BIOLOGICAL CONTROL OF POWDERY MILDEW DISEASE ON SQUASH PLANTS BY *Derxia* sp.

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ABSTRACT

Among the microbial flora of leaf surface of squash (*Cucurbita pepo* L.) plants, an isolate with characters specific to *Derxia* sp. Jensen, Peterson, De and Bhattacharya, was found. As far as we know, it is the first time for *Derxia* sp. to be isolated from living aerial plant parts. Furthermore, results of experiments proved that this isolate could be regarded as a biocontrol agent against the powdery mildew, caused by *Sphaerotheca fuligineae* (Schlect. ex. Fr.) Poll. on squash plants.

Key words: *Derxia* sp., *Sphaerotheca fuligineae*, powdery mildew and biological control.

INTRODUCTION

Derxia sp., free-living, nitrogen-fixing bacteria, is considered as a soil-born microorganism. In this work, *Derxia* sp. could be isolated from leaves of squash (*Cucurbita pepo* L.) plants, and its beneficial role, as a powdery mildew biocontrol agent, was examined.

MATERIALS AND METHODS

This study was conducted to survey the microflora inhabiting the leaves of squash (*Cucurbita pepo* L.) plants in order to search for microbial antagonists against the powdery mildew disease caused by *Sphaerotheca fuliginea*.

1- Sampling of microbial flora:

Leaves of squash plants, at different stages of maturity, were collected from various localities of open field commercial plantations in Kafr El-Sheikh and El-Gharbia governorates, Egypt. Whole blades of healthy as well as powdery mildew infected leaves were separately washed using 20-ml sterilized tap water in sterilized Petri dishes. After well agitating for 1 min., individual aliquots of the washing water (0.2 ml) were then spread onto the surface of potato dextrose agar (PDA) as well as onto nutrient agar (NA) plates. Inoculated plates were incubated at room temperature (28±2°C) and colonies appeared within a week were picked up and re-purified before maintenance on slants of the same isolating medium.

2-Identification:

The sampled microorganisms were submitted to preliminary grouping. The isolated bacterial microorganisms were submitted to further identification according to Bergey's Manual of Systematic Bacteriology (1984).

3-Biological control of powdery mildew on squash plants:

Liquid cultures, grown on potato dextrose medium up to 10 days at static state and room temperature (28±2°C) were tested to inhibit the powdery mildew disease on squash plants under glasshouse and open field conditions.

3-1-*In greenhouse*:

In pots, whole plants and pruned plants (only contain cotyledons) of squash, (cv Balady) of 3 weeks old, were sprayed until runoff using the previously prepared liquid cultures containing $3.2 \cdot 10^{-}$ cfu ml. Check plants were treated with sterilized water Treatments were repeated on the same plants three times with 7 days intervals. Each treatment was represented by five replicates and the experiment was repeated four times in complete randomized design. Older potted plants, with heavy powdery mildew infection, were used as a source of infection. Seven days after each spraying, the disease severity was estimated according to McGrath and Staniszewska (1996), and disease inhibition was calculated.

3-2-*In the open field:*

At the Experimental Farm, Faculty of Agriculture at Kafr El-Sheikh, Tanta University, this work was carried out using randomized complete block design with three replications. Seeds were sown in rows of 1 m. width at 1 m. distance apart and each plot contained 5 plants. Plants were sprayed as previously mentioned but with 2 weeks intervals. Plants treated with the recommended fungicide Topas100EC (10%w/v) Penconazole at concentration of 25 $cm.^3$/100 L of water) were used for comparison and the check treatment (control) was represented by untreated plants. The disease index of the natural infection was estimated 1 week after the 3rd application. The yield was calculated as number of fruits per plant.

4-Statistical analysis:

The obtained data were subjected to ANOVA according to Gomez and Gomez (1984) using the IRRISTAT computer program.

