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Growth Inhibition of *Aspergillus niger* and *Penicillium italicum* by Seed Kernel Oil from Mango (*Mangifera indica* L.)

Adesina, I. A.[1]*, Adebote, V. T.[1], Elehinafe, T. R.[1], and Enerijiofi, K. E.[2]
[1]Department of Science Laboratory Technology, Faculty of Applied Sciences, Rufus Giwa Polytechnic, P.M.B. 1019, Owo, Ondo State, Nigeria
[2]Department of Biological Sciences, College of Basic and Applied Sciences, Samuel Adegboyega University, P.M.B. 001, Ogwa, Edo State, Nigeria
* E-mail of the corresponding author: isaacadesina@gmail.com

Abstract
The present study aims to evaluate the fungal inhibitory activity of seed kernel oil extracted from mango (*Mangifera indica* L.) fruit. Mango is one of the most important tropical fruits that is abundant in Nigeria. Thus, there is an abundant supply of mango seed kernels which are considered as wastes after consumption or industrial processing of mango fruits. The oil was extracted from the mango seed kernels by solvent extraction process. Agar dilution assay was used to evaluate the inhibitory activity of the mango seed kernel oil against *Aspergillus niger* and *Penicillium italicum*. The mango seed kernel oil (MSKO) was found to inhibit growth of the two fungi tested at different concentrations (1.33%, 2.67% and 4.0%). 4% concentration of MSKO in the agar brought about a reduction in the mycelial growth diameter of *A. niger* at Day 7 from 44.0 ± 1.41 mm (control) to 35.5 ± 0.71 mm while the reduction in the mycelial growth diameter of *P. italicum* at Day 7 was from 44.0 ± 14.14 mm (control) to 33.0 ± 0.00 mm. Mango seed kernel oil produced mycelium growth inhibition of *A. niger* and *P. italicum* at 4% concentration, with percentage inhibitions of 19.32% and 25.0%, respectively. The results revealed that *P. italicum* was more sensitive to the mango seed kernel oil than *A. niger*. These results suggest that mango seed kernel oil could have potential applications in the food industry to prevent fungal-associated food spoilage from important pathogenic genera *Aspergillus* and *Penicillium*.
Keywords: Growth inhibition, Mango seed kernel oil, *Aspergillus niger*, *Penicillium italicum*, Inhibitory effect

1. Introduction
Mango (*Mangifera indica* L.) is a member of the *Anarcadiaceae* family which comprises more than 70 genera. Historical records suggest that its cultivation as a fruit tree originated in India more than 4000 years ago (Mukherjee, 1998).
With a growing world production, the mango represents one of the most important tropical fruits and is produced worldwide. Mango production is, however, quite concentrated, since Asia accounts for approximately 77% of global mango production, and America and Africa account for the remaining 23% (FAOSTAT, 2007).
The mango is an important fruit for human nutrition in several parts of the world. It is a tropical fruit widely accepted by consumers throughout the world for its succulence, sweet taste and exotic flavour, being called the King of fruits (Ramteke *et al*., 1999).
It is generally observed that after consumption or industrial processing of mango fruits, considerable amounts of mango seeds are usually discarded as waste. Hence, the utilization of mango by-products especially mango seed may be an economical way to reduce the problem of waste disposal from mango production (Kittiphoom, 2012).
Fungal spoilage of food is a common problem occurring in most parts of the world. Pitt and Hocking (1999) estimated that 5 – 10 % of the world's food production is lost as a result of fungal spoilage. Fungi have the ability to grow in a wide variety of foods, with different genera showing affinity for particular food types. *Aspergillus* and *Penicillium* species are known for the spoilage of stored cereals and other postharvest food products (Filtenborg *et al*., 1996). Araguas *et al*. (2005) reported that *Aspergillus* and *Penicillium* species produce mycotoxins which may be carried over into the finished product.
These spoilage organisms are generally controlled by synthetic chemicals. The increasing consumers demand for a reduced use of chemical preservatives or additives in food or feed has led to our investigation of mango seed kernel oil as potential biological control agent for the control of *Aspergillus niger* and *Penicillium italicum*. In this study, we reported the extraction of seed kernel oil from mango (*Mangifera indica* L.) and its inhibitory effect on growth of *Aspergillus niger* and *Penicillium italicum in vitro*.

