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Moringa oleifera Extraction as Alternative Option for Controlling Citrus Post-harvest Green and Blue Molds *In vetro*

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Abstract:

The post-harvest green and blue molds caused by *Penicillium digitatum* and *Penicillium italicum*, respectively are considered universal diseases that lead to the spoilage of almost all kinds of mature citrus fruits, and cause significant losses in citrus fruits during storage and in the markets greater than what people believes. Several natural products were used to control plant pathogens as alternative of fungicides. *Moringa oleifera* as a natural product was used to treat several plant pathogens. *Penicillium digitatum* and *Penicillium italicum,* were isolated from mature spoiled orange (*Citrus sinensis* L.) and lemon (*Citrus limon* L.) fruits obtained from the local market of Haqle - Saudi Arabia and identified using morphological and physiological characteristics according to the key of Visagie, in addition to molecular methods based on ITS1 sequence analysis. The fungicidal activity of *Moringa oleifera* extract with different concentrations in controlling the growth of the isolates of *Penicillium digitatum* and *Penicillium italicum* was determined *in vitro*. The control activity was highly dependent on *Moringa oleifera* extract concentration. For instance, undiluted *Moringa oleifera* extract showed the highest control activity with no growth as compared to the biotic control without treatment. Diluted *Moringa oleifera* extract 25% and 10% reduced the fungal growth to 46.54 and 29.88%, respectively, for *Penicillium digitatum* and 45.83 and 27.46%, respectively, for *Penicillium italicum*. The results of the recent study show that *Moringa oleifera* extract could successfully control *Penicillium digitatum* and *Penicillium italicum*, the disease agents of green and blue molds as an environmentally friendly product.

Keywords: *Moringa oleifera, controlling citrus, post-harvest, green and blue molds in vetro*

Introduction:

The post-harvest green and blue molds caused by *Penicillium digitatum* and *Penicillium italicum*, respectively are considered universal diseases that lead to the spoilage of almost all kinds of mature citrus fruits (Samson *et al.,* 2004, Duan *et al.,* 2016). Significant losses occurred by these pathogens on citrus fruits in the markets and storage. Farmers can not avid the damages of fruits when they transfer fruits from farms to markets (Smilanick *et al*., 2005, Kawai, *et al.,* 2018). Moreover, farmers still using fungicides to save their products from green and blue molds and other molds fungi (Tripathi and Dubey, 2004, Duan *et al.,* 2016). However, markets demands fruits without fungicides residues (Bhyan *et al*., 2007; Reddy *et al.*, 2010).

There are different natural products used as alternative of fungi-

cides to control plant pathogens (Nithyameenakshi *et al*., 2006; Saravanan *et al*., 2010; Hussein *et al.,* 2011; Ashtiani *et al*., 2018; Chen *et al.,* 2019). Alhussaen (*et al.,* 2011) used successfully garlic extract in controlling *Pythium ultimum in vitro* isolated from tomato seedlings as environmentally friendly product. Other study was carried out by Rongai *et al*., 2017 using pomegranate peel aqueous extract (pae) to control the fusarium wilt of tomato caused by *Fusarium oxysporum,* f. sp. *Lycopersici* and reduced the population of *Fusarium* in soil resulting more healthy plants.

Moringa oleifera is the most widely used species of a monogeneric family, the *Moringa oleifera* ceae, that is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. *Moringa oleifera* was used wildly as medicine in different countries all over the world, different parts of the plant used such as the stem bark, root, bark, fruit, flowers, leaves, seeds, and gum (Compaoré *et al.,* 2011). *Moringa oleifera* was described in several studies and found their nutritional and medicinal properties (Anjorin, Ikokoh, & Okolo, 2010). Anjorin *et al.,* 2010 reported that *Moringa oleifera* leaves have vitamins and essential minerals such as vitamin A, vitamin B, vitamin C, calcium, iron, potassium, essential amino acids, and high protein content.