RESULTS AND DISCUSSION

Survey of microbial flora on leaf surfaces of squash (*Cucurbita pepo* L.) plants grown in the region of Kafr El-Sheikh and El-Gharbia governorates, Egypt, resulted in various isolates of fungi, yeasts, actinomycetes and bacteria (Table 1). On the used media (PDA and NA) the incidence of these microbial groups showed an obvious variation in their frequency. The preliminary identification indicated that the most dominant genera of fungi were *Alternaria*, *Aspergillus*, *Botrytis*, *Cladosporium*, *Epicoccum*, *Mucor*, *Penicillium*, *Stachybotrys* and *Ulocladium* species. Yeast occurred with lower frequency (2.44%), compared with that of fungi (30.5%). On the other hand, the eubacteria as bacilli significantly occurred more than actinomycetes, which rarely frequented. At all, the most frequent microbial group was eubacteria (64.64 %), most of its population (58.54 %) belonged to the aerobic Gram-positive bacilli and some (only 6.10%) belonged to the aerobic, Gram-negative ones.

Interestingly, some bacterial colonies, isolated under the above-mentioned conditions, showed a change in color with aging on plates as well as on slants of NA medium. Their massive slimy colonies grew on plates, as raised as that of some yeast colonies with a pale yellow color which altered to mahogany brown within ~ 10 days. Using the hanging drop technique, rod shaped cells seemed so enormous in size that it might be thought that the tested microorganism is a yeast, solely the obvious motility directed the attention towards thinking about bacteria and not yeast. Gram negative reaction, typical large refractile bodies spreading throughout the whole cell (Fig. 1), and other performed tests (Table 2) affirmed that the bacterium under examination is related to *Derxia* sp. Jensen, Peterson, De and Bhattacharya described in the 1st volume of Bergey's Manual of Systematic Bacteriology (1984). *Derxia* sp, as recorded in this manual, occurred mainly in tropical soils. It was isolated originally by Jensen *et al.* (1960) from a West Bengal soil. Since then, it has been found widely distributed in Brazil (Campêlo and Döbereiner, 1970) and in Indonesia, China, and Southern Africa (Becking, 1981). Campêlo and Döbereiner (1970), found that *Derxia* sp could be more often isolated from root pieces of plant than from soil samples obtained from the same locality.

Based on a numerical analysis of a large number of attributes, Thompson and Skerman (1979) recorded *Derxia gummosa* as a single species within the genus. Roy and Sen (1962) described a new species of *Derxia* from a sample of partially retted jute plants (*Corchorus olitorius*) from Uttar Pradesh, India. However, the described species has not any

cultural or physiological differences from *D. gummosa* to warrant the nomination of a new species

Table (1). Microorganisms sampled from leaves of squash (*Cucurbita pepo* L.) all over the growth season (summer, 2000)

Grouping and form genus	Incidence %
Fungi*	
Alternaria spp.	1.22
Aspergillus spp.	9.76
Botrytis spp.	1.22
Cladosporium spp.	1.22
Epicoccum spp.	1.22
Mucor spp.	3.66
Penicillium spp.	2.44
Stachybotrys spp.	2.44
Ulocladium spp.	1.22
Unknown spp.	6.10
Yeasts	2.44
Bacteria	
Gram	58.54
Gram (including *Derxia* sp.)	6.10
Derxia sp.	1.22
Actinomycetes	2.44

* The fungal genera were identified depending on the morphological characters according to Barnett & Hunter (1972).

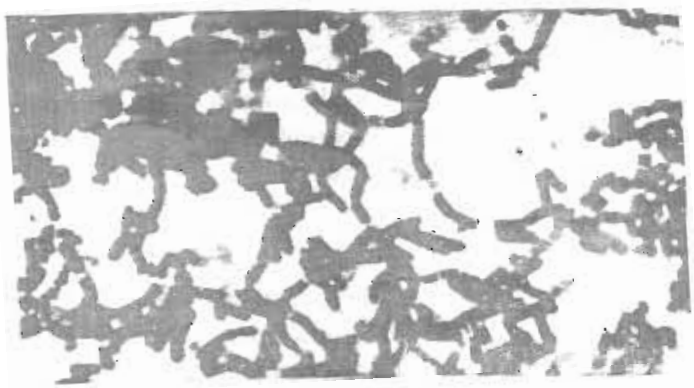


Fig. (1) Gram-stained cells of the tested *Derxia* sp.-like isolate containing the refractile bodies.

