RESULTS

Water analysis:

Physic-chemical properties of water:-

Temperature:

From the data recorded in (table, 1) it is clear that there was no difference in water temperature among freshwater fish ponds during the period of the study. The values of temperature at pond (1) were 28.75 ± 0.09ºC, at pond (2) 28.75 ± 0.09ºC and at pond (3) 28.62 ± 0.08ºC.

Dissolved oxygen (DO):

Table (1) shows that there were variations in the dissolved oxygen content in the three ponds. The values of DO. was 4.40 ± 0.10 mg/l at pond (1), 5.87 ± 0.10 mg/l at pond (2) and 5.47 ± 0.03 mg/l at pond (3).

pH:

Table (1) revealed that there were variations in pH values between the three ponds. pH was 8.6 ± 0.02 at pond (1), 9.3 ± 0.04 at pond (2) and 9.3 ± 0.09 at pond (3).

Secchi disc (visibility) SD.:

Table (1) shows that there were variations in SD. depth varied among the three ponds, 11.0 ± 0.26 cm at pond (1), 8.5 ± 0.42 cm at pond (2) and 9.0 ± 0.0.37 cm at pond (3).

Table (1): Values are average ± of 3 separate determination physical and chemical analysis of water for the pond 1,pond 2 and pond 3, from which algae were isolated.

Analysis	Pond 1	Pond 2	Pond 3
1- Water temp.(ºC)	28.75 ± 0.09	28.75 ± 0.09	28.62 ± 0.08
2- Dissolved oxygen (mg/l)	4.40 ± 0.09	5.87 ± 0.10	5.47 ± 0.03
3- pH value	8.6 ± 0.02	9.3 ± 0.04	9.3 ± 0.09
4- Secchi disk visibility (cm)	11.0 ± 0.26	8.5 ± 0.42	9.7 ± 0.37
5-Total ammonia concentration (mg/l)	0.60 ± 0.02	0.775 ± 0.01	0.70 ± 0.02
6- Nitrite concentration(mg/l)	0.032 ± 0.01	0.051 ± 0.008	0.048 ± 0.007
7- Nitrate concentration (mg/l)	0.495 ± 0.013	0.434 ± 0.021	0.455 ± 0.03
8-Total phosphorus conc.(mg/P_2O_5/l)	0.779 ± 0.058	1.306 ± 0.146	1.19 ± 0.035
9- Chlo "a" Conc.(µg/l)	166.6 ± 10.44	383.65±14.87	284.64 ± 19.23

Total ammonia concentration (NH_4):

Table (1) revealed that there were variations in total ammonia between the three ponds. It was 0.60 ± 0.02 mg/l at pond (1), 0.775 ± 0.01 mg/l at pond (2), and 0.70 ± 0.02 mg/l at pond (3).

Nitrite concentration (NO_2-N):

Table (1) shows the nitrite concentration variation between the three ponds 0.032 ± 0.01 mg/l at pond (1), 0.051 ± 0.0.008 mg/l at pond (2) and 0.048 ± 0.007 mg/l at pond (3).

Nitrate concentration (NO_3-N):

As illustrated in (table, 1) the nitrate concentrations in the three ponds was 0.495 ± 0.013 mg/l at pond (1), 0.434 ± 0.021 mg/l at pond (2) and 0.445 ± 0.03 mg/l at pond (3).

Total phosphorus conc. (T.P.):

Table (1) shows that there were variations in TP. concentrations between the three ponds. It accounts 0.779 ± 0.058 mg/l at pond (1), 1.306 ± 0.146 at pond (2) and 1.19 ± 0.035 mg/l at pond (3).

Chlorophyll "a" conc. (µg/l):

As shown in (table, 1) the highest concentration of chlorophyll (a) was 383.65 ± 0.14.87 µg/l at pond (2) and the lowest conc. was 166.6 ± 10.44 µg/l at pond (1) while at pond (3) the conc. of chlorophyll (a) was 284.64 ± 19.23 µg/l.

Isolation and identification of algae:

The blue green algae (Cyanobacteria):

Cyanobacteria, prokaryotic cells, were the first algae to evolve. Cyanobacteria were the first cells to have two photo systems and to give oxygen as a by-product.

Today, Cyanobacteria are photosynthetic producers in a wide range of freshwater and marine environments, and one of the most common algal groups in terrestrial habitats and in symbiotic associations. Many Cyanobacteria also convert nitrogen gas (N_2) into usable forms of nitrogen through nitrogen fixation. The following is description for algae isolated and purified in an axenic culture.

1-*Oscillatoria curviceps*: is an unbranched filament composed of disk-shaped cells arranged in a single series as (Fig., 3(A, B)). All of the cells in a filament are a like except for terminal cells with a modified shape. *Oscillatoria* often grows in mats of intermoven filaments.

2-*Lyngbya* sp.: is similar to *Oscillatori*a, but its filaments have a firm mucilaginous sheath that normally extends beyond the terminal cell. When a sheath is present, as in *Lyngbya*, the term trichome refers to the series of cells, while the filament includes the sheath and cells.

3-*Anabaena wisconsinense*: is a typical heterocystous Cyanobacterium, filaments in bundles surrounded by a mucilaginous sheath, tapering filaments with heterocyst at their base, *Anabaena* is unbranched filament sometimes give the appearance of a string of beads as shown in (Fig. 4).

The growth curve of *Anabaena wisconsinense* and *Oscillatoria curviceps*:

It is evident from (Fig. 5) that, the growth of *Anabaena wisconsinense* increased exponentially through the first 16 days, there after, decline phase began and continued till the 18th day. On the other hand, *Oscillatoria curviceps* showed an exponential increase through the first 14 days, there after; decline phase began and continued till the 16th day.

