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Original article

Effect of Fungal Infection on the Fatty Acid Composition of Peanut (Arachis hypogaea L.) Oil

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Abstract
Peanut (*Arachis hypogaea* L.) is an important oilseed crop cultivated extensively in many tropical and subtropical regions. Fungal pathogens and root-knot nematodes represent major constraints to peanut production, causing significant yield losses and potentially affecting seed quality. This study investigated the fatty acid composition of peanut oil extracted from healthy seeds and seeds naturally infected with root-rot fungi (*Rhizoctonia solani*, *Fusarium solani*, *Aspergillus niger*, and *Aspergillus flavus*) and root-knot nematode (*Meloidogyne javanica*). Gas chromatographic analysis revealed nine fatty acids in peanut oil, with six identified as palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1n9c), linoleic acid (C18:2n6c), arachidic acid (C20:0), and cis-11-eicosenoic acid (C20:1). The major fatty acids in both healthy and infected seeds were oleic, linoleic, and palmitic acids. Infection resulted in decreased palmitic acid (from 13.14% to 12.75%) and linoleic acid (from 36.60% to 33.87%) content, while oleic acid increased from 40.36% in healthy seeds to 43.14% in infected seeds. These alterations in fatty acid profile may affect oil stability, nutritional quality, and industrial applications. This study provides important insights into how pathogen infection can modify the lipid composition of peanut oil.
Keywords: Peanut Oil, Fatty Acids, Gas Chromatography, *Rhizoctonia solani*, *Fusarium solani*.

Introduction

Peanut (*Arachis hypogaea* L.) is one of the most important oilseed crops globally, cultivated extensively in tropical and subtropical regions. Peanut seeds contain approximately 45-50% oil, making them a valuable source of edible oil and contributing substantially to the agricultural economy in many producing countries (1). The quality and composition of peanut oil are determined by its fatty acid profile, which typically includes palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), arachidic acid (C20:0), and behenic acid (C22:0) (2). Oleic and linoleic acids constitute approximately 80% of the total fatty acids in peanut oil (3). The oleic/linoleic (O/L) ratio is particularly important as it influences oxidative stability, shelf life, and nutritional value of the oil (4,5). Recent studies have demonstrated that high-oleic peanut varieties, containing more than 75% oleic acid with O/L ratios typically greater than nine, exhibit superior oxidative stability and multiple health functions, including lowering blood lipid levels and protecting cardiovascular health (1). Beyond fatty acids, peanut oil contains bioactive components such as tocopherols, with α-tocopherol and γ-tocopherol as the predominant forms, and novel lipid classes like fatty acid esters of hydroxy fatty acids (FAHFAs) that have shown anti-diabetic effects (1,4).

Peanut production faces significant challenges from soil-borne pathogens, particularly root-rot fungi and root-knot nematodes. *Rhizoctonia solani*, *Fusarium* species, and *Aspergillus* species are among the most destructive fungal pathogens causing root rot and seed deterioration (6). Concurrently, root-knot nematodes (*Meloidogyne* spp.) cause substantial damage to peanut root systems, leading to reduced plant vigor, yield losses, and increased susceptibility to fungal pathogens (7). The interaction between these pathogens often results in disease complexes that exacerbate crop losses (8). Recent molecular studies have revealed that fatty acid metabolism plays a crucial role in fungal pathogenesis. In *Fusarium graminearum*, the Δ12 fatty acid desaturase (FgFAD12) is essential for linoleic acid biosynthesis and regulates vegetative growth, conidial germination, and plant pathogenesis (5,10). Deletion of this gene results in the accumulation of oleic acid and reduced linoleic acid, demonstrating the direct link between fungal fatty acid metabolism and pathogenicity (10).