Table (2): Cultural, morphological and biochemical characters of the tested isolate which identified as *Derxia* sp.

Characteristics	Results
Growth on agar medium	Raised slimy massive colonies.
Growth in liquid medium.	Starts as a ring at the glass-liquid interface and develops into a thick surface pellicle.
Aerobic growth	-
Colony color	Pale yellow at first turns to mahogany brown within ~ 10 days.
Motility (swimming)	-
Cell shape	Rods with rounded ends.
Arrangement	Single and short chains.
Size of cells	1- 1.5 µm ×5-25 µm
Gram reaction	-
Presence of refractile bodies in cells	+
Capsule	Present
Catalase test	-
Indole production	-
Nitrate reduction to nitrite	-
Starch hydrolysis.	- (with scant growth)
Growth on nitrogen-free glucose broth.	-
Optimum temperature	25-35°C
Growth on 50°C	-
Optimum pH	7
Growth on pH 4	-

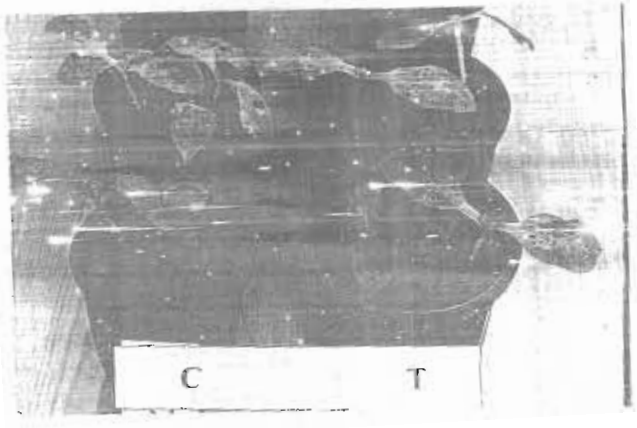
For the available information, the incidence of *Derxia* sp. on living aerial plant parts has not been recorded before (Becking, 1981). In the recent work the frequent occurring of *Derxia* sp. on leaves of squash plants collected from Kafr El-Sheikh and El-Gharbia governorates, Egypt has been proved.

This study revealed that the isolated *Derxia* sp. have an antagonistic effect against the powdery mildew caused by *Sphaerotheca fuliginea* on squash plants under greenhouse and field conditions. In greenhouse, spraying a 10 days old liquid culture of the isolated *Derxia* sp. (containing $3.2 \cdot 10^{7}$ cfu / ml) onto potted squash plants resulted in decreases in disease severity of powdery mildew on cotyledons and expanded leaves. Compared with the untreated check plants, this inhibition reached 99.3% and 81.25%, respectively (Table 3 and Fig. 2). It was noticed that the inhibitory effect

Table (3). Inhibition of powdery mildew disease on squash plants after each of the three applications of the *Derxia* sp. isolate under glasshouse conditions

Time of assessment	% disease inhibition on	
	cotyledons	expanded leaves
1 week after the first application	46.08*	35.66*
1 week after the second application	95.16**	62.50*
1 week after the third application	99.30**	81.25**

