Honeybee venom in control of the microbial contamination of date palm tissue culture stages

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ABSTRACT

***Penicillium* sp., *Aspergillus* niger, Bacillus sp. and Pseudomonas sp. Were isolated from *Phoenix dactylifera* cv. Haiany culturing in vitro in multiplication and rooting stages.**

Six dilutions of honeybee venom (1/10, 1/100, 1/1000, 1/10 000, 1/ 100 000, 1/1000 000 mg L-1) were used to control the Fungal and Bacterial contamination during the date palm tissue culture stages (Multiplication and Rooting stage) without affecting the explants response and development as happened after adding certain antibiotics to the medium. Contaminated explants were cultured on venom containing media for one subculture (4 weeks). After this period explants were subculture onto medium-free venom to study its residual effect on explants contamination.

The results recorded that the first dilution 1/10 has a great effect on controlling the contamination but it reduced the explants response to the media. The dilutions 1/100, 1/1000, 1/10 000 were most effective for controlling the contamination compared with other treatments under investigation without affecting the explants response and development. While the last two dilutions had no effect on decreasing the contamination. When the explants were subculture on to free venom-medium or supplemented with high dilution as 1/1000 and 1/10 000 contamination was recognized in few gars.

Key words: honeybee venom, date palm, tissue culture, contamination

INTRODUCTION

Date palm (*Phoenix dectilefera*) is being propagated traditionally by using seeds or off shoots. Unable to satisfy the extension in new land of the desert as seeds give approximately 50% males and the females are not genetically similar to the mother and off shoot produced in few numbers through life time and along with losing numbers during rearing and transporting, making the tissue culture technique most promising for mass production of the date palm with high quantity and supreme quality especially for rear and expensive species. (veramendi and Navarro,1996) .

Preparing the plant to use in tissue culture needs certain presages precaution since it will carry over organisms from the field or being contaminated accidentally by the staff who undertakes the propagation.

Tissue culture medium is a suitable hostile to various forms of bacteria and fungi, because of high sugar content in it. Under these circumstances certain species of bacteria and fungi are being favored by the optimized growth condition and challenging the value of tissue culture technology. (Barnett and Hunter, 1986)

The use of Clorox and other chlorine containing substance, through effective in controlling contamination have been complained about producing maceration of plant tissues of certain varieties along with the health hazards to the technical staff and environment safety. Therefore, the bee venom as natural antibacterial agent medically approved for human use was searched for in this study to replace the antibiotics in tissue culture

technology to overcome contamination and minimize the hazardous effect of chlorine at the recommended dose of concentration. (Abd-El Kareim *et al.,* 2006)

Bee venom are more friendly than Clorox (sodium hypochlorite Na HOCL) 4.5-5% with no residual effects. And less coasting than it. Using bee venom added 30ps to the total coast of the medium while using Clorox added 60-70ps to medium per litter.

Honeybee venom had an effect Gram-Negative and Gram Positive bacterial species without affecting the plants as happen when using the being recorded with antibiotics (Boman *et al.,* 1989: El-Shaarawy, 2008). Also It had an effect against fungi (Surendra *et al.,* 2011: A-Reum Yu, 2012), which au less sensitive to antibiotics.

And with using of antibiotic widely in controlling of plant tissue culture contamination for about 50 years made resistance to antibiotics beside it affects on the plant health (Katznelson and Sutton, 1951), (Abd-El KareimA.H.*et al.,*2006)

MATERIALS AND METHODS

The present study was carried out during 2012 – 2013 at Central Laboratory of date palm research and development, Agriculture Research Center, Giza, Egypt, and the bee venom was collected from private bee yard from El- Qalubia.

1. Bee venom preparing:

The honeybee venom was collected from bee hive using electric shook device, and stored as powder ready to use. The bee venom was diluted in distilled water to the different concentrations. (El-Shaarawy, 2008)

2. The contamination:

Explants contaminated with fungi and bacteria were collected from two different stages (Multiplication and Rooting) under investigation and then planted into ready potato-dextrose agar (PDA) plates and 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 Dies. Survey Res. Dept. Agric. Res. Center Giza. The isolated bacteria was identified using the description of (Breed *et al.,* 1974) and confirmed at the Bacteria Diseases Res. Dept. Agric. Res. Center Giza.

