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Pink rot of inflorscence: a new disease of date palm in Kuwait

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Abstract

A new bacterial disease of date palm (*Phoenix dactylifera* L.) "Bacterial Pink Rot of Inflorescence" was found in a farmer's palm plantation in Kuwait. The symptoms of this disease were dark brown spots on the spathe cover. Inside these spadices affected flowers, scattered over the inflorescence, acquired pink coloration which at later stages was surrounded by pink mucous. Pink colored colonies of bacteria were isolated from the said mucous. After artificial inoculation of detached spadices with the bacterium, exhibited the symptoms of the said disease on the flowers. Morphological and biochemical studies of bacterium suggested that it belongs to the species *Serratia marcescens*. *In vitro* control measures of bacteria were studied, using streptomycin, copper sulphate and kasumin using various concentrations with success.

Key words: Date palm, inflorescence, Kuwait, new disease, pink rot, *Serratia marcescens*.

Introduction

Date palm (*Phoenix dactylifera* L.) belongs to the family Palmaceae is the major fruit crop of arid, deserts in tropical and subtropical areas of the world including GCC, Middle East, Northern Africa and Southern Asia etc. According to FAO report world date-fruit production reached up to 16.7 million tons (Anonymous 2004). It is main fruit and ornamental tree planted in Kuwait. It is subject to an inflorescence rot caused by fungal complex viz. *Mauginiella scaettae*, *Fusarium moniliforme* and *Thielaviopsis paradoxa* (Anonymous 2006; Djerbi, 1983; Khan, 1989; Najeeb, 2001; Riaz *et al*., 1994).

During the flowering stages in March-April, 2006, on a visit to one of the date palm plantations in Sulaibiyah, spadices of only one tree were found abnormal with light brown spots on the spathe. The spathe was cut open for further studies. The objective of this study was planned to identify the causal organism involved in this pink rot of the inflorescence and its control measures.

Materials and Methods

Collection of specimen

The affected spadics were collected from the date palm tree and brought to the Plant Pathology laboratory for further studies. Almost all the spadics present on the particular male palm tree were remained unopened with light-brown spots.

Symptom studies

The spadices containing brown spots was washed thoroughly with sterilized distilled water, surface sterilized with 0.5% sodium hypochlorite for 2 min and then rinsed with 3-4 washings of sterilized distilled water. The spathe was removed with the help of sterilized scalpel to observe the flower condition.

Isolation of the pathogen

Inoculum from infected flowers was scratched and streaked on Potato Dextrose Agar (PDA) and incubated at 25 °C. Also the pink colored mucilaginous substance was observed under microscope and it was found to be a bacteria, the mucilaginous material was also streaked on Nutrient Agar (NA), incubated for 48-72 h at 30 °C. The pink colored colonies on both the media were separated and purified.

Pathogenecity test

Ten young spadices were used in pathogenecity test in each treatment. Bacterial suspension from 48 hold cultures was collected in sterile distilled water and adjusted to 1×10^6 cfu

mL^{-1} turbid metrically. The inoculum was introduced in two ways:

1. By injecting 2 mL of bacterial suspension (10^6 cfu mL^{-1}) into spadices using 5 ml syringe.
2. Spraying the bacterial suspension on the spathe till runoff.
3. In the control spadices were either sprayed or injected with sterile distilled water.

Spadices were then wrapped with plastic bags to provide humidity for bacterial growth and kept at room temperature in the laboratory.

Re-isolation

After pathogenicity confirmation, isolations of bacterium was performed on NA plates from pink colored pollen grains, infected flowers and flower stalks of artificially inoculated spadices.

Taxonomy / characterization of the pathogen

Taxonomic studies were carried out in the Microbiology laboratory, PAAFR, bacterial culture was studied using various morphological and biochemical tests. Colony morphology and pigment production was determined on NA. Motility was determined using hanging drop technique. Gram stain, acid and gas production from glucose, lactose fermentation, starch hydrolysis, catalase test, indole test, urease test and nitrate reduction test were performed as described by (Harley and Prescott, 2004). Besides this, the bacterium was also identified using the Biomerieux, API 20 E Kit.

Control studies

An antibiotic, a bactericide and a chemical salt at following concentrations in nutrient agar medium were used for *in vitro* control of *Serratia marcescens*.

Streptomycin (0 .25 µg mL^{-1}, 0.50 µg mL^{-1}, 0.75 µg m L^{-1}, 1.0 µg m L^{-1})

Kasumin (a.i. Kasugamycin (¼R, ½R, ¾R, 1R where R=Recommended dose 1 mL / L^{-1})

Copper sulphate (0.025, 0.050, 0.075, 0.100 ppm).

