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Control certain associated fungi of Date fruits under Aswan conditions, Egypt

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Abstract

This study aimed to identify fungi associated with mold development in date fruits. The analysis revealed that *Rhizopus stolonifer*, *Aspergillus niger*, and *Aspergillus flavus* were the most prevalent fungal species isolated from the samples. Specifically, six isolates of *R. stolonifer*, nine isolates of *A. niger*, and five isolates of *A. flavus* were found in association with palm date fruits. The application of ascorbic acid significantly reduced the incidence of these fungal pathogens. These associated fungi pose a substantial health risk, as they can produce hazardous metabolites that affect human health. Given the nutritional value of dates and their importance for human wellness, the findings highlight the urgent need for effective biological control methods to manage fungal diseases in edible fruits.

Key words: date fruit molds, *Rhizopus stolonifer*, *Aspergillus niger*, ascorbic acid, fungal pathogens control.

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1. Introduction

In 2019, the world's annual production of date fruits exceeded 7,600,315 tonnes (Anonymous, 2019). With about 1,465,030 tonnes of dates produced, Egypt is the world's largest date producer (Anonymous, 2019), The date palm is a dioeciously plant with hundreds of variants, technically known as *Phoenix dactylifera* of the monocot family (Kacem-Chaouche et al., 2013). Soft dates, semi–dry dates, and dry dates are all found in Egypt's Nile valley, oases, and desert. Furthermore, due to sexual reproduction, there is a large number of seedling date palms, some of which are invasive. Fruit quality and multiplication of offshoots are both highly desirable (Ragab, 2011). Fresh fruits are susceptible to fungal contamination in the field, during harvest, transportation, and marketing, as well as with the client. Fungi are important in the rotting of fruits because of their pathogenicity to the collected products. Fungi like *Aspergillus* spp. and *Rhizopus* spp. flourish in high-moisture conditions, particularly when gathered after rain or high humidity (Ibrahim & Rahma, 2009). fungal studies of palm date fruits studied previously (Bokhary, 2010; Alghalibi & Shater, 2004; Gherbawy, 2001). *A. flavus* has been found in date palm fruits by several researchers (Ragab et al., 2001; Alghalibi and Shater, 2004). Fungi such as *Aspergillus ochraceus* and *Aspergillus niger* have also been found in date fruits (Ferracin et al., 2009; Heenan et al., 1998). The goal of this study was to identify the related fungi and assess the potential to reduce the occurrence of the fungal diseases.

2. Materials and methods

2.1 Isolation and identification fungi

Dates were collected from the Aswan governorate and gently washed, dried, sprayed with a 1 percent sodium hypochlorite solution, cleaned with sterile distilled water, and dried on sterile filter paper sheet. Samples were cut into small pieces and aseptically put in Petri dishes on medium Potato dextrose agar PDA. Petri dishes were incubated at 27°C and were monitored every day. Fungal colonies were purified on new PDA media Petri dishes using the hyphal tip technique. The morphological properties of the isolated fungi were used to identify them (Leslie and Summerell, 2008). The identity was confirmed by the Plant Pathology Department of Aswan University's Faculty of Agriculture Sciences and Natural Resources in Aswan, Egypt.

2.2 *In vitro* assessment

On PDA medium, in vitro experiments on the mycelial growth of related fungal strains by Ascorbic acid at three doses were conducted. The plates were inoculated with 5 mm diameter 7-day-old mycelia discs of various strains, four petri dishes per replicate, incubated at 25°C, and compared to the control. The length of mycelial growth diameter was measured, as was the reduction in growth diameter. El-Nasr Company for Intermediate Chemicals in Egypt provided all of the tested chemicals.

2.3 *In vivo* assessment

Uninfected date fruits were sprayed with a spore suspension of various fungal strains at a concentration of approximately 1×10^5 spores /mL. The treated fruits were packed in polypropylene punnets and stored at 25 °C with 80–90% relative humidity for 48 hours. The disease severity was evaluated after 6 days, following the methodology described

by Romanazzi et al. (2000).

2.4 Total phenol contents (TPC)

The total phenol concentration was quantified using the Folin-Ciocalteau method with absorbance monitoring at 765 nm after the phenolic components were isolated using the extraction methods described in the preceding section (Ough & Amerine, 1988). The spectrophotometric measurement was repeated twice for each extract, with the average value interpolated onto the gallic acid calibration curve and represented as g of gallic acid per kilogram of material.

