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Control Apple Fruit Fungi Using a Green Biocide Neem (*Azadirachta indica* A. Juss)

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Abstract:

Apple (Malus Domestica Borkh.) is an important fruit which is widely cultivated in the world and also in Egypt. Rotting and decay of the apples were observed regularly. Isolation of associated fungi from apple fruits was carried out on potato dextrose agar medium (PDA).A total of seven fungal pathogens including, *Alternaria alternata*, *Aspergillus niger*, *Colletotrichum gloeosporioides*, *Fusarium* sp., *Penicillium expansum*, *Botryodiplodia theobromae* and *Rhizopus stolonifer* were isolated from decayed fruits. Apple fruit spoilage was most severe under humid environment and was enhanced by wounds on fruit surfaces. Pathogenicity test revealed that all isolates proved pathogenic when artificially inoculated into healthy apple fruits. Neem (*Azadirachta indica* A. Juss) extract is an effective a green biocide (bio-fungicide) against the growth of fungi and spore viability.

Keywords: Apple fruits; Fungi; Azadirachta indica A. Juss

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1. Introduction

Apple (*Malus Domestica* Borkh.) is a highly nutritious fruit containing essential food elements such as carbohydrates, protein, fat and water. Apart from its energy value, apple is a good source of soluble and insoluble fiber [1]. Apples are a widely consumed, rich source of phytochemicals; epidemiological studies have linked the consumption of apples with reduced risk of some cancers, cardiovascular disease, asthma, and diabetes due to strong antioxidant activity [2].Microbes can adhere to surface, invade/penetrate apple fruits surface and multiply within the tissue. Contamination could be from human handling, transport vehicles, insects, dust, and rinse water, harvesting equipment, soil, feces, irrigation water, water used to apply fungicides and insecticides, manure, wild and domestic animals [3]. Besides nutritional merits, apple may get contaminated during growth, harvest, transportation and further processing and handling with microbes from soil, air, water or animal wastes. Handling in stores and retail markets could also add more microorganisms to the surface of the fresh product. The common postharvest and storage fungi of fruits are *Alternaria* spp., *Aspergillus* spp., *Fusarium* spp., and *Penicillium* spp. [4], and the majority of mycotoxins are produced by fungi of the genera, *Alternaria*, *Aspergillus*, *Penicillium* and *Fusarium*[5&6].Among microbes, *Penicillium expansum*, a fungus is a major causative agent of postharvest decay in apple, since it produces patulin, a mycotoxin known to cause harmful effects in humans [7].

Neem (*Azadirachta indica* A. Juss) has anti-fungal and antibacterial. These activities of Neem are because of the presence of compounds like Nimbidin, Nimbin, Sodium intimidates, Gedunin, Nembolide, Cyclic trisulphide, cyclic tetrasulfide, Polysaccharides, NB-II polypeptide glycan [8], as well as immune modulatory properties in different animal species [9&10]. Thus, neem extracts which are cheap and environmentally safe can exhibit considerable control protection of apple against fruit pathogenic fungi with possible improvement quality of economic crops.

This investigation aimed to survey the fungal pathogens in some apple fruits,prevention and control methods ofsome mold fungi affecting apple fruit quality by using a green biocide.

2. Materials and Methods

2.1. Sample collection:

Freshly ripe of apple fruits (Dorsett Golden, Red delicious and Anna apple) were collected from different localities i.e.Cairo, Dakahlia, Kalubia and Sharqia Governorates, Egypt into polyethylene bags and stored at 4°C till used in the laboratory.

2.2. Microbiological analysis:

Microbial contaminants of apple fruits were isolated using the serial dilution and agar plate technique. One gram of naturally infected fruit was added into 90mL of sterilized water resulted in 1\100 dilution then diluted 1\10000. Finally, one milliliter of sample dilutions was pipette to sterilized Petri dishes containing sterilized PDA supplemented with antibiotic penicillin sulfate (at pH 5.5) for enumeration of fungi and incubated at 25± 2°C for five days. All colonies were purified on potato dextrose agar and counted according to[11],then subcultured on PDA slants for further study. The isolated fungi were microscopically examined and identified according to the literatures[12,13,14,15&16]. Fungal frequencies were recorded.