2. Materials and Methods
2.1 *Plant Materials*
Seeds of mango (*Mangifera indica* L.) were collected from fruit orchards around Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria during May to July, 2012.

2.2 *Preparation of Mango Seed Kernel Powder*

The mango seed kernel was separated manually from the enclosed hard cover. The kernel was sundried in the open, until the casing splits and sheds the seeds. The kernels were further dried in the sun in order to reduce its moisture content. Commercial grinder was used to crush the kernels in fine powdered form.

2.3 *Extraction of Oil from Mango Seed Kernel Powder*

The method of AOAC (1990) was used in extracting oil from mango seed kernel. The kernel powder (100 g) was placed in the thimble and about 300 ml of n-hexane was poured into the round bottom flask. The apparatus was heated at 60 ^{o}C and allowed to stay for 5 hrs under continuous extraction using Soxhlet apparatus. At the end of the extraction, the resulting mixture containing the oil was distilled off to recover solvent from the oil.

2.4 *Fungal Isolates*

The food-pathogenic fungi; *Aspergillus niger* and *Penicillium italicum* were obtained from Microbiology laboratory of the Federal University of Technology, Akure, Ondo State; and maintained on Potato Dextrose Agar (PDA) slants in the refrigerator (4 ^{o}C) prior to use.

2.5 *Antifungal Activity*

The inhibitory effect of the mango seed kernel oil was tested by the agar dilution method (Viuda-Martos *et al*., 2008). The oil tested was added to Potato Dextrose Agar medium at a temperature of 45 ^{o}C, and then poured into Petri dishes. Concentrations 1.33%, 2.67% and 4.0% were tested for mango seed kernel oil. The fungi were inoculated as soon as the medium had solidified. A disc (9 mm in diameter) of mycelial material, taken from the edge of five-day-old fungi cultures, was placed at the centre of each Petri dish. The Petri dish with the inoculum was then incubated at room temperature (28 ^{o}C). The efficacy of treatment was evaluated each day during seven days by measuring the diameter of the fungus colonised. The values were expressed in millimetres diameter/day. All tests were performed in duplicate.

2.6 *Statistical Analysis*

Conventional statistical methods were used to calculate means and standard deviations. Data were statistically tested for one-way analysis of variance (ANOVA) using SPSS computer software program and Duncan's multiple range tests were applied for comparing means.

3. Results and Discussion

Mango seed kernel oil at the concentrations assayed all showed the capacity to reduce or inhibit the growth of *A. niger* and *P. italicum*. Tables 1 – 2 show the growth of *A. niger* and *P. italicum*, respectively, during the seven days under study. Figure 1 shows the percentage (%) growth inhibition obtained with the oil at day 7.

There was a statistically significant difference between the mycelial growth of *A. niger* when the control was compared to the different treatments of mango seed kernel oil (Concentrations 1.33%, 2.67%, 4.0%) from Day 2 to Day 7. The different concentrations of mango seed kernel oil show vary degrees of inhibition against *A. niger*, with 4.0% concentration showing the highest mycelial growth inhibition (Table 1). It can be shown from Table 1 that 4.0% concentration of mango seed kernel oil (MSKO) in the agar brought about a reduction in the mycelial growth diameter of *A. niger* at Day 7 from 44.0 ± 1.41 mm (control) to 35.5 ± 0.71 mm.

There was also significant difference between the control and the concentrations used against *P. italicum* from Day 1 to Day 5 but there was no statistical difference between the control and the concentrations on Day 6 and Day7, only slight different was observed (Table 2). It can be shown from Table 2 that 4.0% concentration of mango seed kernel oil in the agar brought about a reduction in the mycelial growth diameter of *P. italicum* at Day 7 from 44.0 ± 14.14 mm (control) to 33.0 ± 0.00 mm.

Mango seed kernel oil produced mycelium growth inhibition of *Penicillium italicum* at 1.33%, 2.67%, 4.0% concentrations, with percentage inhibitions of 23.86%, 26.14% and 25.0%, respectively; while mycelium growth of *Aspergillus niger* had percentage inhibitions of 6.82%, 14.77% and 19.32%, respectively, at the same concentrations (Figure 1).