2.5 Statistical analysis

For statistical analysis, MSTAT-C version 2.10 was employed (1991). The least significant difference (L.S.D.) was used to compare the means of treatments, according to Gomez & Gomez (1984).

3. Results and Discussion

3.1 Isolation and identification

During a survey of fungi associated with mold disease of dates in Aswan governorate, Egypt, six isolates of *Rhizopus stolonifer*, nine isolates of *Aspergillus niger*, and five isolates of *Aspergillus flavus* were identified and isolated. These isolates were recovered from naturally infected date fruits exhibiting typical mold symptoms. The genus *Aspergillus*, comprising five species, was the most frequently isolated group, detected in 45% of the samples. This aligns with findings by Abdel-Sater and Saber (1999), who reported *Aspergillus* as the predominant genus isolated from dates in Egypt. Similarly, Gherbawy et al. (2012) identified *A. niger* and *A. flavus* as the most common species within this genus. Further supporting this, Abu-Zinada and Ali (1982) demonstrated the association of *A. flavus* with various date fruit cultivars in Saudi Arabia. In another study, Nassar (1986) isolated three *Aspergillus* species, including *A. niger*, from dates in Aswan, Egypt. These findings collectively highlight the widespread occurrence of *Aspergillus* species, particularly *A. niger* and *A. flavus*, as significant fungal pathogens associated with date mold diseases in Egypt and neighboring regions.

3.2 *In vivo* assessment

Data in Table (1) reveal that *R. stolonifer*, *A. niger*, and *A. flavus* demonstrated the ability to produce mold symptoms on date fruits. The highest disease incidence was observed with *R. stolonifer* 1 at 37.5%, followed by *R. stolonifer* 2 at 32.4%, while *R. stolonifer* 4 exhibited the lowest incidence at 12.6%. Among the *Aspergillus* species, *A. niger* 1 caused a disease incidence of 18.6%, while *A. flavus* 1 showed an incidence of 22.2%, followed by *A. flavus* 2 with 10.8%. These findings are consistent with those of Shenasi et al. (2002), who reported the presence of *A. flavus* during the early maturation stages of ten date fruit cultivars from the United Arab Emirates, thereby supporting the current results. Similarly, Alghalibi and Shater (2004) identified *Aspergillus* as the most frequently isolated genus from Yemeni date samples, occurring in 75% of the tested samples, with *Rhizopus stolonifer* being the second most commonly isolated fungus. These findings corroborate the significant role of both *Aspergillus* and *Rhizopus* species as key pathogens associated with mold development in date fruits.

Table 1: Disease incidence (%) of fungal strains isolated from naturally infected dates.

Isolates	Disease incidence (%)
R. stolonifer 1	37.5
R. stolonifer 2	32.4
R. stolonifer 3	22.4
R. stolonifer 4	12.6
R. stolonifer 5	0.0
R. stolonifer 6	0.0
A. niger 1	18.6
A. niger 2	0.0
A. niger 3	0.0
A. niger 4	0.0
A. niger 5	0.0
A. niger 6	0.0
A. niger 7	0.0
A. niger 8	10.4
A. niger 9	6.2
A. flavus 1	22.2
A. flavus 2	10.8
A. flavus 3	4.2
A. flavus 4	0.0
A. flavus 5	0.0
L.S.D. $P \geq 0.05$	1.9

3.3 *In vitro* assessment with different concentrations of ascorbic acid

Data in Table (2) indicate that none of the tested concentrations of ascorbic acid (AA) demonstrated significant inhibitory activity against the causal strains of mold (*R. stolonifer* 1, *A. niger*, and *A. flavus* 1). The evaluation was based on growth diameter measurements, which served as an indicator of fungal growth reduction. However, the results showed no notable decrease in growth diameter across all tested concentrations.

Table 2: *In vitro* assessment of growth diameter for date mold causal strains under different ascorbic acid (AA) treatments.

