 2009**), *C. gloeosporioides*, *C. acutatum* and *C. coccodes* (**Harp *et al.*, 2008 and Harp *et al.*, 2013; Nirenberg *et al.*, 2002 and Sharma *et al.*, 2005**). Many *Colletotrichum* species may infect many host crops and induce latent or quiescent infections has led to their classification as some of the most significant postharvest pathogens globally **(Freeman *et al.*, 1998)**. For a variety of fruits grown under artificial conditions, the pathogens' potential for cross-infection has been documented **(Freeman *et al.*, 1998)**. Identify and discover the *Colletotrichum* species that cause anthracnose in Egyptian bell peppers (*Capsicum annuum* L.) **(Ramdial and Rampersad, 2015).** Anthracnose disease, which affects pepper crops, typically manifests as depressed necrotic lesions with concentric rings of acervuli. In some cases, the lesions are brown, which later turn black after the pathogen forms setae and sclerotia (**Roberts *et al.*, 2001 and 2018**). Lesions on the plant's stems and leaves are less obvious; they can be seen as grey spots **(Alexander and Pernezny 2003)**. Developing solutions for plant breeding and disease management requires accurate diagnosis **(Freeman *et al.*, 1998)**.

Many pesticides are used to keep agricultural products free of weeds, insects, and diseases, but their residues pollute the environment and endanger both animals and humans consuming these plants which treated with the pesticide. So, Pesticides used to control anthracnose disease and other pathogens such as bacteria, fungi, and viruses cause many risks at the deferent level of plants and many organisms, also this risk extends to soil and water. Therefore, the use of pesticides in general affects in the environment. Despite the numerous issues that chemical control raises, using pesticides to achieve desired results is still very popular. Now, Large-scale of use novel agrochemicals in the Egyptian agriculture and other countries has led some researcher to investigate the possible genetic material and storage proteins alterations **(Haiba *et al.*, 2011).** The cells of infected plants that are exposed to the pesticide in order to treat the disease by control or eliminating the pathogen are also affected. Pesticides cause many structural or numerical changes in chromosomes or both so it is necessary to use alternatives to these pesticides that are used to control these pathogens and reduce this damage. Potassium sorbate, citric acid, sodium bicarbonate and calcium chloride used as salts but Potassium sorbate and citric acid used as food additives also. So using salts and food additives as substitutes is very important to control *Colletotrichum* species or other pathogens, but they must be tested not only on the pathogen, but also on the cellular levels of treated plants, because the part that is eaten may affect the organism consumed, and this is available because plants are used as a model for such tests. However Chromosomal aberrations have been considered as a dependable indicator of mutagenic activity, since there have been evidence for a correlation between chromosomal damage and toxic effects of a number of pesticides, (**Askin, 2006 and Shehata *et al.*, 2008).** On the other hand, the alteration of Cell division behavior or chromosome structures used for monitoring the mutagenic effects of pesticides and other chemicals. Also These effects included chromosomal abnormalities such as stickiness, laggards, bridges, disturbed, micronuclei and multinuclei **(Haiba *et al.*, 2011).** The agricultural synthetic chemical has been established the genotoxicity in several plant systems via induce chromosomal aberration and decrease cell division **(Al-Ahmadi, 2013).** So this investigation aimed to study the effect of treatments (some salts and food additives as substitute's components of fungicides) on the disease incidence and the disease severity, physical parameters and cytogenetical parameters to reduce yield loss.

2. Material and Methods

This study was carried out during the period from 2021 to 2022, at the laboratories of the Central Lab of Organic Agriculture (CLOA), and Plant Pathol. Res. Inst, Agricultural Research center (ARC), Giza, Egypt, and commercial greenhouse belong to Beheira governorates.

2.1. Plant materials, treatments and isolate

Red sweet pepper (*Capsicum annuum* L) cultivar Canon58 was used as a host plant to study the relation between different treatments such as sodium bicarbonate ($NaHCO_3$), potassium sorbate ($C_6H_7O_2K$), citric acid ($C_6H_8O_7$) and calcium chloride ($CaCl_2$) against the pathogenic fungus

Colletotrichum gloeosporioides, this isolate recorded at GenBank data base under accession number (OP847399).

2.2. Preparation Solutions from Food additives and salts

The spray solutions of tested food additives and salts were prepared at the concentration of 3g/L of tap water as dissolving thoroughly. In every replication, this spray solution was sprayed in each plot of a particular treatment.

2.3. The experimental design

Eight rows, each measuring 0.8 × 70 m in width and length, were divided into 10 parts, each 7 m long. Two meters from each part was used as a boarder not to allow spray interference among the plants in neighbor plots. Each part is considered an experimental plot (a replicate). The complete randomized block design was used for designing and statistical analysis of the experiment. The Canon58 pepper plants were cultivated using conventional methods, such as fertilization, watering, etc. The weeds were physically controlled instead than using insecticides. Grown Canon58 pepper plants were sprayed three times from sodium carbonate, citric acid, potassium sorbate and calcium chloride as bulk materials at a dosage of 3g/L, and repeated this process every 15 days until 2 days before harvesting. Treated plants were sprayed till run of; the control treatment was sprayed with distilled water. After two days following the last application, the fruits from each treatment were picked when they reached maturity and sent to the Plant Pathology Research Institute's Postharvest Diseases Department in the A.R.C. Two groups of Canon58 pepper fruits were established by grading them according to size consistency. Canon58 Pepper fruits from the first group were left without sterilization and used for naturally infected investigations.

Additionally, Canon58 pepper fruits in the second group underwent three rounds of rinsing with sterilized distilled water after being surface sterilized for two minutes with 2% sodium hypochlorite solution. Before being used, the fruits were allowed to air dry. The de-infested Canon58 pepper fruits were injured by perforating their peels on the equator using four sterile steel rods that were 2 mm deep and 0.5 mm in diameter, arranged in 5-mm-diameter circle. The perforated fruits were injected with a conidial suspension of *C. gloeosporioides* (10^5 conidia/mL) using an atomizer for a duration of 30 seconds. The untreated fruits (control) underwent the same preparation, puncturing, and the treatment with water containing Triton X. In the end, each treatment was packaged in carton cartons in a single layer. For every treatment, three boxes containing twelve fruits each were used, and the fruits were stored for four weeks at 10±1°C and 90% relative humidity (RH). Every week from the beginning of the storage period to the end the fruits' physical and chemical changes were evaluated.

