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IMPACT OF USING SOME FUNGICIDES AND ANTIBIOTICS ON CONTROLLING MICROBIAL CONTAMINATION DURING ALL STAGES OF DATE PALM TISSUE CULTURE PROTOCOL

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ABSTRACT

Alternaria sp., Aspergillus niger, Penicillium sp. and *Pseudomonas sp.* were isolated from *Phoenix dactylifera* cv. Zaglol culturing *in vitro* in starting, maturation & germination and multiplication stages. Cupper oxychloride, Vitavax T, Topsin M, Streptomycin, Banucin and Bencid D. were used to reduce the contamination with fungi and bacteria. Using streptomycin at the concentration 250 ppm stages was more effective in increased survival percentage and decreased the degree of contaminated explants by bacteria on starting, maturation & germination and multiplication.

On the other hand, using Cupper oxychloride, vitavax and topsin at 500 ppm for each on starting stage, maturation & germination and multiplication stages recorded no contamination by fungi. Topsin at 250 ppm gave the best results treatments comparing the other treatments results on the explants starting stage it obtained the highest degree of swelling and the lowest degree of browning. Where as, on maturation & germination and multiplication stages treated by using Streptomysin at 50ppm which gave the highest degree of growth vigor and the lowest degree of browning.

INTERODUCION

Date palm *(Phoenix dactylifera)* is generally propagated by using offshoots which produced in low number through the tree whole life time. Thus, rapid propagation of date palm through tissue culture is the most promising techniques for production of sufficient number of date palm offshoots with higher quality and yields. Date palm explants establishment frequently requires special procedures to escape or avoid problems that are associated with contaminations.

Antibiotics have been widely used in control of contamination of micropropagation and plant tissue culture for 40 years or so (Katznelson and Sutton, 1951). Plant tissue culture media are fairly hostile, at least to bacteria and fungi, particularly because of the high sugar concentration of these media. Under these circumstances the bacteria are reluctant to grow or metabolize and are, therefore, largely resistant to antibiotics, it is likely, therefore, the bacteria will remain as persists for the duration of the phase of the culture. It is well recognized that contamination can become evident during later growth phases in less hostile media, for instance where sugar concentrate concentration are lower. Persistence is also likely to be an explanation for this covert contamination. Plant tissue culture media in this paper are complex mixtures of a wide range of compounds including minerals, amino acids, plant hormones and sugars. There are several

possible sources of contaminating organisms. If the plant material was not adequately decontaminated it may carry plant associated organisms from the field. Another possibility is the emergence of human associated organisms derived from the staff who undertake the propagation, breakdown of asepsis is more likely to occur at certain times of the day, such as around break time (Boxus and Terzi, 1987). Coagulase-negative staphylococci, diphtheroids and other microorganisms from the skin or respiratory tract may appear as contaminants at a later stage (Boxus and Terzi, 1987 and 1988).

Streptomyces gresies (waksman) was records as a biocontrol agent to control root rots (Plakshappa *et al.*, 1990). El-Fahl *et al.*, (1982) showed that the fingicides Benlate, Vitavax, Topsin and Thiram were most effective fungicides for controlling the fenugreek diseases under green house conditions. Cupper Oxychloride was utilized in the control of date and ornamental palm diseased (Chase and Broschast, 1991, El-Deeb , 1994 and Rahman *et al.*, 1989)

To mange date palm *(Phoenix dactylifera)* cv. zaglol contamination *in vitro* by fungi such as *Altemaria sp.*, *Aspergillus niger, Penicillium* sp. and bacteria such as *Pseudomonas sp*, Cupper Oxychloride, Vitavax T, Topsin M, Streptomycin, Banucin and Bencid D, were used at different concentrations in starting, maturation & germination and multiplication stages.

MATERIALS AND METHODS

The present study was performed throughout the period from 2004-2005 at the Central Laboratory of Date Palm Researches and Development and Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.

Contaminated explants with bacteria and fungi were collected from different three stages under investigation and then planted on to potato-dextrose agar (PDA) plates and were incubated at 25°C for five days. The isolated fungi were identified using the description of (Barnett and Hunter, 1986). Identification of the isolated fungi was confirmed at the Mycol and plant Dis. Survey Res. Dept. Agric. Res. Center Giza. The isolated bacteria were identified using the description of (Breed et al., 1974) and confirmed at the Bacterial Diseases Res. Dept. Agric. Res. Center Giza.

