Original Research Article

In vitro Potential of Aqueous Extracts of Plant Leaves to inhibit Pathogenic Fungi

Abstract

The in vitro potential of aqueous extracts of plant leaves to inhibit pathogenic fungi was carried out. The effect of leaf extract and concentration on growth inhibition of organism I (*Aspergillus flavus)* in vitro revealed that at concentration of 100g/ml, *Moringa* leaf extract (MLE) gave the highest growth inhibition of (59.14) followed by Neem leaf extract (NLE) (49.70) and Bitter leaf extract (BLE) (45.84) respectively while the least growth inhibition on organism 1 *(Aspergillus flavus)* was by MLE at concentration of 40g/ml (30.11) followed by NLE at 60g/ml (32.11) and BLE at 40 g/ml (40.13). On organism 2 *(Penicillium waksmanii)*, MLE at a concentration of 100g/ml gave the highest growth inhibition of (50.49) followed by NLE (49.01) and BLE (36.72) respectively while the least inhibition on growth of organism 2 in vitro was by BLE at concentration of 60g/ml (16.05) followed by MLE (40.70) and NLE (40.70) at concentration of 80g/ml respectively. On organism 3 *(Botryodiplodia theobromae)*, MLE at concentration of 100g/ml gave the highest inhibition of growth (57.00) followed by NLE (52.71) and BLE (50.15) respectively while the least inhibition on growth of organism 3 in vitro was by BLE at 40g/ml (21.50) followed by MLE at 60g/ml (31.06) and NLE at 40g/ml (41.89). On organism 4 *(Fusarium oxysporum)*, the highest growth inhibition was by MLE at 100g/ml (54.02) followed by NLE at 100g/ml (49.62) and BLE at 100g/ml (44.41) while the least growth inhibition was shown by MLE at 60g/ml (24.04) followed by BLE at 40g/ml (26.60) and NLE at 40g/ml (30.12). The highest grand inhibitory effect of extract concentration on growth inhibition of organism 5 *(Colletotrichum asianum)* in vitro was shown by NLE at 100g/ml (53.68) followed by MLE at 100g/ml (51.51) and BLE at 100g/ml (40.94). The least inhibitory effect on growth of organism 5 in vitro was by BLE at 80g/ml (21.26) followed by NLE at 40g/ml (22.25) and MLE at 40g/ml (32.69). The controls ranged from 2.23 to 4.31 across all extract concentrations and fungal isolates. There were significant differences in growth inhibition between extract concentrations and their controls on all fungal isolates. The use of plant extracts provides alternative means for controlling plant pathogenic fungi.

Keywords: In vitro, pathogenic fungi, aqueous extracts, plant leaves.

1. INTRODUCTION

Diseases caused by fungi result in significant loss of many crops. Fungi generate the greatest impact in terms of reduction in crops productivity [1]. Presently, many researchers are trying to identify effective natural products for controlling diseases thereby replacing synthetic pesticides [2]. The activities of plant extracts have been shown to be environmentally friendly and effective against plant pathogens [3]. Researchers are beginning to show interest in the application of plant products as bio – pesticides [4]. Therefore, there can be a decrease in the use of chemical pesticides

which have undesirable effects on non – target organisms in the environment and on humans through the food crops they consume due to synthetic residues. Plant biopesticides are made from locally available plants, which are non – toxic to humans, are easily degradable and environmentally friendly. There is great demand for them as alternative agents to control plant pathogenic fungi [5]. Due to the development of pathogen resistance as a result of persistent use of synthetics and accumulation of residues, the use of plant products for disease management is considered one of the best alternatives [6]. This study was therefore undertaken to evaluate the in vitro potential of aqueous extracts of plant leaves to inhibit pathogenic fungi.

2. MATERIALS AND METHOD

2.1 Preparation of plant extracts

Forty, sixty, eighty and one hundred grams each of the dried and ground plant leaves were weighed for water extractions. The weighed powdered leaves of each plant species were soaked in 200mls of sterile distilled water for 1 hour after which sieving was done using a muslin cloth into separate beakers for each plant species.

2.2 Extract concentrations

Concentrations of each plant species was prepared to give 40g/ml, 60g/ml, 80g/ml and 100g/ml respectively. Extract concentration of 40g/ml was obtained by dissolving 40g of the plant leaf powder of each plant species respectively in 200mls of sterile distilled water in a beaker. Extract concentration of 60g/ml was obtained by dissolving 60g of the plant leaf powder of each plant species respectively in 200ml of sterile distilled water in a beaker. The same principle was applied to all other extract concentrations.

2.3 Antifungal activity of plant extracts on fungal isolates in vitro.

The pour plate method was used to investigate the efficacy of the extracts on the test fungi in vitro. Three millilitres each of 40g/ml, 60g/ml, 80g/ml and 100g/ml of the extracts for each plant species was dispensed in sterile Petri dishes after which 15 – 20mls of molten PDA was added. The mixture was swirled gently on the work bench and allowed to set. The medium was then inoculated centrally with 4mm discs obtained from 5 – 7 days old cultures of the test fungi. Three replications were set for each experiment. Controls were Petri plates containing PDA with no botanical extract, inoculated with the test fungi. The plates were arranged on laboratory desks in completely randomized design. The Petri plates were incubated at room temperature for 5 – 7 days during which measurement of growth of the fungi colony was carried

out using a meter rule at intervals of 24 hours. Growth inhibition of the fungi was calculated using the formula;

Growth inhibition of fungi $= \frac{R_1 - R_2}{R_1} \times 100$

Where R_1 = radial growth of fungi in control

R_2 = radial growth of fungi in treatment

2.4 Experimental design

3 × 4 × 6 Factorial in Complete Randomized Design

Treatment combinations = 3 × 4 × 6 = 72

Replications = 3

Total plots = 3 × 72 = 216.

3. RESULTS

3.1 Effect of plant leaf extracts on growth inhibition of fungal isolates in vitro

3.1.1 Effect of *Moringa* leaf extract on growth inhibition of test fungi in vitro

The main effect of *Moringa* treatment on organism 1 *(Aspergillus flavus*) in vitro ranged from 22.10 to 48.61 from days 1 to 7 respectively while the control ranged from 0.40 to 5.54 from days 1 to 7 respectively. Significant differences were observed between the treatments and control on all the days observed during the study. The effect of *Moringa* concentration on growth inhibition of organism 1 *(Aspergillus flavus)* in vitro on different days revealed that at 40g/ml, growth inhibition ranged from 3.50 on day 1 to 23.17 on day 7. At 60 g/ml, growth inhibition ranged from 17.70 to 23.80. At 80g/ml, growth inhibition ranged from 8.50 to 29.68 while at 100g/ml, growth inhibition ranged from 15.20 to 40.63. There were significant differences between extract concentrations on days 1, 4, 5, 6 and 7 except on days 2 and 3 as shown in table 1.

Table 1: Main effect of *Moringa* treatment and concentration on growth inhibition of Organism 1 (*Aspergillus flavus*) in vitro.

Treatment	1	2	3	4	5	6	7	(DAYS)
Control	0.40	1.34	2.10	3.00	3.77	4.79	5.54	
Treatment	22.10	43.04	46.20	47.50	43.97	48.83	48.61	
F-LSD (0.05)	**6.78**	**3.84**	**8.92**	**2.64**	**3.03**	**1.74**	**2.68**	
Concentration								
40	3.50	22.35	16.10	16.08	12.77	20.32	23.17	
60	17.70	18.00	23.80	23.02	22.12	21.25	19.40	
80	8.50	24.52	23.30	29.68	25.12	28.15	25.10	
100	15.20	23.90	33.40	32.22	35.48	37.53	40.63	
F-LSD (0.05)	**9.60**	**NS**	**NS**	**3.73**	**4.29**	**2.46**	**3.79**	

NS – No significant difference

The interaction effect of *Moringa* leaf concentration and treatment on growth inhibition of organism 1 *(Aspergillus flavus)* revealed that at 40g/ml, treatment ranged from 6.70 to 43.40 while control ranged from 0.40 to 5.37 respectively. At 60g/ml, treatment ranged from 33.77 cm to 45.40. At 80 g/ml, treatment ranged from 16.70 to 55.97 and at 100g/ml, treatment ranged from 30.00 to 75.35. Significant differences were observed between the treatments and the control on all the days. Also significant differences were observed between treatments at 60g/ml, 80g/ml and 100g/ml on day 1 while on days 4, 5 and 7, significant differences were observed between all concentrations. On day 6, significant differences were observed between concentrations of 100g/ml, 80g/ml and 60g/ml while for days 2 and 3, there were no significant differences among treatments at all concentrations as shown in table 2.