2.4. Evaluation of Anthracnose Disease

To evaluation of Anthracnose Disease must be measure the disease incidence and disease severity;

(a) Disease incidence

The percentage of disease incidence of anthracnose was calculated for each replicate by using this formula:

Disease incidence (%) = (No. Decayed fruits with anthracnose disease /Total no. of fruits) X100 according to **Haggag and El-Gamal (2012).**

(b) Disease severity (%)

The degree of anthracnose on Canon 58 pepper fruits was determined using a modified severity key that was taken from **Ekefan *et al.* (2010)**. 0= no infection (no lesion); 1= law infection (little lesions on 25% of fruits); 2= moderate infection (25- 50% of fruits with small lesions on fruits); 3= severe infection (lesions on 50–70% of fruits); and 4= very severe infection (lesions on ≥70% of fruits). The severity grades were converted to percentages for analysis.

$$\text{Disease severity (\%)} = \frac{\sum \text{no. of diseased fruits of each category xits category}}{4\text{x total number of fruits}} \text{X } 100$$

2.5. Physical properties

a. Fruit weight loss percentage (FWL %)

The formula stated below was applied to determine the weight loss percentage according to **Gad *et al*. (2016);**

FWL% = [(Wi –Ws)/ Wi] x100, Where, Wi = fruit weight at initial period, Ws = fruit weight at sampling period**.**

b. Firmness

To measure hardness of fruit was severed from its two opposing equatorial sides with a blade. The flesh of each fruit was assessed for firmness using an Ametec Firmness Tester (1.5-mm diameter). Kiliogram/cm^2 (Kg/cm^2) was the unit of measurement used for the hardness data **(Gregori *et al.*, 2008).**

c. TSS, total soluble solids

The TSS of fruit juice obtained from 3 Canon 58 pepper fruit tissues of such replicate and filtrated through mesh clothes. TSS was determined using hand refractometer, and data were expressed as TSS (%) (**Gregori *et al.*, 2008).**

2.6. Cytogenetical Parameters

To perform the following tests, tomato flower buds were taken from tomato plants for each treatment, control of water and control of Fenhexamid 50% SC- used with the recommended dose 50cm/100L which used as revealer or monitor for meiotic abnormalities in the studying the cytogenetical parameters only.

2.6.1. Examinations of the meiotic division

1. Randomly selected meiotic specimens from various plants were gathered; unopened floral buds of appropriate sizes were destroyed and fixed in a newly made Carnoy's fixative, which is a mixture of glacial acetic acid, alcohol, and chloroform in a volume ratio of 6:3:1.

2. Anthers were stained using a 1% aceto-orcein staining solution, which was made by mixing 1g of orcein powder with 55 ml of boiling glacial acetic acid. Then the solution was allowed to cool before being filtered and stained with 45 ml of distilled water.

3-After sample was squashed; cell shapes, cell division, at meiosis I and II, cell sizes, and meiocytes were checked (**Kumar and Singhal, 2012).**

2.6.2. Pollen grains analysis

Regarding the fertile (n), fertile (2n unreduced), and sterile of pollen grains

a. Stain-ability tests were used to assess the fertility of pollen grains from each group. Pollens nuclei which stained were considered to be seemingly viable or fertile, but pollen that was shriveled and unstained was tallied as sterile, and assess the bursting PGs also.

b. The loss (%) of pollen grain

Loss PGs (%) = sterile PGs (%) + bursting PGs (%)

c. Unreduced pollen (2n) was defined as pollen grains with a diameter that was 1.5 times greater than that of typical reduced pollen (n). A theoretical frequency for 2n pollen grains was estimated based on the number of triads, tetrads, and dyads that were seen during microsporogenesis.

d. Typically, a triad generated 1 unreduced pollen grain (2n) and 2 reduced pollen grains (n), a dyad produced 2 unreduced pollen grains (2n), and a regular tetrad produced 4 reduced pollen grains (n).

The following formula was used to determine the frequency of 2n pollen grains:

According to **Kumar and Singhal (2012)**, frequency of 2n PG (%) = (2 × dy + tri) / (2 × dy + 3 × tri + 4 × tet) × 100. dy is the observed number of dyads, tri is the number of triads, and tet is the number of tetrads.

2.7. Photographs

Using a digital imaging microscope eyepiece system of HiROCAM (High Resolution Optics Camera), photographs of the freshly prepared desired slides with clear abnormal chromosomes, dyads, triads, tetrads, and pollen grains.

2.8. Statistics Analysis

The complete randomized block design was used for experiment, the data of pre-harvest and cytogenetic statistically analyzed by one-way ANOVA using CoStat Software at $P \leq 0.05$ for compare mean among treatments.

3. Results

3.1. The effect of using food additives and salts as pre-harvest treatments for Canon58 pepper to control anthracnose disease (Disease Incidence (%) and Disease Severity (%)

Canon 58 pepper plants were treated with food additives and salts in pre-harvested stage to prevent Canon 58 peppers from developing anthracnose during the 2021–2022 growing seasons, the disease incidence and severity were significantly reduced in the natural and artificial infected fruits during four weeks of cold storage at 10°C (Tables 1, 2), and extending this duration storage to four weeks at 10°C enhanced the beneficial impact of food additives and salts in control anthracnose disease. The treatments with potassium sorbate and citric acid were completely suppressed anthracnose disease (disease incidence and disease severity) in natural infected in two seasons which storage at 10ºC for four weeks. Sodium bicarbonate and calcium chloride were also important and successful pre-harvest treatments and both reduced disease incidence in natural infection by (11.1%) after four weeks in first season and (22.2%) in second season. Whereas the best treatments were Potassium sorbate and Citric acid which recorded same values in artificial infection during two seasons for reduced disease incidence and disease severity by (33.3%) and (13.3%) respectively after four weeks, compared to control after four weeks at 10ºC which recorded (100%) for disease incidence in two seasons, and for disease severity recorded (80%) in first season and (60%) in second season.

Table (1). Effect of pre-harvest treatments in disease incidence (%) of Canon 58 pepper in two seasons in natural and artificial infection during four weeks storage periods at 10ºC

Treatments	Disease Incidence (%) -Canon58 (cv.)															
	Season 2021								Season 2021							
	Natural infection				Artificial infection				Natural infection				Artificial infection			
	Storage period/ weeks				Storage period / weeks				Storage period /weeks				Storage period /weeks			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Sodium Bicarbonate	0.0	0.0	0.0	11.1	11.1	11.1	22.2	55.5	0.0	0.0	0.0	22.2	0.0	22.2	33.3	55.5
Potassium sorbate	0.0	0.0	0.0	0.0	0.0	0.0	11.1	33.3	0.0	0.0	0.0	0.0	0.0	0.0	22.2	33.3
Citric acid	0.0	0.0	0.0	0.0	0.0	0.0	11.1	33.3	0.0	0.0	0.0	0.0	0.0	0.0	22.2	33.3
Calcium chloride	00	0.0	11.1	11.1	0.0	22.2	22.2	55.5	00	0.0	11.1	22.2	0.0	33.3	33.3	55.5
Control	0.0	11.1	11.1	22.2	33.3	44.4	66.6	100	0.0	11.1	22.2	33.3	11.1	44.4	88.8	100
LSD .0.5%	-	2.1	2.1	1.2	1.3	1.8	2.5	3.5	-	2.1	1.1	1.4	2.1	1.7	3.4	3.5