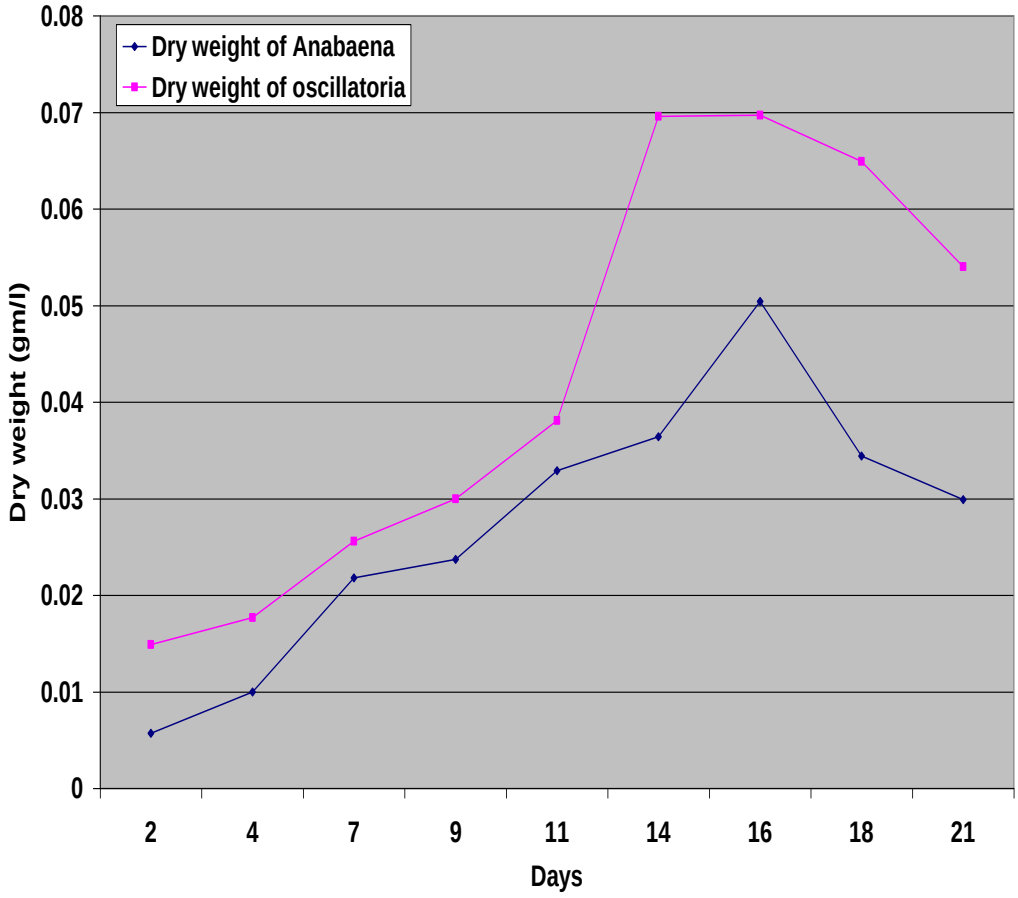


Fig. (5) Growth of *Anabaena wisconsinense* and *Oscillatoria curviceps*.

Isolation and identification of bacteria:

The results of physical and biochemical tests revealed that the bacterial isolates were identified as *Lactobacillus* sp., *Bacillus firmus, Aeromonas hydrophila, Pseudomonas fluorecence* and *Pseudomonas anguilliseptica.*

From (table, 2) the suspected *Lactobacillus* sp was Gram-positive, cocci, coccibacilli and bacilli in shape in the same field, single and in chains. The biochemical tests revealed inability to produce H_2S, indol, gelatine, Voges Proskure reaction VP. It produced acid from glucose, sucrose, sorbitol, xylose, maltose, manitol and galactose. It was isolated from the eye of *O. niloticus*.

The suspected *B. firmus* was Gram-positive rod shape and present single. The biochemical tests revealed inability to produce oxidase, indole, H_2S. It did not oxidative or fermentative. It liquefied gelatine and produced nitrate. Also it produced acid from sorbitol, maltose and manitol. This bacteria has the ability to grow in 3%, 5% and 6.5% NaCl. It was isolated from the stomach of diseased *O. niloticus*.

The suspected *Pseudomonas fluorecence* was Gram-negative, bacilli in shape, single and motile. It produces oxidase, H_2S and catalase. It liquefied gelatine and produce nitrate. It produced acid from glucose, sucrose, sorbitol, xylose, maltose and galactose. It was isolated from the tail of *O. niloticus*.

The suspected *Pseudomonas anguilliseptica* was Gram-negative, motile, bacilli and single in arrangment.The biochemical tests shown that the bacteria produced oxidase, catalase, indol, Voges- Proskauer, gelatin and nitrate. Lycin decarboxylation was negative. It produced

acid from sucrose, xylose, maltose, manitol and galactose. It was isolated from the stomach of *O. niloticus*.

The suspected *A. hydrophila* was Gram-negative, motile, coccibacilli, single in arrangement and fermentative. It produced acid from glucose, sucrose, maltose and galactose. Oxidase, catalase, indol, Voges-Proskauer were positive with it. Lycin decarboxylase was negative. It was isolated from the stomach of *O. niloticus*.

Table (2): Physical and biochemical characters of the isolated bacteria.

Character	*Lactobacills* sp.	*Bacillus firmus*	*Pseudomonas fluorecence*	*Pseudomonas anguilliseptica*	*Aeromonas hydrophilia*
Gram stain	+ve	+ve	-ve	-ve	-ve
Shape	Cocci, bacilli	R.	Bacilli	Bacilli	Cocci, bacilli
Arrangement	Single, short chain	Single	Single	Single	Single
Oxidase	+	-	+	+	-
Catalase	+	+	+	+	+
O/F	O /-	-	O /-	-	F
Motility	-	-	+	+	+
Indol	-	-	-	+	+
V.P.	-	-	.	+	+
M.R.	.	+	.	+	+
H_2S	-	-	+	-	-
Gelatin	-	+	+	+	+
Nitrate	-	+	+	+	+
Arginine ,hyd.	.	+	.	+	+
Ornithine	.	-	+	-	+
Lycin	.	-	-	-	-
	Production of acid from:-				
Glucose	+	-	+	-	+
Sucrose	+	D	+	+	+
Sorbitol	+	+	+	-	-
Xylose	+	-	+	+	-
Maltose	+	+	+	+	+
Manitol	+	+	D	+	-
Galactose	+	D	+	+	+
	Growth on :-				
0.0%NaCl	.	+	+	+	+
3% NaCl	.	+	+	-	+
5%NaCl	.	+	-	-	-
6.5%NaCl	.	+	-	-	-
7.0%NaCl	.	-	.	-	-
Growth at 45ºC	.	+	.	-	-

Isolation and identification of *Aspergillus niger*:

Macroscopic morphology

Colonies on Oxytetracycline glucose yeast extract agar medium at 25°C are initially white, quickly becoming black with conidial production. Reverse is pale yellow as in (Fig. 6 A).

Microscopic morphology

Hyphae are septated, unbranched conidiophores with enlarge tip forming around vesicle covered with flask shaped stigmata that produce chains of round conidia as in (Fig. 6 B).

Antimicrobial activities of *Anabaena wisconsinense* extracts:-

Methanolic extract of *Anabaena wisconsinense* had inhibition zone with the gram negative and gram positive bacteria except *Lactobacillus* species. The diameter of inhibition zones due to methanolic extract were 50, 32, 32 and 30 mm with *Pseudomonas anguilliseptica, A. hydrophila, B. firmus and Pseudomonas fluorecence,* respectively. The inhibition zone with *Aspergillus niger* was 20 mm in diameter in case of methanolic extraction of *Anabaena* while, no response with methanol alone as shown in table 3.