Three different successive experiments were conducted to reduce the contamination in three stages: Experiment I: on starting stage, Experiment II: on maturation & germination stage and Experiment III: on multiplication stage of zaglol date palm cultured *in vitro*.

Three different culture MS (Murashig & Skoog, 1962) media were used at different stage as follows:

1-MS+ 10 mg/l 2,4-D (dichlorophenoxy acetic acid) + 3 mg/l 2iP(6-γ,γ-Dimethylallylamino purine) on starting stage.

2-MS+ 0.1 mg/l NAA (naphthalene acetic acid)+ 2 mg/l 2iP on maturation & germination stage.

3-MS+ 3 mg/l 2iP on multiplication stage.

All different media were autoclaved for 20 min. at 121°C (1.2 Kg/cm²) for each fungicides and antibiotics, Cupper Oxychloride, Vitavax T, Topsin M,

Streptomycin, Banucin and Bencid were used at concentrations 0, 50, 250 and 500 ppm after filter sterilized added to the melton previous autoclaved media. Media of each treatment were distributed in jars 150ml at rate 35 ml/jar.

Explants type:-

1-Sub-shoot tip at two months age cultured on MS media+10 mg/l 2,4-D + 3 mg/l 2iP and contaminated with a low degree of bacteria used as explants on Exp.I.

2-Clusters contained 2-4 embryos cultured on MS media+ 0.1 mg/l NAA+ 2 mg/l 2iP and contaminated with a low degree of bacteria used as explants on Exp.II.

3-Clusters contained 4-5 shoots cultured on MS media+ 3 mg/l 2iP and contaminated with a low degree of bacteria used as explants on Exp.III.

Each treatment contained 10 jars (10 replicates), which were incubated in a growth room at 26±2°C under darkness in Exp.I but in Exp.II and Exp.III in 16hr illumination of 2000 lux (white fluorescent lamps). Subculturing the explants were done onto the same medium every 3weeks and 3subcultures were done on each experiment.

Data were token as follows:

Exp. I: Survival%, contamination by bacteria, contamination by fungi, swelling and browning.

Exp. II and III: Survival%, contamination by bacteria, contamination by fungi, browning, growth vigor, and vitrification.

The later three parameters except survival % were stimulated visually as following:

Negative result (-) =1 Below average results (+) = 2

Average results (++) = 3 Good results (+++) = 4

According to Pottino, (1981).

Survival % was arcsine-transformed and compared using L.S.D. test at 5% as described by (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

1) Survived explants:

The effect of fungicides and antibiotics on survived percentages of explants on starting, maturation & germination and multiplication stages of *Phoenix dactylifera* cv. zaglol after three subcultures are presented in Table (1).

Data clearly indicated that using fungicides and antibiotics at the concentrations 50 and 250 ppm increased the percentage of survived explants of comparing with the control. Among the different treatments, the highest survival percentage (100%) was observed when explants were treated by streptomycin at the concentration 250 ppm on starting, maturation & germination and multiplication stages Also, Banucin and Bencid gave the same result at the same concentration on multiplication stage.

Table(1):Effect of fungicides and antibiotics on percentages of survived explants on (starting, maturation & germination and multiplication stages) of zaglol date palm after three subcultures

Stages	Treatments (ppm)	Cupper Oxychloride	Vitavax	Topsin	Strepto-mycin	Banucin	Bencid
Starting	Control (0.0)	60	60	60	60	60	60
	50	90	80	80	90	90	80
	250	80	90	80	100	90	90
	500	60	70	70	80	90	80
Maturation & germination	Control (0.0)	50	50	50	50	50	50
	50	80	70	80	70	70	60
	250	90	80	80	100	90	80
	500	60	70	60	80	70	60
Multiplication	Control (0.0)	60	60	60	60	60	60
	50	80	70	80	80	70	70
	250	90	80	90	100	100	100
	500	70	60	60	80	70	70

	Starting	Maturation& germination	Multiplication
New L.S.D (A)	6.13	6.15	6.8
New L.S.D (B)	5.01	5.2	5.93
New L.S.D (AxB)	12.27	12.3	12.92

2) Degree of bacteria:

The effect of fungicides and antibiotics on the degree of bacteria contaminated explants on starting, maturation & germination and multiplication stages of *Phoenix dactylifera* cv. zaglol after three subcultures are presented in Table (2).Cleared that, using fungicides and antibiotics at the concentrations 50, 250 and 500 p.pm significant decreased in the degree of contaminated explants by bacteria comparing with the control (MS media without fungicides or antibiotics). The lowest degree contaminated explants by bacteria (1.0) was observed when explants were treated by cupper oxychloride at the concentration 500 ppm on starting stage and treated explants with cupper oxychloride, vitavax, topsin, streptomycin or bencid at the same concentration on maturation & germination stage. Where as using streptomycin at the concentrations 50, 250 or 500, bencid at the concentrations 250 or 500 ppm and vitavax at 500 ppm were more effective decreased the degree of contaminated explants by bacteria on multiplication stage which gave (1.0).

3) Degree of fungi:

Data in Table (3) show that, the degree of fungi contaminated explants on starting, maturation & germination and multiplication stages of zaglol date palm after three subcultures significantly decreased by using fungicides and antibiotics at any concentration used compared to the control (MS media without fungicides or antibiotics). No contamination (0.0) by fungi found when we were using cupper oxychloride at the concentration 250, 500 ppm, vitavax or topsin at 500 ppm on starting stage. On maturation & germination stage treated explants with cupper oxychloride or vitavax at the concentrations 500 ppm gave no contamination by fungi. Also, treated

explants with cupper oxychloride or vitavax at the concentrations 250 or 500 ppm or topsin at 500 ppm gave no contamination by fungi on multiplication stage.

Table (2): Effect of fungicides and antibiotics on the degree of bacteria of explants on (starting, maturation & germination and multiplication stages) of zaglol date palm after three subcultures

Stages	Treatments / (ppm)	Cupper Oxychloride	Vitavax	Topsin	Strepto-mycin	Banucin	Bencid	Mean (B)
Starting	Control (0.0)	3.00	3.00	3.00	3.00	3.00	3.00	3.00
	50	1.67	3.00	2.67	1.33	2.00	2.00	2.11
	250	1.33	2.33	2.00	1.67	2.67	1.67	1.94
	500	1.00	2.00	1.67	1.67	2.33	1.33	1.67
	Mean(A)	1.75	2.58	2.33	1.92	2.50	2.00	
Maturation& germination	Control (0.0)	3.00	3.00	3.00	3.00	3.00	3.00	3.00
	50	1.67	2.33	2.33	1.33	2.67	1.67	2.00
	250	1.67	1.33	1.33	2.00	2.00	1.33	1.61
	500	1.00	1.00	1.00	1.00	2.00	1.00	1.17
	Mean(A)	1.83	1.92	1.92	1.83	2.42	1.75	
Multiplication	Control (0.0)	3.00	3.00	3.00	3.00	3.00	3.00	3.00
	50	2.00	2.00	3.33	1.00	1.33	1.33	1.83
	250	1.33	1.33	3.00	1.00	1.67	1.00	1.56
	500	1.33	1.00	3.00	1.00	1.67	1.00	1.50
	Mean(A)	1.92	1.83	3.08	1.50	1.92	1.58	

	Starting	Maturation & germination	Multiplication
New L.S.D (A)	0.63	0.53	0.53
New L.S.D (B)	0.51	0.43	0.43
New L.S.D (AxB)	1.25	1.06	1.05

Table (3): Effect of fungicides and antibiotics on the degree of fungi of explants on (starting, maturation & germination and multiplication stages) of zaglol date palm after three subcultures

Stages	Treatments / (ppm)	Cupper Oxychloride	Vitavax	Topsin	Strepto-mycin	Banucin	Bencid	Mean (B)
Starting	Control (0.0)	0.33	0.33	0.33	0.33	0.33	0.33	0.33
	50	0.02	0.09	0.13	0.31	0.34	0.29	0.20
	250	0.00	0.05	0.10	0.26	0.22	0.25	0.15
	500	0.00	0.00	0.00	0.11	0.13	0.19	0.07
	Mean(A)	0.09	0.12	0.14	0.25	0.25	0.26	
Maturation & germination	Control (0.0)	0.21	0.21	0.21	0.21	0.21	0.21	0.21
	50	0.11	0.19	0.17	0.20	0.19	0.20	0.18
	250	0.05	0.14	0.10	0.16	0.12	0.15	0.12
	500	0.00	0.00	0.03	0.11	0.10	0.10	0.06
	Mean(A)	0.09	0.14	0.13	0.17	0.15	0.16	
Multiplication	Control (0.0)	0.19	0.19	0.19	0.19	0.19	0.19	0.19
	50	0.11	0.05	0.09	0.14	0.15	0.16	0.11
	250	0.00	0.00	0.03	0.10	0.10	0.13	0.06
	500	0.00	0.00	0.00	0.08	0.10	0.11	0.05
	Mean(A)	0.08	0.06	0.08	0.13	0.14	0.15	

