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Control of Fungi Isolated from Date Palm Fruit in Yola, Adamawa State

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Abstract
Samples of dates were purchased from four (4) major markets in Yola, and transferred to Modibbo Adama University of Technology. Isolation of moulds associated with the rot was carried out on solid Potato Dextrose Agar (PDA) on 9 cm diameter Petri-dish. Four fungal isolates were obtained and proved through pathogenicity test to be the responsible pathogens. These were Aspergillus niger, Fusarium solani, Scopulariopsis brevicaulis, and Rhizopus stolonifer. In-vitro and in-vivo control trials with ash and garlic oil showed effective mycelia growth inhibition at p= 0.001. Inhibition improved with increase in concentration of the garlic oil and ash. Heat treatment equally proved worthwhile with significant inhibition compared to the control at p= 0.001. Increase in temperature (0-550C) was rewarded with better control effect.
Keywords: Date palm, Fungi

1. Introduction

Date palm (*Phoenix dactylifera* L.) is a palm in the genus *Phoenix*, cultivated for its edible sweet fruit (Wikipedia, 2014). Dates are widely distributed facilitated by the fact that dates lend themselves perfectly to being carried along as a high calorie food, with a long-keeping quality (FAO, 2012). Date fruit also can be made into juice, vinegar, wine, beer, sugar, syrup, honey, chutney, pickle, paste, dip, and food flavoring (Barreveld, 1993 and Glasner *et al.*, 2002). It is an ideal food for all age phases, providing the most important essential nutrients like protein, fiber, carbohydrates, fat and minerals (Al-Farsi *et al*., 2005 and Vyawahare *et al.,* 2009).
The plant is affected by various pests and diseases, but the nature of the problem varies with geographic location (Howard *et al.*, 2001). Atia (2011) observed that the date palm fruits are mostly loaded with mixture of microbes: bacteria, moulds and yeast but people go ahead eating after clearing the pericarp, while some eat it whole irrespective of the state of the pericarp. Species of *Alternaria, Aspergillus, Fusarium* and *Penicillium* cause fruit rots of date-palm (Bokhary, 2010).

A report stated that the major agents of date palm spoilage are moulds, followed by bacteria and yeast at all stages of ripening on trees, as well as during storage and processing (Amal *et al.,* 2014). Effects of rot causing organisms along with size, colour, texture, cleanliness, freedom from defects (sunburn, insect damage, sugar migration to surface, fermentation) are the important considerations affecting fruit quality (ChihCheng and Robert, 2007)

Some pathogens and potential mycotoxin producing microbes isolated from date fruits include species of *Aspergillus, Penicillium, Alternaria* and *Fusarium* (Kader, 2007; Hamad, 2008; Hayrettin *et al*., 2012; Aido *et al*., 1996; El-Sherbeeny *et al*., 1985 and Abdulsalam *et al*., 1991).

Hawking of date palm fruits is a common sight especially north of Nigeria were Yola is located. The high patronage this merchandise attracts (both fresh and dried fruits) is a cause for pathological concern in light of reports indicating that some potential mycotoxin producing moulds are implicated. Also, Christensen *et al*. (2007) reported that the agricultural industries sustained huge crop losses as a result of fungal diseases of fruits and plants. The Food and Agricultural Organization of the United Nations (FAO) has estimated that up to 25% of the world's food crops are significantly contaminated with mycotoxins (WHO, 1999). Mycotoxins are highly toxic and cause severe intoxications in human and animals, some of them are carcinogens (Khomutov *et al*., 2011).

Amal *et al*. (2014) observed that methods that will curb the microbial infestation of fruits at post-harvest storage are required and should be made mandatory. The use of fungicides for the control of plant diseases is a common practice all over the world (Wazir, 2013). The use of chemical in control of plant disease poised a risk to the survival of human race (UNEP, 1995). Therefore there is need for other control means to this important menace.

Reports on the successful control of certain moulds associated with postharvest rot of crops are available (Agbeniyi, 1988; Channya and Chimbekujwo, 2002; Bristone *et al*., 2011).