Among these two moulds, *P. italicum* seemed to be more sensitive to the mango seed kernel oil than the *A. niger*. The highest concentration (4.0%) of mango seed kernel oil brought about 25% percentage inhibition upon the mycelial growth of *P. italicum* while 19.32% percentage inhibition was observed in the case of *A. niger*.

Yu *et al*. (2005) reported that mango seed kernel oil is rich in oleic acid, and exhibited 41 – 44% of total fatty acid. Stearic acid is the other major fatty acid in mango seed kernel oil and may account for up to 57% of the total fat (Yu *et al*., 2005). In addition, palmitic and linoleic acids were detected in the oil along with trace amounts of α-linolenic acid (Rao, 1994). This shows that mango seed kernel oil is edible. Edible seed oils are important common food ingredients. Fatty acids are primary nutritional components found in edible seed oils. Growing evidence has suggested that individual fatty acids may play different roles in human health. Diets rich in a specific fatty acid may provide potential prevention of a number of health problems or diseases (Yu *et al*., 2005). Mango seed kernel oil can be developed for medicinal use and in preparation of food items like confectionery.

4. Conclusion

The mango seed kernel oil shows inhibitory activity against the two fungi: *A. niger* and *P. italicum*. The oil is more effective against *P. italicum*. It can be concluded that mango seed kernel oil can be use as natural antifungal in different kind of foods to combat the fungal spoilage caused by *A. niger* and *P. italicum*. This suggests that the mango seed kernels should be further utilized for its oil rather than just discarded as waste.

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Table 1. Inhibitory activity of mango seed kernel oil using agar dilution method upon *Aspergillus niger*

Concentration (%)	Diameter (Mean ± SD n = 2) of mycelial growth (mm) including disc diameter of 9 mm							
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control (0)	9.00 ± 0.00^{a}	14.0 ± 0.00^{a}	28.5 ± 0.71^{b}	32.5 ± 0.71^{b}	36.0 ± 0.00^{b}	38.0 ± 0.00^{c}	40.0 ± 0.00^{b}	44.0 ± 1.41^{c}
1.33%	9.00 ± 0.00^{a}	11.0 ± 0.00^{a}	28.0 ± 0.00^{b}	31.5 ± 0.71^{b}	34.5 ± 0.71^{b}	36.0 ± 1.41^{bc}	37.5 ± 2.12^{ab}	41.0 ± 2.83^{bc}
2.67%	9.00 ± 0.00^{a}	10.0 ± 0.00^{a}	25.0 ± 0.00^{a}	29.0 ± 0.00^{a}	32.0 ± 0.00^{a}	34.0 ± 0.00^{ab}	35.5 ± 0.71^{a}	37.5 ± 0.71^{ab}
4%	9.00 ± 0.00^{a}	10.0 ± 0.00^{a}	25.0 ± 0.00^{a}	27.5 ± 0.71^{a}	30.0 ± 1.41^{a}	32.0 ± 1.41^{a}	33.5 ± 2.12^{a}	35.5 ± 0.71^{a}

NB: Mean with different superscript on the same column are significantly different (P<0.05).

Table 2. Inhibitory activity of mango seed kernel oil using agar dilution method upon *Penicillium italicum*

Concentration (%)	Diameter (MEAN ± SD n = 2) of mycelial growth (mm) including disc diameter of 9mm							
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control (0)	9.00 ± 0.00^{a}	15.5 ± 3.54^{b}	30.0 ± 0.00^{b}	31.5 ± 0.71^{b}	32.5 ± 2.12^{b}	34.5 ± 3.54^{b}	38.0 ± 7.07^{a}	44.0 ± 14.14^{a}
1.33	9.00 ± 0.00^{a}	10.5 ± 0.71^{ab}	18.5 ± 2.12^{ab}	23.5 ± 2.12^{a}	24.5 ± 2.12^{a}	27.5 ± 2.12^{a}	32.5 ± 2.12^{a}	33.5 ± 2.12^{a}
2.67	9.00 ± 0.00^{a}	9.00 ± 0.00^{a}	15.0 ± 7.07^{a}	20.0 ± 0.00^{a}	22.5 ± 0.71^{a}	25.0 ± 0.00^{a}	31.0 ± 0.00^{a}	32.5 ± 0.71^{a}
4.0	9.00 ± 0.00^{a}	9.00 ± 0.00^{a}	15.5 ± 4.95^{a}	22.5 ± 2.12^{a}	24.5 ± 2.12^{a}	26.5 ± 0.71^{a}	32.0 ± 0.00^{a}	33.0 ± 0.00^{a}

NB: Mean with different superscript on the same column are significantly different (P<0.05).