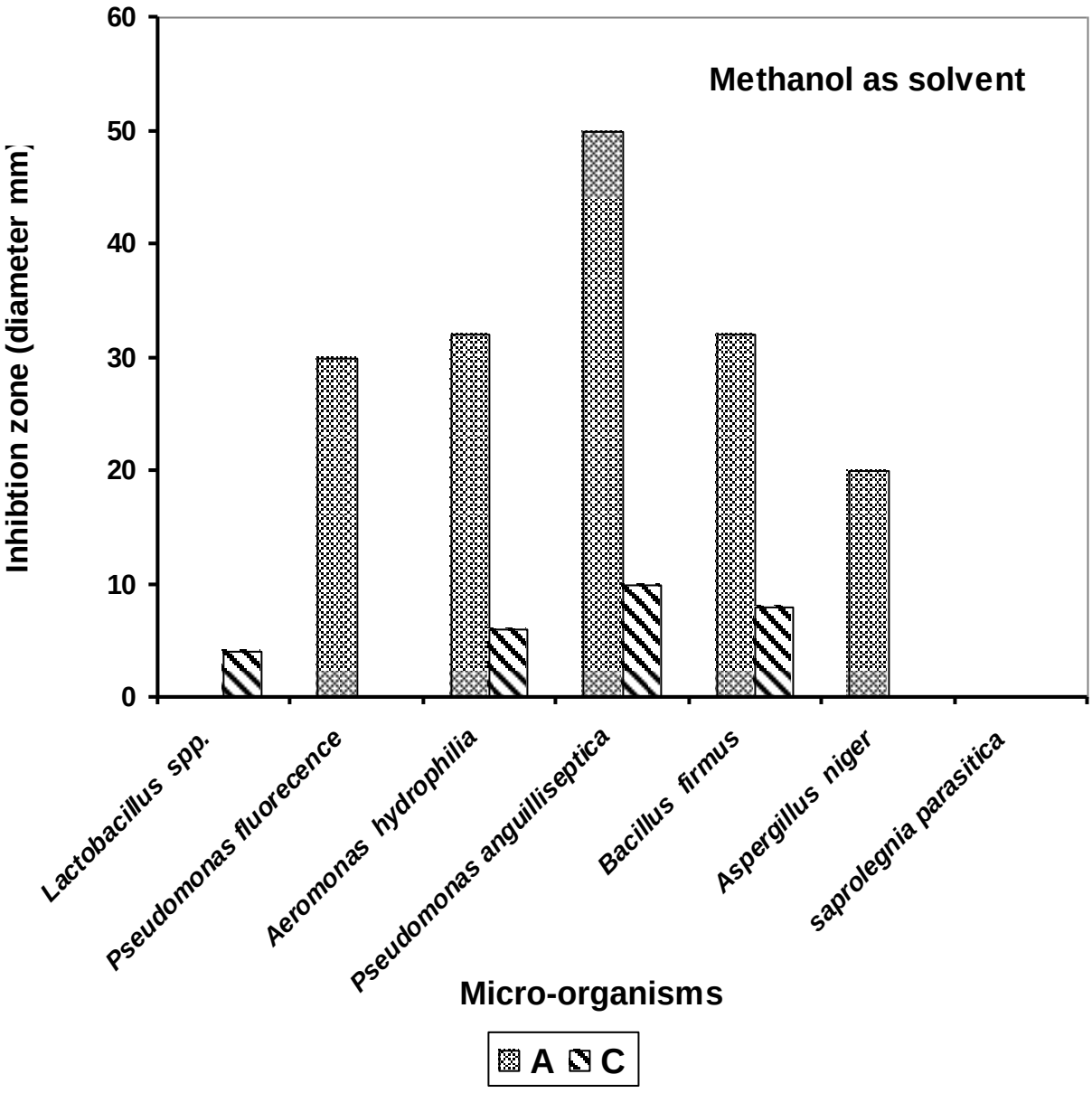
Chloroform extract of *Anabaena wisconsinense* had inhibition zones 30, 20 and 16 mm in diameter with *Pseudomonas fluorecence*, *Pseudomonas anguilliseptica* and *Lactobacillus* sp. respectively, as compared with control table 3.

Ethanolic extract of *Anabaena wisconsinense* gave inhibition zones 34, 24 and 12 mm diameter with *Aeromonas hydrophilia, Bacillus firmus* and *Pseudomonas fluorecence*. Ethanolic extract of *Anabaena wisconsinense* gave inhibition zones 26 mm in diameter with *Aspergillus niger;* while ethanol had no inhibition zone as shown in table 3.

Table (3): The antimicrobial activities of *Anabaena wisconsinense* extracts against different test organisms (inhibition zone measured as mm):

A= the algal extract. C= the control.

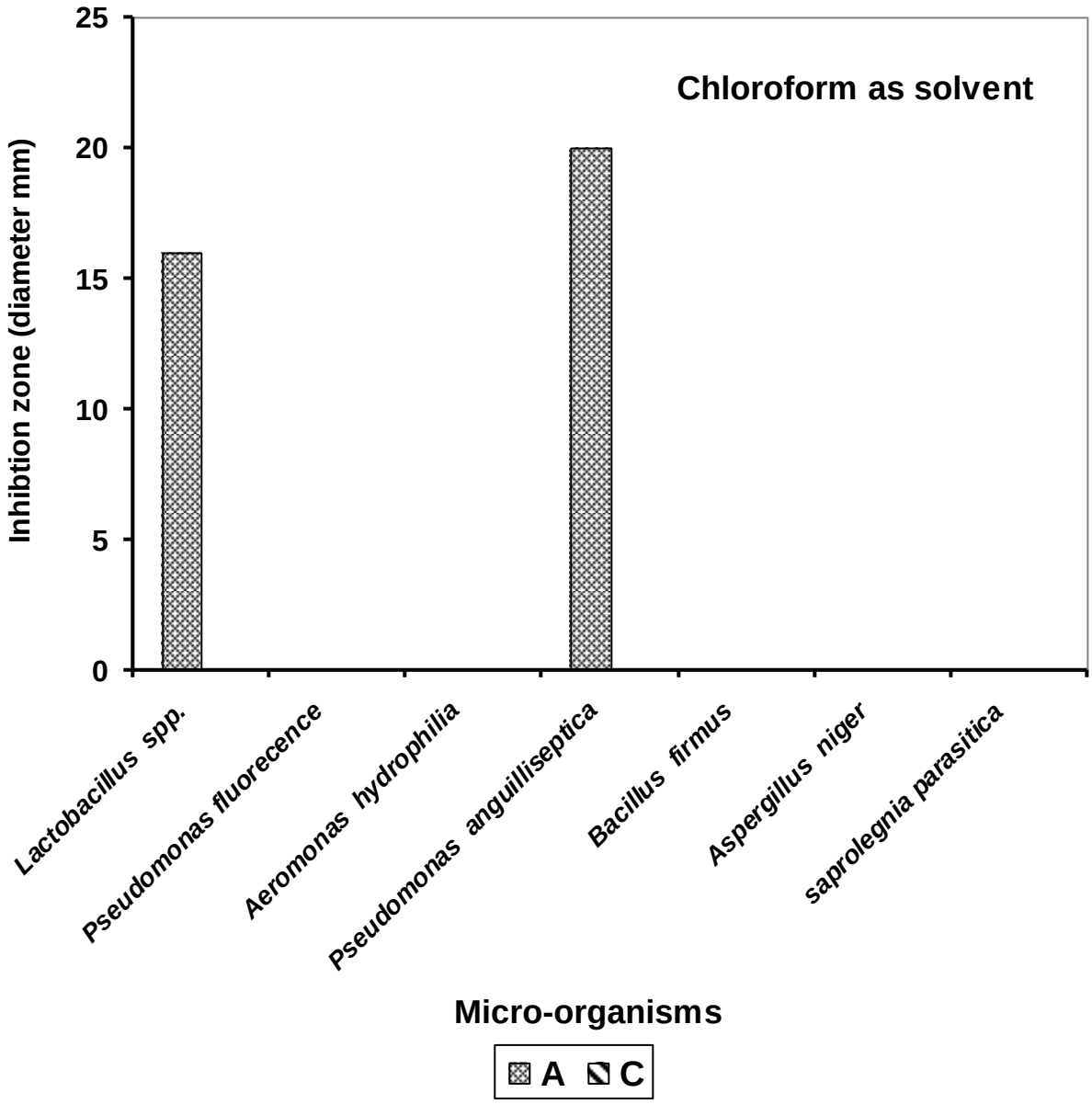
Organisms	Quantity of algeal extract (ml)	Inhibition zone due to the algal extracts					
		Methanol		Chloroform		Ethanol	
		A	C	A	C	A	C
Lactobacillus sp.	30	0.0	4.0	16.0	0.0	0.0	6.0
Pseudomonas fluorecence	30	30.0	0.0	30.0	0.0	12.0	6.0
Aeromonas hydrophilia	30	32.0	6.0	0.0	0.0	34.0	6.0
Pseudomonas anguilliseptica	30	50.0	10.0	20.0	0.0	0.0	6.0
Bacillus firmus	30	32.0	8.0	0.0	0.0	24.0	8.0
Aspergillus niger	250	20.0	0.0	0.0	0.0	26.0	0.0
Saprolegnia parasitica	250	0.0	0.0	0.0	0.0	0.0	0.0