While numerous studies have documented the yield losses caused by these pathogens, limited information is available regarding their impact on seed quality parameters, particularly oil composition. Pathogen infection may trigger biochemical changes in developing seeds, potentially altering lipid metabolism and fatty acid biosynthesis (9). Understanding these modifications is essential for assessing oil quality from infected crops and developing appropriate management strategies. This study aimed to investigate the fatty acid composition of peanut oil extracted from seeds naturally infected with root-rot fungi (*Rhizoctonia solani*, *Fusarium solani*, *Aspergillus niger*, and *Aspergillus flavus*) and root-knot nematode (*Meloidogyne javanica*), compared with oil from healthy seeds.

Materials and Methods

Collection of Plant Material

Peanut plants showing symptoms of root rot and root-knot nematode infection were collected from naturally infested fields. Healthy plants from the same location were collected as controls. Samples were obtained

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approximately 20 days before the harvesting stage and at the harvesting stage. Plants were carefully uprooted, and roots were examined for gall formation and rot symptoms. Seeds were collected from both healthy and infected plants for fatty acid analysis.

Isolation and Identification of Pathogens

Fungi were isolated from infected roots and seeds following surface sterilization with sodium hypochlorite solution (3%) for 3 minutes, rinsing with sterilized distilled water, and plating on potato dextrose agar (PDA) medium. Plates were incubated at 28 ± 2°C for 5-7 days. Fungal colonies were identified based on morphological characteristics according to Barnett (10), Booth (11), and Domsch et al. (12).

Root-knot nematode identification was performed by extracting adult females from galled roots and preparing perineal patterns according to Taylor and Netschert (13). Species identification was based on perineal pattern morphology following Mai and Lyon (14).

Oil Extraction

Peanut seeds were surface sterilized, and crude oil was extracted using a mixture of petroleum ether and diethyl ether according to standard procedures. Seeds were ground, and the oil was extracted by solvent partitioning. The solvent was evaporated under reduced pressure, and the extracted oil was stored in amber vials at -20°C until analysis.

Preparation of Fatty Acid Methyl Esters (FAMEs)

Fatty acid methyl esters were prepared following the method described by Chalvardjian (15). Approximately 50 mg of oil was dissolved in methanol-H_2SO_4 and heated under reflux conditions to achieve complete methylation. The methyl esters were extracted with hexane, washed with distilled water, dried over anhydrous sodium sulfate, and concentrated for gas chromatographic analysis.

Gas Chromatographic Analysis

Fatty acid methyl esters were analyzed using an ACME model 6100 Gas Chromatograph (Young LIN Instrument Co., Korea) equipped with a split/splitless injector and flame ionization detector (FID). Separation was achieved on a 30-m SP-2380 fused-silica capillary column with 0.25 mm internal diameter and 0.2 µm film thickness (Supelco, Bellefonte, PA, USA).

Chromatographic conditions included an injector temperature of 220°C and a detector temperature of 260°C. Nitrogen was used as the carrier gas at a flow rate of 0.5 ml/min with a split ratio of 80:1. The oven temperature was initially maintained at 140°C for 5 minutes, then increased to 240°C at a rate of 4°C per minute, with a total run time of 30 minutes.

Individual fatty acids were identified by comparing retention times with those of authentic standards (Supelco 37 Component FAME Mix). Peak areas were integrated, and relative percentages of fatty acids were calculated based on the total peak area.

Statistical Analysis

Data from gas chromatographic analysis were compiled and analyzed using Microsoft Excel. Descriptive statistics were calculated for fatty acid composition, including mean percentages and standard deviations for each identified fatty acid component in both healthy and infected seed samples.

Results

Pathogen Isolation and Identification

Examination of infected peanut plants revealed the presence of root-knot nematode *Meloidogyne javanica*, identified based on perineal pattern morphology. Fungal isolation from infected roots and seeds yielded four major fungal pathogens: *Rhizoctonia solani*, *Fusarium solani*, *Aspergillus niger*, and *Aspergillus flavus*. The isolation frequency varied depending on plant part and sampling time, with *Fusarium solani* being predominant in roots (78.75% at harvesting stage) and *Rhizoctonia solani* in seeds (33.4% at harvesting stage), as shown in Table 1.