Many spices and herbs exert antifungal activity due to their essential oil fractions. The thyme oil had strong fungicidal effect on *Penicillium* and *Alternaria* (Mironescua and Georgescub, 2008), *Alternaria alternate* (Hadizadeh *et al.,* 2009). The essential oil of *Mentha spicata* showed cidal effect on mycelial growth of *Aspergillus ochraceus*, *Penicillium digitatum*, *Pyricularia oryzae* and *Alternaria alternate* (Singh *et al.*, 2006). Essential oil of *Ocimum gratissimum* inhibited the growth of *Botryosphaeria rhodina*, *Rhizoctonia* spp and two strains of *Alternaria* spp (Terezinha *et al*., 2006). Post-harvest treatment with heat has been used to reduce decay

in several crop including pumpkin, strawberry and apple (Spotts and Chen, 1987).

This study seeks to try out garlic oil, ash and heat treatments as alternative control to this important menace that puts to waste this important crop with an antecedent health risk to consumers.

2. Material and Methods

The research was conducted in 2015. Date palm (*Phoenix dactylifera* L.) showing spoilage was obtained from four major markets in Yola which are Jimeta Old Market, Jimeta Ultra-Modern Market, Jimeta Shopping Complex and Yola Town Market. A sample of 250 fruits were randomly collected from sellers in each market. These were brought to the laboratory of Plant Science Department, Modibbo Adama University of Technology (MAUTECH) Yola, where isolation, pathogenicity test and control trials were carried out.

2.1. Isolation of Fungi from Date Palm Fruits

Under aseptic conditions the diseased sample from date palm fruit showing rot was sectioned into approximately 5 mm square with a heat-sterilized scalpel. The pieces were immersed into 1% sodium hypochlorite contained in a sterile 9 cm diameter Petri- dish for surface sterilization for 30 seconds using sterile forceps. The sterilized pieces were rinsed in three changes of sterile distilled water and then dried between sterile filter papers. With a flamed and cooled pair of forceps, a sterilized piece of date palm was blotted dry between sterile filter papers, then plated aseptically on 9 cm diameter Petri-dish containing sterile solidified Potato Dextrose Agar (PDA) and incubated at room temperature of 33 ± 2^0 C for 7 days. Sub- culturing on fresh sterilize PDA using the method of Klick and Pitt (1988) and Robert *et al*. (1996) was carried immediately when new colonies began to grow through hyphal-tip transfer.

2.2 Identification of Fungi

Microscopic examination was carried out to observe the structure and characteristics of the fungal isolates in addition to macroscopic cultural observations. A sterile needle was used to pick a little portion of the hyphae containing spores and placed on a sterile glass slide stained with Lactophenol cotton blue and examined under the photographic microscope using the method of Fawole and Oso (1995). Micrograph of the isolates showing (conidia etc.) were taken. The morphological and cultural characteristics observed were compared with structures in the identification guides of the International Mycological Institute Kew and of Hunter and Barnet (1998).

2.3. Pathogenicity Test

Healthy date fruits (semi ripe) were obtained and surface-sterilized with 1% sodium hypochlorite for 30 seconds and rinsed in three (3) changes of sterile distilled water according to the method of Chukwuka *et al.* (2010). A sterile cork borer (2 mm in diameter) was used to puncture and inject healthy date fruits with spores' suspension of each isolated fungus in three replicates. Removed tissue was replaced and vasper jelly was smeared to completely seal each hole to avoid contaminant. It was kept at room temperature of $33\pm2\ ^0$C. A similar set up was placed as control using distilled water to complement the fungal inocula. The set up was arranged in a completely randomized design. It was incubated for seven (7) days to allow for possible rot development and the isolates were re-isolated from the new host and shown to be the same as the originally inoculated pathogen.

2.4. Control Trials with Ash, Garlic oil

In-vitro control was carried out using food poisoning method of Raja *et al*. (2009). Different weights (grams) of heat-sterilized tomato leaf ash (at 160^0C for 1 hour in an oven) of 1g, 3g, and 5g were added aseptically using a heat-sterilized spartula on cool sterilized PDA medium in conical flask swirled to mix before pouring into 9 cm Petri-dish, then allowed to solidify, followed by inoculation with 2 mm radius of 7-day old pure culture of each of the isolated fungi sectioned with a sterile cork-borer (*Aspergillus niger*, *Fusarium solani*, *Scopulariopsis brevicaulis* and *Rhizopus stolonifer*). Similar set up was made with ash free to serve as control. The fungi were inoculated at the centre of 9 cm diameter Petri-dish sealed with masking tape. The set up were inoculated for seven (7) days at room temperature of 33 ± 2^0 C in the month of June and radial mycelial growth of each isolate was measured with caliper, rule, and with the aid of hand lens and recorded for analysis.