Table 2: Interaction effect of *Moringa* treatment and concentration on Organism 1 (*Aspergillus flavus*) in vitro.

Concentration	Treatment	1	2	3	4	5	6	7	(DAYS)
40	Treatment	6.70	43.40	30.60	29.93	22.77	36.43	40.97	
60	Treatment	35.00	34.77	45.40	42.93	40.40	38.03	33.77	
80	Treatment	16.70	47.63	44.40	55.97	46.03	51.00	44.33	
100	Treatment	30.00	46.37	64.50	61.17	66.67	69.87	75.35	
	Control	0.40	1.30	1.70	2.23	2.77	4.20	5.37	
F-LSD (0.05)		**13.57**	**NS**	**NS**	**5.28**	**6.07**	**3.48**	**5.36**	

The main effect of *Moringa* treatment on organism 2 (*Penicillium waksmanii*) in vitro ranged from 23.30 to 55.78 across the seven days of observation, while the control ranged between 0.40 to 6.11 from days 1 to 7 respectively. Significant difference was observed between the treatment and control from days 1 to 7. The inhibitory effect of *Moringa* concentration on organism 2 on different days showed that at 40 g/ml, growth inhibition ranged from 16.90 to 28.22. At 60 g/ml, growth inhibition ranged from 15.20 on day 1 to 27.37 on day 7. At 80g/ml and 100g/ml, growth inhibition ranged between 7.70 on day 1 to 29.37 and 37.30 respectively. The highest inhibitory effect of *Moringa* concentration was 37.30 at 100g/ml on day 7. Significant differences between the extract concentrations was on days 4, 5, 6 and 7, while there were no significant differences between concentrations on days 1, 2 and 3 as shown in table 3.

Table 3: Main effect of *Moringa* treatment and concentration on growth inhibition of Organism 2 (*Penicillium waksmanii*) in vitro.

Treatment	1	2	3	4	5	6	7	(DAYS)
Control	0.40	1.20	2.24	3.15	4.14	5.15	6.11	
Treatment	23.30	32.80	46.72	48.83	51.73	55.78	53.31	
F-LSD (0.05)	**10.85**	**5.61**	**3.07**	**1.83**	**1.32**	**2.44**	**0.90**	
Concentration								
40	16.90	19.20	22.68	24.17	25.90	28.22	26.65	
60	15.20	17.90	24.65	25.23	26.88	27.28	27.37	
80	7.70	15.50	24.22	23.60	25.62	29.37	27.52	
100	7.70	15.20	26.37	30.97	33.35	37.00	37.30	
F-LSD (0.05)	**NS**	**NS**	**NS**	**2.58**	**1.87**	**3.45**	**1.27**	

NS – No Significant difference

The interaction effect of *Moringa* treatment and concentration on growth inhibition of organisms 2 in vitro revealed that at concentration of 40g/ml, growth inhibition of treatment ranged from 33.30 to 51.23 while control ranged from 0.40 to 6.07. At 40g/ml, the highest inhibitory effect on growth was 51.23 on day 6. At concentration of 60g/ml, growth inhibition of treatment ranged from 30.00 to 49.60 and the highest inhibitory effect on growth at 60g/ml was 49.60 on day 5. At concentration of 80g/ml, the growth inhibition of treatment ranged from 15.00 to 53.67 and the highest inhibitory effect on growth at 80g/ml was 53.67 on day 6. At concentration of 100 g/ml, the growth inhibition of treatment ranged from 15.00 to 68.87 and the highest inhibitory effect on growth at 100g/ml was 68.87 on day 6. There was significant difference between treatment concentrations and the control on all days. Significant differences were also observed between concentration of 100g/ml and other concentrations on days 4, 5, 6 and 7 while there were no significant differences among treatments at all concentrations on days 1, 2 and 3 as shown on table 4.

Table 4: Interaction effect of *Moringa* treatment and concentration on growth inhibition of Organism 2 (*Penicillium waksmanii*) in vitro

Concentration	Treatment.	1	2	3	4	5	6	7	(DAYS)
40	Treatment	33.30	37.20	43.13	45.17	47.60	51.23	47.23	
60	Treatment	30.00	34.70	47.03	47.37	49.60	49.37	48.63	
80	Treatment	15.00	29.90	46.20	44.10	47.13	53.67	48.90	
100	Treatment	15.00	29.30	50.50	58.70	62.60	68.87	68.47	
	Control	0.40	1.20	2.23	3.17	4.20	5.20	6.07	
F-LSD (0.05)		**NS**	**NS**	**NS**	**3.65**	**2.65**	**4.88**	**1.79**	

NS – No Significant difference

The main effect of *Moringa* treatment and concentration on organism 3 (*Botryodiplodia theobromae)* is shown in table 5. Growth inhibition ranged from 16.90 to 48.79 for treatment and from 0.40 to 5.13 for control. Significant differences were observed between treatment and control on days 1 to 7. The effect of *Moringa* concentration on organism 3 on different days showed that at 40g/ml, growth inhibition ranged from 0.20 to 27.28 from day 1 to 7. At concentration of 60g/ml, growth inhibition ranged from 4.30 to 20.40. At 80g/ml, growth inhibition ranged from 12.70 to 24.15. At 100g/ml, inhibition ranged from 17.40 to 36.92. There were significant differences among concentrations on days 1, 4, 5, 6 and 7 and no significant differences on days 2 and 3.

Table 5: Main effect of *Moringa* treatment and concentration on growth inhibition of Organism 3 (*Botryodiplodia theobromae*) in vitro.

Treatment	1	2	3	4	5	6	7	(DAYS)
Control	0.40	1.20	2.20	2.88	3.65	4.48	5.13	
Treatment	16.90	37.40	46.20	47.64	47.22	48.79	47.21	
F-LSD (P0.05)	**9.96**	**6.51**	**4.32**	**3.37**	**2.43**	**1.58**	**0.58**	
Concentration								
40	0.20	17.40	23.50	25.23	25.43	26.43	27.28	
60	4.30	20.40	20.10	18.60	17.58	19.83	18.80	
80	12.70	18.70	24.10	24.15	22.40	23.37	22.12	
100	17.40	20.60	29.10	33.05	36.33	36.92	36.48	
F-LSD (0.05)	**14.08**	**NS**	**NS**	**4.77**	**3.43**	**2.24**	**0.82**	

NS – No significant difference

The interaction effect of *Moringa* leaf extract and concentration on growth inhibition of organism 3 (*Botryodiplodia theobromae)* is shown in table 6. At 40g/ml, growth inhibition of treatment ranged from 0.00 to 48.37 while control ranged from 0.30 to 6.20. At 60g/ml, growth inhibition of treatment ranged from 8.30 to 39.70. At 80g/ml, growth inhibition of treatment ranged from 25.00 to 46.00. At 100g/ml, growth inhibition of treatment ranged from 34.40 to 69.17. Significant differences between these interactions were on days 1, 2, 3, 4, 5, 6 and 7.

Table 6: Interaction effect of *Moringa* treatment and concentration on growth inhibition of Organism 3 (*Botryodiplodia theobromae*) in vitro.