Table (2). Effect of pre-harvest treatments in disease Severity (%) of Canon 58 pepper in two seasons in natural and artificial infection during four weeks storage periods at 10ºC

Treatments	Disease Severity (%) - Canon58 (cv.)															
	2021								2022							
	Natural infection				Artificial infection				Natural infection				Artificial infection			
	Storage period/ weeks				Storage period/ weeks				Storage period/ weeks				Storage period/ weeks			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Sodium Bicarbonate	0.0	0.0	0.0	2.2	2.2	4.4	6.7	22.2	0.0	0.0	0.0	2.2	2.1	4.4	6.7	22.2
Potassium sorbate	0.0	0.0	0.0	0.0	0.0	0.0	0.0	13.3	0.0	0.0	0.0	0.0	0.0	0.0	2.2	13.3
Citric acid	0.0	0.0	0.0	0.0	0.0	0.0	0.0	13.3	0.0	0.0	0.0	0.0	0.0	0.0	2.2	13.3
Calcium chloride	00	0.0	2.2	6.7	2.2	2.6	4.4	22.2	00	0.0	2.2	6.7	2.2	0.0	4.4	22.2
Control	2.2	4.4	6.7	8.9	4.4	11.11	13.3	80	0.0	4.4	6.7	8.9	4.0	13.3	22.2	60
LSD .0.5%	2.5	4.1	3.5	3.2	5.5	3.2	3.2	5.5	-	4.1	3.5	3.2	5.5	3.2	3.3	3.5

3.2. Physical properties

Assessments and characterization some physical properties for the effect of pre-harvest treatments for Canon58 pepper fruits during storage periods as following;

(a). Fruit weight loss percentage

Results in Table (3) show the effect of pre-harvest treatment of canon58 pepper fruits on weight loss percentage under cold storage conditions during two seasons.

Fruit weight loss percentage increased gradually toward the end of storage period (four weeks) in the control treatment. While all pre-harvest treatments decreased significantly percentage of weight loss compared with untreated fruits of Canon58 pepper during both seasons of this study. After four weeks of cold storage in artificial infection, the fruits of Canon58 pepper which treated by potassium sorbate and Sodium Bicarbonate recorded the lowest value of weight loss (4.1%) and (5.6%) respectively in first season, also citric acid recorded the lowest value (4.2%) in the second season, whereas the highest value of weight loss percentage was found in untreated fruit (control) during two seasons and recorded (15.1%) in first season and (15.3%) in second season, also Calcium chloride gave low value (7.4%) for weight loss during storage period at 10ºC in two seasons compared to control.

(b). Firmness

In control treatment the firmness of canon 58 pepper decreased compared to all pre-harvest treatments which increased the fruit firmness in natural and artificial infection during four weeks storage periods at 10ºC in both seasons. After 4 weeks of storage in natural infection (Table 4) the firmness of Canon 58 pepper plant which treated with potassium sorbate recorded the highest value (4.5kg/cm^2) in the two seasons, then the treatment with citric acid and calcium chloride which both recorded (4.4kg/cm^2) in first season and (4.0kg/cm^2) in second season, while the control recorded (3.5kg/cm^2) in first season and (3kg/cm^2) in second season. Also in artificial infection the fruits treated with citric acid and potassium sorbate recorded (4.2kg/cm^2) for both treatments in two seasons.

Table (3). Effect of pre-harvest treatments in weight loss (%) of Canon 58 pepper in two seasons in natural and artificial infection during four weeks storage periods at 10ºC

Treatment	Weight Loss (%)															
	Season 2021								Season 2022							
	Natural infection				Artificial infection				Natural infection				Artificial infection			
	Storage period/ weeks				Storage period/ weeks				Storage period/ weeks				Storage period/ weeks			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Sodium Bicarbonate	0.4	1.8	3.0	4.7	0.4	1.8	3.5	5.6	0.5	2.0	3.1	4.8	0.6	1.8	3.6	5.8
Potassium sorbate	2.4	4.0	5.8	7.2	1.0	2.4	3.3	4.1	2.5	4.0	5.8	9.0	1.4	2.8	4.4	6.0
Citric acid	3.3	4.8	5.9	7.6	1.2	2.6	4.2	6	3.6	4.8	6.0	7.8	0.5	1.5	2.5	4.2
Calcium chloride	3.9	5.6	7.4	10.5	3.1	4.5	5.4	7.2	4.0	6.0	7.5	10.6	3.2	4.5	5.3	7.4
Control	6.7	8.3	9.5	11.1	3.1	5.0	7.2	15.1	7.0	8.8	9.6	11.3	3.5	5.0	7.5	15.3
LSD .0.5%	0.18	0.85	1.00	0.48	0.48	0.93	0.48	0.83	1.05	1.37	0.98	0.97	0.54	0.76	0.61	0.60

Table (4). Effect of pre-harvest treatments on firmness of Canon 58 pepper in two seasons in natural and artificial infection during four weeks storage periods at 10ºC

Treatment	Firmness (Kg/cm^2)															
	2021								2022							
	Natural infection				Artificial infection				Natural infection				Artificial infection			
	Storage period/ weeks				Storage period/ weeks				Storage period/ weeks				Storage period/ weeks			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Sodium Bicarbonate	4.8	4.5	4.2	4.0	4.5	4	3.5	3.3	4.8	4.5	4.0	4.0	4.5	4	3.5	3.3
Potassium sorbate	5	5.0	4.8	4.5	5	4.5	4.4	4.2	5	5.0	4.8	4.5	5	4.5	4.4	4.2
Citric acid	5	4.8	4.5	4.4	5	4.6	4.4	4.2	5	5.0	4.4	4.0	5	4.6	4.4	4.2
Calcium chloride	5	4.8	4.5	4.4	4.8	4.4	4.2	4.0	5	4.8	4.5	4.0	4.8	4.5	4.2	4.0
Control	4.5	4	3.8	3.5	4.0	3.5	3.0	2.5	4.5	4.2	3.6	3.0	4.2	3.5	3.0	2.5
LSD 0.05	0.96	0.91	0.35	0.97	1.18	0.45	0.7	0.97	0.47	0.64	0.44	0.92	0.99	1.09	1.03	0.95

(c). Total soluble solids percentage (TSS %)

The TSS % of fruits was increased gradually with the advance in cold storage. The differences between pre-harvest treatments and control in natural and artificial infection were recognized during storage periods at 10ºC in the two seasons. After 4 weeks of storage periods, the TSS (%) results of canon 58 fruits in natural and artificial infection which obtained during two seasons were recorded high values then the control as shown in (Table 5). sodium bicarbonate and citric acid recorded (11%) in artificial infection after four weeks at 10ºC during two seasons and the same value for natural infection in second season and Calcium chloride too. While (10.8%) achieved by sodium bicarbonate, potassium sorbate and calcium chloride in natural infection after four weeks during first season and the control recorded (9%).

