The Pharma Innovation Journal 2023; SP-12(10): 1462-1464

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(10): 1462-1464

www.thepharmajournal.com
Received: 27-07-2023
Accepted: 29-09-2023

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Fungi associated with dates in West Bengal, India

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Abstract
On various synthetic media, the fungal counts per gram of air-dried dates from eight different date-palm isolates varied noticeably. The highest fungal counts were found in North 24 Parganas and Hooghly isolates, while the lowest were found in Bankura isolates. Numerous date isolates were typically associated with *Aspergillus flavus, Aspergillus niger, Fusarium* sp., *Pestalotia palmarum, Phytophthora* sp., *Diplodia phoenicum*, and *Cladosporium herbarum*. The fungi appeared to have colonized after the relative humidity was raised to 90% at 25 and 30 °C. The isolated fungi grew best at a glucose concentration of 60% in artificial media.

Keywords: Synthetic media, fungal count, isolate, colonized, relative humidity

Introduction
Dates are an essential and significant food for low-income families due to their high sugar, mineral, and vitamin contents and good stability (Yousif *et al.* 1976) [11]. There is a dearth of information on filamentous fungi related to date fruits. According to Nixon and Moore's 1939 report (Nixon and Neore), fruit spotting, early falling, and rotting were all caused by fungi that thrived during the humid weather conditions of harvest season. In France, Anselme and Balvaskis discovered that *Mauginiella soattae* severely rotted dates with a high commercial value. Michael and Sabet reported a similar rotting of several cultivated date palm varieties for the first time. According to Mrak *et al.* 1942 [8] some species of yeast were to blame for spoilage in California. *Saccharomyces bisporus* was isolated by Al-Bakir from pitted and un-pitted date fruits. There haven't been any prior reports of filamentous fungi colonizing date fruits after harvest (in storage) and causing rotting and spoilage of the fruits.

Materials and Methods
In this study, different isolates were obtained from various date palm plantations in West Bengal's. They were all roughly the same stage of maturity, ripe and soft on all the fruits. Each variety's composite samples underwent a 48-hour air drying period after being washed. The samples were then homogenized in a Waring blender at a rate of 30 grams each. After being suspended in sterile tap water for up to 500 ml, the homogenates were mixed with a mechanical shaker for 25 to 40 minutes. The Waksman (Waksman) dilution plate method was used. The number of fungal colonies per dish was counted, noted, and isolates were made after plated samples were incubated for 3 to 6 days at 28 °C. One week after plating, the plates were examined once more to identify and document any slow-growing fungal colonies.
The fungi were isolated and kept alive on Czapek-Dox agar (CZA), Potato-Dextrose agar (PDA), and Malt extract agar (MEA) media. On PDA, each isolate's total fungal count was determined by using 30, 60, and 90% D-glucose and 5 mg of erythromycin per 100 ml of medium. The media was supplemented with chloramphenicol to prevent bacterial growth. Except where noted, all glassware and media were sterilized before use, and aseptic procedures were followed when isolating fungi.
Magnesium chloride ($MgC1_2$, 6 H_20), magnesium nitrate ($MgN0_3$, 6 H_20), barium chloride ($BaCl_2$, 2 H_20), and ammonium phosphate (NH_4H_2 $P0_4$) were all present in the humidity chambers. At 20, 30, and 38 °C, the first two chemicals maintained relative humidity levels of about 30 and 50%, respectively, while the third chemical and the fourth chemical maintained relative humidity levels of about 90% in both cases, at 20 °C and 25 °C and 30 °C, respectively. After 8 weeks of incubation, final observations on such chambers were recorded.

Results

Fig. 1 displays the total fungal counts as hundreds/g of air-dried samples. The values ranged from 25/g for Bankura isolates to 400 and 680 /g for North 24 parganas and Hooghly isolates, respectively. Czapexdox agar generally had the highest fungal count, with the exception of Bankura isolates.