Concentration	Treatment	1	2	3	4	5	6	7	(DAYS)
40	Treatment	0.00	36.60	44.70	47.23	46.73	47.70	48.37	
60	Treatment	8.30	39.70	38.10	34.70	32.03	35.63	33.07	
80	Treatment	25.00	36.30	46.00	45.60	41.60	42.67	39.70	
100	Treatment	34.40	40.10	56.00	63.03	68.53	69.17	67.70	
	Control	0.30	1.20	2.30	3.23	4.13	5.17	6.20	
F-LSD (0.05)		**19.92**	**13.02**	**8.64**	**6.57**	**4.85**	**3.17**	**1.16**	

The main effect of *Moringa* treatment on organism 4 *(Fusarium oxysporum)* ranged from 18.80 to 39.89 while control ranged from 0.40 to 4.28 from day 1 to 7 respectively. There were significant differences between the treatment and control on all days of the study. The effect of *Moringa* concentration on organism 4 on different days revealed that at 40g/ml, growth inhibition ranged from 12.32 to 20.58. At 60g/ml, growth inhibition ranged from 10.97 to 15.35. At 80g/ml, growth inhibition ranged from 0.20 to 23.47. At 100g/ml, growth inhibition ranged from 10.20 to 36.63. Significant differences among concentrations were observed on days 2, 3, 4, 5, 6 and 7 while there was no significant difference among concentrations on day 1 as shown in table 7.

Table 7: Main effect of *Moringa* treatment and concentration on growth inhibition of organism 4 (*Fusarium oxysporum)* in vitro.

Treatment	1	2	3	4	5	6	7	(DAYS)
Control	0.40	1.24	1.86	2.42	3.01	3.63	4.28	
Treatment	18.80	39.89	38.33	37.63	37.96	37.36	36.68	
F-LSD (0.05)	**4.66**	**3.92**	**2.47**	**2.55**	**1.44**	**2.10**	**2.15**	
Concentration								
40	13.60	20.58	16.92	15.80	14.00	12.32	12.52	
60	14.40	15.35	13.08	12.75	13.03	10.97	12.05	
80	0.20	19.53	22.23	23.47	22.27	23.05	20.73	
100	10.20	26.80	28.15	28.08	32.63	35.65	36.63	
F-LSD (0.05)	**NS**	**5.54**	**3.49**	**3.61**	**2.03**	**2.96**	**3.04**	

NS – No Significant difference

The interaction effect of *Moringa* leaf extract and concentration on growth inhibition of organism 4 is shown in table 8. At 40g/ml, the growth inhibition of treatment ranged from 21.27 to 39.83 while control ranged from 0.50 to 3.77. At 60g/ml, growth inhibition of treatment ranged from 18.77 to 29.57. At 80g/ml, growth inhibition of treatment ranged from 24.10 to 44.07. At 100g/ml, growth

inhibition of treatment ranged 20.00 to 68.23. Significant differences among concentrations within the treatments were observed on days 1, 2, 3, 4, 5, 6 and 7.

Table 8: Interaction effect of *Moringa* treatment and concentration on growth inhibition of Organism 4 (*Fusarium oxysporum)* in vitro.

Concentration	Treatment	1	2	3	4	5	6	7	(DAYS)
40	Treatment	26.70	39.83	32.07	29.33	25.23	21.37	21.27	
60	Treatment	28.30	29.57	24.53	23.37	23.37	18.77	20.37	
80	Treatment	24.10	37.83	42.27	44.07	41.13	42.07	36.87	
100	Treatment	20.00	52.33	54.47	53.77	62.10	67.23	68.23	
	Control	0.50	1.33	1.77	2.27	2.77	3.27	3.77	
F-LSD (0.05)		**2.21**	**7.84**	**4.94**	**5.10**	**2.88**	**4.19**	**4.30**	

The main effect of *Moringa* treatment on organism 5 *(Colletotrichum asianum*) ranged from 19.60 to 45.57 while the control ranged from 0.40 to 4.71. There was no significant difference between treatment and control on days 1 to 7. The effect of *Moringa* concentration on organism 5 *(Colletotrichum asianum*) in vitro revealed that at 40g/ml, growth inhibition ranged from 13.87 to 21.82. At 60g/ml, growth inhibition ranged from 17.70 to 23.55. At 80g/ml, growth inhibition ranged from 0.20 to 25.13. At 100g/ml, growth inhibition ranged from 6.90 to 34.13. Significant differences were observed among concentrations on days 1, 3, 4, 5, 6 and 7 while there was no significant difference on day 2 as shown in table 9.

Table 9: Main effect of *Moringa* treatment and concentration on growth inhibition of Organism 5 (*Colletotrichum asianum)* in vitro.

Treatment	1	2	3	4	5	6	7	(DAYS)
Control	0.40	1.20	2.09	2.77	3.27	4.19	4.71	
Treatment	19.60	37.10	44.97	43.87	40.27	45.57	43.46	
F-LSD (0.05)	**8.00**	**5.03**	**3.22**	**2.48**	**2.61**	**3.03**	**2.38**	
Concentration								
40	15.2	18.80	16.70	15.80	13.87	21.82	20.92	
60	17.70	19.00	23.55	20.88	18.05	21.15	20.07	
80	0.20	19.50	25.13	22.47	21.40	22.78	21.65	
100	6.90	19.30	28.75	34.13	33.75	33.77	33.70	
F-LSD (0.05)	**11.31**	**NS**	**4.56**	**3.50**	**3.69**	**4.28**	**3.37**	

NS – No Significant difference

The interaction effect of *Moringa* leaf concentration and treatment on growth inhibition of organism 5 revealed that at 40g/ml, growth inhibition ranged from 24.93 to 39.40. At 60g/ml, growth inhibition ranged from 32.97 to 44.87. At

80g/ml, growth inhibition ranged from 0.00 to 47.97. At 100g/ml, growth inhibition ranged from 13.30 to 64.93. The control ranged from 0.40 to 4.77. There were significant differences between concentrations of 60g/ml, 80g/ml and 100g/ml on day 1. On day 2, there was no significant difference between treatment concentrations. There were significant differences between concentration of 100g/ml and all other concentrations on days 3 and 4. On day 5, there was a significant difference between 100g/ml and other concentrations and between 80g/ml and 60g/ml. On day 6 and 7 there were significant differences between concentration of 100g/ml and all other concentrations as shown in table 10.

Table 10: Interaction effect of *Moringa* treatment and concentration on growth inhibition of organism 5 (*Colletotrichum asianum)* in vitro.

Concentration	Treatment	1	2	3	4	5	6	7	(DAYS)
40	Treatment	30.00	36.40	31.73	29.33	24.93	39.40	37.07	
60	Treatment	35.00	36.70	44.87	39.03	32.97	38.20	35.43	
80	Treatment	0.00	37.80	47.97	42.17	39.43	41.40	38.67	
100	Treatment	13.30	37.30	55.33	64.93	63.73	63.27	62.67	
	Control	0.40	1.10	1.67	2.27	2.80	4.23	4.77	
F-LSD (0.05)		**16.00**	**NS**	**6.45**	**4.95**	**5.22**	**6.06**	**4.77**	

NS – No Significant difference

3.1.2 Effect of Neem leaf extract on growth inhibition of fungal isolates in vitro

The main effect of Neem leaf treatment and concentration on growth inhibition of organism 1 (*Aspergillus flavus*) in vitro is shown in table 11. Growth inhibition ranged from 25.80 to 51.08 for the treatment while control ranged from 0.40 to 5.18 respectively. Significant differences were observed between the treatment and control on all days under observation. The main effect of Neem concentration on organism 1 (*Aspergillus flavus*) in vitro at different days showed that at 40g/ml, growth inhibition ranged from 15.20 to 30.07. At 60g/ml, growth inhibition ranged from 2.50 to 26.67. At 80g/ml, growth inhibition ranged from 13.27 to 25.90. At 100g/ml, growth inhibition ranged from 11.00 to 34.20. Significant differences were observed among concentrations on days 2, 3, 5, 6 and 7 while there were no significant differences between treatments on days 1 and 4.

Table 11: Main effect of Neem leaf treatment and concentration on growth inhibition of Organism 1 (*Aspergillus flavus*) in vitro

Treatment	1	2	3	4	5	6	7	(DAYS)
Control	0.40	1.10	2.07	3.30	4.16	4.66	5.18	
Treatment	25.80	28.10	36.24	50.04	51.08	48.82	46.07	
F-LSD (0.05)	**10.45**	**5.77**	**4.09**	**2.25**	**1.59**	**1.36**	**1.15**	
Concentration								
40	15.20	20.10	30.07	26.30	25.03	23.53	21.83	
60	8.50	2.50	10.40	26.32	26.67	24.62	23.23	
80	17.70	15.90	13.27	24.83	25.90	24.70	23.22	
100	11.00	20.00	22.88	29.23	32.88	34.12	34.20	
F-LSD (0.05)	**NS**	**8.17**	**5.78**	**NS**	**2.24**	**1.93**	**1.63**	

NS – No Significant difference

The interaction effect of Neem leaf concentration and treatment on growth inhibition of organism 1 *(Aspergillus flavus)* revealed that at 40g/ml, treatment effect ranged from 30.00 to 57.10. At 60g/ml, growth inhibition ranged from 4.20 to 49.40. At 80g/ml, growth inhibition ranged from 24.80 to 47.60. At 100g/ml, growth inhibition ranged from 21.70 to 63.57, while control ranged 0.40 to 5.17. There were significant differences in growth inhibition among treatment concentrations on days 2, 3, 4, 5, 6 and 7 while there was no significant difference on day 1 as shown in table 12.