Moringa oleifera found to have an antifungal activities and was used to control several species of fungi (Nickon *et al*., 2003; Chuang *et al.,* 2007; Moodley *et al.,* 2018). Jabeen *et al.,* 2008 pointed that *Moringa oleifera* extracts were used successfully on bacterial species *Botugytis cinerea* and fungi species *Fusarium oxysporum* and *Mycfosphaerella arachidicola*. Other studies examined the antimicrobial activity of the essential oil of *Moringa oleifera* against *Pseudomonas aeruginosa*, *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus* and different fungal isolates *Penicillium digitatum*, *Penicillium aurantiogriseum*, *Penicillium citrinum*, *Penicillium expansum* and *Aspergillus niger* and found that all microorganisms tested were sensitive to the essential oil (Marrufo *et al.,* 2013). Moreover, extracts of other parts of *Moringa oleifera* have been examined and control successfully bacterial growth of *Streptococcus mutans* and *Staphylococcus aureus* (Elgamily *et al.,* 2016).

The present study was carried out to examine the effect of *Moringa oleifera* extract as an antifungal for green and blue molds caused by *Penicillium digitatum* and *Penicillium italicum*.

Materials and Methods:

Isolate recovery: *Penicillium digitatum* and *Penicillium italicum,* were isolated from mature spoiled orange (*Citrus sinensis* L.) and lemon (*Citrus limon* L.) fruits and identified using morphological and physiological characters. Fruits were obtained from the local market Haqle - Saudi Arabia. Complete media (CM) modified by Al-Najar (2007) was used to obtain optimal growth conditions for the tested fungal isolates. Isolates were grown for 7 to 10 days at 20-25°C on CM plates

(Al-Najar, 2007) to confirm their purity and identity.

Molecular identification: DNA were extracted from the mycelium of the isolated *Penicillium digitatum* and *Penicillium italicum* according to Demirel *et al.,* 2013 and the Internal Transcribed Spacer (IST1) region of the ribosomal nuclear DNA (rnDNA) was amplified using PCR. Standard methods were used to get the ITS1 sequencing and was carried out at a commercial facility (Macrogen Inc., Seoul, South Korea). BLAST search (http://blast.ncbi.nlm.nih.gov) was used to analysis the sequences and ClustalW (http://www.ebi.ac.uk) used to find their relatives of the phylogram of the isolate.

Moringa extraction and *Penicillium* treatment:

The effect of *Moringa oleifera* extract against mycelial growth of *Penicillium digitatum* and *Penicillium italicum* was examined in September 2018. Moringa oleifera was extracted using the method mentioned by Chumarka *et al.,* 2008.

Four concentrations (10, 25, 50 and 100%) of *Moringa oleifera* extract were applied and sterilized distilled water was used as control. Three plates were used for each treatment. Inoculation was carried on by dispersing 1 mL of the *Moringa oleifera* extract from each treatment and added on the surface of each plate. Five millimeter disc was cut from the margin of an actively growing *Penicillium digitatum* and *Penicillium italicum* culture (5 days old) on CM media and incubated in the dark at 25±1°C as an inoculation. Plates with the different treatments were incubated at 25±1°C on the dark. Growth of the mycelium was assessed daily as visual observation and the final assessment was recorded after 5 days of incubation using colony counter on Petri dish, by which the total area of the mycelial growth on each plate was measured.

Data analysis: All treatments were arranged in Completely Randomized Design (CRD) with 3 replicates for each treatment. The growth area was assessed using colony counter on Petri dish of each treatment. General Linear Model (GLM) ANOVA was used to find differences ($p \leq 0.05$) between treatment means (SPSS VER 25).

Results:

Sequence of ITS region of rDNA

The sequences of *Penicillium digitatum* isolates obtained in this study was matched with *Penicillium digitatum* (DQ084021) from Gen-Bank (Table 1). The sequence of isolate *Penicillium digitatum* was 668 bp in length and was 100% identical to the corresponding sequence from *Penicillium digitatum* (DQ084021). Moreover, the sequences of *Penicillium italicum* isolates obtained in this study was matched with *Penicillium italicum* (AY291256) from Gen-Bank (Table 1). The sequence of isolate *Penicillium italicum* was 593 bp in length and was 100% identical to the corresponding sequence from *Penicillium italicum* (AY291256).

Table 1. Sequence length (bp) of ITS region of rDNA for 2 isolates of *Penicillium digitatum* and *Penicillium italicum* from fruits obtained from the local market of Haqle - Saudi Arabia and comparison with sequences in Gen-Bank.