Table (5). Effect of pre-harvest treatments on TSS (%) of Canon 58 pepper in two seasons in natural and artificial infection during four weeks storage periods at 10ºC

Treatment	TSS %															
	2021								2022							
	Natural infection				Artificial infection				Natural infection				Artificial infection			
	Storage period/ weeks				Storage period/ weeks				Storage period/ weeks				Storage period/ weeks			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Sodium Bicarbonate	9.2	9.6	9.8	10.8	9.2	10.2	10.8	11	9.5	10	10.5	11	9.2	10.2	10.5	11
Potassium sorbate	7.6	8.9	10.1	10.8	8	9.2	9.4	10	8.0	9.2	10.4	10.8	8.0	9.0	9.6	10
Citric acid	8.1	8.7	10.2	10.7	8.6	9.4	9.8	11	8.5	9	10.4	11	8.6	9.4	9.8	11
Calcium chloride	8.3	9.3	9.8	10.8	8.4	9	9.4	10	8.5	9.5	10	11	8.4	9.0	9.4	10
Control	7.3	8.5	8.8	9	8.1	8.5	8.9	9.5	7.6	8.7	9.8	10.2	7.8	8.8	9.2	9.7
LSD 0.05	0.37	0.53	0.46	0.84	0.46	0.95	0.29	1.67	0.75	0.86	0.74	1.87	0.96	0.94	0.35	1.06

3.3. Cytogenetical Parameters

Flower buds were obtained from (*Capsicum annuum*) Canon58 pepper plants 2n=2x =24 for all the treatments and control to study the effect of this treatments and fungicide applied (to control *C. gloeosporioides*, which causes anthracnose disease) on the behaviors of meiotic division and the chromosome number (genome) of pollen grains (n), also pollen fertility. Some of these treatments have a negative effect on the behavior of cell division in different stages (meiotic division) and induced abnormal cell, (2n) fertile PGs, bursting, unstained, wrinkle and total sterility in pollen grains in Canon58 pepper plant (Table, 6 and 7) and (Fig 2).

3.3.1. Meiotic division examination

Fungicide was more effective on meiotic division than the other treatments with the recommended doses (50cm/100L) for the Canon58 pepper plant. However, the fungicide treatment showed the highest damage for the behavior of cells during meiosis. Meiocytes, which are type of cells that differentiate into gametes via the meiosis process, the diploid meiocyte (Fig 1- A) divides to form four identical haploid gametes in the normal behaviors via tetrad stage (Fig 1- I, J and K). But used fungicide alteration this behaviors to formed four genetically dissimilar haploid gametes, or to form two haploid gametes and one diploid which resulted from tried stage (Fig 2- R and S), this behaviors resulted from shift chromosomes from the natural path of cell division at the first meiotic division or second meiotic division and may be the first and second division together (Fig 2). The date in Table (6) appeared that The fungicide significantly induced Stickiness (0.53%), Bi-nucleate and cytomixis (1.37%), Enucleate (0.42%), Micronuclei (0.53%), Bridge (0.73%), Laggard (% 4.21), Ring Shape (4.00%) (Fig, 2-b arrow), Fragment (0.42%), Desynchronized (0.63%), another disturbed (8.74%), when sum all type of these abnormal cells in the individual treatment give total abnormal cells for this treatment, so the total abnormal cells recorded (21.58%). But the control was not recorded any of these abnormal behavior. Also potassium sorbate was the second treatment after fungicide and recorded same value (0.63%) for both Micronuclei and Ring Shape, and Desynchronized (3.17%) to give total abnormal cells (4.43%), so this salt which used in numerous variety of foods as antimicrobial agent had genotoxic effects and mutagenic effects this results agreement with **Mpountoukas *et al.* (2008) and Mamur *et al.* (2010)**.

Also citric acid induced abnormal behaviors such as Bi-nucleate and cytomixis (0.30%), micronuclei (0.91%), ring shape (1.21%) and Desynchronized (1.51%) to recorded total abnormal cells (3.93%). This results harmony with **Koca and Turkoglu (2020)** they stated that citric acid and boric acid induced chromosome breakage, stickiness, anaphase bridges and fragment similar to the effect of numerous chemicals.

Table (6). Effect of pre-harvest treatments compared to fungicide in pollen mother cells (PMCs) of Canon58 pepper plant

Treatments	Stickiness	Bi-nucleate and cytomixis	Enucleate	Micronuclei	Bridge	Laggard	Ring Shape	Fragment	Desync-hronized	Another disturbed	Total AC%	Total number of abnormal
Sodiumbicarbonate	0.00	0.31	0.00	0.18	0.00	0.06	0.00	0.00	0.00	0.24	0.79	1639
potassium sorbate	0.00	0.00	0.00	0.63	0.00	0.00	0.63	0.00	3.17	0.00	4.43	1580
citric acid	0.00	0.30	0.00	0.91	0.00	0.00	1.21	0.00	1.51	0.00	3.93	3310
calcium chloride	0.00	0.27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.27	1500
Fungicide	0.53	1.37	0.42	0.53	0.73	4.21	4.00	0.42	0.63	8.74	21.58	950
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1122
LSD 0.05	0.09	0.09	0.06	0.11	0.08	0.17	0.08	0.09	0.12	0.11	0.87	-

These variables were transformed according to this equation$\sqrt{X+1}$, AC= abnormal cells.