Table 12: Interaction effect of Neem leaf treatment and concentration on growth inhibition of Organism 1 (*Aspergillus flavus*)

Concentration	Treatment	1	2	3	4	5	6	7	(DAYS)
40	Treatment	30.00	38.90	57.10	49.00	45.93	42.43	38.47	
60	Treatment	16.70	4.20	19.43	49.40	49.20	44.60	41.30	
80	Treatment	35.00	30.60	24.80	46.43	47.60	44.70	41.27	
100	Treatment	21.70	38.90	43.63	55.33	61.60	63.57	63.23	
	Control	0.40	0.80	1.37	3.23	4.13	4.63	5.17	
F-LSD (0.05)		**NS**	**11.55**	**8.17**	**4.49**	**3.17**	**2.73**	**2.30**	

NS – No Significant difference

The main effect of Neem leaf treatment and concentration on growth inhibition of organism 2 *(Penicillium waksmanii*) in vitro is presented in table 13. For the treatment, growth inhibition ranged from 22.50 to 52.31 while control ranged from 0.40 to 5.68 respectively. There was significant difference between the treatment and control from days 1 to 7. The effect of Neem leaf extract concentration on organism 2 *(Penicillium waksmanii*) in vitro on different days revealed that at 40g/ml, growth inhibition ranged from 15.20 to 27.68. At 60g/ml,

growth inhibition ranged from 11.90 to 27.17. At 80g/ml, growth inhibition ranged from 8.50 to 26.87. At 100g/ml, growth inhibition ranged from 10.20 to 36.08 respectively. Significant differences among concentrations were observed on days 5, 6 and 7 while days 1, 2, 3 and 4 showed no significant differences.

Table 13: Main effect of Neem leaf treatment and concentration on growth inhibition of Organism 2 (*Penicillium waksmanii*) in vitro.

Treatment	1	2	3	4	5	6	7	(DAYS)
Control	0.40	1.21	2.21	3.24	4.12	5.06	5.68	
Treatment	22.50	37.78	42.50	47.80	50.12	52.31	51.79	
F-LSD (0.05)	**15.70**	**2.85**	**4.05**	**1.24**	**0.87**	**0.81**	**1.71**	
Concentration								
40	15.20	18.63	20.02	24.73	26.22	27.68	26.60	
60	11.90	20.03	22.53	25.35	24.97	25.88	27.17	
80	8.50	18.13	23.52	25.40	25.80	26.87	25.10	
100	10.20	21.18	23.35	26.60	31.45	34.30	36.08	
F-LSD (0.05)	**NS**	**NS**	**NS**	**NS**	**1.23**	**1.14**	**2.42**	

NS – No Significant difference

The interaction effect of Neem leaf extract concentration and treatment on growth inhibition of organism 2 (*Penicillium waksmanii*) in vitro showed that at 40g/ml, treatment effect on growth inhibition ranged from 30.00 to 50.30 while control ranged from 0.40 to 5.60 respectively. At 60g/ml, growth inhibition ranged from 23.30 to 48.33. At 80g/ml, growth inhibition ranged from 16.70 to 48.67. At 100g/ml, growth inhibition ranged from 20.00 to 66.63. Significant differences among treatment concentrations were observed on days 5, 6 and 7 while days 1, 2, 3 and 4 revealed no significant differences as shown in table 14.

Table 14: Interaction effect of Neem leaf treatment and concentration on growth inhibition of Organism 2 (*Penicillium waksmanii*) in vitro.

Concentration	Treatment.	1	2	3	4	5	6	7	(DAYS)
40	Treatment	30.00	36.07	37.93	46.30	48.37	50.30	47.60	
60	Treatment	23.30	38.87	42.80	47.47	45.87	46.70	48.33	
80	Treatment	16.70	35.13	44.73	47.50	47.57	48.67	44.60	
100	Treatment	20.00	41.07	44.53	49.93	58.70	63.57	66.63	
	Control	0.40	1.20	2.10	3.17	4.07	5.07	5.60	
F-LSD (0.05)		**NS**	**NS**	**NS**	**NS**	**1.73**	**1.62**	**3.42**	

NS – No Significant difference

The main effect of Neem leaf treatment on growth of organism 3 (*Botryodiplodia theobromae*) in vitro revealed that growth inhibition ranged from 34.00 to 53.79 from days 1 to 7 respectively while the control ranged from 0.40 to 6.02 from days 1 to 7 respectively. There was significant difference between control and treatment on days 1 to 7. The main effect of Neem leaf concentration on growth retardation of organism 3 (*Botryodiplodia theobromae*) in vitro showed that at 40g/ml, growth inhibition ranged from 12.40 to 27.83 respectively. At 60g/ml, growth inhibition ranged from 14.90 to 26.87. At 80g/ml, growth inhibition ranged from 18.60 to 28.60 respectively. At 100g/ml, growth inhibition ranged from 18.60 to 37.30. Significant differences were observed among concentrations on days 5, 6 and 7 while no significant differences were observed on days 1, 2, 3 and 4 as shown in table 15.

Table 15: Main effect of Neem leaf treatment and concentration on growth inhibition of Organism 3 (*Botryodiplodia theobromae*) in vitro.

Treatment	1	2	3	4	5	6	7	(DAYS)
Control	0.40	1.20	2.20	3.14	4.17	5.11	6.02	
Treatment	34.00	35.00	44.90	46.92	51.37	52.92	53.79	
F-LSD (0.05)	**11.59**	**5.40**	**4.35**	**1.68**	**1.52**	**0.65**	**1.15**	
Concentration								
40	12.40	14.80	24.20	25.27	26.47	26.73	27.83	
60	19.40	14.90	21.90	23.50	26.67	26.87	25.88	
80	18.60	20.00	23.80	25.03	26.08	27.40	28.60	
100	18.60	22.50	24.20	26.33	31.85	35.05	37.30	
F-LSD (0.05)	**NS**	**NS**	**NS**	**NS**	**2.14**	**0.92**	**1.63**	

NS – No Significant difference

The interaction effect of Neem treatment and concentration on growth inhibition of organism 3 (*Botryodiplodia theobromae*) in vitro is shown in table 16. At 40g/ml, growth inhibition ranged from 24.40 to 49.47 while control ranged from 0.40 to 5.63. At 60g/ml, growth inhibition ranged from 28.70 to 49.20. At 80g/ml, growth inhibition ranged from 36.70 to 51.07. At 100g/ml, growth inhibition ranged from 36.70 to 68.50. Significant differences in the interaction effect on growth inhibition of test fungi were observed on days 5, 6 and 7 while days 1, 2, 3 and 4 showed no significant differences.

Table 16: Interaction effect of Neem leaf treatment and concentration on growth inhibition of Organism 3 (*Botryodiplodia theobromae*) in vitro.