Different volumes of garlic oil were tested [0ml (control)], 2 ml, 3 ml and 5 ml]. These were added aseptically to cool sterile PDA medium in conical flasks and agitated before pouring into Petri-dishes. Similarly, 2 mm radius of 7-day old pure culture of each of the isolated fungi was inoculated on each of the media. Oils free medium served as control treatment. Petri-dishes were sealed with masking tape and similarly incubated and assessed. All *in-vitro* settings were of completely randomized design replicated thrice.

The method of Channya (1991) was used for the *in-vivo* test, in which a sterile 2 mm diameter cork borer was used to puncture and inject sterilized healthy semi-ripe fruits with spores' suspension of 7-day old pure culture of each of the isolated fungi using sterile needle. Weight of 0.5 g, 1.0 g and 1.5 g of ash was subjected to

the wounded date palm fruits. Inoculated fruits were put in sterile transparent polythene bags and incubated as for the *in-vitro* set up. Similar set up was made with ash free to serve as control. Three replicates for each treatment was used and plated at the centre of the 9 cm diameter Petri-dish sealed with masking tape. Radial rot of fruits was measured with caliper and rule and with the aid of hand lens.

A sterilized sample of date palm fruit was punctured with a sterile 2 mm diameter cork borer and spores' of the isolated fungi were inoculated to it as stated above. Similarly, 0.2, 0.3, and 0.4 ml of garlic oil were injected respectfully into the wounded date palm fruits. Oil free medium served as control. The set up was a completely randomized design. The set up were inoculated for seven (7) days at room temperature 33 ± 2^0 C and radial rot of each fruit was measured with caliper and rule, and with the aid of hand lens.

2.5 Control using heat

Effect of heat on the fungal isolates was carried out using the method of Harrak and Chetto (2002) and Harrak (2003). An electric oven was set at four temperature regimes of room temperature of 33^0 C (control), 45^0 C, 50^0 C and 55^0 C for 3 hours. The isolates was inoculated into healthy semi-ripe date palm fruits by using a sterile 2 mm diameter cork borer to puncture and inject sterilized fruit with spores' suspension of the isolated organisms using 2.0 ml syringe. Samples were transferred into open glass Petri-dishes (9 cm in diameter) with a filter paper inserted to the lower lid, then incubated in an electric oven according to the above-stated temperatures. After treatment on hot air, samples were inoculated for seven (7) days and the filter paper was moistened with sterile distilled water once daily throughout the period. The set up was a completely randomized design in three replicates. The radial rot of each fruit was measured with caliper and rule, and with the aid of hand lens.

Results were analysed using Statistical Analysis System (SAS) version 7 and the means that were significant were separated using the Least Significant Difference (LSD).

3. Result and Discussion

Aspergillus niger, *Fusarium solani*, *Scopulariopsis brevicaulis* and *Rhizopus stolonifer* were the isolates that were associated with the rot of date palm fruits in Yola markets. The pathogenicity tests on these isolates proved they were the rot pathogens.

Analysis of variance for the efficacy of tomato ash on these moulds ($p<0.0001$) was very significantly different from the control set up for both *in-vitro* and *in-vivo* tests. The performance of ash at different levels is shown in Tables 1 and 2 respectively. There was however a considerably lower effect of ash on *Scopulariopsis brevicaulis* than the other moulds. Higher quantity of ash produced a corresponding better control. This collaborates reports by Channya and Chimbekujwo (2002) that wood ash effectively controlled fungal rot of plantains (*Musa parasidiaca* L.). Agbeniyi (1988) also reported that when kola nut (*Cola nitida*) was treated with ash of *Vernonia* spp, *Tectonia grandis* and *Cassia siemia* before storage, it reduced the incidence of moulds. Bristone *et al*. (2011) reported that when tubers of sweet potatoes were treated with wood ash, rot caused by *Rhizopus stolonifer* and *Penicillium expansum* was reduced to minimal level. Agbeniyi (1988) and Channya (1991) adduced the performance of ash to pH of ash that is highly alkaline. The ash of tomato was found to have a pH of 9.