2.3. Control methods

2.3.1- Effect of a green biocide neem extract (NE) on the linear growth rate of the isolated fungi *in vitro*

For evaluation of *in vitro* antifungal activity of a green biocide (plant extract of *A. indica*) obtained from Grace Company of pesticides, Cairo, Egypt. Commercial neem extract (NE) was dissolved in sterilized PDA at 1, 5, 10 and 20 concentrationper 100ml PDA, then poured it into 9 cm diameter of sterilized Petri dishes. One disc 0.5 cm diameter of the isolated fungal i. e. *A. alternata, Aspergillus niger, Fusarium* sp. and *P. expansum* was cut from 1-week-old cultures on the PDA plates then placedin thecenter ofthe dish according to[17,18&19]. Three Petri dishes were used as replicates per treatment then incubated at 25 ± 2°C.Colony diameters was measured after incubation period [20]. Medium without phytoextract served as control. Percent growth inhibition (%) was calculated according to the formula

(C-T)/C×100 Where C = growth in control, T = growth in treatment [21& 22].

2.3.2- Effect of a green biocide neem extract (NE)on the mycelial dry weight of the isolated fungi *in vitro*

Neem extract (NE) was dissolved in sterilized Potato dextrose broth (PDB) at rates 1, 5, 10 and 20ml concentration per 100ml PDB medium. One disc from the isolated fungi (0.5cm) 1-week-old cultures was cut from PDA plates then placed in the liquid media and incubated at 25 ± 2°C for 15 days. After incubation period were dried at 80^0c for 24h. Effect of neem extract on the mycelial dry weight was calculated and recorded for each fungus.

2.3.3-Effect of neem extract on spore viability

A green biocide (neem extract) was tested on spore germination (viability) of the tested pathogens including *A. alternata, Aspergillus niger, Fusarium* sp. and *P. expansum*. A disc of 0.5 cm diameter of 7 days old PDA fungal culture was placed at the center of agar plates and incubated at 25±2ºC (3 replication for each concentration). The sporulations of tested fungi were studied separately by collecting spore suspension of the respective fungi after passing the culture filtrate through amuslin cloth. To harvest spores, 10 mL of sterile water were poured over the plateand the concentration was adjusted to 1 $x10^2$ conidia/ml. Each plate was inoculated with 0.1 mL of spore suspension that was spread evenly over the plate. Each treatment had three replicate plates that were incubated at 25ºC in the dark. After 48 h of incubation, the proportion of spores that had germinated was calculated [23].

2.3.4. Fruit treatment (Fungal decontaminants)

Conidial suspension of the pathogen was harvested by flooding the dishes (7-day-old culture) with steriledistilled water containing 0.01% Tween-80 and adjusted to 10^4conidia per ml. Healthy apple fruits were surface-sterilized with 70% ethanol and air evaporation then wounded with a sterilized nail at 2 points (2 mm deep 5 mm wide) on the equator of each fruit. All tested apple fruits were dipped in the commercial neem extract (NE) with each concentration for 1 minutes and then 1ml of the conidial suspension was injected into each of thewounded sites. Untreated fruits (control) were dipped in sterile distilled water. Three replicates were used per treatment. Both treated and untreated (control) fruits were kept in plastic containers and incubated at 25± 2°C., with 95% RH. Disease severity was estimated as lesion diameter on the fruit and recorded at the indicated times[24].

3. Results

A total of 1290 fungal colonies (1110 molds &180 yeasts)were isolated from different rotten apple fruits collected from four different Governorates. e. Cairo, Dakahlia, Kalubia and Sharqia . Also, data indicated that Sharqia Governorate sample had the highest infection, followed by Kalubia and Dakahlia while Cairo Governorate had the least infection (Table 1).

On the other hand, the highest total fungal count was found in the Dorsett Golden apple fruit compared to others, which record 461fungal coloniesequal 41.5% followed by Red delicious apple fruit 404 fungal colonies equal 36.4%. Anna apple gave 245 fungal colonies equal 22.1%.

