Inhibition of Fungal Colonization of Stored Peanut Kernels with Products from Some Medicinal/Culinary Plants

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ABSTRACT

Products from five medicinal/culinary plants—*Citrus aurantifolia* fruit peel oil, *Cymbopogon nardus* leaf oil, *Ocimum gratissimum* leaf powder, *Xylopia aethiopica* fruit powder, and *Syzigium aromaticum* clove powder—were tested for activity against fungal colonization of stored peanut. The natural microflora of kernels were supplemented with a norsolorinic acid (NOR) mutant of *Aspergillus parasiticus* before treatment with the various plant products. Treated kernels were stored at 5.7% moisture content in mini-polyethylene bags for 11 mo. Of the five plant products, the *Syzigium* clove powder proved to be most efficacious. After 4 mo storage, a low colony-forming unit (CFU) value of 0.601 log units of NOR *A. parasiticus* was recorded per gram of kernels treated with the powder. Significantly higher CFU values ($P \leq 0.05$) were associated with kernels that received no plant product (3.099 log units) and kernels treated with the other plant products(1.459-2.930 log units). CFU of total fungi, superficial fungal growth, internal kernel discoloration, and fungal growth/sporulation on the internal surfaces of the cotyledons also were suppressed by the *Syzigium* powder after 11 mo. The *Citrus* oil and the *Ocimum* leaf powder were moderately effective, being similar to each other in several storage parameters. The *Cymbopogon* leaf oil was the least effective of the plant materials tested. In a test tube experiment, the *Syzigium* and *Ocimum* powders were more efficacious when mixed with stored peanut kernels than when separated from kernels with a piece of mosquito-proof screen. The optimum rates of the two powders for preventing superficial fungal growth on kernels at 8% moisture in mini-polyethylene bags at 28 C were 150 and 100 g/kg of kernels, respectively, for *Syzigium* and *Ocimum*. At these rates, 93 and 56% of kernels treated with the *Syzigium* and *Ocimum* powders, respectively, were free from superficial fungal growth after 4 mo. These results point to the potential of the two powders, especially *Syzigium*, for preventing mold growth and possibly aflatoxin production in stored peanut.

Key Words: *Arachis hypogaea*, fungal inhibition, *Ocimum gratissimum*, storage fungi, *Syzigium aromaticum*.

Peanut (*Arachis hypogaea* L.) is an important food and oil crop in Ghana. The crop is grown in all regions of the country, but a substantial proportion of production originates from the upper east, upper west and northern regions. The total national production of peanut was estimated at 113,000 mt in 1990. Yield during the same year was estimated to average 1.4 mt/ha (Anon, 1991). In listing some of the constraints to peanut production in Ghana, Atuahen-Amankwa *et al.* (1988) indicated that peanut kernels stored in the humid areas of the country are prone to colonization by fungi. Awuah and Kpodo (1996) reported that peanuts sold on the Ghanaian market were contaminated by a variety of fungi including *Aspergillus flavus* Link. ex Fries and *A. parasiticus* Speare. Aflatoxin levels ranging from 5.7 to 22,168 µg/kg were associated with damaged/moldy/shrivelled kernels, but aflatoxins were either absent or occurred at extremely low levels in visibly healthy kernels. Mintah and Hunter (1978) assayed 80 peanut samples from markets within the Accra metropolitan area and reported that approximately half of the samples had aflatoxin levels above the 30 µg/kg hazard level recommended by the Food and Agricultural Organization and the World Health Organization. The study also showed that undamaged and visibly healthy kernels generally had low levels of aflatoxins. A similar study by Beardwood (1964) also pointed to the importance of the aflatoxin problem in Ghana.

Aflatoxigenic fungi and aflatoxins in peanut and peanut-based products are recognized as an important issue in Ghana because aflatoxins are known to be responsible for a number of disease conditions in humans and animals (Enomoto and Saito, 1972; Hendrickse *et al.*, 1982; Oyelami *et al.*, 1997). Among the post-harvest measures thought to be useful for managing aflatoxigenic fungal growth and aflatoxin production is drying the crop to moisture levels of *ca.* 10%. Due to the general lack of artificial drying facilities, most Ghanaian farmers are unable to adopt this

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recommendation. Thus, the crop may be stored at moisture levels conducive to fungal growth and aflatoxin production especially in areas where harvesting and storage coincide with frequent rainfall. Chemical treatment is not recommended due to environmental and health risks, and it is impractical due to the high cost.