Concentration	Treatment	1	2	3	4	5	6	7	(DAYS)
40	Treatment	24.40	28.60	46.20	47.37	48.77	48.37	49.47	
60	Treatment	38.30	28.70	41.80	43.97	49.20	48.67	46.13	
80	Treatment	36.70	38.90	45.50	46.87	48.00	49.67	51,07	
100	Treatment	36.70	43.60	46.20	49.50	59.50	64.97	68.50	
	Control	0.40	1.00	2.10	3.03	4.13	5.07	5.63	
F-LSD (0.05)		**NS**	**NS**	**NS**	**NS**	**3.03**	**1.30**	**2.30**	

NS – No Significant difference

The main effect of Neem treatment on growth inhibition of organism 4 *(Fusarium oxysporum)* in vitro revealed that inhibition ranged from 28.90 to 49.04 while control ranged from 0.40 to 5.30. There were significant differences between the control and the treatment on days 1 to 7. The effect of Neem concentration on growth inhibition of organism 4 *(Fusarium oxysporum)* in vitro showed that at 40g/ml, growth inhibition ranged from 11.60 to 20.80. At 60g/ml, growth inhibition ranged from 13.47 to 27.15. At 80g/ml, growth inhibition ranged from 12.70 to 27.35. At 100g/ml, growth inhibition ranged from 6.80 to 39.12. There were significant differences among concentrations on days 4, 5, 6 and 7 while days 1, 2 and 3 revealed no significant differences as shown in table 17.

Table 17: Main effect of Neem leaf treatment and concentration on growth inhibition of Organism 4 (*Fusarium oxysporum*) in vitro.

Treatment	1	2	3	4	5	6	7	(DAYS)
Control	0.40	1.09	1.67	3.03	4.00	4.68	5.30	
Treatment	28.90	33.98	29.07	47.09	49.04	46.83	43.63	
F-LSD (0.05)	**11.41**	**3.69**	**3.70**	**2.46**	**1.55**	**1.89**	**1.84**	
Concentration								
40	20.80	18.05	16.83	16.98	16.35	13.63	11.60	
60	18.20	15.85	13.47	26.30	27.15	27.10	24.60	
80	12.70	16.73	15.85	25.80	27.35	24.55	22.55	
100	6.80	19.52	15.35	31.17	35.23	37.73	39.12	
F-LSD (0.05)	**NS**	**NS**	**NS**	**3.48**	**2.19**	**2.67**	**2.60**	

NS – No Significant difference

The interaction effect of Neem concentration and treatment on growth inhibition of organism 4 *(Fusarium oxysporum)* in vitro is shown in table 18. At 40g/ml, growth inhibition ranged from 19.23 to 41.10 while the control ranged from 0.40 to 5.27. At 60g/ml, inhibition ranged from 25.40 to 50.03. At 80g/ml, inhibition ranged from 25.00 to 50.33. At 100g/ml, growth inhibition ranged from

13.30 to 71.93. There were significant differences among treatments at different concentrations on days 4, 5, 6 and 7 while days 1, 2 and 3 showed no significant differences.

Table 18: Interaction effect of Neem leaf treatment and concentration on growth inhibition of Organism 4 (*Fusarium oxysporum*) in vitro.

Concentration	Treatment	1	2	3	4	5	6	7	(DAYS)
40	Treatment	41.10	34.97	31.90	31.57	29.67	23.77	19.23	
60	Treatment	36.10	30.73	25.40	49.37	50.03	49.03	43.53	
80	Treatment	25.00	32.43	30.03	48.43	50.33	44.30	39.83	
100	Treatment	13.30	37.80	28.97	59.00	66.13	70.20	71.93	
	Control	0.40	1.03	1.67	3.17	4.37	4.80	5.27	
F-LSD (0.05)		**NS**	**NS**	**NS**	**4.91**	**3.09**	**3.77**	**3.67**	

NS – No Significant difference

The main effect of Neem treatment and concentration on growth inhibition of organism 5 (*Colletotrichum waksmanii*) in vitro is shown in table 19. For Neem treatment, growth inhibition ranged from 20.80 to 42.29 while control ranged from 0.40 to 4.58 respectively. The main effect of Neem concentration on organism 5 showed that at 40g/ml, growth inhibition ranged from 6.90 to 18.90. At 60g/ml, inhibition ranged from 12.52 to 22.80. At 80g/ml, growth inhibition ranged from 4.30 to 25.48. At 100g/ml, growth inhibition ranged from 14.40 to 36.62. There were significant differences among the concentrations on days 3 to 7 while days 1 and 2 recorded no significant differences.

Table 19: Main effect of Neem leaf treatment and concentration on growth inhibition of Organism 5 (*Colletotrichum asianum*) in vitro.

Treatment	1	2	3	4	5	6	7	(DAYS)
Control	0.40	1.10	2.00	2.62	3.56	4.12	4.58	
Treatment	20.80	37.70	37.50	37.28	42.29	39.50	37.46	
F-LSD (0.05)	**13.19**	**8.47**	**4.56**	**3.28**	**2.75**	**2.43**	**2.29**	
Concentration								
40	6.90	18.90	9.80	9.07	15.47	13.00	12.80	
60	16.90	22.80	22.20	17.50	15.60	13.90	12.52	
80	4.30	15.40	23.30	21.32	25.48	23.72	22.38	
100	14.40	20.60	23.80	31.92	35.15	36.62	36.38	
F-LSD (0.05)	**NS**	**NS**	**6.45**	**4.64**	**3.89**	**3.44**	**3.24**	

NS – No Significant difference

The interaction effect of Neem leaf treatment and concentration on growth inhibition of organism 5 *(Colletotrichum asianum)* revealed that at 40g/ml, growth inhibition ranged from 13.30 to 36.60 while control ranged from 0.40 to 4.03. At 60g/ml, inhibition of growth ranged from 21.10 to 44.40. At 80g/ml, inhibition of

growth ranged from 28.30 to 47.00. At 100g/ml, growth inhibition ranged from 28.30 to 68.20. Significant differences were observed on days 3 to 7 while no significant differences were seen on days 1 and 2 as shown in table 20.

Table 20: Interaction effect of Neem leaf concentration and treatment on growth inhibition of Organism 5 (*Colletotrichum asianum*) in vitro.

Concentration	Treatment	1	2	3	4	5	6	7	(DAYS)
40	Treatment	13.30	36.60	17.90	16.07	27.83	22.47	21.57	
60	Treatment	33.30	44.40	42.40	32.57	28.27	24.40	21.10	
80	Treatment	28.30	29.70	44.40	39.97	47.00	42.93	39.90	
100	Treatment	28.30	40.10	45.30	60.53	66.07	68.20	67.27	
	Control	0.40	1.20	1.60	2.07	3.10	3.53	4.03	
F-LSD (0.05)		**NS**	**NS**	**9.12**	**6.56**	**5.50**	**4.87**	**4.58**	

NS – No Significant difference

3.1.3 Effect of Bitter leaf extract on growth inhibition of fungal isolates in vitro.

The main effect of bitter leaf treatment and concentration on growth inhibition of organism 1 (*Aspergillus flavus)* in vitro is shown in table 21. For the treatment, inhibition ranged from 35.00 to 67.70 while the control ranged from 1.00 to 5.90 from days 1 to 7 respectively. Significant difference between the treatment and control were observed on days 1 to 7. The main effect of bitter leaf concentration on growth inhibition of *Aspergillus flavus* in vitro showed that at 40g/ml, growth inhibition ranged from 11.80 to 39.90. At 60g/ml, growth inhibition ranged from 20.30 to 37.30. At 80g/ml, growth inhibition ranged from 20.90 to 31.70. At 100g/ml, growth inhibition ranged from 19.10 to 28.40. There were no significant differences between the extract concentrations on growth inhibition of test fungi on all the days observed in this study except on day 6.

Table 21: Main effect of Bitter leaf treatment and concentration on growth inhibition of Organism 1*(Aspergillus flavus*) in vitro

Treatment	1	2	3	4	5	6	7	(DAYS)
Control	1.00	2.50	3.50	4.30	4.80	5.30	5.90	
Treatment	67.70	35.70	37.70	40.30	41.90	35.00	41.60	
F-LSD (0.05)	**7.69**	**8.37**	**6.83**	**7.04**	**13.28**	**5.17**	**12.87**	
Concentration								
40	39.90	17.70	14.80	19.00	24.90	11.80	25.60	
60	37.30	25.80	22.80	22.90	21.40	20.80	20.30	
80	31.70	13.80	23.10	22.80	21.90	21.30	20.90	
100	28.40	19.10	21.90	24.60	25.30	26.80	28.20	
F-LSD (0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**7.31**	**NS**	

NS – No Significant difference

The interaction effect of concentration and bitter leaf treatment on growth inhibition of *Aspergillus flavus* in vitro revealed that at 40g/ml, inhibition ranged from 18.50 to 78.60 while the control ranged from 1.20 to 5.60 respectively. At 60g/ml, inhibition ranged from 34.60 to 73.50. At 80g/ml, growth inhibition ranged from 25.20 to 62.60. At 100g/ml, inhibition of growth ranged from 35.60 to 55.90. Significant differences were observed between treatment concentrations on day 6 only while days 1, 2, 3, 4, 5, and 7 showed no significant difference among treatment concentrations as shown in table 22.