2. Percentage of fungal frequency

Percentage of fungal frequency isolated from apple fruits were recorded in Table (2). Data show that seven fungal genera were isolated and identified from the three types of apple fruits(Dorsett Golden, Red delicious and Anna apple cvs.). These are *Alternaria alternata, Aspergillus niger, Colletotrichum gloeosporioides, Fusarium* sp., *Penicillium expansum, Botryodiplodia theobromae* and *Rhizopus stolonifer*. Also, data in this table show that *A. alternata* was the most fungal frequency which records 27.8% followed by *P. expansum* 24.3%, *Aspergillus niger* 16.8%, *Fusarium*sp.,13.2%,*Botryodiplodiatheobromae* 12.1%, and

Rhizopus stolonifer 3.7%. Less fungal frequency was recorded with Colletotrichum fungus which gave 2.1%.

Table 1:Percentage of total colonies forming isolated from apple fruits collected from different Governorates

Microorganisms		Location												Total
		Sharqia			Kalubia			Dakahlia			Cairo			
Apple Cvs		D	R	A	D	R	A	D	R	A	D	R	A	
Type of colony														
Fungi	T	207	128	90	96	105	68	94	86	59	64	85	28	1110
	%	16.1%	9.9%	6.9%	7.4%	8.1%	5.3%	7.3%	6.7%	4.6%	4.9%	6.6%	2.2%	86.04%
Yeast	T	20	17	12	15	23	11	10	30	9	10	18	5	180
	%	1.5%	1.3%	1.0%	1.2%	1.8%	0.9%	0.8%	2.3%	0.7%	0.8%	1.4%	0.4%	13.96%
Total		227	145	102	111	128	79	104	116	68	74	103	33	1290
%		17.6%	11.2%	7.9%	8.6%	9.9%	6.1%	8.1%	9.0%	5.3%	5.7%	8.0%	2.6%	100%

D=Dorestt Golden R= Red delicious A = Anna apple

Table 2:Percentage of apple fruits associated fungal frequency:

Fungi	Location												T.C apple			T.C
	Sharqia			Kalubia			Dakahlia			Cairo						
	D	R	G	D	R	G	D	R	G	D	R	G	TD	TR	TG	
Alternaria	50	43	25	40	35	20	20	24	20	12	10	10	122	112	75	309
	16.2	13.9	8.09	12.9	11.3	6.5	6.5	7.8	6.5	3.9	3.25	3.2	39.5	36.2	24.3	27.8
Penicillim	60	28	20	20	20	15	24	30	15	10	20	8	114	98	58	270
	22.2	10.4	7.4	7.4	7.4	5.6	8.9	11.1	5.6	3.7	7.4	2.9	42.2	36.3	21.5	24.3
Botryodiplodia	25	23	15	0	10	0	10	9	11	12	9	10	47	51	36	134
	18.7	17.2	11.2	0	7.5	0	7.5	6.7	8.2	8.9	6.7	7.5	35.1	38.1	26.9	12.1
Aspergillus	30	16	20	11	17	10	20	20	13	10	20	0	71	73	43	187
	16.1	8.6	10.7	5.9	9.1	5.3	10.7	10.7	6.9	5.3	10.7	0	37.9	39.1	22.9	16.8
Rhizopus	20	0	0	5	3	0	0	3	0	0	10	0	25	16	0	41
	48.7	0	0	12.2	7.3	0	0	7.3	0	0	24.3	0	60.9	39.1	0	3.7
Fusarium	20	18	10	20	12	23	10	0	0	20	13	0	70	43	33	146
	13.7	12.3	6.9	13.7	8.2	15.8	6.9	0	0	13.7	8.9	0	47.9	29.5	22.6	13.2
Colletotrichum	2	0	0	0	8	0	10	0	0	0	3	0	12	11	0	23
	8.7	0	0	0	34.8	0	43.5	0	0	0	13.1	0	52.1	47.8	0	2.1
Total	207	128	90	96	105	68	94	86	59	64	85	28	461	404	245	1110
	18.6	11.5	8.1	8.6	9.5	6.1	8.5	7.7	5.3	5.8	7.6	2.5	41.5	36.4	22.1	100

D =Dorestt Golden R= Red delicious A = Anna apple

3. Fungal control:

3.1. Effect of neem extract on the linear growth rate of the tested fungi

The antifungal activity of neem extract (NE), on the linear growth rate of tested fungi i. e. *A. alternata, A. niger, Fusarium* and *P. expansum* were recorded in Table 3. Data presented that, the linear growth rate of all tested fungi was decreased with all different concentrates compared with untreated control. The effect of NE on tested fungi was increasing with the increase of NE concentration. Also, NE was more effective against *Fusarium* than other tested fungi followed by *A. alternata* . Neem extract (NE)was more effective at 20 cons. than others ml.

