ISSN: 3064-9846
Review Article
Journal of Agricultural, Earth and Environmental Sciences

Fungi Associated with Avocado (Persea Americana) Post-harvest Fruit Bio Degeneration: Occurrence, in Vitro Susceptibility to Leaf Extracts of Aloe Vera, Neem and Pear Seed Oil and Attendant Effects of the Fungi on Nutritional Composition of the Pear Fruit

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Submitted: 11 June 2025 **Accepted:** 19 June 2025 **Published:** 26 June 2025

https://doi.org/10.63620/MKJAEES.2025.1088

Citation: *Ijato, J. Y., Oluwafemi, T. E., & Ojo, B. O. (2025). Fungi Associated with Avocado (Persea Americana) Post-harvest Fruit Bio Degeneration: Occurrence, in Vitro Susceptibility to Leaf Extracts of Aloe Vera, Neem and Pear Seed Oil and Attendant Effects of the Fungi on Nutritional Composition of the Pear Fruit. J of Agri Earth & Environmental Sciences, 4(3), 01-07.*

Abstract

Wounds have been reported to be the major pre-disposing factor of fruits and vegetables to both transit and storage microbial attack. The variation in fungal loads of avocado fruits observed in this study can be adduced to the differences in the level of post-harvest sanitation. Fungal isolates from rotten avocado fruits in Ado Ekiti were Colletotrichum acutatum, Cercospora purpurea, Sphaceloma persea, Botryosphaeria dothiorella, Pestalotiopsis guipinii, Aspergillus niger and Rhizopus nigricans. The study revealed antifungal effects of Aloe vera, neem and pear seed (oil extracts) at different concentrations on fungal isolates from avocado. This study also revealed that neem oil extract was the most inhibitive on the fungal isolates, this was followed by Aloe vera oil extract and pear seed extract at different concentrations. Higher concentrations supported higher antifungal effects of all the oil extracts.

All the tested isolates were found to be pathogenic on the avocado fruits. Pestalotiopsis guipinii was found to be most pathogenic with diameter of the spoiled area of 44 mm while Sphaceloma persea recorded 23.00 mm. The results of the study suggest the need for developing appropriate strategy to control prevalent post-harvest fungal diseases of avocado fruits.

Keywords: Bio-control, Aloe Vera Oil, Pear Seed Oil, Neem Oil, Fungal Pathogens, Avocado Pear.

Introduction

Production of avocado (Persea americana) within several agro-ecological zones in most African countries especially Nigeria is dominated by smallholder farmers (85%). Johnny et al., (2019y) [1]. reported they are mainly produced for export market, mainly to the European Union for dietary value and the remainders are sold in the local markets.

In addition to food use, consumption of avocados has been reported to associate with various health benefits, such as reduction in cholesterol and decreased risk of cardiovascular disease (Duarte et al., 2016) [2]. Pear fruit contains twice as much protein compared to any other fruit, is very rich in minerals and vitamins Persea americana is attacked by many diseases, causing relatively short shelf-life.

Ewekeye et al, (2016) [3]. Reported that infections reduce the quality (health hazards through the production of toxins) and

sometimes cause completely unmarketable produces that fail in meeting the international standards for exports. O'Brien, (2017) [4]. Reported that losses due to infection pose a major threat to global food production annually. Sangeetha et al, (2013) [5]. Reported that rot caused by fungal pathogens provokes severe losses of agricultural and horticultural crops every year

Materials and Methods

Samples Collection

Avocado pear fruit samples were purchased from Ado-Ekiti market, Nigeria. These were transported in sterile polythene bags for fungal isolation and further analyses.

Samples Processing

The surface sterilized fruits showing symptoms of diseases were sliced into 2mm² pieces and plated onto sterile PDA supplemented with 250 mg chloramphenicol in Petri dishes to inhibit bacterial contamination. The plates were incubated at room temperature for 4-5days and observed for fungal growth and later sub cultured into fresh PDA medium. Obtained pure isolates of fungi were identified on the bases of macro and micro morphological characteristics. Morphological characteristics of the fungi (mycelium coloration or pigmentation, presence or absence of septate, spore morphology) were recorded. In some cases, the infected tissues were stained by cotton blue and lactophenol (McClenny, 2005) and observed under microscope [6].

