

ORIGINAL ARTICLE

First report of powdery mildew caused by *Podosphaera xanthii* on *Luffa cylindrica* in Egypt and its control

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Abstract

Luffa cylindrica M. Roem, is commonly called sponge gourd or Egyptian cucumber. In September 2018, several plants showing symptoms of powdery mildew were observed in some fields at different locations in Egypt. Identification and pathogenicity tests indicated that the causal fungus of powdery mildew disease of luffa *cylindrica* was *Podosphaera xanthii*. Results of surveyed luffa plants grown at different field localities of northern Egypt, for powdery mildew disease incidence revealed that the maximum record (57.33%) of disease occurrence was recorded in some fields belonging to Beheira governorate followed by, Alexandria and Sharqia (53.67% and 48.00%, respectively). Meanwhile, fewer occurrences were observed in Kafer El-Sheekh governorate (45.33%). We applied biocontrol agents as a foliar spray against powdery mildew *in vitro* and under field conditions. The effects of some essential oils, organic acid and bioproducts were also studied. All treatments significantly reduced *P. xanthii* compared to untreated plants. *Chaetomium globosum* and *Saccharomyces cerevisiae* alone or grown on rice straw and/or bagas showed highly reduced disease incidence compared to the other treatment. From the present study it could be suggested that the usage of biocontrol formulated on rice straw might be used as an easily applied, safe and cost effective control method against powdery mildew diseases.

Keywords: biocompost, biocontrol, essential oil, *Luffa cylindrica*, *Podosphaera xanthii*, powdery mildew

Introduction

Luffa cylindrica M. Roem, commonly known as sponge gourd, vegetable sponge, Egyptian cucumber or bath sponge, is an annual plant species, belonging to the Cucurbitaceae family. *Luffa cylindrica* is cultivated in different agro-ecological zones of Egypt. There are many reports about its uses. Silva *et al.* (2015) reported its use in medicine, industry, beauty and house cleaning products in many countries. Also, the dry fiber of luffa is very useful as packing material, bathroom sponge, sound proof linings, and it is a component of shock absorbers (Davis and DeCourley 1993; Oboh and Aluyor 2009). In the medical field a lot of scientists have reported the benefits of extracts of various parts of *L. cylindrica* as being an emetic or, expectorant, and having anti-inflammatory, antifungal, antimicrobial,

antiprotozoal, anthelmintic, stomachic, antipyretic, anti-myocardial ischemia and hepatoprotective properties (Bailey 1989; Parkash *et al.* 2002; Muthumani *et al.* 2010; Indumathy *et al.* 2011; Ng *et al.* 2011; Pal and Manoj 2011; Partap *et al.* 2012; Sanjaya and Acharya 2016). *Cylindrica* have been reported to contain phytochemicals such as: alkaloids, tannins, saponins, sterols, glycosides, carbohydrates, and flavonoids (Fahima *et al.* 2014). Luffa plants are infected with many diseases. The two major constraints are: powdery mildew and downy mildew which grow well on luffa. Powdery mildew is one of the most aggressive diseases that affect leaves in cucurbits (McGrath 2017). Infection is evident by the development of white mycelia and conidia, mainly on leaves and stems. It also

can affect fruits and floral structures. Severely infected leaves may become chlorotic, or necrotic and brittle. Consequently, it decreases the photosynthetic potential, and concomitantly lowers fruit quality and yield (Stadnik and Bettiol 2001).

Podosphaera xanthii is commonly found in tropical and subtropical regions, whereas *Golovinomyces orontii* is primarily seen in temperate climates (Cohen *et al.* 2004; Naruzawa *et al.* 2011). Physiological races and pathotypes are well documented for both species (Lebeda *et al.* 2016). Therefore, the aim of the present work was to evaluate the effects of some essential oils, organic acids and bioagents alone or mixed with organic waste against powdery mildew disease caused by *P. xanthii* on luffa plants under laboratory and field conditions.