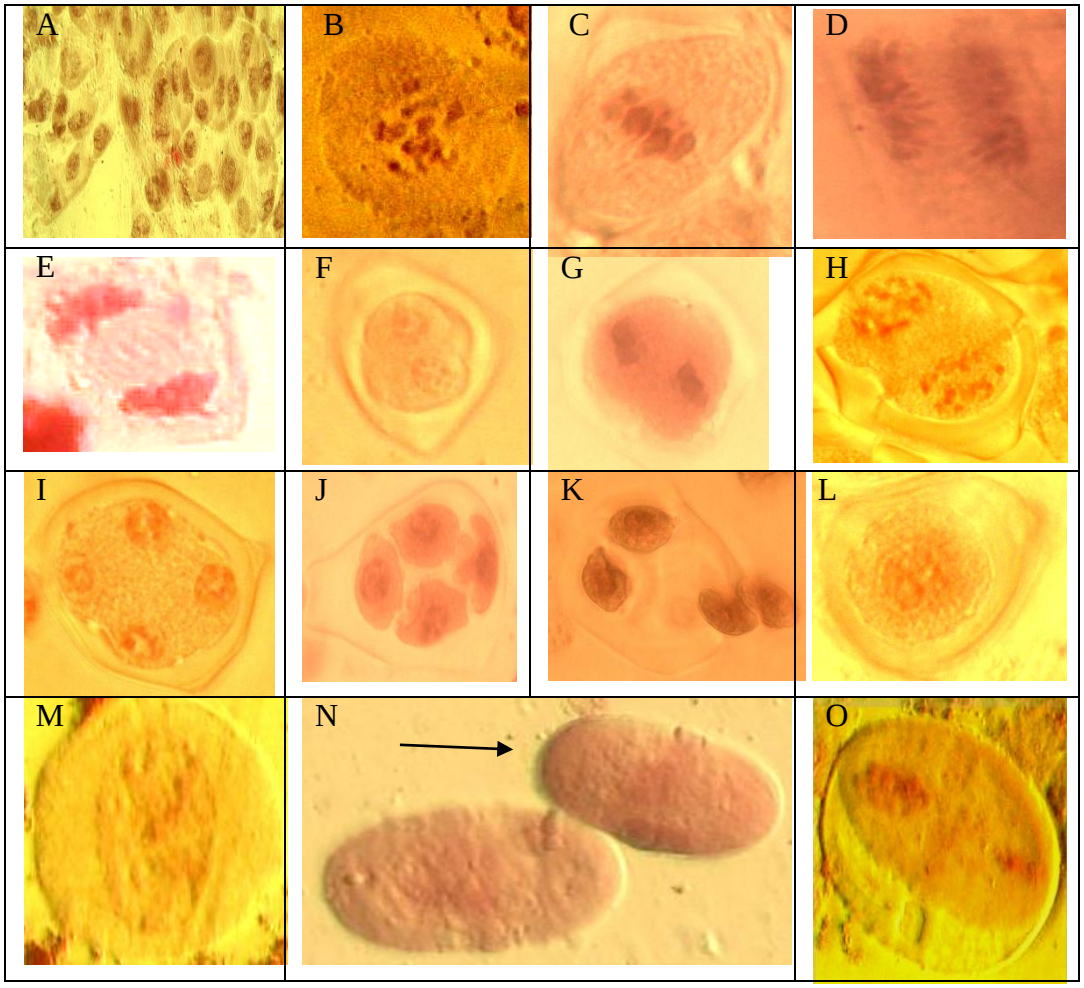

Fig. (1). Meiocytes with normal cell division in Canon58 pepper plant from a to K; (A) Tapetal cells, (B) Diakinesis, (C) Metaphase I, (D) Anaphase I, (E) Late anaphase I, (F) Dyad, (G) Metaphase II; (H) Early anaphase II; (I) Telophase II; (J) Tetrad; (K) Tetrad release gametes (microspores), (L and M) Microspores immature pollen grains, (N) Fertile (mature) n pollen grain (small) arrow (reduce), (O) Mature pollen grain with generative nucleus and vegetative (pollen tube) nucleus, Scale bars = 40µm.