Certain plants commonly used in traditional medicinal and culinary preparations in Ghana have been found to be fungicidal (Awuah, 1989, 1990, 1994; Awuah and Kpodo, 1996). For example, extracts from *Ocimum gratissimum* L., *Xylopia aethiopica* (Dunal) Rich, *Monodera myristica* Gaerth, *Cinnamomum verum* Presl., *Piper nigrum* L., *Syzigium aromaticum* (L.) Merr. & Perr., and *Cymbopogon citratus* (D.C.) Stapf have inhibited synthesis of norsolorinic acid (NOR), a precursor in the aflatoxin biosynthesis pathway (Awuah and Kpodo, 1996). It may be feasible to utilize products from such plants during bag-storage of shelled peanut in Ghana to inhibit fungal colonization of kernels. Substances released by these plant products into the storage environment may either exert a direct fumigant effect on kernels or suppress the activities of storage fungi as in modified atmosphere packaging (Landers *et al.*, 1967; Ellis *et al.*, 1993, 1994).

This paper reports investigations on the use of powders from *O. gratissimum* leaves, *X. aethiopica* fruits, and *S. aromaticum* cloves; oils from the fruit peel of lime [*Citrus aurantifolia* (Christm.) Swingle]; and leaves of citronella [*Cymbopogon nardus* (L.) Rendle] to inhibit growth of aflatoxigenic and other fungi in stored peanut.

Materials and Methods

Evaluation of Efficacies of Five Plant Products. A shelled peanut kernel sample of 5.7% moisture content was obtained from a local market in Kumasi and the visibly healthy kernels sorted from shrivelled/moldy/damaged kernels for subsequent experiments. To ensure that the initial kernel inoculum was sufficiently high, conidia from a 7-d-old culture of *A. parasiticus* ATCC 98106 (NOR mutant; wh, nor-1) on potato-dextrose agar with chloramphenicol at 500 mg/mL (CPDA) were harvested in 200 mL sterile distilled water and the conidial concentration adjusted to 1.3×10^5 conidia/mL. A total of 100 mL of the conidial suspension was atomized onto 5 kg of peanut kernels and incubated in a dark enclosure (24-28 C). After 3 d, the success of the inoculation with the *A. parasiticus* NOR mutant was found to be high through biopsy assays of 50 randomly selected kernels on CPDA. Additionally, the number of propagules was determined by homogenizing (1 min) 20 g of inoculated kernels in 100 mL sterile water with a Waring blendor (low speed). The homogenate (0.2 mL) was serially diluted and assayed on CPDA. The number of colony forming units (CFU) was determined and expressed as CFU/g kernel sample.

Plant products used for storing peanut kernels are listed in Table 1. Citrus peel and *Cymbopogon* leaf oils were obtained from commercial dealers, whereas other materials were either obtained from the local market or from backyard plantings. When a plant product was used in a powdered form, 30 g of the powder was thoroughly mixed with 150 g inoculated kernels, placed in a high density polyethylene bag (approx. 350 cm^2 in size), and sealed with a Pifco polyethylene bag sealer. The height of the head space in each bag was about 11 cm. Three-replicate bags with each plant product were prepared and enclosed in another bag (approx. 1240 cm^2 in size) made of interlaced polypropylene fabric before storage in a dark enclosure (24-28 C). When an oil was used, 10 mL was incorporated into 2.5-g absorbent cotton wool and packaged with kernels. Inoculated peanut samples stored without any plant product served as the control.

Table 1. Anti-fungal activity of five medicinal/culinary plant products against colonization of stored peanut kernels by a norsolorinic acid mutant of *Aspergillus parasiticus* and other fungi.

Treatment	Rate/kg of kernels	CFU/g of peanut kernels[a] 4 mo storage[b]	11 mo storage[c]
Untreated		3.099 a	6.097 a
Xylopia powder	200 g	2.930 a	5.959 b
Cymbopogon oil	66.7 mL	2.910 a	6.097 a
Ocimum powder	200 g	2.217 ab	5.949 b
Citrus oil	66.7 mL	1.459 bc	5.708 c
Syzigium powder	200 g	0.601 c	3.837 d

[a]Kernels were inoculated with a conidial suspension (1.3×10^5 conidia/mL) of a norsolorinic acid mutant of *A. parasiticus* prior to storage. Data are the means of three replicates. Each mean is a log [colony-forming units (CFU) + 1] transformation of CFU/g of peanut kernels. Means in a column followed by the same letter(s) are not significantly different (Duncan's multiple range test; $P \leq 0.05$).