Sequence length (bp)	Match from Gen-Bank (Location)	Gen-Bank accession number	Identities (%)	Gaps
668	*Penicillium digitatum* 2005 (USA)	DQ084021	100	0
593	*Penicillium italicum* 2003 (Sweden)	AY291256	100	0

Effect of *Moringa oleifera* extract on the growth of *Penicillium digitatum*

Moringa oleifera extract was found to be effective to control *Penicillium digitatum* isolated from orange and lemon fruits obtained from the local market of Haqle - Saudi Arabia under *in vitro* conditions (Figure 1, Table 2). One hundred percent concentration of *Moringa oleifera* extract showed the highest growth inhibition activity. At this level, the fungus has no growth compared with the control (56.72 cm^2) (Figure 1, Table 2). Moreover, 50% diluted *Moringa oleifera* extract reduced the fungal growth to 93.79% (3.52 cm^2) compared with the control. The 25% diluted *Moringa oleifera* extract reduced the fungal growth to 81.89% (10.52 cm^2) compared with the control. The last diluted *Moringa oleifera* extract (10%) reduced the growth to 52.68% (26.84 cm^2) compared with the control.

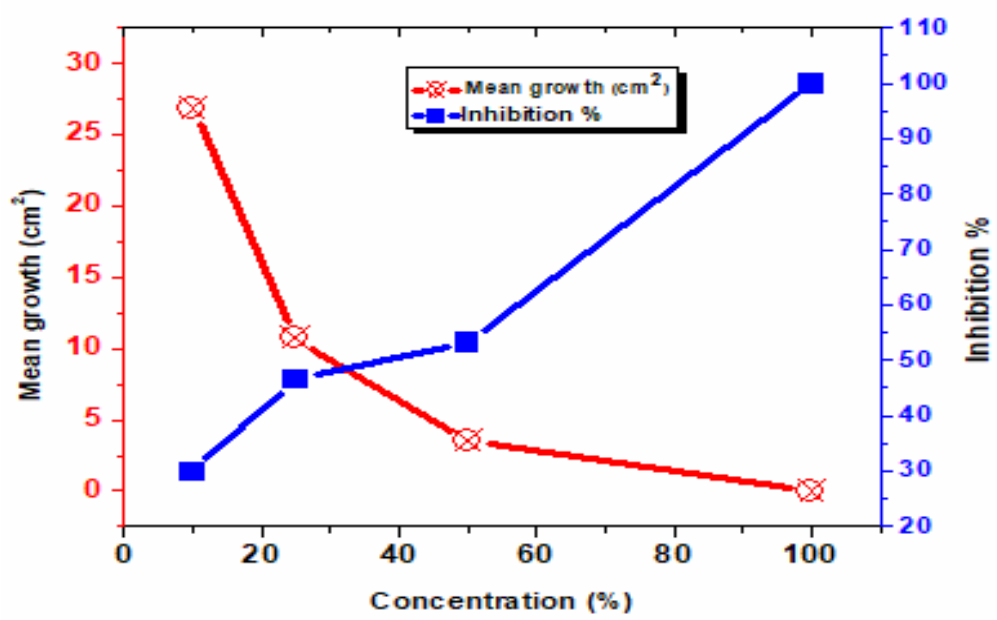

Figure 1: Mean growth area (cm^2) and the percentage of growth inhibition area of *Penicillium digitatum* due to treatment with different *Moringa oleifera* extract concentrations.

Table 2. Mean growth area (cm^2) and the percentage of growth inhibition area of *Penicillium digitatum* due to treatment with different *Moringa oleifera* extract concentrations.

Morin extract conc.	Mean growth (cm2)	Inhibition percentage (%)
Full concentration 100%	00.00[a]	100.0
50%	03.52[b]	93.79
25%	10.27[c]	81.89
10%	26.84[d]	52.68
Control	56.72[e]	0.00

On the other hand, *Penicillium italicum* isolated from orange and lemon fruits obtained from the local market of Haqle - Saudi Arabia were controlled by *Moringa oleifera* extract under *in vitro* conditions (Table 3). One hundred percent concentration of *Moringa oleifera* extract showed the highest growth inhibition activity. At this level, the fungus has no growth compared with the control (56.72 cm^2) (Figure 2, Table 3). The 50% concentration of the *Moringa oleifera* extract reduced the fungal growth to 92.82% (4.07 cm^2) compared with the control. The other diluted extract 25% reduced the fungal growth to 80.80% (10.89 cm^2) compared with the control. The final diluted *Moringa oleifera* extract (10%) reduced the growth to 48.41% (29.26 cm^2) compared with the control.