A=algal extract

C=control

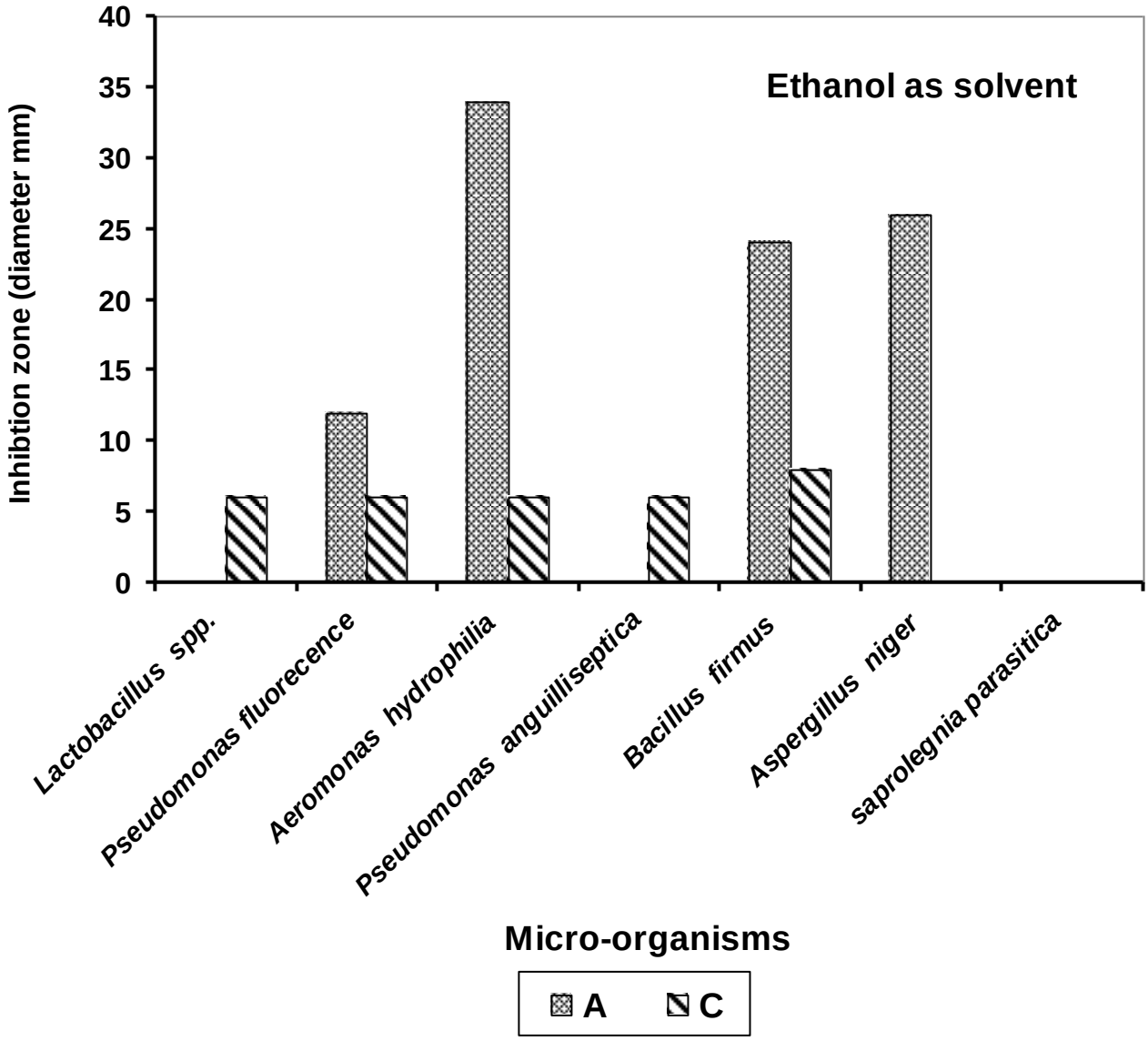
Fig. (7): The effect of methanolic extract of *Anabaena wisconsinense* on growth of micro-organisms (measured as diameter of inhibition zone (mm)).



A=algal extract

C=control

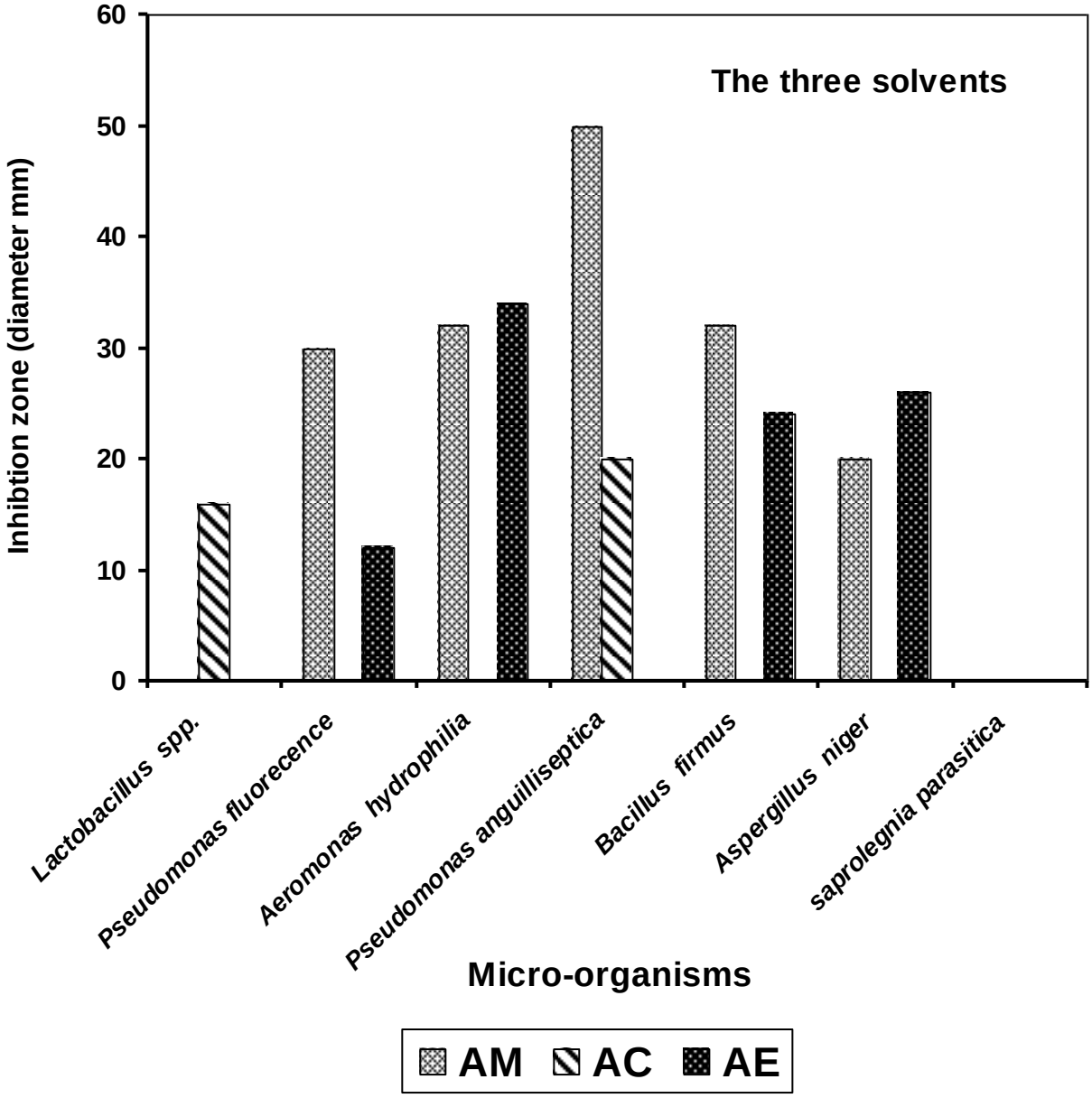
Fig. (8): The effect of chloroformic extract of *Anabaena wisconsinense* on growth of micro-organisms (measured as diameter of inhibition zone (mm)).