	Starting	Maturation& germination	Multiplication
New L.S.D (A)	0.08	0.03	0.03
New L.S.D (B)	0.07	0.02	0.02
New L.S.D (AxB)	0.16	0.05	0.05

4) Swelling and browning on starting:

Effect of fungicides and antibiotics on swelling and browning of explants on starting stage of *Phoenix dactylifera* cv. zaglol after three subcultures are presented in Table (4). It was evident that using, Topsin at 250 ppm, Banucin at 500 ppm or Bencid at 500ppm gave the highest degree of swelling (2.67). Where as the lowest degree of browning (2.33) obtained by using Topsin at (50 or 250ppm), Streptomysin at 50 ppm or Bencid at 250 ppm.

Table (4): Effect of fungicides and antibiotics on the degree of swelling and browning of explants on starting of zaglol date palm after three subcultures

	Treatments (ppm)	Cupper Oxychloride	Vitavax	Topsin	Strepto-mycin	Banucin	Bencid	Mean (B)
Swelling	Control (0.0)	1.33	1.33	1.33	1.33	1.33	1.33	1.33
	50	1.33	2.00	2.67	2.33	1.67	2.67	2.11
	250	1.33	1.67	2.67	2.00	2.33	2.33	2.06
	500	1.33	1.67	2.00	1.67	2.67	2.67	2.00
	Mean(A)	1.33	1.67	2.17	1.83	2.00	2.25	
Browning	Control (0.0)	3.67	3.67	3.67	3.67	3.67	3.67	3.67
	50	2.67	2.67	2.33	2.33	2.67	3.33	2.67
	250	3.00	3.33	2.33	3.33	3.00	2.33	2.89
	500	3.00	3.00	3.33	4.00	3.67	3.00	3.33
	Mean(A)	3.08	3.17	2.92	3.33	3.25	3.08	

	Swelling	Browning
New L.S.D (A)	0.48	0.56
New L.S.D (B)	0.39	0.45
New L.S.D (AxB)	0.96	1.11

5) Growth vigor, browning and vitrification on maturation and germination stage:

Effect of fungicides and antibiotics on growth vigor, browning and vitrification of explants on maturation & germination stage of zaglol date palm after three subcultures are presented in Table (5). Growth of date palm embryos was significantly increased by the addition of fungicides or antibiotics at the concentrations 50 ppm but decreased when the concentration increased to 500ppm. Streptomysin at 50 ppm gave the highest degree (3.67) of growth vigor followed by Banucin at 50 ppm which gave (2.67) with significant differences between them. The lowest degree of browning (1.67) observed by using Streptomysin or Banucin at 50 ppm. At general using fungicides and antibiotics at any concentration used insignificantly increased vitrification.

6) Growth vigor, browning and vitrification on multiplication stage.

Comparing the effect of different studied on multiplication stage, it was found in Table (6) that the most positive responses, i.e. more growth vigor, less browning and vitrification were obtained by using Bencid at the concentration 250ppm which gave the highest value of growth (4.0), followed by Streptomysin at 50ppm which gave (3.67) with no significant differences between them compared with the control or with the other treatments.