Materials and Methods

Disease survey and detection

In Egypt, the cultivated area of *L. cylindrica*, estimated to be about 3,692 feddan, produced 24,503,000 luffa fruits «cone» (Anonymous 2009). A survey of the incidence of powdery mildew on luffa plants was carried out from September to December 2018, at different

field localities of northern Egypt (Beheira, Alexandria, Kafer El-Sheekh and El-sharqia governorates). Disease symptoms include white mycelia and conidia, mainly on leaves, but fruits and floral structures can also be affected. Severely infected leaves may become chlorotic, or even necrotic and brittle (Figs. 1, 2).

Pathogenicity test

Pathogenicity was confirmed by dusting conidia on healthy plants of *P. xanthii*. Non-inoculated plants served as controls. A sterile brush was used to transfer conidia from the affected leaves to fully expanded leaves of healthy plants. A plastic bag was placed around each plant for a week and then removed. Non-inoculated controls were stroked with a sterile brush, placed in a plastic bag and kept separate in the greenhouse. The fungus was re-identified to fulfill Koch's postulates (Fig. 3).

Molecular identification of the fungal isolate

The selected fungus was identified according to its morphological characteristics using molecular biology techniques. The morphological characteristics were examined using a light microscope (Olympus c×41).



Fig. 1. Symptoms of powdery mildew growth on the lower surface of the luffa leaf (right)



Fig. 2. Symptoms of powdery mildew growth on the upper surface of the luffa leaf



Fig. 3. Pathogenicity test of *Podosphaera xanthii* on *Luffa cylindrica*

For molecular identification, the fungal biomass was obtained by cultivation of fungal spores on small, sterilized, slices of luffa leaves for 10 days. The total genomic DNA was extracted using CTAB protocol (Eida *et al.* 2018). The cultivated fungal biomass was powdered using liquid nitrogen and then DNA was isolated. DNA of the fungal isolate was amplified by polymerase chain reaction (PCR) using ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') designed for sequencing (Elshahawy *et al.* 2018; Hasanin *et al.* 2019).

Identification was achieved by comparing the contiguous DNA sequence with data from the reference and type strains available in public databases GenBank using the BLAST program (National Centre for Biotechnology Information) (<http://www.ncbi.nlm.nih.gov/BLAST>). The obtained sequences were aligned using Jukes Cantor

Model and the isolate was registered in Gen Bank (Kheiralla *et al.* 2016; Darwesh *et al.* 2019).

Source of the pathogens

Luffa leaves affected by *P. xanthii* showing typical symptoms of powdery mildew disease were collected from different locations in Egypt (Beheira, Alexandria, Kafer El-Sheekh and El-sharqia governorates).

Sources of bio-agents

Seven different isolates of fungi, bacteria and yeast were used to study their antagonistic effects against *P. xanthii* *in vitro*. Three isolates of fungi (*Trichoderma harzianum*, *T. viride* and *Chaetomium globosum*) were local fungal strains isolated from Egyptian soil. They

were identified in the Plant Pathology Department of the National Research Center, Giza, Egypt. The strain was kept on potato dextrose agar medium and stored at 4°C. Three isolates of bacteria (*Bacillus subtilis*, *Pseudomonas fluorescens* and *P. lindbergii*) and one isolate of yeast (*Saccharomyces cerevisiae*) were obtained from the Plant Pathology Department, National Research Center, Doki, Giza and used as antagonistic microorganisms.

Sources of essential oils

Five essential oils: cinnamon, thyme, neem, chili and sour almond were obtained from Oils Extract Unite, National Research Center, Giza, Egypt.

Laboratory experiments

Inoculum preparation of the pathogens

Luffa leaves affected by *P. xanthii* showing typical symptoms of powdery mildew disease were collected from different locations previously mentioned. Profusely growing conidia were collected for artificial inoculation by using a brush and suspended in sterile distilled water containing 0.01% tween 80 (Poly oxyethylene). The conidial suspension was then centrifuged at 3,000 rpm for 5 min twice, in order to separate conidia from conidiophores (Kitao and Doazan 1989). The concentration of the conidia was adjusted to 3×10^4 conidia · ml⁻¹ using a haemocytometer. This suspension was sprayed using an atomizer on the healthy leaves of 60 day old luffa plants maintained in a foam tray. After inoculation, the foam trays were covered with polythene bags for 24 h to maintain high humidity for disease development.