Morphological identification of fungi was based on the fungal culture colony or hyphae, the characteristics of the spores and reproductive structures (Agrios, 2005) [7]. The colonies that developed were counted and sub-cultured repeatedly on PDA plates to obtain pure cultures. They were later stored on PDA slants for identification and characterization.

Collection and Preparation of Plant Materials

The plant materials (Aloe vera, neem and pear seed) were obtained from Ado-Ekiti environment. The extraction process followed the procedure described by Handa et al, (2008) [8]. The leaves were washed under tap water, rinsed in three changes of sterile distilled water and dried using sterile blotting paper. They were then placed and dried at a temperature of 40°C for three weeks inside hot air oven. The seeds were also washed under tap water and rinsed in three changes of sterile distilled water. They were blotted dry using sterile blotting papers, cut into smaller pieces and placed in the oven at the same temperature for three weeks. All the plant materials were pulverized with sterile mortar and pestle so as to rupture the leaf tissues and cell structures to release the active cell contents.

These were stored in airtight glass containers protected from sunlight until required for analysis. Forty grams (40g) dried powder of each plant were weighed separately and transferred into conical flask for extract preparation as given below.

Extraction of Aloe Vera, Neem and Pear Seed Oils

The ground plant material was placed in the extraction thimble. The weighed amount was placed in an extraction chamber which was suspended above the flask containing the solvent n-hexane and below a condenser. The flask was heated and the evaporated n-hexane was moved into the condenser where it was converted into a liquid that trickled into the extraction chamber containing the plant materials. The extraction was designed so that when the solvent surrounding the sample exceeded a certain level it overflowed and trickled back down into the boiling flask. At the end of the extraction process, the flask containing the n-hexane extract was removed and n-hexane was evaporated by using rotary evaporator.

Preparation of Inoculum

The fungal inoculum was prepared from 5-day old culture grown on potato dextrose agar medium. The Petri dishes were flooded with 8 to 10ml of distilled water and the conidia were scraped using sterile spatula. The spore density of each fungus was adjusted with spectrophotometer (A595 nm) to obtain a final concentration of approximately 105 spores/ml according to Cheesbrough (2002) [9].

Effects of Crude Plant Extracts on Growth of Fungal Mycelia

The effectiveness of the crude oil extracts in controlling rots was evaluated with the seven fungal isolates, namely: Cercospora purpurea, Botryosphaeria dothiorella, Sphaceloma persea, Colletotrichum acutatum, Pestalotiopsis guepinii, Aspergillus niger and Rhizopus nigricans. They were isolated from avocado pear fruit and characterized.

Determination of the Effects of the Crude Extracts on the Fungal IsoArticle Accepted for Publication Lates

The method of Amadioha and Obi (1999) [10]. was adopted to determine the effects of the crude oil extracts on the fungi. Different concentrations of the crude oil extracts were prepared by weighing separately 10-1, 10-2 and 10-3 of oil extract of (Aloe vera, neem and pear seed) respectively. Each powder was dissolved in 1ml sterile distilled ethanol and water to form solutions of different concentrations.

The Muller Hinton agar medium was prepared according to manufacturer instruction and autoclaved at the 121°C for 15min. The media was poured into each Petri dish and set aside to solidify under the laminar hood. After solidifying the media, the sterile glass spreader was used to spread the inoculums throughout the medium uniformly. Then, 100µl of each extract adjusted to the same concentration (50mg/ml) and perforated filter paper (disc) were soaked for two hours before placing them on the agar plate. The agar plate was allowed to stay for 1 hour under the incubator later at 37°C for one daytime.