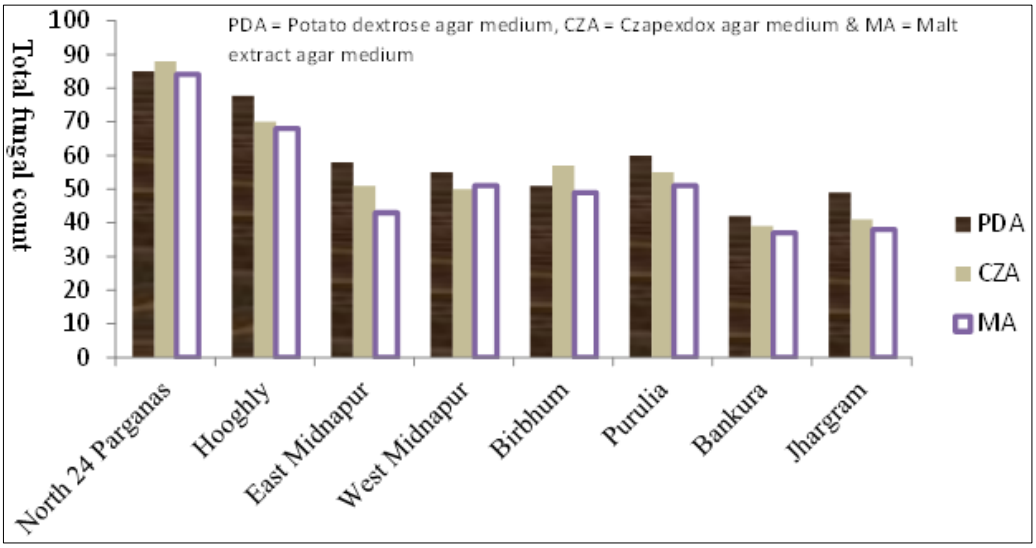

Fig 1: Fungal counts of dates when tested with three different media

The lowest was on Malt extract agar with the exception of North 24 parganas, Hooghly, Birbhum, Purulia isolates which showed lower counts when PDA was used. A few yeast colonies occasionally appeared, especially when using West Midnapur dates isolates. Although these colonies were counted as part of the total, it should be noted that no attempts were made to isolate them at this point in the work. Future research will focus on these elements.

Table 1 lists isolated fungi for all date varieties in order of their abundance. With the exception of West Midnapur isolate, all of the different date isolates were completely colonized when relative humidity was increased to 90% and temperatures were between 30 and 40 °C (Table 2). Isolates from the West Midnapur, however, exhibited no discernible growth in these circumstances. On PDA enriched with 30, 60, and 90% glucose, the growth of all date varieties showed distinct differences. For example, (a) on 30%, the maximum count was observed for the East Midnapur, West Midnapur, Birbhum, Bankura and Jhargram, isolates; (b) on 60%, the maximum count was observed for the West Midnapur, Birbhum, Bankura and Jhargram isolates; and (c) on 90%, all Isolates responded, differently.

Discussion

According to the results, total fungal counts differ noticeably between different date varieties and growth media. The abundance of *Fusarium* sp, *Phytophthora* sp. *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigates and Pestalotia palmarum* isolated from various date varieties appeared to be associated with the optimal sugar concentration of the varieties and also indicated that these strains of the fungi were osmotolerant. The differences in fruit sugar content may also be the cause of variations in the colonization of these fungi on various date varieties.

The West Midnapur isolate's organisms grew poorly on both its host and synthetic media. This disease may be caused by an antifungal substance found in dates or by an organism producing an antifungal substance that may be harmful to the organism's own growth.

Additionally, the growth activity of the fungi increased when these organisms were cultured on a medium with a high sugar concentration, indicating that the antifungal component the organism may produce was neutralized by the presence of sugar in the medium. *Alternaria brassicicola* was able to proliferate because Dunn *et al*. (Dunn *et al.*) demonstrated that D-glucose hindered the fungicidal activity of ethylene thiuram disulfide (ETD) *in vitro*.