Table 1. Fungi isolated from infected peanut plants at harvesting stage

Isolated Fungus	Isolation Percentage (%)			
	Infected Plants		Healthy Plants	
	Roots	Seeds	Roots	Seeds
Rhizoctonia solani	6.25	33.4	0.00	0.00
Fusarium solani	78.75	33.2	0.00	0.00
Aspergillus niger	8.00	11.1	0.00	0.00
Aspergillus flavus	7.00	22.3	0.00	0.00

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Fatty Acid Composition of Peanut Oil

Gas chromatographic analysis of peanut oil extracted from healthy and naturally infected seeds revealed the presence of nine fatty acids, six of which were identified as known fatty acids (Table 2, Figure 1). The identified fatty acids included palmitic acid (C16:0), a saturated fatty acid; stearic acid (C18:0), a saturated fatty acid; oleic acid (C18:1n9c), a monounsaturated fatty acid (omega-9); linoleic acid (C18:2n6c), a polyunsaturated fatty acid (omega-6); cis-11-eicosenoic acid (C20:1), a monounsaturated fatty acid; and arachidic acid (C20:0), a long-chain saturated fatty acid. Three additional peaks remained unidentified and were designated as unknown fatty acids.

Table 2. Fatty acid composition of healthy and naturally-infected peanut seeds (cv. Giza 6)

Fatty Acid	% in Healthy Seeds	% in Infected Seeds
Palmitic acid (C16:0)	13.14	12.75
Stearic acid (C18:0)	2.51	2.91
Oleic acid (C18:1n9c)	40.36	43.14
Linoleic acid (C18:2n6c)	36.60	33.87
Unknown 1	0.42	—
cis-11-Eicosenoic acid (C20:1)	1.24	1.37
Arachidic acid (C20:0)	1.32	1.31
Unknown 2	2.79	2.84
Unknown 3	1.62	1.73
Total	100.00	99.92
Oleic/Linoleic Ratio	1.10	1.27

Figure 1. Peanut plants are naturally infected with Rhizoctonia (1) and Root-knot nematode (2).

Comparison of Fatty Acid Profiles Between Healthy and Infected Seeds

The fatty acid composition of oil from healthy and infected peanut seeds showed distinct differences (Table 2). The major fatty acids in both healthy and infected seeds were oleic acid (C18:1n9c), linoleic acid (C18:2n6c), and palmitic acid (C16:0). These three fatty acids constituted approximately 90% of the total fatty acids in both samples.

Quantitative analysis revealed several differences between healthy and infected seeds. Palmitic acid (C16:0) decreased from 13.14% in healthy seeds to 12.75% in infected seeds, representing a reduction of approximately 3.0%. Stearic acid (C18:0) increased from 2.51% in healthy seeds to 2.91% in infected seeds, an increase of 15.9%. Oleic acid (C18:1n9c) increased from 40.36% in healthy seeds to 43.14% in infected seeds, representing a 6.9% increase. Linoleic acid (C18:2n6c) decreased from 36.60% in healthy seeds to 33.87% in infected seeds, a reduction of 7.5%. cis-11-Eicosenoic acid (C20:1) showed a slight increase from

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1.24% to 1.37%, while arachidic acid (C20:0) remained relatively stable, changing from 1.32% to 1.31%. The three unknown peaks showed minor variations between healthy and infected samples.

The oleic/linoleic (O/L) ratio increased from 1.10 in healthy seeds to 1.27 in infected seeds, representing a 15.5% increase. Total saturated fatty acids (palmitic + stearic + arachidic) remained constant at 16.97% in both healthy and infected seeds. Total unsaturated fatty acids (oleic + linoleic + eicosenoic) showed a slight increase from 78.20% in healthy seeds to 78.38% in infected seeds. The overall saturated/unsaturated ratio remained relatively constant despite individual fatty acid changes.

Effect of Infection on Yield Parameters

The high disease incidence observed in the field correlated with substantial yield losses. At 20 days before harvest, infected plants showed reductions of 78.2% in shoot fresh weight, 48.4% in root fresh weight, 92.8% in pod fresh weight, 93.7% in seed fresh weight, and 85.6% in 100-pod fresh weight compared to healthy plants. Seed dry weight was reduced by 86.55%, and pod number decreased by 76.3% (Table 3).