Table (5): Effect of fungicides and antibiotics on growth vigor, browning and vitrification of explants on maturation & germination stage of zaglol date palm after three subcultures

	Treatments (ppm)	Cupper Oxychloride	Vitavax	Topsin	Strepto-mycin	Banucin	Bencid	Mean (B)
Growth vigor	Control (0.0)	1.67	1.67	1.67	1.67	1.67	1.67	1.67
	50	2.00	2.33	2.00	3.67	2.67	2.33	2.50
	250	1.67	2.00	1.67	2.00	2.00	1.67	1.83
	500	1.00	1.67	1.33	1.33	1.33	1.33	1.33
	Mean(A)	1.58	1.92	1.67	2.17	1.92	1.72	
Browning	Control (0.0)	3.00	3.00	3.00	3.00	3.00	3.00	3.00
	50	2.67	2.67	2.00	1.67	1.67	1.33	2.00
	250	2.67	2.67	2.33	2.00	2.33	2.67	2.44
	500	3.67	3.33	3.33	2.33	2.67	3.33	3.11
	Mean(A)	3.00	2.92	2.67	2.25	2.42	2.58	
Vitrification	Control (0.0)	1.33	1.33	1.33	1.33	1.33	1.33	1.33
	50	1.33	1.33	1.00	1.33	2.33	1.00	1.39
	250	1.00	2.67	1.00	1.67	3.00	2.33	1.94
	500	1.00	2.67	1.00	1.00	3.33	2.33	1.89
	Mean(A)	1.17	2.00	1.08	1.33	2.50	1.75	

	Growth vigor	Browning	Vitrification
New L.S.D (A)	0.42	0.50	0.45
New L.S.D (B)	0.34	0.41	0.37
New L.S.D (AxB)	0.84	1.00	0.90

Table(6):Effect of fungicides and antibiotics on growth vigor, browning and vitrification of explants on multiplication stage of zaglol date palm after three subcultures

	Treatments (ppm)	Cupper Oxychloride	Vitavax	Topsin	Strepto-mycin	Banucin	Bencid	Mean (B)
Growth vigor	Control (0.0)	1.67	1.67	1.67	1.67	1.67	1.67	1.67
	50	1.67	2.67	2.00	3.67	3.00	3.67	2.78
	250	1.67	2.33	2.00	3.33	3.00	4.00	2.72
	500	1.00	1.67	1.00	2.00	2.00	2.67	1.72
	Mean(A)	1.50	2.08	1.67	2.67	2.42	3.00	
Browning	Control (0.0)	2.67	2.67	2.67	2.67	2.67	2.67	2.67
	50	3.33	3.33	3.00	2.33	2.00	2.33	1.72
	250	3.33	3.67	3.67	2.00	2.67	1.33	2.78
	500	2.67	4.00	4.00	2.67	3.00	1.67	3.00
	Mean(A)	3.00	3.42	3.33	2.42	2.58	2.00	
Vitrification	Control (0.0)	1.67	1.67	1.67	1.67	1.67	1.67	1.67
	50	2.67	1.00	2.67	1.00	1.67	1.00	1.67
	250	2.67	1.00	3.00	1.33	1.00	1.00	1.67
	500	3.00	1.33	2.00	1.67	1.00	1.00	1.67
	Mean(A)	2.50	1.25	2.33	1.42	1.33	1.17	

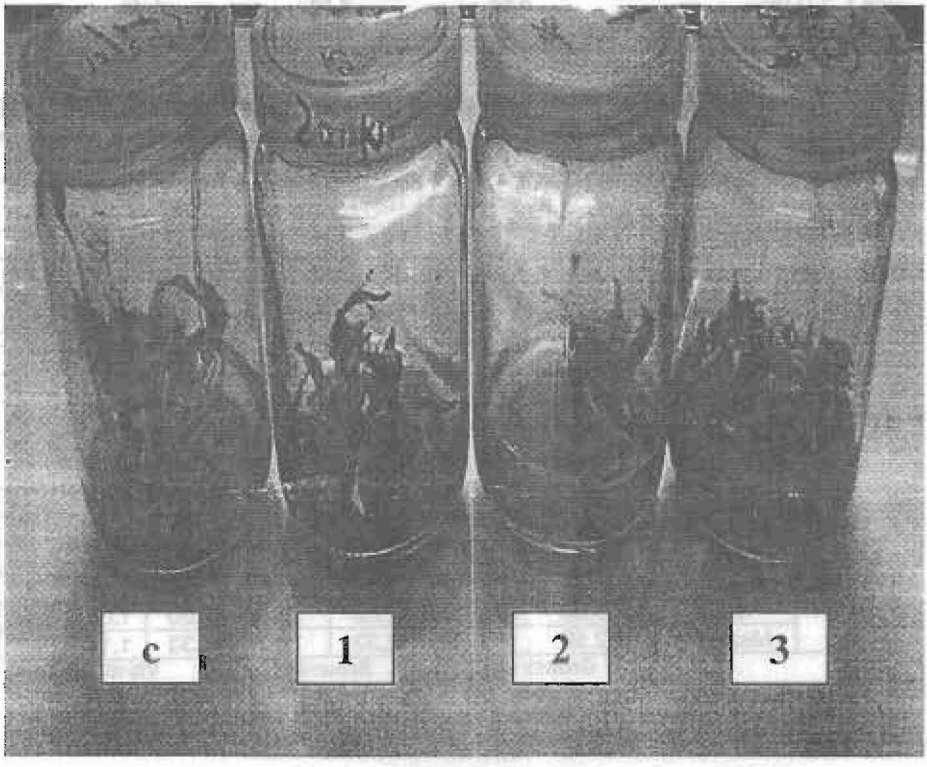
	Growth vigor	Browning	Vitrification
New L.S.D (A)	0.53	0.47	0.39
New L.S.D (B)	0.43	0.38	0.32
New L.S.D (AxB)	1.06	0.94	0.77