The sensitivity of the test microorganisms was found by assessing the diameter of the zone of inhibition in which significant susceptibility was taken as mm in diameter. The sensitivity of the test microorganisms was found by assessing the diameter of the zone of inhibition percentage was taken thus: Growth inhibition (%) = $\frac{(DC - DT}{DC} \times \frac{100)}{1}$

Where DC = Average diameter of colony with control
DT = Average diameter of colony with treatment

Pathogenicity Test

Pathogenicity test was carried out using the techniques described by Akintobi, et al, (2011), Ijato et al. (2023) [11, 12]. Healthy avocado pear fruit samples were obtained from the market with zip lock bag and transported to microbiology Lab. The avocado

pears were then washed under running tap to eliminate dirt from their surfaces. They were surface sterilized in 1% NaCl for three minutes. Thereafter, they were rinsed in three changes of sterile distilled water and wiped dry using a sterile blotting paper. A sterile inoculating needle containing fungal spore was used to punch the avocado pear fruit. The isolated fungal pathogens and the inoculating needle were used to inoculate the healthy avocado pear. The negative control was also set in the same manner. Disease development was checked after 24hour. The point of inoculation of each type of fungus was examined and recorded. The diameter of the rotten portion of the fruits was measured and the fungi were later re-isolated from the inoculated samples and compared with the initial isolates.

Proximate Analysis

The proximate composition was determined according to AOAC, (2000) [13].

Moisture Content Determination

Two grams (2.0g) of the sample(s) were placed in an oven maintained at 100 - 103°C for 16 hours with the weight of the wet sample and the weight after drying noted. The drying was repeated until a constant weight was obtained. The moisture content was expressed in terms of loss in weight of the wet sample.

$$\% \text{ moisture content} = \frac{\text{weight of moisture}}{\text{weight of sample}} \times 100$$

Ash Content Determination

Two grams (2.0g) of each of the oven-dried samples in powder form were accurately weighed and placed in crucible of known weight. These were ignited in a muffle furnace and ashed for 8hours at 550°C. The crucible containing the ash was then removed, cooled in a desiccator and weighed and the ash content expressed in term of the oven-dried weight of the sample.

$$\% \text{ Ash content} = \frac{\text{weight of ash}}{\text{weight of sample}} \times 100$$

Protein Content Determination

The protein nitrogen in 1g of the dried samples was converted to ammonium sulphate by digestion with concentrated H_2SO_4 and in the presence of $CuSO_4$ and Na_2SO_4. These were heated and the ammonia evolved was steam distilled into boric acid solution. The nitrogen from ammonia was deduced from the titration of the trapped ammonia with 0.1M HCl with Tashirus indicator (double indicator) until a purplish pink color was obtained. Crude protein was calculated by multiplying the value of the deduced nitrogen by the factor 6.25mg.

Crude Fibre Content Determination

Two grams (2.0g) of each sample was weighed into separate beakers, the samples were then extracted with petroleum ether by stirring, settling and decanting 3 times. The samples were then air dried and transferred into a dried 100ml conical flask. A measure of 200cm3 of 0.127M sulphuric acid solution was added at room temperature to the samples. The first 40cm3 of the acid was used to disperse the sample. This was heated gently to boiling point and boiled for 30 minutes. The contents were filtered to remove insoluble materials, which was then washed with distilled water, then with 1% HCI, next with twice ethanol and finally with diethyl ether. Finally, the oven-dried residue was ignited in a furnace at 550oC. The fibre contents were measured by the weight left after ignition and were expressed in term of the weight of the sample before ignition.

Fat Content Determination

The lipid content was determined by extracting the fat from 10g of the samples using petroleum ether in a Soxhlet apparatus. The weight of the lipid obtained after evaporating off the petroleum ether from the extract gave the weight of the crude fat in the sample.

Carbohydrate Content Determination

The carbohydrate content of the samples was determined as the difference obtained after subtracting the values of protein, lipid, ash and fibre from the total dry matter (AOAC, 2009) [14].

Results

Table 1 shows the percentage occurrence of the fungal isolates: Rhizopus nigricans 12(27.90%), Cercospora purpurea 10(23.20%), Aspergllus niger 8(18.60), Pestalotiopsis guepinii 5(11.62%), Botryosphaeria dothiorella 4(9.30%), 3(6.97%) and Colletotrichum acutatum 1(2.31%). Table 2 shows the prevalence occurrence of the fungal isolates from avocado pear. The fungal isolates from avocado pear: Rhizopus nigricans (27.90%) had highest percentage occurrence while Colletotrichum acutatum (2.31) had the least percentage occurrence.