On the other hand, data indicated that after 3 days the linear growth rate of *A.alternata* was 35.66mm with untreated (control) which decreased to12.00, 9.66, 8.00 and 6.66 mm when treated with 1, 5, 10 and 20ml of NE cons. equal 66.3, 72.9, 77.6 and 81.3% reduction respectively. The linear growth rate of *Fusarium* was decreased from 44.66mm with untreated (control) to 22.33, 13.00, 11.00 and 10.33mm when treated with 1, 5, 10 and 20ml of NE cons. equal 50.0, 70.9, 75.4 and 76.9% reduction respectively. The linear growth rate of both *A. niger* and *P. expansum* were 70.00mm with untreated (control) which decreased to 17.00, 13.66, 13.00 and 11.00mm with *A. niger* equal 74.7, 80.5, 81.4 and 84.3% reduction when treated with the same cons. While, *P. expansum* was found to be decreased to 17.33, 17.00, 12.00 and 11.33mm equal 75.2, 75.7, 82.9 and 83.8% reduction respectively.

After 7 days the linear growth rate of *A.alternata* was 70.00mm with untreated (control) which decreased to 25.33, 13.33,11.00and 9.00 mm when treated with 1, 5, 10 and 20ml of NE cons. equal 63.8, 80.9, 84.3 and 87.1% reduction respectively. The linear growth rate of *Fusarium* was decreased from70.00 mm with untreated (control) to 47.00, 23.66, 19.66and 18.33mm when treated with 1, 5, 10 and 20ml of NE cons. equal 32.9, 66.2, 71.9 and 73.8% reduction respectively. The linear growth rate of both *A. niger* and *P. expansum* were 70.00mm with untreated (control) which decreased to 17.00, 12.66, 13.00 and 11.00mm with *A. niger* equal 75.7, 81.9, 81.4 and 84.3% reduction when treated with the same cons. While*P. expansum* was decreased to 17.33, 13.66, 11.33and 11.33mm equal 75.2, 80.5, 83.8and 83.8% reduction respectively. After 10 days the linear growth rate of *A. alternata* was 70.00mm with untreated (control) which decreased to 29.66, 15.33,12.00and 8.66 mm when treated with NE cons. equal 57.6, 78.1, 82.9 and 87.6% reduction respectively. The linear growth rate of *Fusarium* was decreased from70.00 mm with untreated (control) to 54.00, 22.33, 21.66and 19.33mm when treated with NE cons. equal 22.9, 68.1, 69.1 and 72.4% reduction respectively. The linear growth rate of both *A. niger* and *P. expansum* were 70.00mm with untreated (control) which decreased to 11.33, 10.66, 10.00 and 10.00mm with *A. niger* equal 83.8, 84.7, 85.7 and 85.7% reduction when treated with the same cons. While, *P. expansum* was found to be decreased to 10.66, 10.66, 10.00and 10.00mm equal 84.8and 85.7% reduction respectively.

After 15 days the linear growth rate of *A. alternata* was 70.00mm with untreated (control) which decreased to 40.66, 25.00,17.33and 9.00 mm when treated with NE cons. equal 41.9, 64.3, 75.2 and 87.1% reduction respectively. The linear growth rate of *Fusarium* was decreased from70.00 mm with untreated (control) to 62.33, 34.33, 30.66and 23.66mm when treated with NE cons. equal 10.9, 50.9, 56.2 and 66.2% reduction respectively. The linear growth rate of both *A. niger* and *P. expansum* were 70.00mm with untreated (control) which decreased to 10.66, 10.33, 10.00 and 10.00mm with *A. niger* equal 84.8, 85.2, 85.7 and 85.7% reduction when treated with the same cons. While*P. expansum* was found to be decreased to 10.66, 10.66, 10.33and 10.00mm equal 84.8, 84.8,85.2and 85.7% reduction respectively.