Spore suspension preparation of the bio-agents

Preparation of fungal suspensions

The medium was poured before solidification into sterilized Petri plates, then the cooled and solidified plates were inoculated with equal discs (5 mm φ) of each of the tested isolates and incubated at 25 ± 2°C for 7 days (Last and Hamley 1956) under an alternating light and darkness regime (12 h/12 h) in an automatic incubator to enhance spore production. Three plates were used for each isolate as replicates. After the elapse of the incubation period, the plates were flooded with 10 ml of sterilized distilled water and brushed thoroughly with a rubber brush. The suspension was filtered through three layers of cheesecloth to remove mycelia residues. The number of spores · ml⁻¹ was counted in the collected spore suspension using a Spencer haemocytometer slide, then adjusted to about 2.5×10^5 spores · ml⁻¹. The prepared spore suspension was used as follows: *B. subtilis*, *P. lindbergii* and

P. fluorescens were grown on nutrient broth medium and incubated at 30°C for 48 h and then cell suspension was adjusted to 1.8×10^8 , 2.3×10^8 and 2.8×10^8 cfu (Kiraly *et al.* 1970).

Effect of bio-agents on powdery mildew caused by *Podosphaera xanthii*

Six bio-agents, previously mentioned, were tested for controlling powdery mildew disease on luffa leaves caused by *P. xanthii* under *in vitro* conditions.

The efficacy of the bio-agents was assessed based on the appearance of the powdery mildew disease caused by *P. xanthii* employing the detached leaf method of Varalakshmi *et al.* (1999). Fresh luffa leaves (60 days old) were washed in sterile distilled water and surface disinfected with 70% ethyl alcohol and then air dried. Spore suspensions of the tested bio-agents were sprayed on the surface of the leaf using an atomizer and allowed to air dry. The treated leaves were sprayed with conidial suspension of *P. xanthii* (3×10^4 conidia · ml⁻¹) 24 hours after the treatment with bio-agents. Leaves smeared with the conidial suspension without tested materials served as a control. In each treatment, three leaves were placed in a foam tray having moist tissue paper and packed in polyethylene bags, then incubated at 25 ± 2°C. Each treatment was replicated thrice. After 72 h, powdery mildew growth was observed on the inoculated leaves. Leaves were carefully examined to estimate the severity of downy mildew infection based on a score chart of 0 to 5 (0 – no infection; 1 – 1–10; 2 – 10.1–15; 3 – 15.1–25; 4 – 25.1–50 and 5 – more than 50% of the leaf area being covered with mildew growth) as described by Singh *et al.* (1994). The following equation was applied:

$$P = \Sigma (n \times y) / SN \times 100 [\%],$$

where: *P* – disease severity, *n* – number of infected leaves in each category, *y* – numerical values of each category, *S* – the highest rating value and *N* – total number of the infected leaves. On the other hand, the efficacy of each treatment was calculated as follows:

Efficacy = (Control – Treatment/Control) × 100 [%]
and number of spores using a haemocytometer slide.

Effect of essential oils and organic acids on powdery mildew caused by *Podosphaera xanthii*

Five essential oils and three organic acids, previously mentioned, were tested for controlling powdery mildew disease on luffa leaves caused by *P. xanthii* under *in vitro* conditions.

The efficacy of the essential oils and organic acids was assessed based on the appearance of the powdery

mildew disease caused by *P. xanthii* employing the detached leaf method of Varalakshmi *et al.* (1999). Fresh luffa leaves (60 days old) were washed in sterile distilled water and surface disinfected with 70% ethyl alcohol and then air dried. Solutions of the tested essential oils at concentrations of 3% or organic acids at concentrations of 1% were sprayed on the surface of the leaf using an atomizer and allowed to air dry. The leaves treated with essential oils or organic acids were sprayed with conidial suspension of *P. xanthii* (3×10^4 conidia \cdot ml⁻¹) 24 h after the treatment with essential oils or organic acids. The leaves smeared with the conidial suspension without tested materials served as a control. In each treatment, three leaves were placed in a foam tray having moist tissue paper and packed in polyethylene bags, then incubated at $25 \pm 2^\circ\text{C}$. Each treatment was replicated thrice. After 72 h, powdery mildew growth was observed on the inoculated leaves. Leaves were carefully examined to estimate the severity of downy mildew infection based on the previously mentioned score chart.