3. Preparing of the media:

Four kinds of media were prepared two for each stage:

Multiplication stage: a) MS + 30 gm L-1 sucrose + 3 mg L-1 2ip + 3 gm active charcoal b) MS + 30 gm L-1 sucrose + 3 mg L-1 2ip + 3 gm active charcoal + venom.

Rooting stage: a) MS + 1 mg L-1 NAA + 30 gm L-1 sucrose + 3 gm active charcoal b) MS + 1 mg L-1 NAA + 30 gm L-1 sucrose + 3 gm active charcoal + venom.

These media were autoclaved for 20 min. at 121°C (1.2 kg cm-1) in flasks. Let the flasks to cool down to 40°C inside the laminar flow then added the different concentrations of bee venom using syringe filter with 0.02 micron then the flasks were shaken well for perfect distribution of bee venom dilutions 1/10, 1/100, 1/1 000, 1/10 000, 1/100 000, 1/1 000 000 mg L-1 .

The media were distributed in jars 40 ml / jar then incubated for 4 days before using (planting).

4. Choosing the plants:

Contaminated explants with bacteria were collected from the two different stages under investigation. There were 20 jars for every concentration of venom from 1/10 to 1/1 000 000in every stage of the palm tissue culture with 20 jars as control replanted on the same medium (with total of 240 jars). All chosen jars had a medium bacterial contamination with suitable healthy plants.

The explants were first cleaned up in distal sterilized water then planted on the media. Explants were incubated in growth room at 26 ± 2°C in 16 hr. illumination of 2000 lux (white flour cent lamps). Sub culturing the explants were done every 3 weeks 5 jars on free venom medium and the rest on the tested medium to determine the effect of bee venom on the contamination and the response and the growth of the explants.

Data were taken as follows: Survival %, Contamination by bacteria.

RESULTS AND DESCUTION

The effect of bee venom on the contamination:

I: The multiplication stage:

Data present in table (1) showed that effect of bee venom difference concentrations against the bacterial and fungal contamination in the multiplication stage. The first three dilutions 1/10, 1/100 and 1/1000 gave a good effect on the contamination on the tissue culture jars as there were no growths of the bacteria or fungi in the jars even when transplanting some of the explants on free venom medium (control). 1/10 000 concentration was effective as he present in the medium in the three subculture but when the explants planted on free medium some jars got contaminated in the 2nd and 3rd sub. The last two concentrations 1/100 000 and 1/1000 000 were not effective on the bacterial or fungal contamination as it appear badly on them.(Fig 1 and 2)

II: The rooting stage:

Data in table (2) shows the effect of bee venom on with the rooting stage. In the four concentrations 1/10, 1/100, 1/1000 and 1/10 000 there were no growth of bacteria in the tubs and after replanting them on the control medium too. But with the last two concentrations 1/100 000 and 1/1 000 000 the venom was not effective on the bacteria and there were many contaminated tubs. But it can be observed that the contaminations are less in rooting stage than multiplication stage. (Fig 3 and 4)

The privies results of bee venom are in agreement with many authors (Boman *et al.,* 1989; El-Gizawy 2006; El-Shaarawy 2008). They all founded that some of the honeybee venom got an effect on the growth of bacteria. And with Mulfinger, 1990 and Kaviani *et al.,* 1995 found that big dilutions of bee venom become not effective on growth some kind of bacteria.

But with the observation the plants in dilution 1/10 were affected by the bee venom and there growth was less than normal in the other dilutions or in control.

From the previous results, it can demonstrated that, the best bee venom concentration to use in date palm tissue culture are 1/100 and 1/1000 concentrations as it control the bacterial or fungal contamination and not does affect the plant vigor.

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Tables

Table (1): The effect of bee venom on contamination of multiplication stage contamination jars of date palm tissue culture.