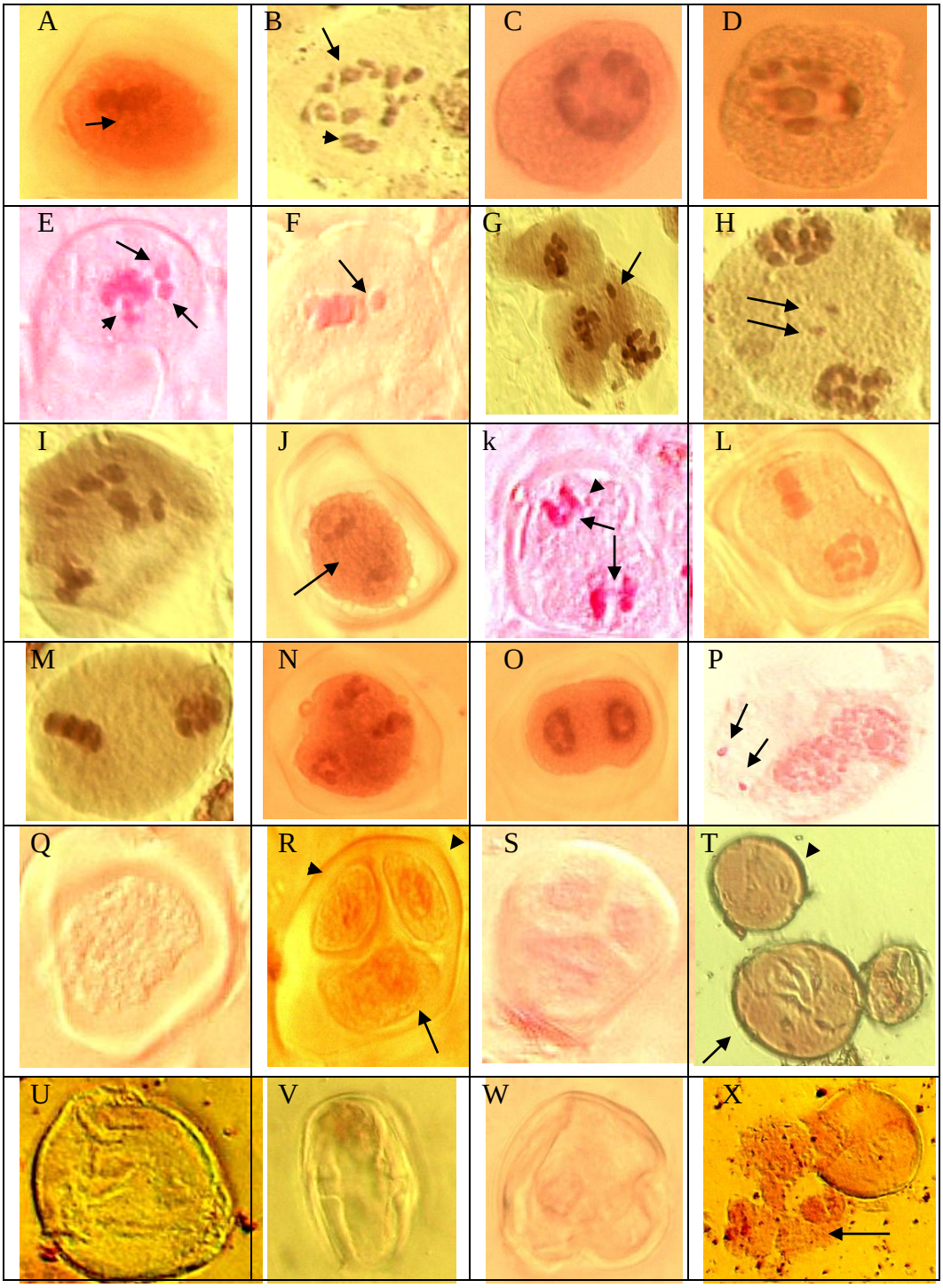

Fig. (2). Abnormal meiocytes; (A) Metaphase I with chromatin arrow, (B) ring shape for chromosomes arrow and infinity shape arrow head, (C) Chromosomes positioned towards the periphery of cell, (D) Chromosomes form 5 group, (E) Sticky metaphase I with lagging chromosomes arrowhead and two micronuclei arrow, (F) Metaphase I with micronuclei arrow, (G) Cytomixis and Lagging chromosome arrow, (H) telophase I with two fragments arrow, (I) Unoriented distribution in metaphase I, (J) multibridges in anaphase I arrow, (K) Bridge in anaphase II arrow and Lagging chromosome arrowhead, (L, M, N) Desynchronized meiosis at second meiotic division, (O) Chromosomes positioned towards the periphery in telophase II, (P) Binucleate formed from Cytomixis between cells also two migration chromosomes arrows, (Q) Enucleate cell, (R) Triad with two small gametes (microspore) arrowheads and one big arrow, (S) Abnormal Triad, (T) Fertile (mature) pollen grain 2n (big) arrow (unreduced) and Fertile n arrowhead, (U, V) Sterile pollen grain, (W) Wrinkled (unstained) Sterile pollen, (X) Bursting pollen release Pollen Cytoplasmic Granules (PCG) arrow. Scale bars = 40µm.

3.3.2. Pollen grains analysis

Some treatments may be induced cells to shift from the normal division (Fig, 1) and resulted to form abnormal cells (Fig, 2). This distortion extends to the stages of pollen development to form sterile pollen, unreduced mature fertile pollen grain (2n), Wrinkled pollen and bursting pollen (Pollen release cytoplasmic granules (PCG)).

Table (7). Effect of pre-harvest treatments compared to fungicide in pollen grains (PGs) fertility % and sterility % of Canon58 pepper plant

Treatments	Dyads	Triads	Tetrads	Sterility %			Bursting	Fertility %			T. N. PGs
				Unstained PGs	Wrinkle PGs	Total sterility		(n) PGs	(2n) PGs	Total fertility	
Sodium Bicarbonate	0	0	0	0.00	0.00	0.00	0.00	100.00	0.00	100.00	7600
potassium sorbate	40	22	140	18.94	6.06	25.00	0.00	68.94	6.06	75.00	1320
citric acid	25	38	26	11.90	0.74	12.65	0.15	81.55	5.65	87.20	6720
Calcium chloride	0	0	0	0.00	0.00	0.00	0.00	100.00	0.00	100.00	3800
fungicide	16	78	93	17.58	3.85	21.43	17.58	36.26	24.73	60.99	1820
Control	0	0	0	0.00	0.00	0.00	0.00	100.00	0.00	100.00	7160
LSD 0.05	-	-	-	0.09	0.07	0.11	0.01	0.11	0.15	0.07	-

These variables were transformed according to this equation$\sqrt{X+1}$, T. N. = total number of pollen grain, PGs = pollen grains, n= haploid and 2n= diploid.

Total fertility estimate from fertile haploid and fertile diploid pollens, the results in (Table, 7) shown that no significant difference between Sodium Bicarbonate (100%) and calcium chloride (100%) compared to control (100%) for total fertility% (Fig 3- a), while other treatments such as citric acid, potassium sorbate and fungicide recorded (87.20%), (75%) and (60.99%) respectively, and appeared significant difference with control. However, fungicide significantly increased unreduced fertile pollens (2n) (Fig 2- T arrow) and recorded (24.73%) then the treatments with potassium sorbate (6.06%) and citric acid (5.65%) without significant different between them. Also percentage of total sterility includes various types of pollens such as un-stained pollens (Fig 2- U and V) and wrinkled pollen (Fig, 2- W). And must not ignorance the bursting pollen grains, bursting pollens also increased the loss percentage of pollen grain (Fig, 2- X), fungicide recorded the highest percentage of bursting pollens (17.58%) addition to the unstained pollens (17.58%) and wrinkle pollens (3.85%), consequently the loss of pollens increased in the treatment with fungicide and recorded (39.01%) (Fig 3- c). Un-stained pollens recorded in the treatment with potassium sorbate (18.94%), and wrinkled pollens (6.06%) so the total sterile pollens (25%). Subsequently the increase in fertility was attendant to decrease in the sterility, and contrarily the decreased in fertility attendant to increase in sterility.