A=algal extract

C=control

Fig. (9): The effect of ethanolic extract of *Anabaena wisconsinense* on growth of micro-organisms (measured as diameter of inhibition zone (mm)).



AM= Methanolic extract of *Anabaena wisconsinense*

AC= Chloroformic extract of *Anabaena wisconsinense*

AE= Ethanolic extract of *Anabaena wisconsinense*

Fig. (10): The effect of the three extractions (AM, AC and AE) of *Anabaena wisconsinense* on growth of micro-organisms (measured as diameter of inhibition zone (mm)).

Antimicrobial activities of *Oscillatoria curviceps* extracts:-

Methanolic extract of *Oscillatoria curviceps* had inhibition zone 34, 16 and 4 mm in diameter with *Lactobacillus* sp., *Pseudomonas anguilliseptica* and *A. hydrophila* respectively. The inhibition zone with *Aspergillus niger* was 20 mm in diameter in case of methanolic extraction of *Oscillatoria* while, no response with methanol alone. Neither methanolic extract of *Oscillatoria* nor methanol alone had effect on *Saprolegnia parasitica* as shown in (table, 4).

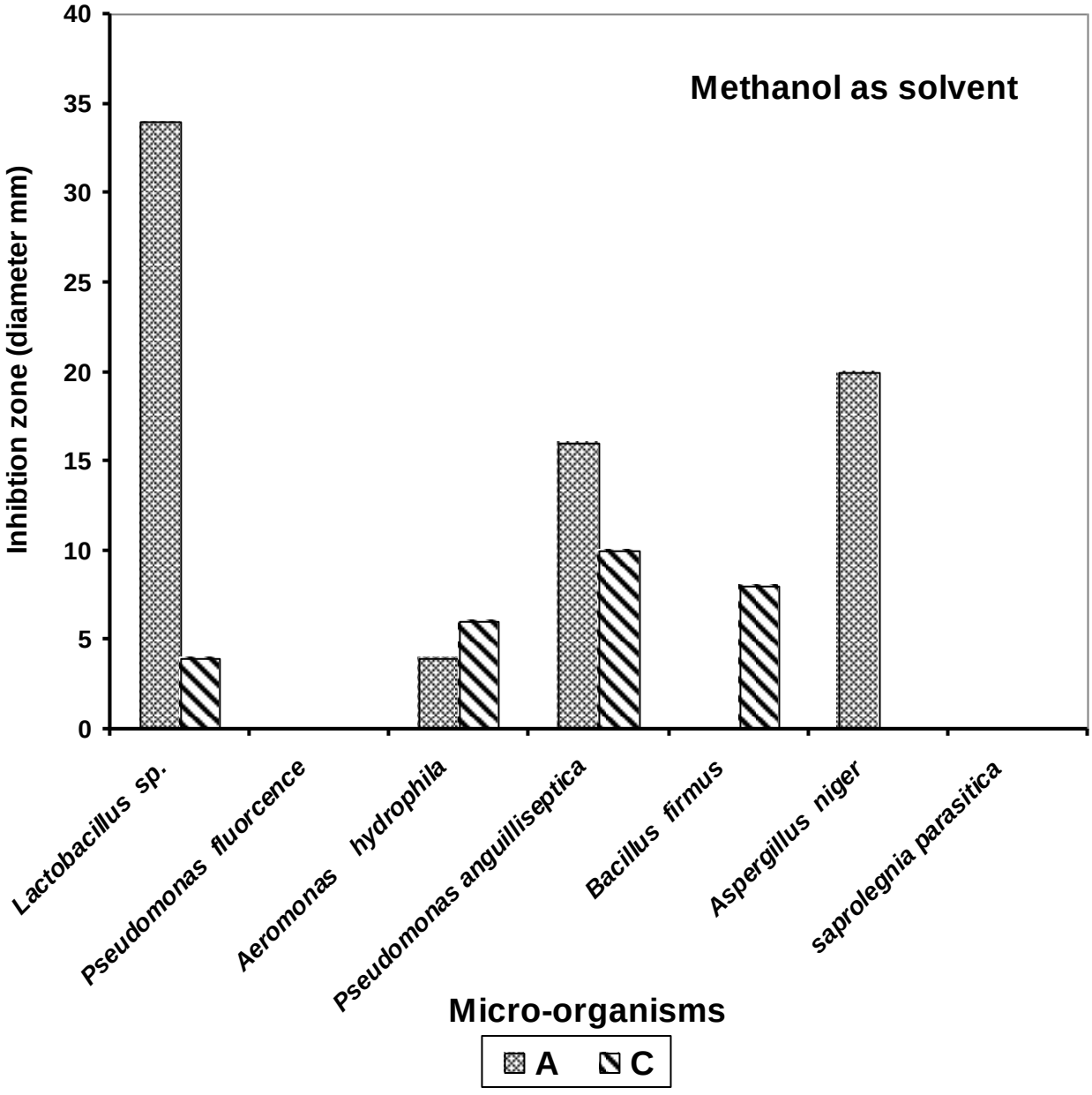
Chloroform extract of *Oscillatoria curviceps* had inhibition zones 14, 10, 10, and 6 mm in diameter with *Pseudomonas anguilliseptica, Pseudomonas fluorecence*, *A. hydrophila* and *Lactobacillus* sp., respectively. The control did not give any effect as shown in (table, 4).

Chloroform extract of *Oscillatoria curviceps* had inhibition zones 94 and 8 mm in diameter with *Saprolegnia parasitica* and *Aspergillus niger*, respectively.

Ethanolic extract of *Oscillatoria curviceps* had inhibition zones 30, 30, 20, 14 and 6 mm diameter with *Lactobacillus sp., A. hydrophilia, Pseudomonas anguilliseptica, B. firmus* and *Pseudomonas fluorecence*, respectively. Ethanolic extract of *Oscillatoria curviceps* gave inhibition zones 28 mm in diameter with *Aspergillus neiger*; while ethanol had no inhibition zone. Neither ethanolic *Oscillatoria* extract nor ethanol alone had effect on *Saprolegnia parasitica* as shown in (table, 4).

Table (4): The antimicrobial activities of *Oscillatoria curviceps* extracts against different test organisms (inhibition zone measured as mm):
A= the algal extract C= the control.