Control trials with garlic oil *in-vitro* and *in-vivo* produced even a better growth inhibition of all the moulds at $p<0.0001$. Tables 3 and 4 show the effect of the different concentrations on the mycelial radial growth of the moulds with *Aspergillus niger* being the most inhibited compared to the control while *Scopulariopsis brevicaulis* been the least affected by garlic oil *in vitro*. *In-vivo* test result however shows that *Scopulariopsis brevicaulis and Rhizopus stolonifer* were the most susceptible to garlic oil control. Inhibition improved with increase in concentration of garlic oil for both *in-vitro* and *in-vivo* tests. The result is also similar to that of Atia (2011) who reported that increased in oil concentration resulted in reducing percentage of mycelia growth of the tested fungi. Garlic oil (*Allium sativum*) contain diallyl thiosulfinate as an active compound (Burt, 2004; Zaika, 1988; Ceylan, 2004). *Allium sativum* (garlic) juice extract belongs to such non-traditional treatments and among the natural fungicide substances, it has been found most active against many fungal species (Curtis *et al*., 2004; Slusarenko *et al*., 2008). Allicin is the most important biologically active substance of *A. sativum* crude extract; it is formed from its precursor, alliin, by the action of allinase enzyme (Josling, 2003). Pharmacologically, allicin is the most important and the most active substance and it is found in the fresh extract of Allium (Vasile *et al*., 2012).s

Alice and Rao (1987) observed that *A. sativum* extracts significantly reduced seed infection by *Drechslera* on rice and treated seeds had significantly higher viability. On barley in green house and field experiments, allicin used as elicitor was as effective as fungicide against the leaf spot severity caused by *Bipolaris sorokiniana* (Silva *et al*., 2001; Rodrigues *et al.,* 2002; Rodrigues and Bach, 2003; Antoniazzi *et al*., 2008). Garlic extract treatment of wheat seeds significantly reduced the incidence of seed-borne fungi, increased seed germination, the number of healthy seedlings and vigour index (Grozav and Foarce, 2005; Khalaf *et al*., 2011).

Table 1: Effect of Ash on Fungal Pathogens of Date Fruit *In-vitro*

	Means for mycelial radial growth (mm)			
Concentration (g)	*A. niger*	*F. solani*	*S. brevicaulis*	*R. stolonifer*
1	8.26	8.23	12.53	30.21
3	7.75	6.65	12.01	28.61
5	6.62	5.51	8.90	16.80
0	17.28	17.18	13.98	40.23
LSD (0.0001)	1.21	1.26	1.53	4.47

Table 2: Effect of Ash on Fungal Pathogens of Date Fruit *In- vivo*

	Mean of mycelial radial growth (mm)			
Concentration (g)	*A. niger*	*F. solani*	*S. brevicaulis*	*R. stolonifer*
0.5	8.89	6.10	6.52	6.52
1.0	7.09	5.63	3.93	5.78
1.5	3.59	4.94	1.49	4.72
0	10.43	6.12	8.26	7.94
LSD (0.0001)	1.19	0.56	1.31	0.70

A report by Hassan *et al.* (2005) showed that garlic completely controlled the intensity of *B. sorokiniana* and *Fusarium* spp after the treatment of wheat seeds. It is generally accepted that the essential oil components act on the functionality and the structure of the cell membrane (Viuda-Martos *et al*., 2008). Low concentrations result in changes of the cell structure, inhibiting respiration and changing the permeability of the cell membrane, whereas high concentrations lead to severe membrane damage, loss of homeostasis and cell death (Carson *et al*., 2002).

Conner and Beuchat (1984) suggest that the antifungal activity is the product of essential oil components' interaction with enzymes responsible for energy production and the synthesis of structural compounds of the cell. On the other hand, Omidbeygi *et al*. (2007) suggested that the essential oil components pass through the cell membrane, integrating with enzymes and proteins of membranes, causing loss of macromolecules from the interior of the cell, leading to changes in the cell and ultimately to its death. Cristani *et al*. (2007) indicate that the antifungal activity of terpene relates to their ability to act not only on the permeability, but also on other functions of cell membranes. These components can pass through the cell membrane and interact with intracellular structures. Daferera *et al.* (2000) state that fungitoxic effect of essential oil is a consequence of hydrogen bonds formation between hydroxyl groups of phenolic compounds and active sites of cellular enzymes.