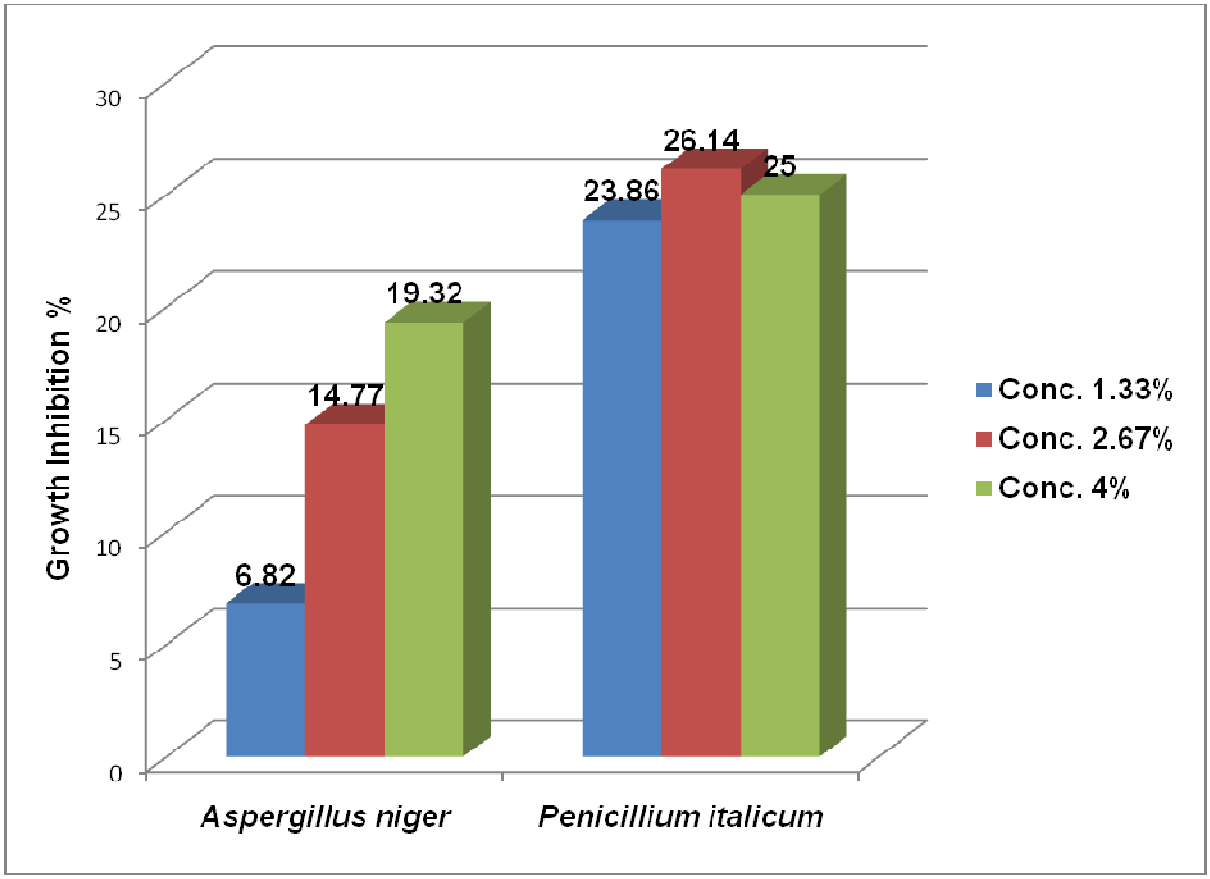

Figure 1. Percentage inhibition of mango seed kernel oil upon the growth of *Aspergillus niger* and *Penicillium italicum* at day 7