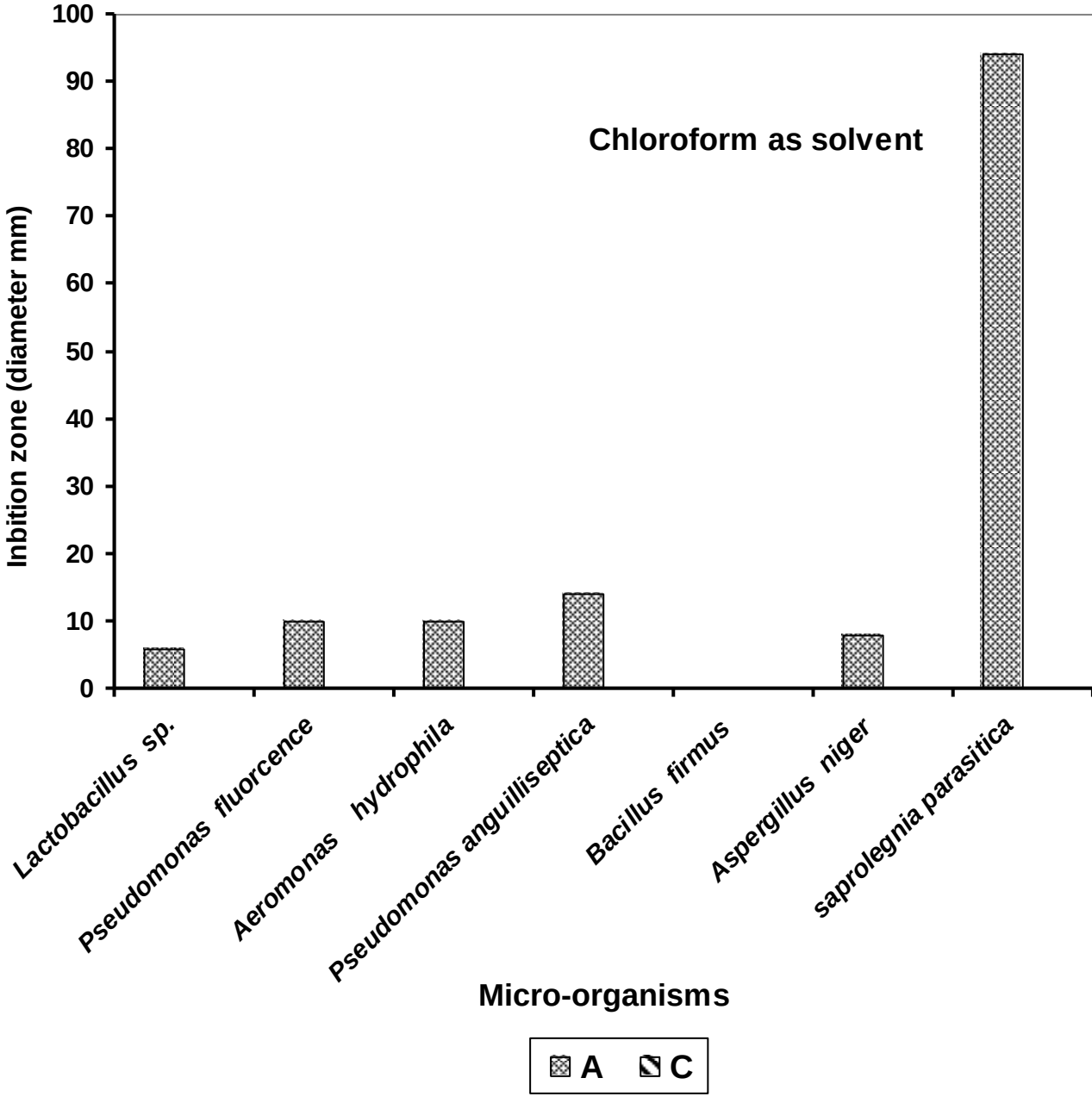
Organisms	Quantity of the extract (ml)	**Inhibition zone due to the algal extracts**					
		Methanol		Chloroform		Ethanol	
		A	C	A	C	A	C
Lactobacillus sp.	30	34.0	4.0	6.0	0.0	30.0	6.0
Pseudomonas fluorcence	30	0.0	0.0	10.0	0.0	6.0	6.0
Aeromonas hydrophila	30	4.0	6.0	6.0	0.0	30.0	6.0
Pseudomonas anguilliseptica	30	16.0	10.0	14.0	0.0	20.0	6.0
Bacillus firmus	30	0.0	8.0	0.0	0.0	14.0	8.0
Aspergillus niger	250	20.0	0.0	8.0	0.0	28.0	0.0
Saprolegnia parasitica	250	0.0	0.0	94.0	0.0	0.0	0.0



A=algal extract

C=control

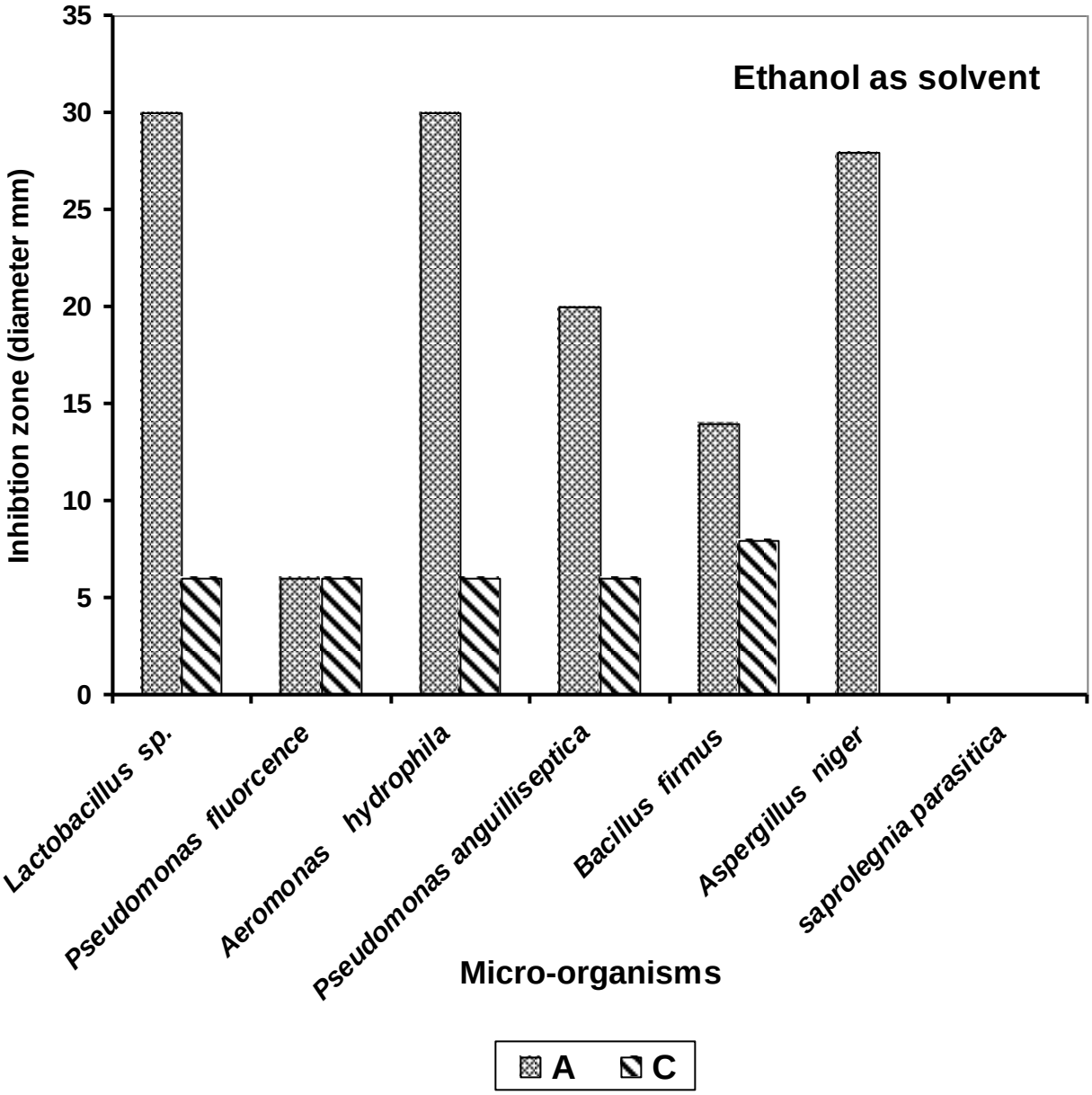
Fig. (11): The effect of methanolic extract of *Oscillatoria curviceps* on growth of micro-organisms (measured as diameter of inhibition **zone** (mm)).