Table 22: Interaction effect of Bitter leaf extract and concentration on growth inhibition of Organism 1(*Aspergillus flavus*) in vitro.

Concentration	Treatment.	1	2	3	4	5	6	7	(DAYS)
40	Treatment	78.60	32.80	26.30	33.90	45.30	18.50	45.50	
60	Treatment	73.50	49.20	42.10	41.20	37.70	36.10	34.60	
80	Treatment	62.60	25.20	42.50	41.20	38.80	37.20	35.90	
100	Treatment	55.90	35.60	40.00	44.90	45.70	48.30	50.50	
	Control	1.20	2.60	3.30	4.10	4.60	5.10	5.60	
F-LSD (0.05)		**NS**	**NS**	**NS**	**NS**	**NS**	**10.34**	**NS**	

NS – No Significant difference

The main effect of bitter leaf treatment and concentration on growth inhibition of organism 2 (*Penicillium waksmanii*) in vitro is shown in table 23. For the treatment, inhibition of growth ranged from 13.90 to 30.49 while the control ranged from 0.30 to 5.60 respectively from days 1 to 7. There was significant difference between the treatment and the control from days 1 to 7. The main effect of bitter leaf concentration on growth inhibition of organism 2 in vitro revealed that 40g/ml, growth inhibition ranged from 0.10 to 14.60. For 60g/ml, growth inhibition ranged from 7.02 to 22.70. For 80g/ml, growth inhibition ranged from 2.60 to 24.38. For 100g/ml, growth inhibition ranged from 6.70 to 28.32. There was no significant difference between the concentrations on days 1 and 2, while days 3 to 7 showed significant differences among concentrations.

Table 23: Main effect of Bitter leaf treatment and concentration on growth inhibition of Organism 2 (*Penicillium waksmanii)* in vitro.

Treatment	1	2	3	4	5	6	7	(DAYS)
Control	0.30	1.90	3.00	3.62	4.53	5.11	5.60	
Treatment	27.10	13.90	28.00	23.98	30.49	28.96	29.42	
F-LSD (0.05)	**17.11**	**6.70**	**6.14**	**3.34**	**2.33**	**1.82**	**1.96**	
Concentration								
40	0.10	14.60	14.60	8.60	12.20	9.67	10.17	
60	22.70	7.80	7.40	5.40	7.02	7.40	8.18	
80	20.80	2.60	18.60	19.57	24.38	23.83	23.37	
100	11.20	6.70	21.20	21.65	26.45	27.23	28.32	
F-LSD (0.05)	**NS**	**NS**	**8.69**	**4.73**	**3.29**	**2.58**	**2.77**	

NS – No Significant difference

The interaction effect of concentration and treatment on growth inhibition of organism 2 in vitro showed that at 40g/ml, growth inhibition ranged from 0.00 to 27.30 while the control ranged from 0.20 to 4.67 respectively. At 60g/ml, growth inhibition ranged from 7.87 to 45.00. At 80g/ml, inhibition ranged from 3.30 to 43.23. At 100g/ml, growth inhibition ranged from 11.30 to 49.97. No significant difference was observed between treatment concentrations on days 1 and 2 while significant differences were observed on days 3, 4, 5, 6, 7 as shown in table 24.

Table 24: Interaction effect of Bitter leaf extract concentration and treatment on growth inhibition of Organism 2 (*Penicillium waksmanii)* in vitro.

Concentration	Treatment	1	2	3	4	5	6	7	(DAYS)
40	Treatment	0.00	27.30	26.90	14.17	20.87	15.17	15.67	
60	Treatment	45.00	13.70	12.40	7.87	10.60	10.83	11.93	
80	Treatment	41.10	3.30	33.90	34.87	43.23	41.50	40.10	
100	Treatment	22.20	11.30	38.90	39.03	47.27	48.33	49.97	
	Control	0.20	1.90	2.40	3.03	3.53	4.17	4.67	
F-LSD (0.05)		**NS**	**NS**	**12.29**	**6.69**	**4.65**	**3.65**	**3.92**	

NS – No Significant difference

The main effect of bitter leaf treatment and concentration on growth inhibition of organism 3 (*Botryodiplodia theobromae*) in vitro is shown in table 25. For the treatment, growth inhibition ranged from 20.30 to 45.72 while the control ranged from 0.50 to 6.14 from day 1 to 7 respectively. There was significant difference between the treatment and the control from days 1 to 7. The main effect of bitter leaf concentration on growth inhibition of organism 3 (*Botryodiplodia theobromae*) showed that at 40g/ml, growth inhibition ranged from 5.11 to 27.50. At 60g/ml, growth inhibition ranged from 16.80 to 27.70. At 80g/ml, growth

inhibition ranged from 3.80 to 29.70. At 100g/ml, growth inhibition ranged from 10.90 to 35.42. There were no significant differences between the concentrations on day 2 while days 1, 3, 4, 5, 6 and 7 showed significant differences.

Table 25: Main effect of Bitter leaf treatment and concentration on growth inhibition of Organism 3 (*Botryodiplodia theobromae*) in vitro.

Treatment	1	2	3	4	5	6	7	(DAYS)
Control	0.50	1.10	2.57	3.69	4.67	5.74	6.14	
Treatment	39.80	20.30	38.00	43.33	41.64	45.72	45.58	
F-LSD (0.05)	**10.46**	**8.84**	**4.10**	**2.55**	**1.48**	**1.09**	**1.23**	
Concentration								
40	27.50	9.20	5.11	8.67	6.50	13.18	13.53	
60	27.70	19.00	16.80	25.43	25.93	27.32	26.50	
80	9.90	3.80	27.93	28.70	29.70	27.93	28.00	
100	15.40	10.90	31.30	31.25	30.48	34.48	35.42	
F-LSD (0.05)	**14.80**	**NS**	**5.80**	**3.61**	**2.09**	**1.54**	**1.74**	

NS – No Significant difference

The interaction effect of bitter leaf concentration and treatment on growth inhibition of organism 3 in vitro showed that at 40g/ml, inhibition ranged from 8.75 to 54.40 while the control ranged from 0.50 to 42.57. At 60g/ml, inhibition ranged from 31.17 to 54.70. At 80g/ml, inhibition ranged from 6.70 to 54.17. At 100g/ml, inhibition ranged from 20.50 to 64.07. There was no significant difference between the treatment concentrations on growth inhibition of test fungi on days 1 and 2 while days 3, 4, 5 and 7 showed significant differences between treatment concentrations as shown in table 26.

Table 26: Interaction effect of Bitter leaf extract concentration and treatment on growth inhibition of Organism 3 (*Botryodiplodia theobromae*) in vitro.

Concentration	Treatment	1	2	3	4	5	6	7	(DAYS)
40	Treatment	54.40	17.50	8.75	15.13	10.03	22.17	22.50	
60	Treatment	54.70	36.60	31.17	46.73	46.73	48.37	46.30	
80	Treatment	19.40	6.70	52.70	53.20	54.17	49.70	49.47	
100	Treatment	30.50	20.50	59.40	58.27	55.63	62.63	64.67	
	Control	0.50	0.80	1.47	2.20	2.97	4.20	4.57	
F-LSD (0.05)		**NS**	**NS**	**8.21**	**5.11**	**2.95**	**2.17**	**2.46**	

NS – No Significant difference

The main effect of bitter leaf treatment on growth inhibition of organism 4 *(Fusarium oxysporum)* in vitro ranged from 14.60 to 45.43 and the control ranged from 0.40 to 5.55 from days 1 to 7 respectively. There was significant difference between the

treatment and the control from days 1 to 7. The main effect of bitter leaf concentration on growth inhibition of organism 4 *(Fusarium oxysporum)* in vitro showed that at 40g/ml, growth inhibition ranged from 1.80 to 19.92. At 60g/ml, growth inhibition ranged from 2.00 to 24.23. At 80g/ml, growth inhibition ranged from 17.60 to 26.08. At 100g/ml, growth inhibition ranged from 9.60 to 33.50. There were no significant differences between the concentrations on day 1 while days 2, 4 and 5 showed significant differences as shown in table 27.