Treatments	Growth diameter (mm)		
	R. stolonifer 1	*A. niger* 1	*A. flavus* 1
AA 1000 ppm	98	100	94
AA 500 ppm	96	100	96
AA 250 ppm	100	100	100
Control	100	100	100
L.S.D. $P \geq 0.05$	-	-	2.04

3.4 *In vivo* assessment with different concentrations of ascorbic acid on date mold

Ascorbic acid (AA) significantly suppressed mold incidence on dates, as shown in Table (3). The highest concentration of AA (1000 ppm) reduced the incidence of *R. stolonifer* 1 to 12.4%, while the lowest concentration (250

ppm) resulted in a suppression of 22.2%, compared to the control, which showed an incidence of 40.6%. For *A. niger* 1, AA at 1000 ppm reduced the incidence to 6.8%, whereas 250 ppm showed lower suppression at 14.6%, compared to the control, which had an incidence of 34.8%. Similarly, AA at 1000 ppm achieved the highest suppression of *A. flavus* 1, reducing its incidence to 2.8%, while 250 ppm resulted in a suppression of 4.4%, compared to the control, which exhibited an incidence of 18.4%. These results align with findings from previous studies, which suggest that ascorbic acid can enhance plant resistance mechanisms, improving their ability to combat fungal infections. For instance, Khan et al. (2011) and Singh et al. (2020) reported that ascorbic acid treatments can activate resistance responses in plants, such as strawberries, making them more resilient to fungal pathogens.

Table 3*: In vivo* disease incidence of date mold under different ascorbic acid (AA) treatments.

Treatments	Disease incidence (%)		
	R. stolonifer 1	*A. niger* 1	*A. flavus* 1
AA 1000 ppm	12.4	6.8	2.8
AA 500 ppm	16.4	18.4	3.2
AA 250 ppm	22.2	14.6	4.4
Control	40.6	34.8	18.4
L.S.D. $P \geq 0.05$	0.0	0.0	0.0

3.5 Biochemical analysis evaluation under different treatments of ascorbic acid

3.5.1 Total phenolics estimation of dates

Results in Table (4) show that total phenolic content was estimated for both control and treated samples of date fruits after six days of treatment with specific concentrations of ascorbic acid (AA). Significant differences were observed among treatments. For *R. stolonifer* 1, the control exhibited the highest total phenolic content at 7.24, followed by treatments with AA at 250 ppm (6.34) and 500 ppm (5.82). For *A. niger* 1, the control also had the highest total phenolic content at 6.14, followed by AA at 250 ppm (5.2) and 500 ppm (4.94). In contrast, potassium phosphite at 500 mg/L resulted in the lowest total phenolic content at 4.35. For *A. flavus* 1, the control recorded the highest phenolic content at 8.68, while AA at 1000 ppm resulted in the lowest phenolic content at 1.86. The increase in phenolic content, observed in some treatments, is consistent with the hypothesis that AA stimulates a defensive metabolic shift in date fruits. Additionally, previous studies have shown that nanoparticle treatments, such as Cr NPs, can activate plant metabolism to enhance phenolic synthesis, providing stronger antioxidant and defense responses in conjunction with increased PPO activity (Lipșa et al., 2020; Bayat et al., 2019; Xie, 2016). These findings highlight the complex biochemical interactions influencing phenolic accumulation under different treatment conditions.

Table 4*: In vivo* disease incidence of date mold under different ascorbic acid (AA) treatments.

Treatments	Total phenolic content (mg/g)		
	R. stolonifer 1	*A. niger* 1	*A. flavus* 1
AA 1000 ppm	3.22	2.98	1.86
AA 500 ppm	5.82	4.94	2.34
AA 250 ppm	6.34	5.2	7.66
Control	7.24	6.14	8.68
L.S.D. $P \geq 0.05$	1.64	1.64	1.64

4. Conclusion

The findings of this study demonstrate that specific fungi are associated with mold development on date fruits, and experimental evidence confirmed their role in causing mold. The application of ascorbic acid directly to date fruits proved highly effective in reducing the incidence and spread of mold caused by these fungi. These results highlight the importance of employing safe and effective resistance agents, such as ascorbic acid, to combat microorganisms associated with mold on date fruits. This approach not only helps preserve the quality of the fruits but also plays a crucial role in safeguarding human health.

References

Abdel-Sater MA, Saber SM, 1999. Mycoflora and mycotoxins of some Egyptian dried fruits. Bulletin of the Faculty of Science, Assiut University **28**(1-D): 91–107.

Abu-Zinada AH, Ali MI, 1982. Fungi associated with dates in Saudi Arabia. Journal of Food Protection **45**(9): 842–844.

Alghalibi SM, Shater ARM, 2004. Mycoflora and mycotoxin contamination of some dried fruits in Yemen Republic. Assiut University Bulletin for Environmental Research **7**(2): 19–25.