Table 3. Effect of natural infection on the yield parameters of peanut cv. Giza 6 at 20 days before harvesting

Parameter	Healthy Plants	Diseased Plants	Reduction (%)
Shoot fresh weight (g)	2115.56	461.97	78.2
Root fresh weight (g)	91.85	47.39	48.4
Pods fresh weight (g)	650.02	46.82	92.8
Number of pods	223	52.90	76.3
100 pods fresh weight (g)	322.63	46.60	85.6
Seeds fresh weight (g)	263.59	16.60	93.7
Seeds' dry weight (g)	123.42	16.60	86.55
Number of galls	—	241	—
Number of egg masses	—	237	—

At the harvesting stage, infected plants showed reductions of 74.6% in shoot fresh weight, 60.7% in root fresh weight, 88.1% in pod fresh weight, 89.7% in seed fresh weight, and 75.8% in 100-pod fresh weight. Seed dry weight was reduced by 90.5%, and pod number decreased by 81.9% (Table 4).

Table 4. Effect of natural infection on yield parameters of peanut cv. 6 at the harvesting stage

Parameter	Healthy Plants	Diseased Plants	Reduction (%)
Shoot fresh weight (g)	1993.19	505.59	74.6
Root fresh weight (g)	103.61	40.77	60.7
Pods fresh weight (g)	727.32	86.93	88.1
Number of pods	259	47	81.9
100 pods fresh weight (g)	356.12	86.16	75.8
Seeds fresh weight (g)	405.04	41.85	89.7
Seeds' dry weight (g)	262.95	25.02	90.5
Number of galls	—	235	—
Number of egg masses	—	444	—

Discussion

This study demonstrates that infection by root-rot fungi and root-knot nematodes significantly alters the fatty acid composition of peanut oil. The observed changes in fatty acid profiles, particularly the decrease in linoleic acid and increase in oleic acid, have important implications for oil quality, stability, and nutritional value. The fatty acid profile of healthy peanut seeds observed in this study (oleic acid 40.36%, linoleic acid 36.60%) is consistent with previously reported values for conventional peanut cultivars. Recent chromatographic analysis of ten peanut varieties revealed oleic acid concentrations ranging from 43.13% to 50.71% and linoleic acid from 26.01% to 33.63%, with palmitic acid between 11.24% and 12.19% (7). Similarly, studies on peanut oil bodies from high-oleic cultivars have demonstrated variability in fatty acid profiles, with some cultivars exhibiting high oleic acid and monounsaturated fatty acids with reduced linoleic acid content (1).

The alteration in fatty acid composition likely results from pathogen-induced modifications in lipid metabolism within developing seeds. Fungal and nematode infections can trigger defense responses in plants, including the activation of lipoxygenase pathways and the production of reactive oxygen species (9). These stress responses may affect the activity of key enzymes involved in fatty acid biosynthesis and desaturation. One of the universal mechanisms of plant resistance to abiotic and biotic stress factors is an increase in the proportion of unsaturated fatty acids in membrane phospholipids, mediated by desaturase enzymes that facilitate the formation of double bonds in fatty acids (2). This increase in unsaturated fatty

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acid content enhances membrane plasticity and viscosity while potentially influencing plant-pathogen interactions (2).

The decrease in linoleic acid (18:2) and concomitant increase in oleic acid (18:1) suggest possible inhibition of oleate desaturase (Δ12-desaturase), the enzyme responsible for converting oleic acid to linoleic acid (16). This enzyme introduces a second double bond into oleic acid to produce linoleic acid. Stress conditions, including pathogen attack, can downregulate desaturase activity, leading to accumulation of oleic acid and reduced linoleic acid content (17). Interestingly, recent research on *Fusarium graminearum* has shown that the fungal Δ12 fatty acid desaturase gene (FgFAD12) is essential for linoleic acid biosynthesis in the pathogen itself, and deletion of this gene results in defective vegetative growth, reduced conidial germination, and impaired plant pathogenesis (5,10). The ΔFgfad12 mutant accumulated large amounts of oleic acid while linoleic acid production was restrained, mirroring the fatty acid changes observed in infected peanut seeds in the present study (10). This suggests that fungal infection may involve complex interactions between host and pathogen fatty acid metabolism pathways.