A=algal extract

C=control

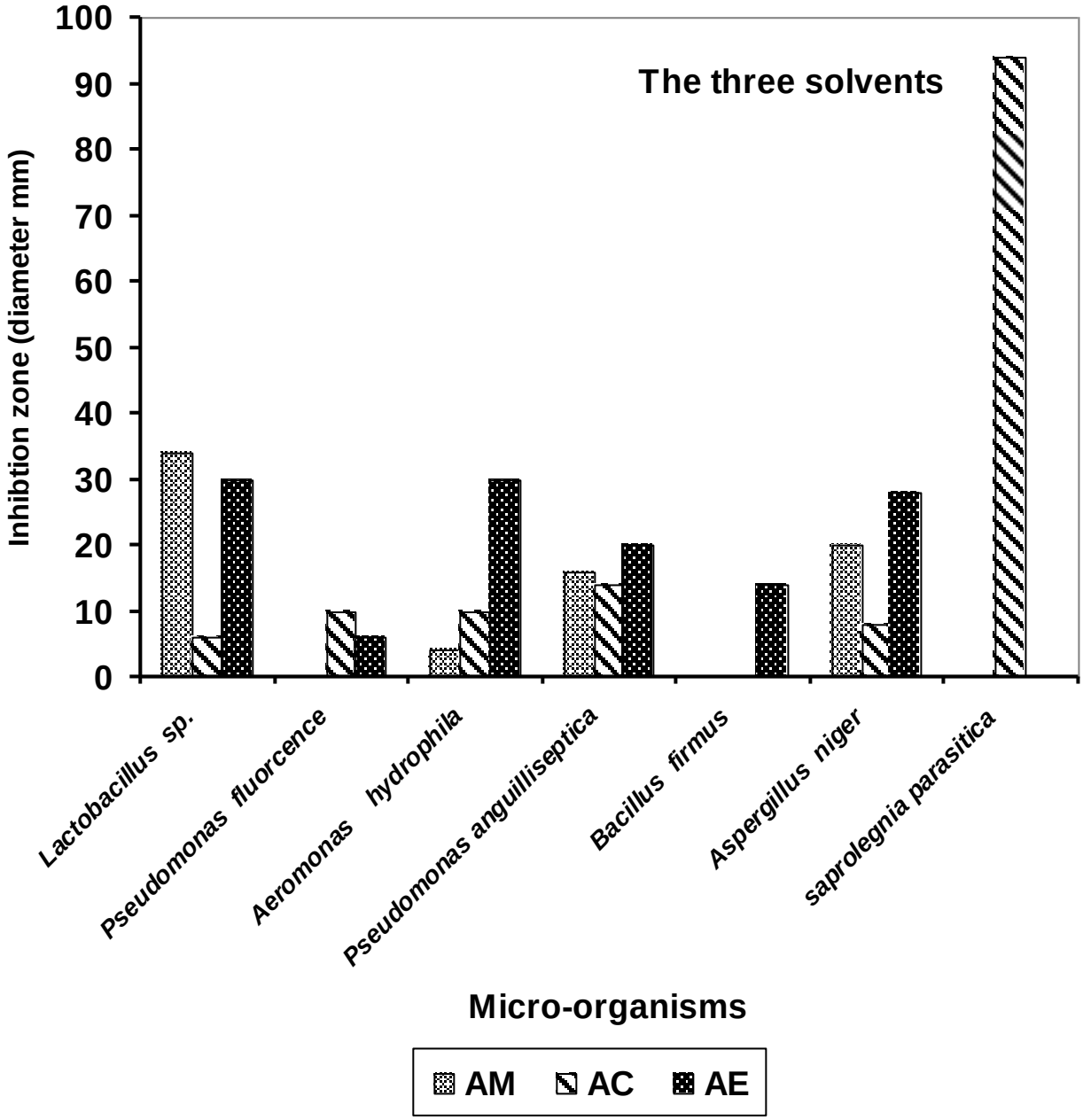
Fig. (12): The effect of chloroformic extract of *Oscillatoria curviceps* on growth of micro-organisms (measured as diameter of inhibition zone (mm)).



A=algal extract

C=control

Fig. (13): The effect of ethanolic extract of *Oscillatoria curviceps* on growth of micro-organisms (measured as diameter of inhibition zone (mm)).



AM= Methanolic extract of *Oscillatoria curviceps*.

AC= Chloroformic extract of *Oscillatoria curviceps.*

AE= Ethanolic extract of *Oscillatoria curviceps.*

Fig. (14): The effect of the three extractions (AM, AC and AE) of *Oscillatoria curviceps* on growth of micro-organisms (measured as diameter of inhibition zone (mm)).

Experimental studies of *Pseudomonas anguilliseptica*:

The experimental injection of *Pseudomonas anguilliseptica* revealed that *Pseudomonas anguilliseptica* was pathogenic for *O. niloticus* at 0.2 ml of 10^7 cells / ml by intrapretonial injection (I/P) route givining 50% mortality rate and control did not give any mortality as shown in (table, 5).

The experimental infection of *O. niloticus* showed clinical signs and postmortem lesion as in (Fig. 17). Clinical signs were hemorrhages all over the body and fins and scall loss. The postmortem findings showed septicemia, hemorrhages in the liver, intestine and kidneys, necrotic foci in liver.

Efficiency of *Anabaena wisconsinense* methanolic extract against the pathogenic *Pseudomonas anguilliseptica* among *Orechromis niloticus:*

Table (6) showed that the intrapretonial inoculation (I/P) of 0.2×10^7 cells /ml of *Pseudomonas anguilliseptica* caused mortality 50% among *Orechromis niloticus*, while the treated *Orechromis niloticus* with methanolic extract of *Anabaena wisconsinense* had mortality 12.5%. The other two groups did not show any mortalities or clinical signs.

Table (5): Pathogensity test of *Pseudomonas anguilliseptica* among *Oeriochromis niloticus.*

Items	***Pseudomonas anguilliseptica***	**Control**
No, of examined fish	20	20
Dose	0.2×10^{7} cells /ml	0.2 ml sterile saline
Route of injection	I/P	I/P
Mortality rate	(50%)	(0%)

Table (6): Efficiency of *Anabaena wisconsinense* methanolic extract against the pathogenic *Pseudomonas anguilliseptica* among *Orechromis niloticus.*

Bac. = Bacteria Ex. = Extract I/P = Intrapretonial inoculation

parameters	***Pseudomonas anguilliseptica* (Bac.)**	***Anabaena* methanolic extract (Ex.)**	**Bac.+ Ex.**	**Control**
No. of examined fish	20	20	20	20
Dose	0.2×10^{7} cells/ml	0.5 ml	0.2 ml from bact.+0.5 ml from Ex.	0.2 ml saline
Route of injection	I/P	I/P	I/P	I/P
Mortality rate	50%	0.0	12.5%	0.0

Fig. (3): **A, B** Photographs Showing *Oscillatoria curviceps.* (A, 400x) , (B,1000x).