Table 27: Main effect of Bitter leaf treatment and concentration on growth inhibition of Organism 4 (*Fusarium oxysporum*) in vitro.

Treatment	1	2	3	4	5	6	7	(DAYS)
Control	0.40	1.00	2.80	3.55	4.27	5.10	5.55	
Treatment	23.90	14.60	45.43	44.64	42.56	42.47	41.67	
F-LSD (0.05)	**13.31**	**5.17**	**3.59**	**3.40**	**3.00**	**1.36**	**1.25**	
Concentration								
40	9.90	1.80	14.78	19.07	19.92	18.68	18.35	
60	4.40	2.00	24.23	21.68	20.02	18.77	17.90	
80	24.00	17.60	26.08	25.45	23.68	25.18	24.68	
100	10.20	9.60	31.37	30.18	30.03	32.50	33.50	
F-LSD (0.05)	**NS**	**7.31**	**5.08**	**4.81**	**4.24**	**1.92**	**1.76**	

NS – No Significant difference

The interaction effect of concentration and treatment on growth inhibition of organism 4 *(Fusarium oxysporum)* in vitro showed that at 40g/ml, growth inhibition ranged from 2.60 to 36.37 while the control ranged from 0.30 to 4.80 respectively from days 1 to 7. At 60g/ml, growth inhibition ranged from 3.00 to 45.53. At 80g/ml, growth inhibition ranged from 34.30 to 48.97. At 100g/ml, growth inhibition ranged from 18.40 to 61.23. There was no significant difference between the treatments at all concentrations on day 1. Day 2 showed significant differences at all treatment concentrations while day 3 showed significant differences at treatment concentrations of 40g/ml, 80g/ml and 100g/ml. Days 4, 5, 6, and 7 showed significant differences at treatment concentrations of 60g/ml,80g/ml and 100g/ml respectively as shown in table 28.

Table 28: Interaction effect of Bitter leaf treatment and concentration on growth inhibition of Organism 4 (*Fusarium oxysporum*) in vitro.

Concentration	Treatment	1	2	3	4	5	6	7	(DAYS)
40	Treatment	19.40	2.60	27.63	35.30	36.37	33.03	31.90	
60	Treatment	8.30	3.00	45.53	39.97	35.97	32.87	30.70	
80	Treatment	47.80	34.30	48.97	46.77	42.33	44.27	42.83	
100	Treatment	20.00	18.40	59.60	56.53	55.57	59.70	61.23	
	Control	0.30	1.00	1.93	2.83	3.47	4.33	4.80	
F-LSD (0.05)		**NS**	**10.34**	**7.18**	**6.80**	**6.00**	**2.72**	**2.49**	

NS – No Significant difference

The main effect of bitter leaf treatment on growth inhibition of organism 5 (*Colletotrichum asianum*) in vitro showed that the treatment ranged from 12.92 to 39.03 and the control ranged from 0.50 to 5.21 from days 1 to 7 respectively. There was significant difference between the treatment and the control from days 1 to 7. The effect of bitter leaf concentration on growth inhibition of organism 5 (*Colletotrichum asianum*) in vitro showed that at 40g/ml, growth inhibition ranged from 9.45 to 21.38. At 60g/ml, growth inhibition ranged from 1.85 to 19.67. At 80g/ml, growth inhibition ranged from 5.80 to 16.98 and at 100g/ml, growth inhibition ranged from 3.60 to 32.05. There was no significant difference between the concentrations on days 1 and 3 while day 2 showed significant differences at concentrations of 60g/ml and 80g/ml. Days 4 and 5 showed significant differences at concentrations of 40g/ml, 80g/ml and 100g/ml while days 6 and 7 showed significant differences at 40g/ml, 80g/ml and 100g/ml respectively as shown in table 29.

Table 29: Main effect of Bitter leaf treatment and concentration on growth inhibition of Organism 5 (*Colletotrichum asianum*) in vitro

Treatment	1	2	3	4	5	6	7	(DAYS)
Control	0.50	1.36	2.30	3.04	3.97	4.66	5.21	
Treatment	16.00	12.92	28.00	28.07	36.59	38.23	39.03	
F-LSD (0.05)	**9.42**	**4.09**	**8.54**	**3.21**	**1.43**	**2.96**	**2.57**	
Concentration								
40	10.20	9.45	15.20	17.25	21.38	19.92	19.78	
60	13.30	1.83	11.40	8.88	14.43	17.67	19.67	
80	5.80	10.22	9.90	8.82	14.50	16.80	16.98	
100	3.60	7.03	24.10	27.27	30.82	31.40	32.05	
F-LSD (0.05)	**NS**	**5.79**	**NS**	**4.54**	**2.02**	**4.19**	**3.64**	

NS – No Significant difference

The interaction effect of bitter leaf concentration and treatment on growth inhibition of organism 5 in vitro showed that at 40g/ml, growth inhibition ranged from 20.00 to 38.63. At 60g/ml, growth inhibition ranged from 2.23 to 34.07. At 80g/ml, growth inhibition ranged from 11.10 to 29.67. At 100g/ml, growth inhibition ranged from 6.70 to 58.13. There was no significant difference between the treatments at all concentrations on days 1 and 3 while day 2 showed significant difference at treatment concentrations of 60g/ml and 100g/ml. Days 4 and 5 showed significant differences at concentrations of 40g/ml, 80g/ml and 100g/ml respectively while day 6 showed significant difference at concentrations of 40g/ml, 80g/ml and 100g/ml. Day 7 showed significant difference at concentrations of 60g/ml,80g/ml and 100g/ml respectively as shown in table 30.

Table 30: Interaction effect of Bitter leaf concentration and treatment on growth inhibition of Organism 5 (*Colletotrichum asianum*) in vitro

Concentration	Treatment.	1	2	3	4	5	6	7	(DAYS)
40	Treatment	20.00	17.47	28.20	31.57	38.63	35.10	34.33	
60	Treatment	26.10	2.23	20.60	15.13	25.47	30.97	34.07	
80	Treatment	11.10	19.40	18.10	15.13	25.77	29.67	29.60	
100	Treatment	6.70	12.57	45.10	50.43	56.50	57.20	58.13	
	Control	0.50	1.43	2.30	2.93	4.13	4.73	5.23	
F-LSD (0.05)		**NS**	**8.19**	**NS**	**6.42**	**2.86**	**5.92**	**5.15**	

NS – No Significant difference

3.2 Grand inhibitory effect of leaf extracts on growth of fungal isolates in vitro

The grand inhibitory effect of leaf extracts on growth inhibition of fungal isolates in vitro revealed that on organism 1 *(Aspergillus flavus)*, the highest growth inhibition was by bitter leaf extract (BLE) (35.03) followed by *Moringa* leaf extract (MLE) (34.83) and Neem leaf extract (NLE) (33.35). On organism 2 (*Penicillium waksmanii*), the highest growth inhibition was by MLE (36.35) followed by NLE (35.42) and BLE (21.35) respectively. On organism 3 (*Botryodiplodia theobromae*), the highest growth inhibition was by NLE (37.08) followed MLE (33.12) and BLE (31.83). On organism 4 (*Fusarium oxysporum*), the highest growth inhibition was by NLE (32.26) followed by BLE (29.13) and MLE (28.63). On organism 5 *(Colletotrichum asianum*), the highest growth inhibition was by MLE (32.26) followed by NLE (29.32) and BLE (23.33). There were significant differences on growth inhibition of fungal isolates in

vitro among leaf extracts as shown in table 31.

Table 31: Grand effect of leaf extracts on growth inhibition of fungal isolates in vitro.