The effects of waste biorice straw and bagas on powdery mildew caused by *Podosphaera xanthii*

Modified medium rice straw and bagas according to El-Gamal and Hamed (2003) were prepared and inoculated with *Ch. globosum* and/or *S. cerevisiae*, and incubated at $28\text{--}30^\circ\text{C}$ for 15 days. To prepare the biostraw and bagas are used as a carrier. Cut rice straw or bagas were moistened with water until it reached 70% humidity. Five kilogram of rice straw and/or bagas was packaged in thermal bags; 5% soil and 5% molasses were enclosed in the bags and sterilized. They were then inoculated with 5% from the culture of *Ch. globosum* and/or *S. cerevisiae* which was grown on a fermentation medium and incubated for 15 days. To obtain biostraw and bagas extract biostraw former outfitted soaked in a 5 l of water for 24 h and then taken filtrate.

Field experiments for controlling powdery mildew disease

These experiments were done during the 2018 growing season at Kafer El-dwar (Behaira) governorate, to study the effects of biocontrol agents, essential oils at concentrations of 3%, organic acids at concentrations of 1%, and 3% compost T, on disease severity of powdery mildew on *L. cylindrica*. Powdery mildew disease severity was determined under natural field conditions according to the previously mentioned adopted scale.

Foliar application

The field was divided into plots 6×6 m². Each plot consisted of two rows and each row contained three hills on the eastern side. Two seeds/hill were sown. The seeds were sown on April 1, 2018. The experimental treatments previously mentioned were applied three times, the first one 60 days after planting, the second one, a month after the first spray while, the final spray was done 30 days after the second spray. Disease severity was recorded 15 days after the last spray.

Statistical analysis

Tukey test for multiple comparisons among means was utilized as described by Neler *et al.* (1985).

Results and Discussion

Disease survey and detection

Results of luffa plants grown at different field localities of northern Egypt (Beheira, Alexandri, sharqia and Kafer El-Sheekh governorates), surveyed for powdery mildew disease (Table 1) showed that the largest record of disease occurrence occurred in some fields of Beheira (57.33%) followed by Alexandria (53.67%) and Sharqia (48.00%) governorates. Fewer occurrences of powdery mildew were observed in Kafer El-Sheekh governorate (45.33%).

Fungal strain isolation and selection

Isolate number CF2 showed the highest infection activity on luffa plants, selected for identification.

Phenotypic identification of the isolated fungi

The isopropanol method was used in the genomic DNA isolating process and purifying (Darwesh *et al.* 2015). Polymerase chain reaction (PCR) technology was used to amplify internal transcribed spacer (ITS) genes and their sequences. Using the BLAST program (<http://www.ncbi.nlm.nih.gov/BLAST>) the sequences obtained were compared to the sequences provided in GenBank and showed that the similarity percentage of this isolate was 98.83% with strain *P. xanthii*. The sequences of the obtained strain were submitted to GenBank and recorded under accession number MN620385. The phylogenetic tree illustrated that these strains were very close to the type strains of *Podosphaera* genus deposited in the culture collection center of the National Center for Biotechnology Information (Fig. 4). These strains were named *P. xanthii* SUB6476157 Seq.MN620385.