According to Sharma and Tripathi (2006), the active components cause loss of integrity of the cell wall, and thus the loss of cytoplasmic constituents from the cells of hyphae. Lucini *et al.* (2006) indicated that the inhibition of mycelial growth of fungi is caused by monoterpenes. These components can increase the concentration of lipid peroxides, such as hydroxyl, alkoxyl and alkoperoxy radicals, leading to the destruction of cells.

It has been observed that application of essential oils for postharvest disease control of fresh product, as a novel emerging alternative to hazardous anti-fungal treatments will allow a safer and environmentally more acceptable management of post-harvest diseases (Hadizadeh *et al*., 2009). The antifungal activity of the essential oils is different, depending on the mould type (Mironescua and Georgescub, 2008). Suwitchayanon and Kunasakdaku (2009) reported that the inhibitory effects of plant oils might be regarded as cidal agent against fungal growth and showed abnormal conidia and malformations as swollen, often septated and pale colour of hypha.

Table 3: Effect of Garlic Oil on Fungal Pathogens of Date Palm Fruit Rot (*In- vitro)*

	Mean of radial mycelia growth (mm)			
Concentration (ml)	*A. niger*	*F. solani*	*S. brevicaulis*	*R. stolonifer*
2	10.35	12.65	13.05	35.03
3	9.55	10.24	9.93	33.61
5	7.23	4.71	6.10	27.51
0	17.28	17.18	13.98	40.23
LSD (0.0001)	1.21	1.43	4.24	1.24

Table 4: Effect of Garlic Oil on Fungal Pathogens of Date Palm Fruit Rot (*In- vivo*)

	Mean of radial mycelia growth (mm)			
Concentration (ml)	*A. niger*	*F. solani*	*S. brevicaulis*	*R. stolonifer*
0.2	7.16	4.62	3.90	3.23
0.3	4.33	3.39	3.39	1.19
0.4	3.40	2.04	1.29	0.91
0	10.43	6.12	8.26	7.94
LSD (0.0001)	1.38	0.74	1.20	1.16

Treated date fruit with heat showed effective growth inhibition as compared with control at p=0.0001 for all the pathogens. Temperature increase within the range of 45-55 ^{0}C had a corresponding improvement in mycelial growth inhibition. *Scopulariopsis brevicaulis* and *Fusarium solani* were the most susceptible to temperature control (Table 5). These temperature regimes did not change the physiology of the fruit. This is also similar with the result of Atia (2011) whose treatment on Deglate and Elak varieties of date reduced the number of date fruits infected by fungi compared with the untreated controls. Similarly, Harrak and Chetto, (2002) and Harrak, (2003) reported that heat treatment at 60 for 1-1.5 h lead to complete control other organisms on stored date and did not change its physical or chemical characters. Kader and Hussein (2009) also reported that, drying the dates to 20% moisture or lower greatly reduced incidence of moulds and yeasts.

Table 5: Effect of Heat on Fungal Agents of Date palm Fruit Rot

	Mean of radial mycelia growth (mm)			
Temperature (^{0}C)	*A. niger*	*F. solani*	*S. brevicaulis*	*R. stolonifer*
45	6.64	3.51	2.63	3.27
50	6.01	1.86	0.76	2.97
55	5.98	1.12	0.47	0.58
0	10.43	6.12	8.26	7.94
LSD (0.0001)	1.01	0.86	1.41	1.11

4. Conclusion

Fungal contamination is a direct relationship with both the physical initial dates and environmental conditions of the premises including the storage temperature and humidity with consequent decrease on the market value. The sensitivity of dates to fungal spoilage is related to its poor conservation of places of production and storage which is a big challenge to operators. This alteration is particularly important as the storage conditions and storage are inadequate

Therefore ash, garlic oil and heat have the potential to reduce the date loss and consumer exposure to mycotoxin poisoning and these are more environmentally friendly than fungicides.

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