3.2- Effect of neem extract on the mycelial dry weight of the tested fungi

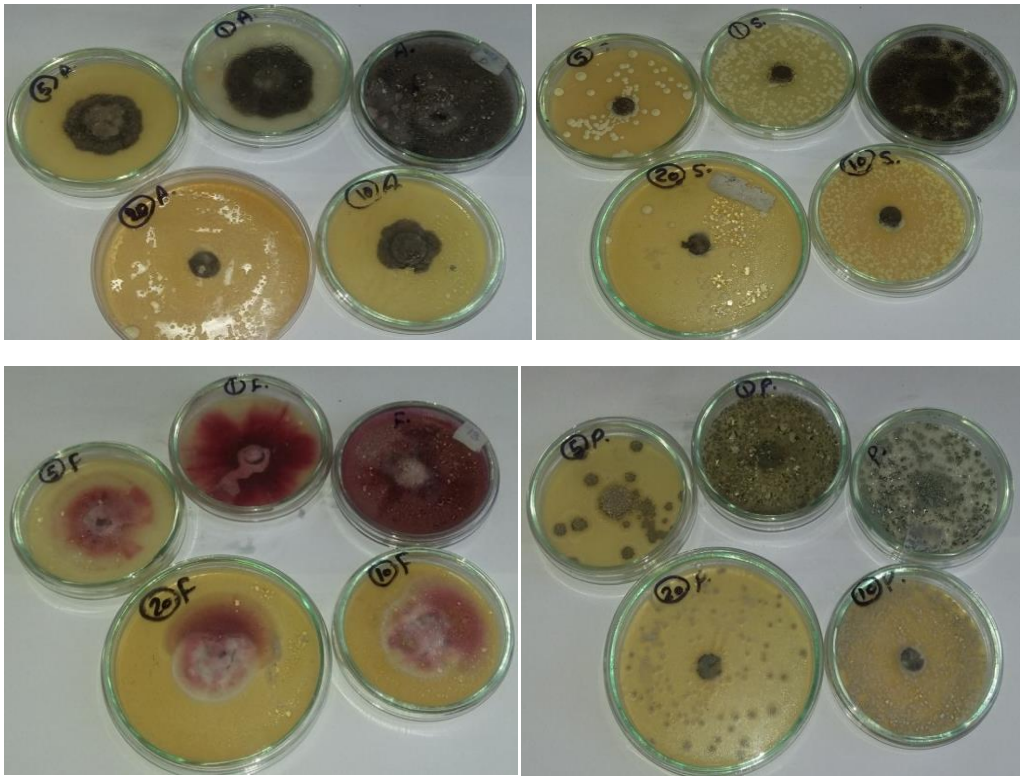
Effect of neem extract on the mycelial dry weight was calculated and tabulated in (Table 4). Data presented that, neem extract was found to reduce all mycelial dry weight of all tested fungi (*A. alternata, A. niger, Fusarium* and *P. expansum*) with all concentrated used comparing with untreated control. Also, data show that, increasing the reduction percent with increasing the concentration of NE. The mycelial dry weight of *A. alternata* was 4.8g with untreated (control) then decreased to 0.5g when treated with NE at 20 ml concentration equal 89.5% reduction.

The mycelial dry weight of *A. niger* 1.1g with untreated (control) then decreased to 0.2g when treated with NE at 20 ml concentration equal 81.8% reduction. The mycelial dry weight of *Fusarium* 3.9g with untreated (control) then decreased to 0.2g when treated with NE at 20 ml concentration equal 94.9% reduction. The mycelial dry weight of *P.expansum* 2.1g with untreated (control) then decreased to 0.2g when treated with NE at 20 ml concentration equal 90.4% reduction