Extract	1	2	3	4	5	(Test fungi)
MLE	34.83	36.35	33.12	28.63	32.26	
NLE	33.35	35.42	37.08	32.26	29.32	
BLE	35.03	21.35	31.83	29.13	23.33	
F-LSD (0.05)	**4.25**	**3.18**	**233**	**3.09**	**3.06**	

Key: MLE – *Moringa* Leaf Extract, **NLE** – Neem Leaf Extract, **BLE** – Bitter Leaf Extract

3.3 Grand effect of concentration on growth inhibition of fungal isolates in vitro.

The grand effect of extract concentration on growth inhibition of fungal isolates in vitro showed that on organism 1 *(Aspergillus flavus)*, the highest growth inhibition was at concentration of 100g/ml (51.56) followed by 80g/ml (40.94), 60g/ml (38.55), 40g/ml (37.78) and 0g/ml (3.18) respectively. On organism 2 *(Penicillium waksmanii)*, the highest growth inhibition was at concentration of 100g/ml (45.41), followed by 80g/ml (38.46), 40g/ml (34.34), 60g/ml (33.93) and 0g/ml (3.05). For organism 3 *(Botryodiplodia theobromae)*, the highest growth inhibition was at concentration of 100g/ml (53.28) followed by 80g/ml (40.66), 60g/ml (39.28), 40g/ml (33.91) and 0g/ml (2.92) respectively. For organism 4 *(Fusarium oxysporum)* and 5 *(Colletotrichum asianum)*, the highest growth inhibition was at concentration of 100g/ml (49.35) and (48.71) respectively followed by 80g/ml (39.14) and (30.88), 60g/ml (30.90) and (30.63), 40g/ml (28.23) and (28.09) and 0g/ml (2.41) and (3.21) respectively. There were significant differences in growth inhibition of all fungal isolates among extract concentrations as shown in table 32.

Table 32: Grand effect of extract concentration on growth inhibition of fungal organisms in vitro.

Concentration	1	2	3	4	5	(Test fungi)
0	3.18	3.05	2.92	2.41	3.21	
40	37.78	34.34	33.91	28.23	28.09	
60	38.55	33.93	39.28	30.90	30.63	
80	40.94	38.46	40.66	39.14	30.88	
100	51.56	45.41	53.28	49.35	48.71	
F-LSD (0.05)	**5.48**	**4.10**	**3.01**	**3.99**	**3.95**	

3.4 Grand interaction effect of extract and concentration on growth inhibition of fungal isolates in vitro

The grand interaction effect of leaf extract and concentration on growth inhibition of organism I (*Aspergillus flavus)* in vitro revealed that at concentration of 100g/ml, *Moringa* leaf extract (MLE) gave the highest growth inhibition of (59.14) followed by Neem leaf extract (NLE) (49.70) and Bitter leaf extract (BLE) (45.84) respectively while the least growth inhibition on organism 1 *(Aspergillus flavus)* was by MLE at concentration of 40g/ml (30.11) followed by NLE at 60g/ml (32.11) and BLE at 40 g/ml (40.13). On organism 2 *(Penicillium waksmanii)*, MLE at a concentration of 100g/ml gave the highest growth inhibition of (50.49) followed by NLE (49.01) and BLE (36.72) respectively while the least inhibition on growth of organism 2 in vitro was by BLE at concentration of 60g/ml (16.05) followed by MLE (40.70) and NLE (40.70) at concentration of 80g/ml respectively. On organism 3 *(Botryodiplodia theobromae)*, MLE at concentration of 100g/ml gave the highest inhibition of growth (57.00) followed by NLE (52.71) and BLE (50.15) respectively while the least inhibition on growth of organism 3 in vitro was by BLE at 40g/ml (21.50) followed by MLE at 60g/ml (31.06) and NLE at 40g/ml (41.89). On organism 4 *(Fusarium oxysporum)*, the highest growth inhibition was by MLE at 100g/ml (54.02) followed by NLE at 100g/ml (49.62) and BLE at 100g/ml (44.41) while the least growth inhibition was shown by MLE at 60g/ml (24.04) followed by BLE at 40g/ml (26.60) and NLE at 40g/ml (30.12). The highest grand inhibitory effect of extract concentration on growth inhibition of organism 5 *(Colletotrichum asianum)* in vitro was shown by NLE at 100g/ml (53.68) followed by MLE at 100g/ml (51.51) and BLE at 100g/ml (40.94). The least inhibitory effect on growth of organism 5 in vitro was by BLE at 80g/ml (21.26) followed by NLE at 40g/ml (22.25) and MLE at 40g/ml (32.69). The controls ranged from 2.23 to 4.31 across all extract concentrations and fungal isolates. There were significant differences in growth inhibition between extract concentrations and their controls on all fungal isolates as shown in table 33.

Table 33: Grand interaction effect of leaf extract and concentration on growth inhibition of fungal isolates in vitro.

Extract	Concentration	1	2	3	4	5	(Test fungi)
	0	2.57	3.22	3.22	2.23	4.31	
MLE	**40**	30.11	43.52	38.33	27.97	32.69	
	60	38.62	43.81	31.06	24.04	37.46	

	80	43.71	40.70	35.98	34.89	35.34
	100	59.14	50.49	57.00	54.02	51.51
	0	3.19	3.09	3.18	2.31	2.28
	40	43.11	42.37	41.89	30.12	22.25
NLE	**60**	32.11	41.94	42.40	40.60	32.35
	80	38.62	40.70	45.23	38.62	36.04
	100	49.70	49.01	52.71	49.62	53.68
	0	3.78	2.85	2.37	2.67	3.03
	40	40.13	17.14	21.50	26.20	29.33
BLE	**60**	44.93	16.05	44.37	28.06	22.09
	80	40.48	34.00	40.76	43.89	21.26
	100	45.84	36.72	50.15	44.41	40.94
F-LSD (0.05)		**9.49**	**7.11**	**5.22**	**6.91**	**6.84**

Key: MLE – *Moringa* Leaf Extract, **NLE** – Neem Leaf Extract, **BLE** – Bitter Leaf Extract

4. DISCUSSION

The aqueous extracts of leaves of *Moringa oleifera*, *Azadirachta indica* and *Vernonia amygdalina* were found to be effective in inhibiting the radial growth of the fungal organisms in vitro, with inhibition varying from one extract to another. This is in agreement with Gwa and Akombo [7] who reported that *Piper nigrum, Zingiber officinale, A. indica, C. Papaya* and *N. tabacum* had significant effect on mycelia growth of *A. flavus* in vitro. Also, Sangoyomi *et al.* [8] demonstrated the fungitoxic effect of extracts obtained from *Allium sativum, Ocimum gratissimum, Cassia alata, Azadirachta indica* and *Hibiscus rosasinensis.* They showed that the extracts were able to inhibit the microbial growth and reduce the production of conidia in the four major fungi associated with yam rot during storage. *M. oleifera, A. indica* and *V. amygdalina* leaf extracts at various concentrations were found to be effective against the growth of fungi organisms. This agrees with Tijjani *et al.* [9] who reported significant inhibitory property of Neem *(A. indica*) and *Moringa* (*M. oleifera)* extracts on mycelia growth of *Rhizopus stolonifer.* Akpa *et al.* [10] also reported a significant inhibitory property of Neem extract on growth of *Colletotrichum graminicola.* This also agrees with the results of Amadioha [11] who reported the efficacy of *C. Papaya, A.ciliata* and *C. odirata* among other extracts in reducing the growth of *Colletotrichum capsid.* During the study, there was a general increase in growth inhibition with an increase in extract concentration. This finding agrees with

Ghangaonkar [12] who reported that extracts of *Polyalthia longifolia* and *Tridax procumbens* inhibited the growth of *Fusarium oxysporum* and *Aspergillus niger* on cowpea seed with increasing concentration. Also, Okoro [13] reported that there was increased antifungal activity of *V. amygdalina* on *A. flavus* and *B. theobromae* with increasing concentration. The increase in extract concentrations implied an increase in the active ingredients of the solutions which act on the fungus thereby affecting its physiological processes and consequently lowering the growth of the fungi. Liamngee *et al.*, [14].

5. CONCLUSION

The results of this study have established that plant extracts possess antifungal potential and have the ability to inhibit the growth of pathogenic fungi in vitro. This is an important step in developing plant based biopesticides as ideal treatments for future plant disease management programmes. These botanicals are not only environmentally friendly, cost effective, easy to produce and easy to apply formulations, they are also safe for consumers and provide alternative means for controlling plant pathogenic fungi.

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