** and * refere to the significance of treatments compared with control at $P = 0.01$ and 0.05 respectively



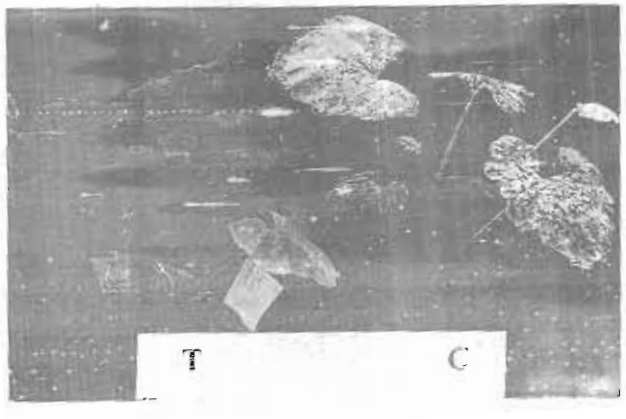


Fig. (2). Biological control of powdery mildew disease on cotyledons and leaves of squash plants (C= control, T= treated plants)

was as clears as the application was repeated and the disease was severe (on cotyledons). In the open field, three applications of the tested bioagent resulted in 93.33 % disease inhibition, and in the same time, using the fungicide Topas100EC inhibited the disease by 92.21 % compared with the untreated plants. The total yield, expressed as the total number of fruits, increased by 72.55 % and 28.21 % (compared with check treatment) using the liquid culture of *Derxia* sp. and the fungicide Topas100EC, respectively (Table 4).

Table (4): Inhibition of powdery mildew disease on squash plants and potential yield using the *Derxia* sp. isolate and the fungicide under field conditions.

Treatments	% disease inhibition	% increase in number of fruits/plant
Derxia sp. isolate	93.33**	72.55**
Fungicide	92.21**	28.21*

** and * refere to the significance of treatments compared with control at P.= 0.01 and 0.05, respectively.

Based on the obtained results, *Derxia* sp. could be recognized as an antagonist against the powdery mildew disease on squash besides the previously known ones. Such well-known antagonists are *Ampelomyces quisqualis* (Elad *et al.*, 1998), *Verticillium lecanii* (Verhaar *et al.*, 1997), *Cladosporium* spp. (Minuto *et al.*, 1991), *Trichoderma harzianum* (Elad *et al.*, 1998), *Rhodotorula* spp. (Yohalem 2000), *Tilletiopsis* spp. (Hoch and Provvidenti, 1979) *Sporothrix* spp. (Yohalem 2000), *Pseudomonas* (Vogt and Buchenauer, 1997), and *Bacillus subtilis* (Bettiol *et al.* 1997). Ease of growth in mass on synthetic media, and ability to persist in the same habitat where the pathogen acts candidate the tested isolate to have advantages to be used as a practical biocontrol agent against the powdery mildew disease on squash.

It was noticed that in some replicates (data not shown), infection with whatever degrees took place before the first application. In such cases, the powdery mildew slightly developed after treating with the *Derxia* sp. liquid culture compared with intensive disease development on the untreated plants. This may lead to assumption that our antagonist has a prophylactic and not curative effects. However, the nature of the mechanism of action of *Derxia* sp., needs further studies.

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الملخص العربي

المقاومة الحيوية لمرض البياض الدقيقي على نباتات الكوسة باستخدام البكتيريا *Derxia* sp.

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من بين الفلورا الميكروبية التي تقطن سطح أوراق نباتات قرع الكوسة أمكن الحصول على عزلة بكتيرية تشابه في صفاتها البكتريا المعروفة باسم *Derxia* sp. وفي حدود المعلومات المتاحة، تعتبر هذه أول مرة يتم عزل هذه البكتيريا من أجزاء هوائية لنباتات حية. زيادة على ذلك واعتماداً على نتائج التجارب التي تم إجراؤها فإنه يمكن اعتبار البكتريا المعزولة والتي تم تعريفها كـ *Derxia* sp. عاملاً حيوياً مضاداً لمرض البياض الدقيقي على نباتات قرع الكوسة حيث أدى رش نباتات قرع الكوسة بالمزرعة السائلة له تحت ظروف الحقل الى تقنين المرض بنسبة ٩٣,٣٣ % وزيادة عدد الثمار بنسبة ٧٢,٥٥ % مقارنة بمعاملة الكنترول.