The oleic/linoleic (O/L) ratio is a critical determinant of peanut oil quality and oxidative stability. Oils with higher O/L ratios exhibit greater resistance to autoxidation and rancidity, resulting in extended shelf life (4,5). Linoleic acid, with two double bonds, is more susceptible to oxidation than oleic acid, which has only one double bond. The oxidation rate of oleic acid is approximately one-tenth that of linoleic acid, so high-oleic acid raw materials and their products show good oxidation stability (1). The increased O/L ratio observed in infected seeds (from 1.10 to 1.27) theoretically suggests improved oxidative stability. However, this apparent benefit must be considered alongside other factors. Studies on peanut oil blending have demonstrated that during storage, notable changes occur in both fatty acid profiles and tocopherol levels, with oxidative stability decreasing as polyunsaturated fatty acid content increases (3,6,8). Pathogen infection may also introduce hydrolytic enzymes, lipases, and oxidative enzymes that can promote oil degradation during storage (18). Fungal contamination, particularly by *Aspergillus* species, raises concerns about mycotoxin production, which can compromise oil safety regardless of fatty acid composition (19).

The reduction in linoleic acid, an essential omega-6 fatty acid, may have nutritional implications. Linoleic acid is required for human health and must be obtained through diet (20). Polyunsaturated fatty acids play significant roles in regulating cell membrane structure, fluidity, signal transduction, and immune responses (10). However, modern diets often contain excessive omega-6 relative to omega-3 fatty acids, and the health implications of reduced linoleic acid content are complex. The increase in oleic acid is generally considered beneficial, as monounsaturated fatty acids are associated with reduced cardiovascular disease risk (21). Recent research has also identified novel lipid classes in nut oils, including fatty acid esters of hydroxy fatty acids (FAHFAs), which have shown anti-diabetic effects in mammalian systems (4). The impact of pathogen infection on these minor but bioactive lipid components warrants further investigation.

The severe yield reductions observed (78-93% in various parameters) indicate that the pathogen complex (*R. solani*, *F. solani*, *A. niger*, *A. flavus*, and *M. javanica*) caused extensive damage to peanut plants, affecting not only yield quantity but also seed quality parameters including oil composition. The dominance of *Fusarium solani* in infected roots (78.75% at harvest) and *Rhizoctonia solani* in infected seeds (33.4% at harvest) suggests that different pathogens may preferentially colonize different plant tissues, potentially exerting tissue-specific effects on lipid metabolism. The presence of three unknown fatty acids in both healthy and infected samples warrants further investigation using advanced analytical techniques such as liquid chromatography coupled with mass spectrometry, which has proven effective for comprehensive lipid profiling in nut oils (4).

Conclusion

This study investigated the effect of fungal and nematode infection on the fatty acid composition of peanut oil by comparing healthy seeds with those naturally infected by root-rot fungi (*Rhizoctonia solani*, *Fusarium solani*, *Aspergillus niger*, and *Aspergillus flavus*) and root-knot nematode (*Meloidogyne javanica*). Gas chromatographic analysis revealed nine fatty acids in peanut oil, with six identified as palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1n9c), linoleic acid (C18:2n6c), arachidic acid (C20:0), and cis-11-eicosenoic acid (C20:1), with oleic, linoleic, and palmitic acids comprising approximately 90% of total fatty acids. Infection significantly altered the fatty acid profile, decreasing palmitic acid from 13.14% to 12.75% and linoleic acid from 36.60% to 33.87%, while increasing oleic acid from 40.36% to 43.14% and stearic acid from 2.51% to 2.91%, resulting in an increased oleic/linoleic ratio from 1.10 to 1.27.

Conflict of interest

The authors declare no conflict of interest.

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