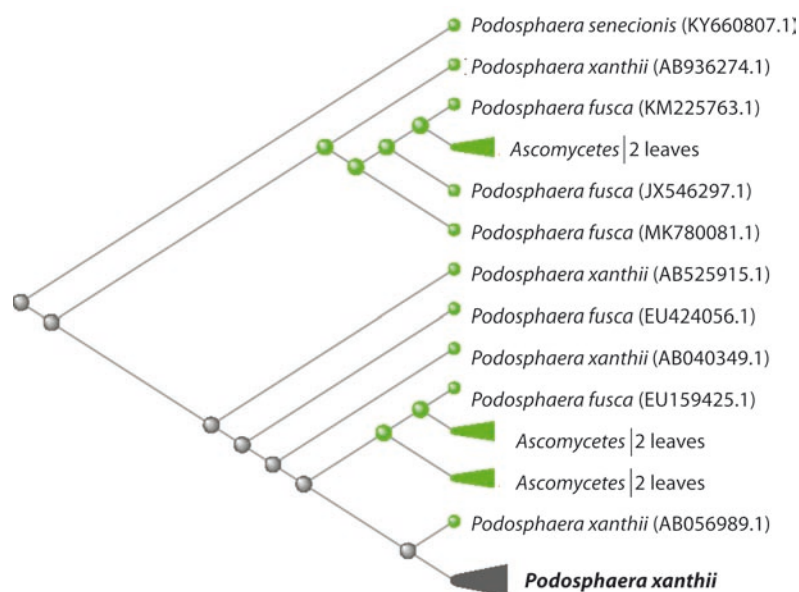


Fig. 4. Neighbor-joining phylogenetic representation of the strain obtained and their closest ITS sequence of strain *Podosphaera xanthii* and their related strains in GenBank

Table 1. Survey of powdery mildew of *Luffa cylindrica* at different locations in four governorates during October 2018

Governorates (localities)	Powdery mildew of <i>Luffa cylindrica</i> [%]
Beheira	57.33* a
Alexandria	53.67 b
Sharqia	48.00 c
Kafer El-Sheekh	45.33 d

*these percentages are the means of surveyed fields; the same letters in each column are not significantly different ($p \leq 0.05$)

Evaluation of some treatments of luffa plants with different bio-agents, essential oils, organic acids and biocompost on the development of powdery mildew disease caused by *Podosphaera xanthii* (in vitro)

Our results illustrated that all treatments reduced disease severity compared to untreated controls (Table 2). Application with both *Ch. globosum* and *S. cerevisiae* showed a high reduction in disease severity compared to other bio-agents. The percent of disease incidence with powdery mildew with bio-agent treatment ranged between 3.11 and 5.0% but in the case of *T. viride*, and *B. subtilis* there was a moderate reduction in powdery mildew incidence and ranged between 30.5 and 10.2%. However, with *T. harzianum*, *P. lindbergii* and *P. fluorescens* there was a weak effect (75.67, 60.5 and 50.33%, respectively). Also, treatment with neem oil and chili oil caused a significant reduction of disease severity of powdery mildew compared to other oils and control. Treatment with organic acids (benzoic acid, citric acid and boric acid) reduced disease severity by 10.5, 15.0

and 15.0%, respectively. On the other hand, the most effective treatment on disease severity was obtained with bioproducts *Ch. globosum* and *S. cerevisiae* which was formulated on rice straw (3.0 and 8.11%, respectively). This is consistent with Papavizas and Lumsden (1980), who stated that biological control proved to be successful for controlling various plant pathogens. Biological control is still cheap and easy to apply. In addition, its application is safe, not hazardous for humans and avoids environmental pollution (Sivan and Chet 1989).

The effects of some treatments of luffa with different bioagents, essential oils, organic acids and bioproducts on the development of powdery mildew diseases caused by *Podosphaera xanthii* under field conditions

In the present study, spraying luffa with the bioagents, i.e. *Ch. globosum* and *S. cerevisiae* alone or formulated on rice straw and bagas effectively reduced powdery mildew compared to other treatments and control. Deore *et al.* (2004) used culture filtrates of different species of *Trichoderma* for the management of powdery mildew of cluster bean caused by *Leveillula taurica*. They revealed that culture filtrates of *Trichoderma* spp. alone or in combination were highly effective against powdery mildew. Also, *T. harzianum* T39 (TRICHODEX) spray reduced powdery mildew severity caused by *Sphaerotheca fusca* on greenhouse cucumber by up to 97%.