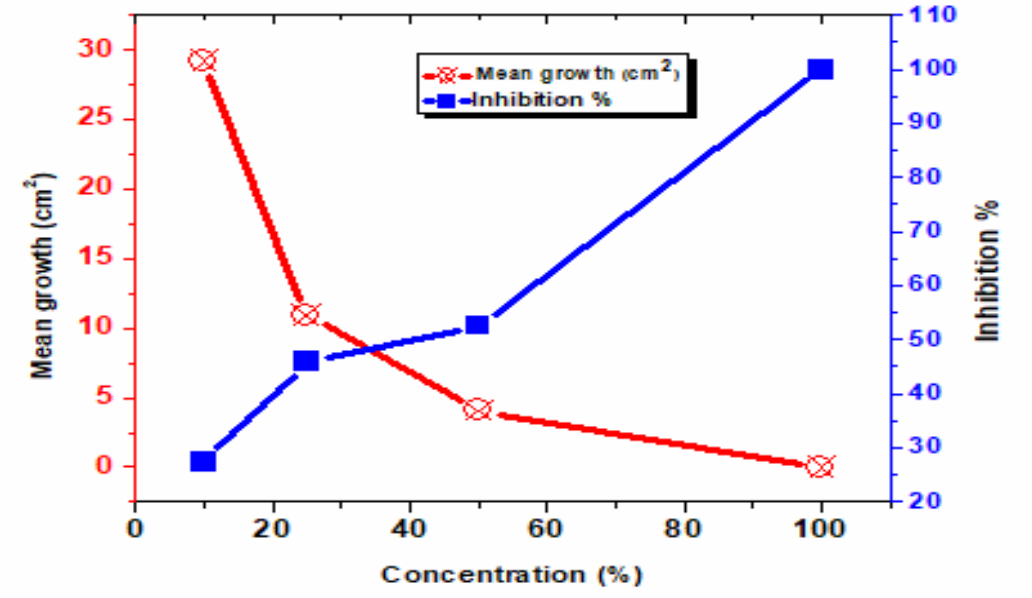

Figure 2: mean growth area (cm^2) and the percentage of growth inhibition area of *Penicillium italicum* due to treatment with different *Moringa oleifera* extract concentrations.

Table 3. Mean growth area (cm^2) and the percentage of growth inhibition area of *Penicillium italicum* due to treatment with different *Moringa oleifera* extract concentrations

Morin extract conc.	Mean growth (cm2)	Inhibition percentage (%)
Full concentration 100%	00.00[a]	100.0
50%	04.07[b]	92.82
25%	10.89[c]	80.80
10%	29.26[d]	48.41
Control	56.72[e]	00.00

Discussion:

Identification of plant pathogens is very important to find effective disease management methods. The incorrect identification could lead to ineffective control strategies and money losses. The traditional identification of fungi is using morphological characteristics but when some species have the same characteristics, the identification difficult will be (Agrios, 2005). In some fungi such as *Pythium* spp. features are similar among the groups as result of the environmental effect (Van Os, 2003).

Molecular methods have been used to identify plant pathogens and to understand the variation between the population by using several DNA methods (Levesque and de Cock, 2004; Drenth *et al*., 2006). The sequences of the Internal Transcibed Spacer (ITS1) region of the ribosomal DNA (rDNA) for *Penicillium* isolates were obtained for identification to species level. *Penicillium digitatum* isolate was found to be 100% identical to *Penicillium digitatum* (DQ084021) in the Gene-Bank database (Table 1). Moreover, isolate of *Penicillium italicum* was found to be 100% identical to *Penicillium italicum* (AY291256) in the Gen-Bank (Table 1). Identification by using sequences of its was confirm the identification by using the morphological and physical characterization.

Moringa oleifera extract was found to be effective in this study when used to control *Penicillium italicum* and *Penicillium digitatum in vitro* and reduced the growth. Bukar *et al*., 2010 found that the *Moringa oleifera* leaf extract was effective in controlling bacteria species of *Escherichia coli, Salmonella typhimurium* and fungi of *Mucor* and *Rhizopus*. Moreover, other study found *Moringa oleifera* extract have an antifungal activity against the phytopathogenic fungi *Fusarium solani, Fusarium oxysporum, Colletotrichum musae and Colletotrichum gloesporioides in vitro* (Gifoni *et al.,* 2012). Furthermore, Moringa oleifera extract was found to be effective against the plant pathogenic of *Fusarium solani, Fusarium oxysporum*, *Alternaria solani, Alternaria alternaria*, *Rhizoctonia solani* in vitro (Riad, *et al*., 2014).