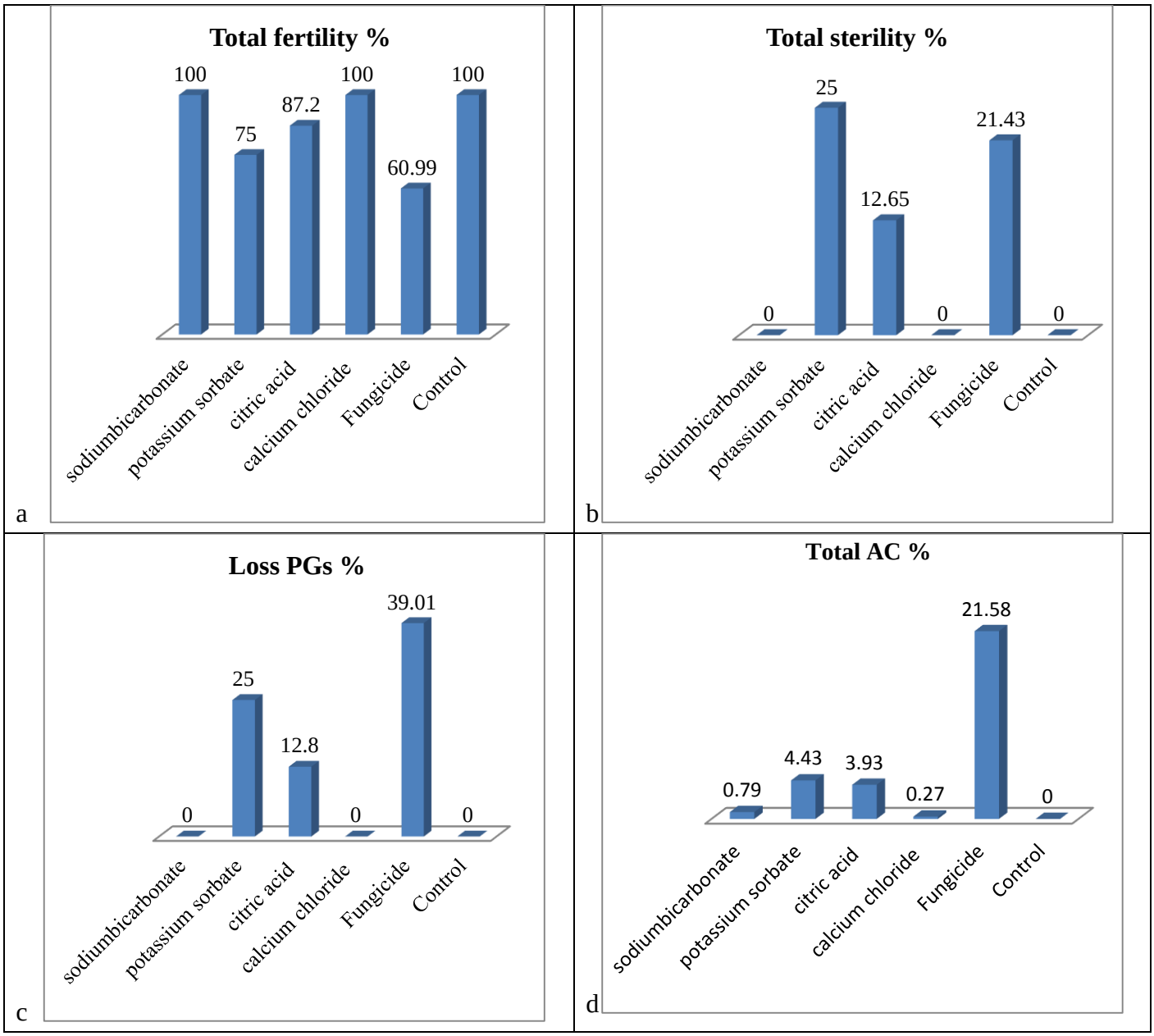

Fig (3). Diagram for effect of different treatments in; (a) Total fertile PGs %, (b) Total sterile PGs %, (c) Loss (PGs) % and (d) The total abnormal cell (AC) % during cell division in different stages.

4. Discussions

4.1. Anthracnose Disease and physical properties

Using salts and food additive as Alternative treatments to control anthracnose disease of pepper fruit decreased the disease incidence and disease severity in two seasons in the naturally and artificial infected during four weeks of cold storage at 10°C.

Potassium sorbate at 1-5% concentration showed complete inhibition of spore germination of *C. gloeosporioide*s (**Fallik *et al.*, 1997) and** reported the potassium sorbate and sodium carbonate were inhibited pathogen growth, this inhibition probably due to reduction of fungal cell turgor pressure which resulted in collapse and shrinkage of hyphae and spores, consequently, an inability of the fungus to sporulate. Also **Fadda *et al.* (2015)** reported that 2000 mg/L PS had fungistatic activity against *Penicillium expansum* and reduced blue mold on apple. Similarly, **Palou *et al.* (2002**) found

that 3% Sodium carbonate had fungistatic activity and reduced the activities of both *P. digitatum* and *P. italicum*. **Türkkan *et al.* (2017**) confirmed that carbonate salts have broad-spectrum antimicrobial properties. also potassium sorbate significantly decreased *Botrytis*, *Rhizoctonia*, *Alternaria* and Anthracnose tomato fruit rots **Jabnoun-Khiareddine *et al. (*2016)**. While **Palou *et al.*, (2002)** studied the inhibition effect of a wide range of food additives against *P. digitatum and P. italicum*-inoculated on orange and lemon, and reported that Potassium sorbate is among the most effective compounds tested in decreasing *P. digitatum*. In another study, potassium sorbate exhibits the greatest antimicrobial activity and inhibit growth of several pathogens **Arsian *et al*. (2009)**. The treatment with Potassium sorbate played important role in improve the physical properties of pepper plants due to the metabolic processes of potassium and activation numerous enzymes promoting photosynthesis.

The treatments increased fruit firmness in two seasons during cold storage at 10^{o}C for 4 weeks, this result agree with **Mandour *et al*. (2019)** and attributed to increase cell wall strengthens and mainly the middle lamella by holding the cells altogether, thus reduced ripening, so calcium enhance fruit firmness which reduced the ability of pathogens causing decay to penetrate plant tissues so disease incidence and disease severity decreased. Also **Miles *et al.* (2009) and Abou-EL-Hassan *et al.* (2020)** reported that calcium plays a role in defense against to pathogens by increasing the hardness of cell walls, making them more resistant to deleterious enzymes produced by fungi, also this hardness of the cell wall reduces the loss of water from the cells and reducing evaporation, so decreases the weight loss. TSS% was increased gradually due to continuous water loss during storage period while all treatments in this study decreased weight loss % compared to control; additionally, the treatments decreased the chemical changes in the juice content so the TSS% increased. The cold storage for four weeks at 10^{o}C and all treatments led to increase the shelf life and all the pre-harvest treatments reduced the weight loss %, disease incidence % and disease severity %, and increased the firmness and TSS % during two seasons in natural and artificial infections thus increasing the yield.

4.2. Cytogenetical Parameters

The use of fungicides is one of the reasons that lead to chromosome structural and number changes, the structural change occurs in the chromosome, such as deletion, duplication, translocation, and inversion. Deletion is the loss or cut a piece of the chromosome, including the genes it contains, and these missing genes may be essential genes for the life of the organism. In this case, the original individuals die as a result of this loss. But if the loss contains other genes, it will produce surviving individuals, but with some deformities. In plants, deletion affects the fertility of pollen grains (male gametes) and female gametes. This missing piece of the chromosome (fragment- Fig 2- H) may move to other chromosome (unilateral transfer). Any kind of unilateral or bilateral chromosomal segment transfer from chromosome to another is referred to as a translocation.

Reciprocal translocations, often referred to as segmental interchanges, are a significant class of translocations with evolutionary relevance that entail the shared exchange of chromosomal segments between two pair of non-homologous chromosomes. All forms of both unilateral and bilateral transfer of segments from one chromosome onto other are considered translocations. Translocation heterozygotes can remain pair up their chromosome forming across but when segregate resulted to form two types of gametes, half of these gametes have not all genes and other gametes have duplicated genes.

Translocation heterozygote's cytology; Heterozygote translocation occurs when a translocation affects one of the two sets of chromosomes. It will be impossible for the chromosomes involved in translocation to pair normally into bivalents in such a plant. At pachytene, a cross-shaped structure containing four chromosomes will be seen as a result of pairing between homologous portions of chromosomes, and in diakinesis the ring shape appear and easy seen in the metaphase I, so there are three possible orientations for this configuration of four chromosomes during anaphase I: one of them Alternate disjunctional, the chromosomes in opposite orientation will point in the same direction towards the pole. Put otherwise, adjacent chromosomes will point in the directions of opposing poles. This will be made feasible by the infinity shape forming. As shown in (Fig 2- B arrowhead) the

cytological effect of the occurrence of the translocation is the appearance of the infinity shape and two possible orientations form ring shape (Fig 2- B arrow) which resulted from adjacent disjunctional by two ways of adjacent, and finally the functional gametes resulted from alternate disjunctions, the gametes formed by two ways of adjacent disjunctions, which would carry duplications or deficiencies as a result establish would be sterile (Table 7) (Fig 2- U and V) or non-functional. Therefore, in a plant having a translocation in heterozygous condition, there will be considerable pollen sterility as shown in (Fig 3- b) which appeared effect of treatment in pollen grains sterility %.