Foliar application of different essential oils on luffa showed significant reduction in disease severity compared to controls (Table 3). Neem and chili were the

Table 2. The effects of some treatments of luffa with different bioagents and chemical inducers on the development of powdery mildew diseases caused by *Podosphaera xanthii* (*in vitro*)

Treatments	Concentration	Disease severity [%]	Reduction
Biocontrol agents			
<i>Chaetomium globosum</i>	2.5×10^5 spores · ml ⁻¹	3.11	96.15
<i>Trichoderma harzianum</i>	2.5×10^5 spores · ml ⁻¹	75.67	6.23
<i>Trichoderma viride</i>	2.5×10^5 spores · ml ⁻¹	30.50	62.21
<i>Bacillus subtilis</i>	1.8×10^8 cfu	10.20	87.36
<i>Pseudomonas lindbergii</i>	2.3×10^8 cfu	60.50	25.03
<i>Pseudomonas fluorescens</i>	2.8×10^8 cfu	50.33	37.63
<i>Saccharomyces cerevisiae</i>		5.00	93.80
Essential oils			
Cinnamon oil	3.0%	25.50	68.40
Thyme oil	3.0%	45.33	43.82
Sour almond oil	3.0%	55.33	31.43
Chili oil	3.0%	17.33	78.53
Neem oil	3.0%	15.00	81.41
Organic acids			
Benzoic acid	1.0%	10.5	86.99
Citric acid	1.0%	15.00	81.41
Boric acid	1.0%	15.00	81.41
Compost tea			
Rice straw + <i>Chaetomium globosum</i>		3.00	96.82
Rice straw + <i>Saccharomyces cerevisiae</i>	3%	8.11	89.95
Bagas + <i>Saccharomyces cerevisiae</i>	3%	40.50	49.81
Bagas + <i>Chaetomium globosum</i>	3%	20.33	74.81
Control	–	80.70	00.00

Table 3. The effects of some treatments of luffa with different bioagents and chemical inducers on the development of powdery mildew disease caused by *Podosphaera xanthii* under field conditions

Treatments	Disease severity	Reduction
Biocontrol agents		
<i>Chaetomium globosum</i>	0.0 f	100.0
<i>Trichoderma harzianum</i>	50.67 a	91.29
<i>Trichoderma viride</i>	10.60 d	80.90
<i>Bacillus subtilis</i>	3.50 e	93.69
<i>Pseudomonas lindbergii</i>	34.33 b	38.14
<i>Pseudomonas fluorescens</i>	28.50 bc	48.65
<i>Saccharomyces cerevisiae</i>	1.33 f	97.60
Essential oils		
Cinnamon oil	24.00 c	56.76
Thyme oil	30.50 b	45.05
Sour almond oil	4.80 e	91.35
Chili oil	4.55 e	91.80
Neem oil	8.67 d	84.37

Table 3. The effects of some treatments of luffa with different bioagents and chemical inducers on the development of powdery mildew disease caused by *Podosphaera xanthii* under field conditions – continuation

Treatments	Disease severity	Reduction
Organic acids		
Benzoic acid	3.50 e	93.69
Citric acid	49.67 a	10.50
Boric acid	17.00 d	69.37
Compost tea		
Rice straw + <i>Chaetomium globosum</i>	0.00 f	100.0
Rice straw + <i>Saccharomyces cerevisiae</i>	1.00 f	98.19
Bagas + <i>Saccharomyces cerevisiae</i>	13.25 d	76.13
Bagas + <i>Chaetomium globosum</i>	7.33 e	86.79
Control	55.50 a	00.00

Figures with the same letters in each column are not significantly different ($p \leq 0.05$)

most effective (81.41 and 78.53%, respectively). Cinnamon oil had a moderate effect (68.04%). Thyme and sour almond were the least effective (43.82 and 31.43%, respectively). Foliar application with benzoic, citric and boric acids significantly decreased the percent of disease severity. However, benzoic acid caused significant decrease compared to controls and other treatments.

Application of essential oils for the control of powdery mildew demonstrated the potential use of natural substances for its management. Sturchio *et al.* (2014) found that application of clove oil and rosemary oil alone or in combination reduced disease incidence and severity.

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