[b]Only colonies of NOR *A. parasiticus* were estimated. Initial CFU/g of kernels before storage = 3.4661 log units.

[c]Data are CFU of total fungi/g of kernels. Fungi included species of *Aspergillus, Penicillium, Fusarium, Rhizopus,* and several unidentified genera.

After 4 and 11 mo of storage, kernels from each storage bag were assayed for CFU of fungi as previously described. The NOR *A. parasiticus* population was assayed at 4 mo, but total fungi (represented by species of *Aspergillus, Penicillium, Fusarium, Rhizopus,* and some unidentified genera) were assayed at 11 mo. Additionally, representative kernels from the various treatments were visually examined for the following: (a) residual scent of the plant product used, (b) discoloration of the embryo and cotyledon, (c) fungal growth/sporulation on the internal surfaces of the cotyledons, (d) crispness, (e) wrinkles on the testa, (f) departure of testa from original color, and (g) superficial fungal growth.

Optimal Rates of* Syzigium *and* Ocimum *Powders. Because powders from *S. aromaticum* and *O. gratissimum* were effective in inhibiting fungal growth, they were selected for further studies. To determine the optimum rates, visibly healthy kernels (1400 g) were inoculated with 50 mL of a 3.9×10^5 conidial suspension of NOR *A. parasiticus*. After 3 d incubation at 28 ± 2 C, *A. parasiticus* colonization of kernels was determined (as described previously) and 20 g kernel samples (8.0% moisture) placed in 70-cm^2 high density polyethylene bags. The *Syzigium* and *Ocimum* powders were separately mixed with the samples at rates of 0, 6.25, 12.5, 25, 50, 100, 150, and 200 g/kg of kernels (i.e., 0, 0.625, 1.25, 5, 10, 15 and 20% of kernel weight). Four-replicate bags of kernels treated at each level were sealed with a polyethylene bag sealer and stored in a dark incuba-

tor at 28 C (± 2 C). After 4 mo, kernels from each of the bags were visually examined and classified based on the extent of superficial fungal growth.

Contact vs. Noncontact Action of* Syzigium *and* Ocimum *Powders. Healthy appearing kernels were immersed in tap water to adjust the moisture content to 26% and then inoculated with a conidial suspension of *A. parasiticus* NOR mutant. Kernels were incubated in darkness at 28 ± 2 C for 3 d and the success of colonization determined. *Syzigium* and *Ocimum* powders at 3 g each were separately placed at the bottom of large test tubes (15 × 2.3 cm) and a piece of mosquito-proof screen fitted tightly into each tube approximately 1 cm over the plant powder. A 15-g sample of inoculated peanut kernels was placed on the screen and the open end of the tube sealed with three layers of parafilm M (American National Can, Greenwich, CT). Additional treatments included 15 g of inoculated kernels mixed with 3 g of each powder, a phostoxin treatment (1.2-g phostoxin tablet per 15 g infected kernels) and an untreated control. For each treatment, three replicate test tubes were maintained. After 7 wk of storage at 28 ± 2 C, kernels from each test tube were visually examined and classified based on the extent of superficial fungal growth.

Data Analysis. Data on colony forming units were transformed to log (CFU/g kernel + 1) and percentage data were subjected to arcsine transformation before performing an analysis of variance. The significance of treatment means was assessed by Duncan's multiple range test.

Results

A low CFU value of 0.601 log units of *A. parasiticus* (NOR mutant)/g kernel was associated with kernels treated with the *Syzigium* powder after 4 mo storage. This was significantly lower ($P \leq 0.05$) than the CFU of 3.099 log units/g of untreated kernels. Values associated with other plant products ranged from 1.459 log units (*Citrus* peel oil) to 2.930 log units (*Xylopia* fruit powder) (Table 1). After 11 mo, the CFU value for total fungi remained significantly low ($P \leq 0.05$) on kernels treated with the *Syzigium* powder. The NOR *A. parasiticus* was not detectable in most of the bagged kernel samples after 11 mo.

The *Syzigium* powder also suppressed growth of kernel surface mycoflora, kernel discoloration (embryo and cotyledon), and growth/sporulation of fungi on the internal surfaces of the cotyledons (Table 2). The *Citrus* oil and the *Ocimum* leaf powder were moderately effective based on these criteria. The *Cymbopogon* leaf oil was the least effective of the plant products.