Results

Symptom studies

Light brown to dark brown spots of different sizes and shapes were observed on the spathe (Plate-1). When spathe was removed, some pink colored flowers were observed. The actual color of the flowers was changed from white creamy to shiny pink. At some places inner surface of spathe also exhibited pink colored slime layer.

Bacterial isolation from spathe

Isolations on PDA and NA from the infected flowers resulted in pink colonies of bacteria. Purification of the bacterium was carried out on NA and kept at 4 °C for further studies.

Pathogenicity test

After seven days, spadices were unwrapped and the inflorescence was opened by removing spathe. The pink colored flowers were prominent here and there on the bunch / inflorescence. Affected pollens were present inside the cover of the spathes at different places. Shining pink color growth was observed on pollens and stalks of the flowers as well as on base and stalk of the inflorescence.

Re-isolation

Isolations were made on nutrient agar from different parts of the inflorescence namely affected flowers, pollen grains and flower stalks. All the parts exhibited similar type of pink colored bacterial colonies.

Identification

Morphological and biochemical properties were similar to initially isolated bacterial culture as well as to the re-isolated bacteria after pathogenicity test.

Characterization of the pathogen/Taxonomy

On the basis of morphological and biochemical tests, the colonies of this microbe were pink in appearance, circular and have an entire margin. The bacterium was Gram negative and rod shaped (Table 1). Besides the results shown in Table 1 and on the basis of Biomereux API 20E kit test results the bacterial agent isolated from date palm flower was identified as *Serratia marcescens.*

In vitro vontrol of *serratia marcescens*

To control this pathogen different concentration of kasumin (bactericide, active ingredient kasugamycin), streptomycin (antibiotic) and copper sulfate were tested in the laboratory with the following results. Kasumin was used with 1R, ¾R, ½R and ¼R (Recommended dose = 1 mL L^{-1} of nutrient agar medium) and control (without kasumin) the maximum bacterial growth was observed in control, followed by ¼R and ½. There was no growth found in ¾R and 1R. In case of streptomycin, maximum growth was observed in control. Very little growth was observed in 25 µg mL^{-1} and 50 µg mL^{-1} and no growth in 75 µg mL^{-1}, whereas in case of Copper sulphate treatments, there was maximum bacterial growth recorded in

control followed by 0.025 and 0.050 ppm. There was no growth observed in 0.075 ppm (Table 2).

Discussion

In this study the bacterium organism isolated from date palm spathe resembled morphologically and biochemically to *S. marcescens* (Karampour *et al*., 1984). During experimental period the inoculation of bacteria using spray method failed to cause any disease symptoms suggesting that either the bacterium got entry into spadices through cracks or by insect transmission. This bacterial agent caused a pink coloration of flowers after one week of injection into spathe. The isolation (as per Koch;s postulates) from pink flowers resulted in identification of *S. marcescens*. Similar symptoms of date palm spathe associated with *S. marcescens* have not been reported in Kuwait's list of date palm diseases.

Bacterial suspension inoculation into spadices using syringe technique resulted in consistent infection of flower and inner part of spadices all the treatments. Initially the color was pink, and after 2-3 weeks it turned brown. *In vitro* control of the pathogen by different chemicals concentrations resulted in differences in growth on NA. Similar findings have also been reported by Loper *et al.,* (1991) for *Erwinia amylovora*. Higher concentration of all the chemicals resulted in bactericide activities. These results indicates that the risk of severe infection of inflorescence will be high in case the environmental conditions are favorable for proliferation of the bacterium.

Conclusions

So far inflorescence/flower rot caused by bacteria in date palm has not been reported in Kuwait. Therefore, bacterial pink rot of male flowers of date palm is a new disease in Kuwait.

Table 1: Morphological and biochemical characteristics of *S. marcescens*

Characters	**Results**
Colony color	Dark pink
Colony morphology	Smooth
Gram reaction	Negative
Spore formation	Negative
Motility	Positive
Lactose utilization	Negative
Acid and Gas production from glucose	Positive
Acid and Gas production from lactose	Negative
Starch hydrolysis	Negative
Catalase test	Positive
Indole test	Negative
Urease test	Positive
Nitrate reduction	Positive

Table 2: *In vitro* control studies of *S. marcescens*

Kasumin	Control	1/4 R	1/2R	3/4R	R
	+++	++	+	-	-
Streptomycin	Control	25 µg / ml	50 µg / ml	75 µg / ml	
	+++	++	+	-	
$CuSO_4$	Control	.025ppm	.050ppm	.075ppm	
	+++	++	+	-	

- No growth
+ Rare growth
++ Moderate growth
+++ Excellent growth

Plate 1:- Opened spathe showing pink infected flowers

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