Table (3): Effect of neem extract on the linear growth rate

Time/day	Fungi		Diameter\mm					LSD 5% between period time
			control	1ml	5ml	10ml	20ml	
3	*A. alternata*	L	35.66	12.00	9.66	8.00	6.66	21.31 C
		R	-	66.3	72.9	77.6	81.3	
	A.niger	L	70.00	17.66	13.66	13.00	11.00	
		R	-	74.7	80.5	81.4	84.3	
	Fusarium	L	44.66	22.33	13.00	11.00	10.33	
		R	-	50.0	70.9	75.4	76.9	
	P. expansum	L	70.00	17.33	17.00	12.00	11.33	
		R	-	75.2	75.7	82.9	83.8	
7	*A. alternata*	L	70.00	25.33	13.33	11.00	9.00	27.73 B
		R	-	63.8	80.9	84.3	87.1	
	A.niger	L	70.00	17.00	12.66	13.00	11.00	
		R	-	75.7	81.9	81.4	84.3	
	Fusarium	L	70.00	47.00	23.66	19.66	18.33	
		R	-	32.9	66.2	71.9	73.8	
	P. expansum	L	70.00	17.33	13.66	11.33	11.33	
		R	-	75.2	80.5	83.8	83.8	
10	*A. alternata*	L	70.00	29.66	15.33	12.00	8.66	27.31 B
		R	-	57.6	78.1	82.9	87.6	
	A.niger	L	70.00	11.33	10.66	10.00	10.00	
		R	-	83.8	84.7	85.7	85.7	
	Fusarium	L	70.00	54.00	22.33	21.66	19.33	
		R	-	22.9	68.1	69.1	72.4	
	P. expansum	L	70.00	10.66	10.66	10.00	10.00	
		R	-	84.8	84.8	85.7	85.7	
15	*A. alternata*	L	70.00	40.66	25.00	17.33	9.00	30.28 A
		R	-	41.9	64.3	75.2	87.1	
	A.niger	L	70.00	10.66	10.33	10.00	10.00	
		R	-	84.8	85.2	85.7	85.7	
	Fusarium	L	70.00	62.33	34.33	30.66	23.66	
		R	-	10.9	50.9	56.2	66.2	
	P. expansum	L	70.00	10.66	10.66	10.33	10.00	
		R	-	84.8	84.8	85.2	85.7	
LSD 5% between conc. of Neem extract			66.27 A	25.31 B	16.04 C	13.83 D	11.85 E	
LSD 5% between fungi			*A. alternata*=24.91 B *A.niger*=23.60 C *Fusarium* = 34.41 A *P. expansum*=23.71 C					

L = linear growth rate R = Reduction%



Figs. (1, 2, 3 & 4): Effect of neem extract on the linear growth rate of *A. alternata*, *A. niger*, *Fusarium* and *P. expansum* at different concentration respectively.

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Table (4):Effect of NE on the mycelial dry weight of pathogens

Organisms	Conc.\ ml	Fresh wt \g	Dry wt.\g	Reduction%
A.alternata	1	9.3	1.9	60.4
	5	8.5	1.6	66.7
	10	5.6	0.8	83.3
	20	3.9	0.5	89.5
	control	13	4.8	100
A. niger	1	8.2	0.9	18.2
	5	7.9	0.8	27.3
	10	5.6	0.7	36.4
	20	3.3	0.2	81.8
	control	9.6	1.1	100
Fusarium	1	7.2	1.7	56.4
	5	6.9	1.6	59
	10	5.2	0.4	89.7
	20	3.4	0.2	94.9
	control	11.3	3.9	100
P.expansum	1	8.8	1.9	9.5
	5	7.4	1.3	38.1
	10	5.7	0.4	80.9
	20	3.9	0.2	90.4
	control	9.1	2.1	100

3.3- Effect of NE on spore production

NE reduced spore-forming *A. alternata, A. niger, Fusarium* and *P. expanse* at the same concentration compared with untreated control.

Table (5): Effect of NE on spore production

Organisms		NE concentration \ml				
		control	1	5	10	20
A.alternata	sp	$6x10^3$	$4x10^3$	$32x10^2$	$24x10^2$	$12x10^2$
	R	100	33.3	46.7	60	80
A.niger	sp	$13x10^4$	$5x10^4$	$4x10^4$	$2x10^4$	$1x10^4$
	R	100	61.5	69.2	84.6	92.3
Fusarium	sp	$16x10^4$	$13x10^4$	$11x10^3$	$8x1o^3$	$6x1o^3$
	R	100	18.8	93.1	95	96.3
P.expansum	sp	$2x10^5$	$16x10^4$	$7x10^4$	$6x10^4$	$2x10^4$
	R	100	20	65	70	90

Sp = spore production R = Reduction%

3.4- Control Apple fruit pathogens by using NE *in vivo*:

Effect of NE on Apple fruit pathogens was clearly observed at two weeks after the artificial inoculation. All apple fruit pathogens were found to be reduced by using NE at the 20ml concentration used, as well as increasing the reduction percent with both two apple fruit cultivars i. e. Dorsett Golden and Red delicious . The ID on the fruits treated with NE and inoculated with *A.alternata* on Dorsett Golden was only 51.1% of the control fruit after the inoculation with fungus only. While on red apple was 65.1%. The ID on treated fruits and inoculated with *A.niger* on yellow apple was only 68.5%, while on red apple the ID of treated fruits was 65.8%. The ID on treated fruits and inoculated with *Fusarium* on Dorsett Golden was only 58.3% while on red apple the ID of treated fruits was 59.4%. The ID on treated fruits and inoculated with *P.expansum* on yellow apple was only 50.7% while on red apple the ID of treated fruits was 54.5%.

Finally, the result indicated that there was no significant difference between cultivars. While there was theasignificant difference between the fungi since, *A.niger* represent the highest one (Table 6).

Table (6): Effect of NE on Apple fruit pathogens

Organisms	Diameter of rotten fruits \cm						LSD 5% between fungi
	Dorsett Golden			Red delicious			
	U	T	R	U	T	R	
A.alternata	4.9d-f	2.4g-i	51.1	8.3b	2.9f-i	65.1	4.65 B
A.niger	16.2a	5.1c-e	68.5	15.5a	5.3cd	65.8	2.40 A
Fusarium	3.6d-g	1.5hi	58.3	3.2e-i	1.3i	59.4	5.36 B
P.expansum	6.9bc	3.4d-h	50.7	7.7b	3.5d-g	54.5	10.50 C
LSD 5% between cvs	5.49 A			5.97 A			

U = Un-treated T = Treated R= Reduction%

4. Discussion

Apple (*Malus Domestica* Borkh.) is a highly nutritious fruit as they contain antioxidants, vitamins, and minerals that are essential for human being and play important role in the prevention of heart diseases, cancer, and diabetes. Seven fungal genera were isolated and identified from the three types of apple i. e. Dorsett golden, Red delicious and Anna apple. These are *Alternaria alternata*, *Aspergillus*

niger, *Colletotrichum gloeosporioides*, *Fusarium* sp., *Penicillium expansum*, *Botryodiplodia theobromae* and *Rhizopus stolonifer*. *Penicillium expansum*causes a soft rot of apples. This particular species has a special significance because of its ability to produce the mycotoxinpatulin which has been detected as a contaminant in apple. Commercially produced and properly handled bread generally lacks sufficient amounts of moisture to allow for the growth of any microorganisms except molds.

[25] reported that contaminated foods are the source of various foodborne conditions due to gastroenteritis in human. The consumption of foods could have both positive and negative effect onthe part of consumers. The focus of this study was to compare the quality of commercially available food by assessing their microbial load and the presence of pathogenic fungi. Accordingly, a variety of chemical, biological and physical strategies had been developed to control the mycotoxigenic pathogens.

Neem (*Azadirachta indica* A. Juss) has anti-fungal and antibacterial activities due to the presence of compounds like Nimbidin, Nimbin, Sodium intimidates, Gedunin, Nembolide, Cyclic trisulphide, cyclic tetrasulfide, Polysaccharides, NB-II polypeptide glycan [8,9&10]. Thus, neem extracts which are cheap and environmentally safe can exhibit considerable control protection of apple against fruit pathogenic fungi with possible improvement quality of economic crops. The in vivo and in vitro antifungal activity of neem extract in the fruit reported in our study are in agreement with other reporters [26&27].

From our obtained results we might conclude that apple rotted fruit caused by fungi are responsible for the economic loss. *A. alternata*, *Aspergillus niger*, *Fusarium* sp., and *Penicillium expansum* were the most frequent fungi. Neem (*A. indica*) extract is an effective a green biocide (bio-fungicide) against the growth of fungi and spore viability. Disinfection of apple fruits with neem extract (at 1, 5, 10 and 20% concentrations) had the potential for suppressing fungal contamination which is mainly associated with these fruits which agrees with other previous reports [8,9,10&24]. This has gone a long way in providing a better alternative to the over dependency on synthetic fungicides. Using of neem extract in this study was easily available, with the aneasy method of extraction; it can be exploited in the control of apple fruit decay caused by fungi, can be used as fungicidal fruit treatments for the control fungal association.

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