Table 1: Frequency of Occurrence of Fungal Isolates from Avocado Pear

Pathogens	Frequency	Percent
Cercospora purpurea	10	23.20
Botryosphaeria dothiorella	4	9.30
Sphaceloma persea	3	6.97
Colletotrichum acutatum	1	2.31
Pestalotiopsis guepinii	5	11.62
Aspergillus niger	8	18.60
Rhizopus nigricans	12	27.90
Total	43	100.00

Table 2 shows fungicidal effect of Aloe vera oil extract at different concentrations (mg/mL), it is evident that concentrations at 10-3 (mg/mL), Pestalotiopsis guepinii (30.30) was most inhibited, this was closely followed by Sphaceloma persea (28.60). Colletotrichum acutatum (22.00) was least inhibited at concentrations 10-3 (mg/mL). Increase in concentration of Aloe vera oil extract favoured increase in inhibitory effect.

Table 2: Antifungal activities of Aloe vera oil extract at various concentrations (mg/mL)

Isolates	Diameter of zones of inhibition (mm)			
	10^{0}	10^{-1}	10^{-2}	10^{-3}
Cercospora purpurea	10.00[b]	14.20[c]	22.00[b]	25.40[c]
Botryosphaeria dothiorella	11.00[ab]	17.10[b]	20.30[b]	22.10[d]
Sphaceloma persea	12.00[a]	20.00[a]	22.20[b]	28.60[b]
Colletotrichum acutatum	10.00[b]	15.20[c]	18.90[bc]	22.00[d]
Pestalotiopsis guepinii	11.12[ab]	18.00[b]	26.10[a]	30.30[a]
Aspergillus niger	8.00[c]	14.00[c]	19.40[bc]	25.80[c]
Rhizopus nigricans	10.00[b]	14.00[c]	17.30[c]	25.00[c]
Control	0.00	0.00	0.00	0.00

Values are mean ± standard error of the mean for bioassay conducted in triplicate. Means followed by the same letter(s) are not significantly different (multivariate analysis, Fisher's protected LSD at ≤ 0.05).

Table 3 shows fungicidal effects of different concentrations (mg/mL) of neem oil extract. It could be inferred from the Table that concentrations at 10-3 (mg/mL), Sphaceloma persea (30.10) was most inhibited, this was closely followed by Pestalotiopsis guepinii (28.80). Aspergillus niger (20.50) was least inhibited at concentrations 10-3 (mg/mL). Increase in concentration of neem oil extract favoured increase in inhibitory effect.

Table 3: Antifungal activities of neem oil extract at various concentrations (mg/mL)

Isolates	Diameter of zones of inhibition (mm)			
	10^{0}	10^{-1}	10^{-2}	10^{-3}
Cercospora purpurea	12.00[a]	16.00[ab]	19.60[b]	23.80[c]
Botryosphaeria dothiorella	8.00[c]	10.50[c]	15.80[c]	21.00[d]
Sphaceloma persea	10.00[b]	17.40[c]	23.10[a]	30.10[a]
Colletotrichum acutatum	11.00[ab]	16.00[ac]	21.60[ab]	27.90[b]
Pestalotiopsis guepinii	12.00[a]	18.70[a]	21.90[ab]	28.80[b]
Aspergillus niger	10.00[a]	13.30[cd]	20.50[b]	20.50[d]
Rhizopus nigricans	00.00[d]	8.00[d]	17.10[c]	23.20[c]
Control	0.00	0.00	0.00	0.00

Values are mean ± standard error of the mean for bioassay conducted in triplicate. Means followed by the same letter(s) are not significantly different (multivariate analysis, Fisher's protected LSD at <0.05).

Table 4 shows fungicidal effects of pear seed oil extract at different concentrations (mg/mL), it could be deduced that concentrations at 10-3 (mg/mL), Pestalotiopsis guepinii (28.00) was most inhibited, this was closely followed by Botryosphaeria dothiorella (25.00). Aspergillus niger (10.20) was least inhibited at concentrations 10-3 (mg/mL). Increase in concentration of pear seed oil extract favoured increase in inhibitory effect.