Venom dilutions	Contaminated jars	1st sub		2nd sub*		3rd sub	
		Free◊	Cont.□	Free	Cont.	Free	Cont.
Control	20	-	20	-	-	-	-
1/10	20	20	-	15	-	10	-
Control				5	-	10	-
1/100	20	20	-	15	-	10	-
Control				5	-	10	-
1/1000	20	20	-	15	-	10	-
Control				5	-	10	-
1/10000	20	20	-	14	1	8	2
Control				3	2	5	2
1/100000	20	15	5	6	4	4	2
Control				-	5	-	-
1/1000000	20	10	10	-	5	-	-
Control				-	5	-	-

◊Free = no. of free contamination jars □ Cont. = no. contaminated jars
*Sub: re culture after 45 days on the same midia

Table (2): The effect of bee venom dilutions on contamination of rooting stage of date palm tissue culture.

Venom dilutions	Contaminated jars	1st sub		2nd sub		3rd sub	
		Free◊	Cont.□	Free	Cont.	Free	Cont.
Control	20	-	20	-	-	-	-
1/10	20	20	-	15	-	10	-
Control				5	-	10	-
1/100	20	20	-	15	-	10	-
Control				5	-	10	-
1/1000	20	20	-	15	-	10	-
Control				5	-	10	-
1/10000	20	20	-	14	1	8	2
Control				3	2	2	2
1/100000	20	15	5	7	3	2	2
Control				1	4	1	2
1/1000000	20	12	8	5	2	1	2
Control				1	4	-	2

◊ Free = no. of free contamination jars □ Cont. = no. contaminated jars

Figures

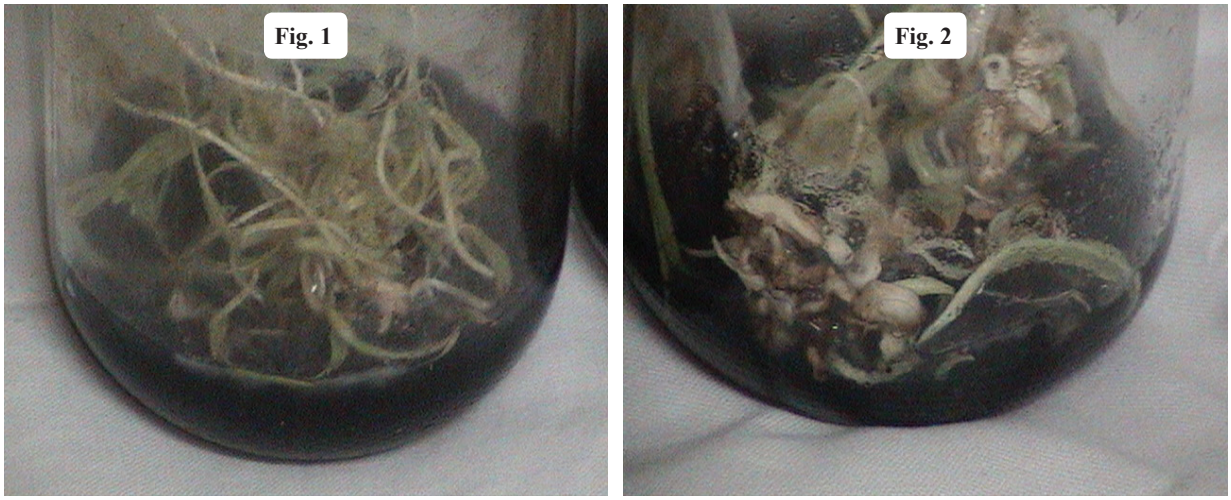

Fig. 1. Contaminated date palm plants before replanting on bee venom medium (multiplication stage).
Fig. 2. Contaminated date palm plants after replanting on bee venom medium (multiplication stage).

Fig. 3. Contaminated date palm plant before replanting on bee venom medium (rooting stage)

Fig. 4. Contaminated date palm plant after replanting on bee venom medium (rooting stage).