From the above results we can concluded that, contamination with fungi or bacteria were caused deterioration on growth and development of

explants (Fig. 1). (Using streptomycin at the concentration 250 ppm on starting, maturation & germination and multiplication stages were more effective in increased survival percentage and decreased the degree of contaminated explants by bacteria. This results can be bake to that, the antibiotics least likely to be phytotoxic are those acting at sites such as the bacterial cell wall rather than those which act on the ribosome or DNA mycoplasmas (Bove and Garnier, 1997) and possibly L-forms (Paton, 1987) may cause significant contamination.

On the other hand, using Cupper oxychloride, vitavax or topsin at the concentration 500 ppm on starting stage, maturation & germination and multiplication stages gave no contamination by fungi. Topsin at 250 ppm gave the best treatments comparing the other treatments on the starting stage it obtained the highest degree of swelling and the lowest degree of browning. Where as, on maturation & germination and multiplication stages treated by Streptomysin at the concentration 50ppm was the most positive responses it gave the highest degree of growth vigor and the lowest degree of browning. At general using fungicides and antibiotics at any concentration used insignificantly increased degree of vitrification.

Fig. (1): Effect of date palm contamination on *in vitro*



C : control . 1,2,3 : contamination by fungi and bacteria .
note : deterioration the tissue in 1,2,3 .
1, 2, 3, : contaminated tissues buy fungi an bacteria

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استخدام بعض المبيدات الفطرية والمضادات الحيوية للتحكم في تلوث مزارع أنسجة نخيل البلح

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تم عزل فطريات الالترناريا ، اسبرجلس نيجر ، بنيسيليوم وكذلك بكتريا بسيدوموناس من مزارع نخيل البلح في المعمل في مرحلة البداية ومرحلة التطور والإنبات ، ومرحلة التضاعف . وقد استخدمت بعض المطهرات الفطرية مثل أوكس كلورور النحاس ، فيتا فاكس ثيرام ، توبسن م٧٠ وبعض المضادات الحيوية وهي ستربتومايسين وبانيوسين وينسيد ط م وذلك لتقليل التلوث الناتج من الفطر والبكتريا في المراحل المذكورة سابقا . وكانت النتائج المتحصل عليها من خلال استخدام مادة استربتو مايسين هي زيادة نسبة بقاء الأنسجة حية عن استخدامه بتركيز ٢٥٠ جزء في المليون خلال مراحل البداية ، التطور ، الإنبات والتضاعف وقد انخفضت نسبة التلوث البكتيري عند هذا التركيز .

من ناحية أخرى وجد أن استخدام أوكس كلورور النحاس وفيتا فاكس ثيرام والتوبسن في تلك المراحل السابقة عند تركيز ٥٠٠ جزء في المليون منع حدوث التلوث بالفطر إلا أن التوبسن أعطى أفضل النتائج عند تركيز ٢٥٠ جزء في المليون بالمقارنة بالمعاملات الأخرى . وفي مرحلة البداية كانت أفضل استجابة في إنتفاخ الجزء النباتي وأقل درجة للتلون البني عند نفس التركيز للتوبسن بينما أدى استخدام الاستربتوميسين بتركيز ٥٠ جزء في المليون إلى الحصول على أعلى معدل للنمو وأقل نسبة للتلون البني في مرحلتي التطور والتضاعف .