Fig. (4): Photographs showing *Anabaena wisconsinense.* (400x)

Fig (5 A): Macroscopic photograph of *Aspergillus niger.* Colonies on Oxytetracycline glucose yeast extract agar medium are initially white, quickly becoming black with conidial production and reverse is pale yellow.

Fig (5 B): Microscopic photograph of *Aspergillus niger* (1000x) Hyphae are septated, unbranched conidiophores with enlarge tip forming around vesicle covered with flask shaped.

Fig. (15 A): A photograph of a plate showing the effect of methanolic extract of *Anabaena wisconsinense* on growth of *Pseudomonas fluorecence* (measured as diameter of inhibition zone (mm)).
D: sterilized paper disc impregnated with 30 µl of *Anabaena* methanolic extract.
A: inhibition zone.
Ps.: *Pseudomonas fluorecence* growth on tryptic soy agar medium**.**

Fig. (15 B): A photograph of a plate showing the effect of methanolic extract of *Anabaena wisconsinense* on growth of *Pseudomonas anguilliseptica* (measured as diameter of inhibition zone (mm)).

D: sterilized paper disc impregnated with 30 µl of *Anabaena* methanolic extract.

A: inhibition zone.

Ps.: *Pseudomonas anguilliseptica* growth on tryptic soy agar medium**.**

Fig. (16A): A photograph of a plate showing the effect of chloroform extracts of *Oscillatoria curviceps* on growth of *Saprolegnia parasitica* on Oxytetracycline glucose yeast extract agar medium (measured as diameter of inhibition zone (mm)).

Fig. (16 B): A photograph of a plate showing the effect of ethanolic extract of *Oscillatoria curviceps* on growth of *Aeromonas hydrophilia* (measured as diameter of inhibition zone (mm)).

D: sterilized paper disc impregnated with 30 µl of *Oscillatoria* ethanolic extract.

A: inhibition zone.

Ah: *Aeromonas hydrophilia* growth on tryptic soy agar medium .

Fig. (17): A photograph showing *Oeriochromis niloticus* infected experimentally I/P by *Pseudomonas anguilliseptica* and showed (A) hemorrhages all over the body and fins, (B) congestion in the internal organs and (C) necrosis in liver

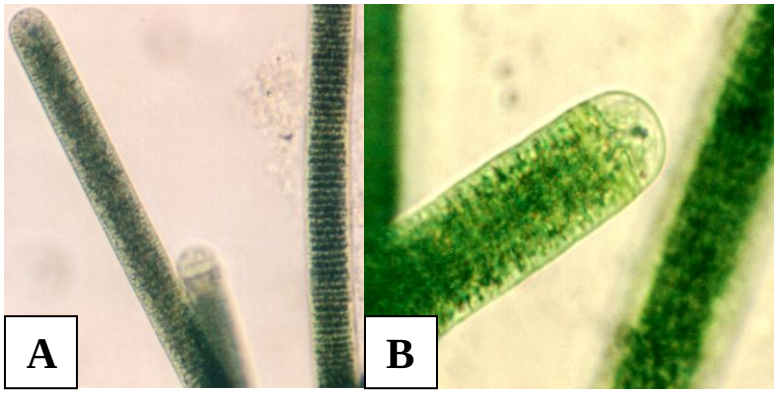


Fig. (3)

Fig. (4)

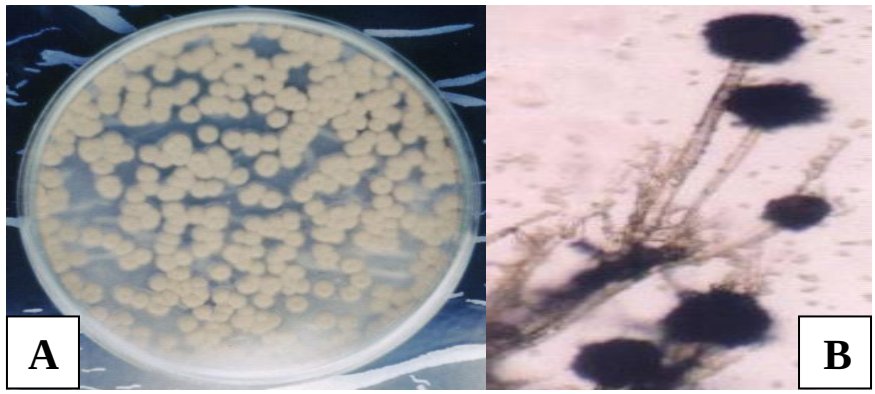


Fig. (5)

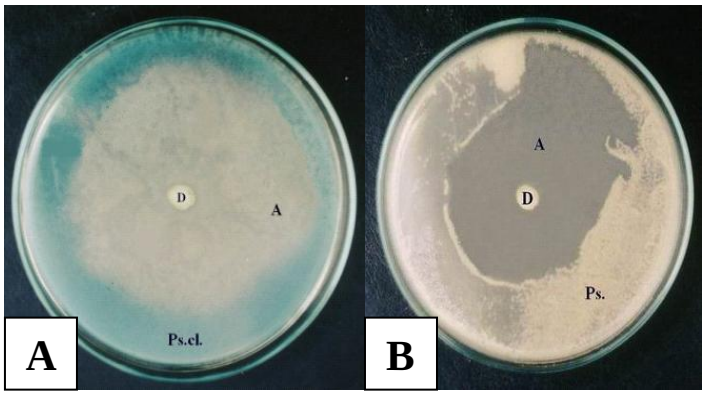


Fig. (15)

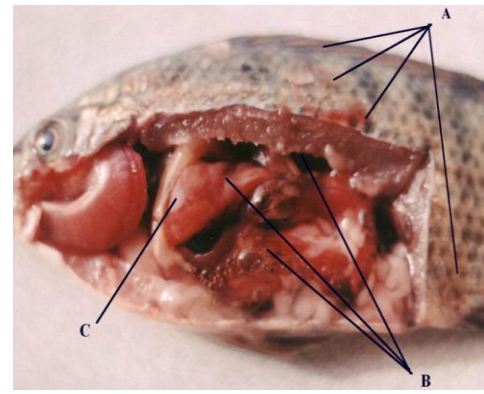


Fig. (17)

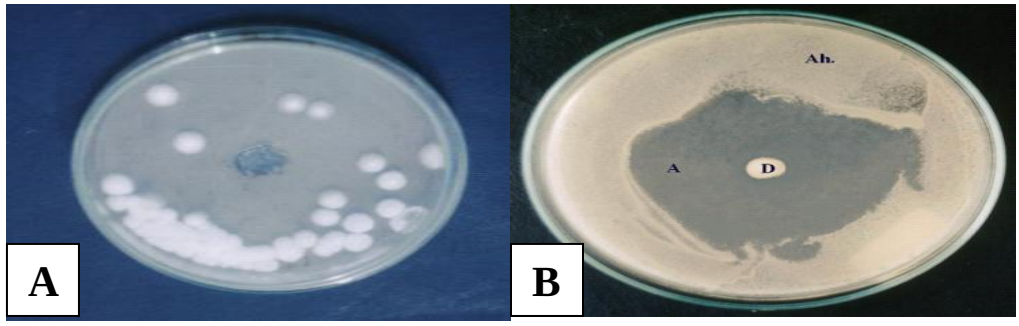


Fig. (16)