The optimum rates of the *Syzigium* and *Ocimum* powders for preventing fungal growth on kernels stored in polyethylene bags at approximately 28 C were 150 and 100 g/kg of kernels, respectively (Table 3). At these rates, 93 and 56% of kernels, respectively, were devoid of superficial fungal growth. At lower rates of each powder, significantly higher percentages of kernels had slight to abundant levels of superficial fungal growth .

When peanut kernels were stored in test tubes, more effective suppression of fungal colonization was obtained when a plant powder was thoroughly mixed with the kernels than when the kernels were separated from the powder by a piece of mosquito-proof screen fitted in the tube over the powder. All kernels thoroughly mixed with the *Syzigium* powder, for example, were free from superficial fungal growth whereas only 42% of kernels that were separated from the powder had no superficial fungal growth (Table 4). When mixed with kernels, the inhibitory effect of the *Syzigium* powder was comparable to that of phostoxin.

Discussion

Medicinal/culinary plants, notably *O. gratissimum* and *S. aromaticum*, were previously reported to suppress fungal growth *in vitro* (Awuah, 1990; Awuah and Kpodo, 1996) and this activity was confirmed by studies reported here. Of the five plant products tested, the powder from *S. aromaticum* proved to be most effective in suppressing fungal growth on peanut kernels at 5.7% moisture even after 11 mo storage in polyethylene bags. Kernels treated with the *Syzigium* powder were devoid of discoloration, superficial fungal growth, and fungal growth/sporulation on the inner surfaces of the cotyledons. They also did not

Table 2. Characteristics of peanut kernels after 11 mo storage with five medicinal/culinary plant products.

Treatment	Discoloration[a]		Crispness[b]	Wrinkles[a]	Departure from original color[a]	Scent[c]	Surface[a] mycoflora	Internal growth/ sporulation[a]
	Embryo	Cotyledon						
Untreated	+++	+++	+	++	+(+)	NA	+++	+++
Xylopia powder	++	++	++	++	++	-	+(+)	++
Cymbopogon oil	+++	+++	+	++	++	(+)	+++	+++
Ocimum powder	+(+)	+(+)	++	++	+(+)	-	-	+
Citrus oil	+(+)	++	++	+	+(+)	(+)	(+)	+
Syzigium powder	-	(+)	+++	(+)	+	-	-	-

[a]- = None; + = slight; ++ = moderate; +++ = extensive; (+) = rating approaching next level; internal growth/sporulation refers to fungal growth and sporulation on the internal surfaces of the cotyledons.

[b]Crispness of kernels: + = soft ; ++ = moderately crisp; +++ = very crisp.

[c]Scent of plant product picked up by kernels during storage: - = none; + = slight; NA = not applicable.

Table 3. Effect of different rates of *Syzigium* clove and *Ocimum* leaf powders on fungal colonization of stored peanut kernels after 4 mo storage at 28 C[a].

Gram of powder/kg of kernels	Incidence of fungal growth on kernels[b]					
	None		Slight		Abundant	
	Syzigium	*Ocimum*	*Syzigium*	*Ocimum*	*Syzigium*	*Ocimum*
0	0 c	0 b	0 c	0 c	100 a	100 a
6.25	0 c	0 b	6 c	8 bc	94 a	92 ab
12.5	0 c	0 b	3 c	3 bc	97 a	97 ab
25	0 c	0 b	9 c	6 bc	91 a	94 ab
50	0 c	0 b	29 b	32 ab	71 b	68 b
100	9 b	56 a	70 a	39 a	21 c	5 c
150	93 a	49 a	7 c	51 a	0 d	0 c
200	91 a	58 a	9 c	42 a	0 d	0 c

[a]Data are the percent of kernels averaged across four replications having 43 to 48 kernels each. Data were arcsine transformed for statistical analysis. Means in a column followed by the same letter(s) are not significantly different (Duncan's multiple range test; $P \leq 0.05$).

[b]Incidence of fungal growth was rated visually and included species of *Aspergillus, Penicillium, Fusarium, Rhizopus* and several unidentified genera.

Table 4. Extent of superficial fungal growth on high moisture peanut kernels stored with *Syzigium* clove and *Ocimum* leaf powders.