Low seed set and semi-sterility are indicators of the existence of translocation heterozygosity. The formation of quadrivalents during meiosis can then verify this. Alternate disjunction leads to the formation of only two types of functioning gametes. Three different kinds of progeny will result from the functional gametes: (i) normal, (ii) translocation heterozygote, and (iii) translocation homozygote. These three types would be obtained in 1: 2: 1 ratio.

Also the chromosome bridges (Fig 2- J and K) were recorded at fungicide and it produced due to chromosomal breakage and joining of incorrect ends **(Maity and Maitra, 2019).** Some treatments and fungicide also maybe induced changes in chromosome number, such as restitution nuclei which formed from inability for univalent chromosomes to segregate via anaphases at meiosis I and II and produced dyads and triads respectively which produced two types of apparently fertile pollen grains (n) and (2n) pollens (Table 7). Therefore, one cell of pollen mother cells (PMC) (Fig 1- a) gave four haploid male gametes at telophase II from tetrad (Fig 1- I, J and K), and from triads gave two haploid (n) (Fig 2- R arrowheads) and one diploid (2n) (Fig 2- R arrow) also from dyads gave two diploid (2n) male gametes.

This behavior considered type of ploidy called Euploidy which refers to organisms have duplication of whole set of chromosomes, cause complete nondisjunction and interspecies crosses and may lead to formation new species. When fertilize male gamete (2n) the female gamete (n) produce triploidy (3n) offspring (three sets of chromosomes) **(Elagamey *et al.*, 2023)**, and that have been induced by crossing and widely found in the commercial fruit to improved growth, size, weight and maybe pathogen resistance and tolerated for environmental conditions. But the plant which has this type of euploidy perhaps appear to be morphological normal but it is classified as abnormal in chromosomal number. While, added or deleted one or a few chromosomes from the normal chromosomal number refers to Aneuploidy, as shown in (Fig 2- E arrowhead, G arrow and K arrowhead), the lagging chromosome loss from the set of chromosome for male gamete to produce monosomic (2n-1) offspring when fertilize this abnormal male gamete the normal female gamete (n) or this lagging associated with other male gamete to produce trisomic (2n+1) offspring, also Fig.(2- P arrows) clarified two migration chromosomes when associated with other male gamete produce tetrasomic (2n+2) offspring or produce double trisomic (2n+1+1) offspring, when fertilize these abnormal male gametes the normal female gamete (n) also, while loss these chromosomes produce nullisomic (2n-2) offspring or double monosomic (2n-1-1) offspring.

The development of aneuploidys may have arisen by a process called non-disjunction, which occur when paired chromosomes do not separate either during meiosis I or meiosis II. The direct result of this event is that gametes develop have too few or too many chromosomes, if this occurs during meiosis I normal gametes are not developed, if it occurs during meiosis II half of the gametes will be normal and the other half will be abnormal. So this defect maybe effects in the development immature pollens to form mature pollens, therefore, the result is the production of sterile pollen. Also abnormality in the formation of the spindle in meiosis II affected the post-meiotic products, depending on which type of irregularity that occurs in the cell, the spindles may group or separate homologous chromosomes, and result in meiocytes with tri-nucleate in telophase II, or even with cells presenting the division of non-equational cytoplasm due to the unbalanced number of chromosomes. Pollen grains formed in two sizes **(Haridy, 2016 and Haridy *et al.*, 2022),** due to the disordered meiotic behavior, resulting in unreduced (2n) pollen grain creation. These seemingly productive pollen grains have been classified into two categories based on size: n (22.48–27.98µm × 19.27–24.77µm, normal

reduced) and 2n unreduced (29.82–33.49µm × 25.23–32.56µm) according to **Kumar and Singhal (2012)** (Fig 2- T arrow).

However, in this study 2n pollen grains were estimated from triads, and dyads. The widespread phenomenon in the plant kingdom is 2n pollen grains formation, while 2n gametes perhaps originate from various cytological abnormalities **(Xue *et al.*, 2011)**. This behavior appears a major amount of heterogeneity in chromosomes number and different type of ploidy, in context, the plant genomes may exhibit great cytogenetic differences, in consistency with **Kumar and Singhal (2012)** who confirmed that 2n pollen grains were formed from dyads and triads stages. So diploid gametes probably result from multiple different of cytological abnormalities, which due to five main cytological mechanisms of 2n gametes formation; premeiotic chromosome doubling during the process from mitosis to meiosis, meiosis disturbances, abnormal cytokinesis during first or second meiosis resulted in dyads and triads formation, respectively, followed by 2n pollen formation, desynaptic mutants and abnormal spindles of meiosis, parallel or tripolar spindles during metaphase II of microsporogenesis are responsible for the formation of 2n pollen grains. On the other side **Souza *et al.* (2012)** suggested that the sterility occurred during the gametophytes development, probably starting in the microspore stage and are aborted in the beginning of their development. This agrees with **Salehi *et al.* (2021**) he reported that the earliest stress induced developmental defects such as chromosomal separation occur during meiosis leading to unbalanced chromosome separation and then pollen sterility. **Cali (2008)** found that pesticides could give rise to mutation by changing genetic structure of plants.

Fungicides application stimulated mutation similar to the mutation occurred by mutagens chemical substance which resulted from chromosomal changes. These reasons decreased the percentage of fertile pollen grains in the treated plants using fungicides compared with the control can be attributed to their toxic effects on pollens. In fact, this toxic effect becomes more evident for use of fungicides. This behavior may have a negative effect on productivity and quality of fruits at the long run. A decrease in pollen fertility was reported by **Tort *et al.* (2005), Cali and Candan (2010)** and **Souza *et al.* (2012).** Pollen grains fertility, in this study, is seriously affected by fungicide, potassium sorbate and citric acid which used to control this fungal disease that affects pepper plants, this lead to a decrease in fruits productivity.

Conclusion

Using potassium sorbate, citric acid, sodium bicarbonate and calcium chloride as pre-harvest treatments to reduced disease incidence and severity of Anthracnose and improved color sweet pepper quality or physical Parameters as well as decreased the weight loss %, and increased fruit firmness and TSS% on the shelf for four weeks. While in the Cytogenetical levels the treatments with potassium sorbate and citric acid recorded negative effect on cells behavior or cell division, microspores development, pollen fertility and sterility, so when the fertility rate decreases, the rate of fruit formation decreases and subsequently reduces yield production.

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