Anonymous, 2019. Global production statistics of dates. Faostat, Food and Agriculture Organization of the United Nations. Available at: http://www.fao.org/faostat.

Bayat M, Chudinova E, Zargar M, Lyashko M, Louis K, Adenew FK, 2019. Phyto-assisted green synthesis of zinc oxide nanoparticles and its antibacterial and antifungal activity. Research on Crops **20**(4): 725–730.

Bokhary HA, 2010. Seed-borne fungi of date-palm, *Phoenix dactylifera* L. from Saudi Arabia. Saudi Journal of Biological Sciences **17**(4): 327–329.

Ferracin LM, Frisvad JC, Taniwaki MH, Iamanaka BT, Sartori D, Schapovaloff ME, Fungaro MHP, 2009. Genetic relationships among strains of the *Aspergillus niger* aggregate. Brazilian Archives of Biology and Technology **52**(SPE): 241–248.

Gherbawy YA, 2001. Use of RAPD-PCR to characterize *Eurotium* strains isolated from date fruits. Cytologia **66**(4): 349–356.

Gherbawy YA, Elhariry HM, Bahobial AAS, 2012. Mycobiota and mycotoxins (aflatoxins and ochratoxin) associated with some Saudi date palm fruits. Foodborne Pathogens and Disease **9**(6): 561–567.

Gomez KA, Gomez AA, 1984. *Statistical*

Procedures for Agricultural Research. 2nd ed. John Wiley and Sons, New York, USA, 680 pp.

Heenan CN, Shaw KJ, Pitt JI, 1998. Ochratoxin A production by *Aspergillus carbonarius* and *A. niger* isolates and detection using coconut cream agar, Journal of Food Mycology **1**: 67–72.

Ibrahim S, Rahma MA, 2009. Isolation and identification of fungi associated with date fruits (*Phoenix dactylifera* Linn.) sold at Bayero University, Kano, Nigeria. Bayero Journal of Pure and Applied Sciences **2**(2): 127–130.

Kacem-Chaouche N, Dehimat L, Meraihi Z, Destain J, Kahlat K, Thonart P, 2013. Decommissioned dates: chemical composition and fermentation substrate for the production of extracellular catalase by an *Aspergillus phoenicis* mutant. Agriculture and Biology Journal of North America **4**: 41–47.

Khan TA, Mazid M, Mohammad F, 2011. Role of ascorbic acid against pathogenesis in plants. Journal of Stress Physiology & Biochemistry **7**(3): 222–234.

Leslie JF, Summerell BA, 2008. *The Fusarium Laboratory Manual*. John Wiley & Sons, New York, USA, 388 pp.

Lipșa FD, Ursu EL, Ursu C, Ulea E, Cazacu A, 2020. Evaluation of the antifungal activity of gold–chitosan and carbon nanoparticles on *Fusarium oxysporum*. Agronomy **10**(8): 1143.

Nassar MSM, 1986. Mycoflora associated with dates in Aswan area. MSc Thesis. Department of Botany, Faculty of Science, Assiut University, Egypt.

Ough CS, Amerine MA, 1988. *Methods for Analysis of Musts and Wines*. John Wiley & Sons, New York, USA.

Ragab WS, Ramadan BR, Sorour MA, Ahmed NA, 2011. Physical and chemical changes in fruits of three date palm (*Phoenix dactylifera* L.) cultivars grown in South Valley, Egypt. Journal of Food and Dairy Sciences **2**(11): 605–615.

Ragab WSM, Ramadan BR, Abdel-Sater MA, 2001. Mycoflora and aflatoxins associated with saidy date as affected by technological processes. *The Second International Conference on Date Palms*, UAE University, Al Ain, UAE, pp. 409–421.

Shenasi M, Aidoo KE, Candlish AAF, 2002. Microflora of date fruits and production of aflatoxins at various stages of maturation. International Journal of Food Microbiology **79**(1–2): 113–119.

Singh P, Singh J, Ray S, Rajput RS, Vaishnav A, Singh RK, Singh HB, 2020. Seed biopriming with antagonistic microbes and ascorbic acid induces resistance in tomato against *Fusarium* wilt. Microbiological Research **237**: 126482.

Xie J, Ming Z, Li H, Yang H, Yu B, Wu R, Yang ST, 2016. Toxicity of graphene oxide to white rot fungus *Phanerochaete chrysosporium*. Chemosphere **151**: 324–331.