Treatment[b]	Rate/kg of kernels	Incidence of fungal growth on kernels[a]					
		None		Slight		Abundant	
		No./total	%	No./total	%	No./total	%
Syzigium							
Mixed	200 g	26/26	100 a	0/26	0 b	0/26	0 c
Unmixed	"	11/26	42.3 c	4/26	15.4 a	11/26	42.3 ab
Ocimum							
Mixed	200 g	20/27	74 b	1/27	4 b	6/27	22 bc
Unmixed	"	5/27	18.5 c	8/27	29.6 a	14/27	51.9 ab
Phostoxin	80 g	25/25	100 a	0/25	0 b	0/25	0 c
Untreated		3/27	11 c	7/27	26 a	17/27	63 a

[a]Incidence of fungal growth was rated visually and included species of *Aspergillus, Penicillium, Fusarium, Rhizopus,* and several unidentified genera. Data were taken after 7 wk storage of 26% moisture kernels at 28 C. They are the means of three replications having 25 to 27 kernels each. Percent data were arcsine transformed for statistical analysis. Means in a column followed by the same letter(s) are not significantly different (Duncan's multiple range test; $P \leq 0.05$). No./total = kernels belonging to the category/total no. of kernels.

[b]Kernels were either thoroughly mixed with a plant powder (mixed) or separated from the powder (unmixed) with a piece of mosquito-proof screen fitted inside the storage tubes over the powder. The kernels were placed on top of the screen.

pick up the scent of the powder after 11 mo. El-Maraghy (1995) previously reported the antifungal properties of cloves when used to store lentil seeds over an 8-wk period. In the present studies, fungal growth was suppressed on peanut for up to 11 mo with the *Syzigium* clove powder at 200 g/kg of kernels. At this rate, the *Ocimum* leaf powder was moderately suppressive to fungal growth. It also did not leave any scent on the kernels. Due to their potential use as fungal inhibitors in the bag storage of peanut, the *Ocimum* and *Syzigium* powders have been selected for further studies.

While the results on fungal suppression are encouraging, aflatoxin levels in peanut kernels stored with the various plant products were not determined due to lack of analytical facilities. By suppressing the growth of fungi including the aflatoxigenic species on kernels during storage, levels of aflatoxins are likely to be reduced (Batt *et al.*,1983; Badii and Moss, 1988). The low moisture levels of peanut kernels used for some of the experiments were not favorable for aflatoxin synthesis (Dickens and Pattee, 1966). Therefore, cooperative studies are in progress to test the effects of *Syzigium* clove and *Ocimum* leaf powders on both fungal colonization and aflatoxin levels of peanut under storage conditions similar to those used by Ghanaian farmers.

The optimal application rates of the *Syzigium* and *Ocimum* powders for polyethylene bag-storage of peanut at 28 C and 8% moisture were 150 and 100 g/kg of

kernels, respectively. At these application rates, 93% of kernels treated with the *Syzigium* powder and 56% of those treated with the *Ocimum* powder showed no visible evidence of fungal growth after 4 mo. These rates may be higher than needed under field conditions in Ghana wherein temperatures, especially during the night, could be below the storage temperature of 28 C used in the current study. In Ghana, stored peanut kernels are exposed to freely circulating air which facilitates further drying during storage. The interaction between kernel moisture content and temperature as determinants of efficacies of the two plant products needs to be investigated.

The major and biologically active components of *O. gratissimum* are reported to be thymol (Sofowora, 1970; Sainsbury and Sofowora, 1971) and eugenol (Tripathi *et al.*, 1986) while those of *S. aromaticum* are eugenol and acetyl eugenol (Hassanali *et al.*, 1990). These compounds possess antimicrobial properties (El-Said *et al.*, 1969; Mansour, 1996) and have fumigant properties. To achieve a high degree of efficacy, it appears that the plant powders must be evenly distributed among the kernels to facilitate optimal release of their antifungal components into the storage environment.

Many chemicals, especially at high rates in their pure form, may pose health risks. However, the content of eugenol and thymol, in *S. aromaticum* and *O. gratissimum*, respectively, may be sufficiently low to minimize risks when products from the two plants are used to store peanut kernels. This is supported by the fact that both plants are used routinely as spices in certain Ghanaian cuisines in addition to their utility in some medicinal preparations that are administered by the oral route. Since the safety of these products can only be speculated, there may be the need to assay for residues of thymol and eugenol on peanut kernels treated with the two plant products.

In conclusion, these studies have shown that *Syzigium* clove and *Ocimum* leaf powders could be used to inhibit growth of fungi and possibly aflatoxin synthesis in the storage of peanut. The *Syzigium* clove powder particularly has more potential. Field studies, and food safety determinations need to be carried out before commercial use patterns can be recommended to farmers, retailers and end-users. This approach may offer a cost-effective means of providing a partial solution to the peanut aflatoxin problem in Ghana.

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