Table 4: Antifungal activities of pear seed oil extract at various concentrations (mg/mL)

Isolates	Diameter of zones of inhibition (mm)			
	10^{0}	10^{-1}	10^{-2}	10^{-3}
Cercospora purpurea	10.00b	15.10[b]	19.90b	23.70abc
Botryosphaeria dothiorella	10.00b	14.80[b]	20.30b	25.00a
Sphaceloma persea	0.00c	8.00[c]	11.90d	16.20e
Colletotrichum acutatum	11.00a	9.20[c]	17.20b	20.90d
Pestalotiopsis guepinii	12.00a	19.10[a]	23.10a	28.00a
Aspergillus niger	0.00c	0.00[d]	8.00e	10.20f
Rhizopus nigricans	0.00c	8.00c	13.60c	16.90e

Control	0.00	0.00	0.00	0.00

Values are mean ± standard error of the mean for bioassay conducted in triplicate. Means followed by the same letter(s) are not significantly different (multivariate analysis, Fisher's protected LSD at < 0.05).

Table 5 shows the nutritive composition of avocado affected by fungi. The result obtained indicated that moisture content ranged between 73.22 and 74.60mm with Pestalotiopsis guepinii having the highest value while Sphaceloma persea having the least moisture content. The value of dry matter ranged between 33.12 and 35.90 with Cercospora purpurea having the highest value while Colletotrichum acutatum had the least value. Ash content of the cabbages ranged between ranged between 0.76 and 0.98 with Cercospora purpurea having highest value while Sphaceloma persea was least. Result of crude fibre content revealed that it ranged between 3.32 and 4.03 with Colletotrichum acutatum having the highest value while Sphaceloma persea had the least value for crude fibre. Fat content ranged between 15.21 and 17.21 with Sphaceloma persea having highest value while Botryosphaeria dothiorella has the least value of fat content. Crude protein ranged between 1.66 and 2.06 with Sphaceloma persea having the highest value while Pestalotiopsis guepinii has the least value. Carbohydrate content of the pear ranged between 5.35 and 6.01 with Cercospora purpurea having the highest value while Pestalotiopsis guepinii had the least value.

Table 5: Result of proximate analysis of avocado pear infected with various fungal pathogens

Parameters (%)	Fungal pathogens							
	Control	cp	bd	sp	ca	pg	an	rn
Moisture content	69.25^{b}	73.77^{a}	74.08^{a}	73.22^{a}	73.59^{a}	74.60^{a}	72.90^{a}	74.00^{a}
Dry matter	30.65^{c}	35.90^{a}	35.12^{a}	34.07^{a}	33.01^{b}	35.75^{a}	35.05^{a}	34.10^{a}
Ash content	1.00^{a}	0.98^{a}	0.86^{a}	0.76^{b}	0.79^{b}	0.91^{a}	0.80^{b}	0.95^{a}
Crude fibre	4.75^{a}	3.79^{b}	3.50^{b}	3.32^{c}	4.03^{a}	3.90^{b}	3.74^{b}	3.62^{c}
Fat content	17.87^{c}	16.60^{ab}	15.21^{c}	17.21^{a}	16.87^{ab}	15.30^{c}	16.56^{bc}	15.57^{bc}
Crude protein	2. 66^{a}	1.81^{b}	1.76^{b}	2.06^{a}	1.80^{b}	1.66^{c}	1.91^{b}	1.88^{b}
Carbohydrate	6.64^{a}	6.01^{a}	5.90^{a}	5.95^{a}	5.45^{b}	5.35^{b}	5.92^{a}	5.70^{b}

Keys
CP: Cercospora Purpurea
BD: Botryosphaeria Dothiorella
SP: Sphaceloma Persea
CA: Colletotrichum Acutatum
PG: Pestalotiopsis Guepinii
AN: Aspergillus Niger
RN: Rhizopus Nigricans

Discussion

Amadi et al., (2014) [15]. Reported some of the fungal isolates in this study as post-harvest deterioration organisms of many fruits and vegetables in the tropics. reported that contamination of agricultural product is a function of many factors including infestation in the field prior to harvest, handling during harvesting and methods of packaging and transportation of the product to the market. Johnny et al (2019z) [16]. Reported various activities of plant extracts against plant pathogens. Several reports showed the implication of A. niger in spoilage of many fruits and vegetables (Tafinta et al., 2013) [17]. This study also revealed that neem oil extract was the most effective on the fungal isolates, followed by Aloe vera oil extract and pear seed extract at different concentrations.