Table 1: Fungal genera and species isolated from different dates varieties on Potato dextrose agar (PDA), Czapexdox agar (CZA) and Malta extract agar (MA) media

	North 24 parganas	Hooghly	East Midnapur	West Midnapur	Birbhum	Purulia	Bankura	Jhargram
Potato dextrose agar (PDA) medium								
Fusarium sp.	+	+	-	-	-	-	-	+
Phytophthora sp.	+	+	-	-	+	+	-	+
Diplodia phoenicum	-	+	+	+	-	+	-	-
Alternaria sp.	-	-	-	-	-	-	-	+
Aspergillus niger	+	+	+	+	+	+	+	-
Aspergillus flavus	+	-	+	-	-	+	+	-
Aspergillus fumigatus	+	+	-	-	-	-	+	-
Pestalotia palmarum	-	-	-	-	-	+	+	+
Cladosporium herbarum	-	+	-	+	+	-	-	-
Czapexdox agar (CZA) medium								
Fusarium sp.	+	+	+	-	-	-	+	+
Phytophthora sp.	+	+	-	+	-	-	-	-

Diplodia phoenicum	-	+	-	-	-	-	-	-
Alternaria sp.	+	+	-	+	-	+	-	+
Aspergillus niger	+	+	-	+	-	-	-	+
Aspergillus flavus	+	+	-	-	-	+	+	-
Aspergillus fumigatus	+	-	-	+	-	+	-	-
Pestalotia palmarum	-	+	+	-	-	-	-	-
Cladosporium herbarum	-	-	-	-	--	-	-	-
Malta extracto agar (MA) media								
Fusarium sp.	+	+	-	-	-	-	+	-
Phytophthora sp.	-	-	-	-	-	+	+	+
Diplodia phoenicum	-	+	-	+	+	-	-	-
Alternaria sp.	+	+	-	-	-	-	-	+
Aspergillus niger	+	+	-	-	+	+	-	+
Aspergillus flavus	-	+	+	+	-	+	-	-
Aspergillus fumigatus	-	-	-	-	-	-	-	+
Pestalotia palmarum	+	+	+	+	+	+	+	-
Cladosporium herbarum	+	-	+	-	-	+	+	-

+=Fungus present & - = Fungus absent

Table 2: Fungal colonization and keeping quality of different date isolates incubated at various temperatures and relative humidities

Isolates	30% Relative humidity			60% Relative humidity			90% Relative humidity		
	20 °C	25 °C	30 °C	20 °C	25 °C	30 °C	20 °C	25 °C	30 °C
North 24 parganas	Good	Good	Bad	Good	Worse	Worse	Bad	Worse	Worse
Hooghly	Good	Good	Good	Good	Good		Good	Worse	Worse
East Midnapur	Good	Worse	Worse	Good	Good	Good	Good	Worse	Worse
West Midnapur	Good	Good	Worse	Good	Good	Good	Bad	Good	Worse
Birbhum	Good	Good	Bad	Good	Good	Bad	Worse	Worse	Worse
Purulia	Good	Good	Bad	Good	Good	Bad	Bad	Bad	Worse
Bankura	Good	Good	Good	Good	Good	Good	Good	Worse	Worse
Jhargram	Good	Good	Good	Good	Good	Good	Bad	Bad	Bad

Good = Date not decayed with no visible colonization,
Worse = Colonized and decayed by fungi.
Bad = Slightly decayed but not colonized by fungi

Conclusion

El-Abyad and Ismail also noted that adding glucose to organic soil caused some fungi's germ tubes to grow longer and considerably reduced the partial suppression of soil Fungistasis. Attention should be paid to the isolation of A. flavus from all tested date isolates. It is generally known that this fungus contributes to the creation of Aflatoxin (Allcroft *et al*. 1963, and Lancaster *et al*. 1961) [2, 6].

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