In the present study, *Moringa oleifera* extract as low as 10% showed a good potential for reducing mycelial growth area to 26.84 cm^2 of *Penicillium digitatum* and 29.26 cm^2 of *Penicillium italicum* compared with the control (Table 1 and 2). These results demonstrate that *Moringa oleifera* extract even in low

concentrations could be used to control green and blue molds caused by *Penicillium digitatum* and *Penicillium italicum*. Moreover, other *Moringa oleifera* extract concentrations used in the present study showed from slight reduction to complete inhibition of growth of the mycelia. These results agreed with the results of Maqsood *et al.,* 2017 who study and explore *In vitro* antifungal activity of *Moringa oleifera* leaves against Aspergillus fumigatus, *Aspergillus niger* and *Candida albicans* at four different concentrations (50-300 mg/ml) by ager well diffusion method. The maximum zone of inhibition was recorded in the case of methanolic leaves extract (16 mm) against *Aspergillus niger* at a concentration of 300 mg/ml, which was at par to the standard antibiotic. Methanolic extract showed the highest MIC value (70 mg/ml) against *Aspergillus niger*.

Conclusion:

Moringa oleifera is a natural product was used to treat plant pathogens of *Penicillium digitatum* and *Penicillium italicum* isolated from mature spoiled orange and lemon fruits. Results of the present study highly recommended using *Moringa oleifera* extract in controlling *Penicillium italicum* and *Penicillium digitatum*.

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Conflict of interest: the author declare no conflict of interest

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استخدام نبات المورنقا أوليفيرا كخيار بديل للسيطرة على أمراض الأعفان الخضراء والزرقاء بعد الحصاد على الحمضيات

رياض أبوبكر باصهي

قسم الأحياء، الكلية الجامعية بحقل، جامعة تبوك، السعودية

الملخص:

تعتبر أمراض ما بعد الحصاد الأعفان الخضراء والزرقاء الناتجة عن *Penicillium digitatum* و *Penicillium italicum* ، على التوالي ، من الأمراض العالمية التي تؤدي إلى تلف كميات كبيرة من الحمضيات الناضجة، وتسببت في خسائر كبيرة في ثمار الحمضيات أثناء التخزين وفي الأسواق أكبر مما يعتقده الناس. تم استخدام العديد من المنتجات الطبيعية للسيطرة على مسببات الأمراض النباتية كبديل للمبيدات الفطرية. المورينغا أوليفيرا منتج طبيعي يستخدم لعلاج العديد من مسببات الأمراض النباتية. تم عزل *Penicillium digitatum* و *Penicillium italicum*، من البرتقال الناضج (*Citrus sinensis* L.) وثمار الليمون (*Citrus limon* L.) وتم الحصول عليها من السوق المحلية في محافظة حقل – منطقة تبوك– المملكة العربية السعودية وتم التعرف عليها باستخدام الخصائص المورفولوجية والفسيولوجية، بالإضافة إلى الطرق الجزيئية القائمة على تحليل تسلسل ITS1. تم تحديد نشاط مبيد الفطر في مستخلصات المورينغا أوليفيرا بتركيزات مختلفة في التحكم في نمو عزلات *Penicillium digitatum* و *Penicillium italicum* في المختبر. كان نشاط المكافحة يعتمد بدرجة كبيرة على تركيز مستخلص المورينغا أوليفيرا. على سبيل المثال، أظهر مستخلص المورينغا أوليفيرا غير المخفف أعلى نشاط تحكم مع عدم وجود نمو للفطريات بالمقارنة مع الشاهد، في حين أن مستخلص المورينغا أوليفيرا المخفف ٢٥ و ١٠٪ قلل من نمو الفطريات إلى ٤٦,٥٤ و ٢٩,٨٨٪ على التوالي بالنسبة إلى *Penicillium digitatum* و ٤٥,٨٣ و ٢٧,٤٦٪ على التوالي ل *Penicillium italicum* أظهرت نتائج الدراسة الحديثة أن مستخلص المورينغا أوليفيرا يمكن أن يسيطر بنجاح على *Penicillium digitatum* و *Penicillium italicum* كعوامل مرضية للأعفان الخضراء والزرقاء كمنتج صديق للبيئة.