Pathogens associated with stem end rot (SER) in various locations of the world were as follow, in Italy, Collectotrichum gloeosporioides, C. fructicola and Diaporthe foeniculacea or D. sterilis (Guarnaccia et al., 2016) [18], in California, Neofusicocum luteum and Phomopsis perseae were reported by Twizeyimana et al., (2013) [19]. While in South Africa, Pestalotiopsis versicolor, Rhizopus stolonifer, Fusarium sambucinum and Fusarium solani (Kimaru et al., 2018) [20]. Ferro et al., (2003) [21]. Reported antibacterial effect of Aloe vera leaf gel on two-gram positive bacteria: Shigella flexneri and Streptococcus progenes. Aloe vera juice had been reported to have antimicrobial activity against Mycobacterium smegmatis, Klebisella pneumoniae, Enterococcus faecalis, Micrococcus luteus, Candida albicans and Bacillus sphricus (Suleyman and Sema, 2009), against urinary tract infection (Bukhari et al., 2017). Casian et al., (2007) [22, 23]. Reported antimycelial effect of hydroalcoholic extracts of fresh leaves of Aloe vera against Botrytis gladiolorum, Fusarium oxysporum, Heterosporium pruneti and Penicillium gladioli.

The Aloe vera plant-based ointment tremendously repress the growth of examine microbes as contrast with other antimicrobial properties containing plants and fungi pathogen show more resistant as compared to bacterial pathogens against Aloe vera ointment (Dharajiya et al., 2015) [24].

The nutritive compositions of infected pear indicate that moisture content ranged between 73.22 and 74.60% with Pestalotiopsis guepinii with the highest value while Sphaceloma persea had the least moisture content (Table 6). The value of dry matter ranged between 33.12 and 35.90%. Cercospora purpurea and Colletotrichum acutatum infected pear had the highest and least value respectively.

Ash content of the pear ranged between 0.76 and 0.98%. Cer-

cospora purpurea and Sphaceloma persea infected pear had the highest and least value respectively. The value obtained for the affected avocado pear is compared with the value range of 5.50 - 16.10% was reported by Udousoro and Ekanem (2013) for twelve edible vegetables in Nigeria [25].

Result of crude fibre content revealed that it ranged between 3.32 and 4.03%. Colletotrichum acutatum and Sphaceloma persea infected pear had the highest and least value respectively. The values obtained from these findings are similar to values reported by (Gotruvalli et al. 2016) [26]. Who reported low ash contents for the leaves of Amaranthus viridis and Alternanthera sessilis. Hanif et al. (2006) [27]. Reported that the presence of high fiber contributes significantly to nutritive value as fiber lowers body cholesterol level and consequently decreases the risk of cardiovascular diseases.

Fat content ranged between 15.21 and 17.21%. Sphaceloma persea and Botryosphaeria dothiorella infected pear had the highest and least value respectively. High crude fat composition has been reported in green vegetables (Okezie et al., 2017) [28].

Crude protein ranged between 1.66 and 2.06%. Sphaceloma persea and Pestalotiopsis guepinii infected pear had the highest and least value respectively. The values obtained in this study were lower compared with the crude protein range of 25.06 - 30.02% reported of some medicinal leaves (Aborisade et al., 2017) [29]. These results correlated with the values reported by Gotruvalli et al. (2016) [30, 31]. for the leaves of Alternanthera sessilis (4.5%) and Amaranthus viridis (2.11%). This study indicated that the vegetables investigated had moderately low amount of lipid. Carbohydrate content of the pear ranged between 5.35 and 6.01%. Cercospora purpurea and Pestalotiopsis guepinii infected pear had the highest and least value respectively [32].

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