

Results

The antimicrobial activity of twenty five tested plant oils and extracts against the test organisms which belonged to six Gram-positive, six Gram-negative bacterial species, in addition to two yeast species and five fungal species is presented in Tables (1-6) and illustrated in figures (1-15).

1- Effect of different plant oils and extracts against E.coli:

Data in tables (1-5) and illustrated in figure (1) indicate the effect of different concentrations of plant oils and extracts (namely, crude, 50%, 25%, 12.5% and 6.25%) against the test organism E.coli.

The effect of crude oils and plant extracts on E.coli, as estimated by the width of the inhibition zone, could be arranged in a descending order as follows: Caraway (31 mm.) which was the most effective, followed by thyme (28 mm.), pine (22 mm), clove (20 mm), aniseed (18 mm), marjoram (15 mm), lemongrass (14 mm), Jasmine (12 mm.) basil (12 mm.) , rosemary (10 mm), coriander (10 mm.) cumin (10 mm), fennel (10 mm.) , rose (8 mm.), dill (8 mm.), geranium (8 mm.), pelargonium (6 mm.) and peppermint (6 mm.) . While the crude oils or extracts of castor, chamomile, celery, spearmint, onion, drosera and salvia were found to be non-effective against E.coli.

Table (2): Effect of 50 % concentration of plant oils and extracts on different test organisms..

Test Organisms		Diameter of inhibition zone in m.m. related to:																								
		Aniseed	Caraway	Celery	Coriander	Cumin	Dill.	Fennel	Jasmin.	Chamomile	Drosera.ex.	Geranium	Pelargonium	Onion ex.	Basil	Marjoram	Peppermint	Spearmint	Rosemary	Salvia ex.	Thyme	Pine.	Rose	Clove	Castor	Castor oil
Gram-negative	<u>Escherichia coli</u>	12	10	0	8	8	0	8	10	0	0	6	0	0	10	12	0	0	8	0	16	16	0	14	0	0
	<u>Klebsiella aerogenes</u>	7	12	8	7	12	10	8	14	8	0	12	14	0	18	12	12	8	8	0	34	22	8	18	0	0
	<u>Klebsiella pneumonia</u>	10	14	8	10	10	10	10	14	8	0	10	10	0	12	16	0	8	9	0	22	10	8	17	0	0
	<u>Pseudomonas aeruginosa</u>	14	10	8	8	8	8	12	32	12	0	12	6	8	14	14	0	14	8	0	32	24	10	22	0	0
	<u>Pseudomonas fluorescens</u>	6	8	0	0	6	0	8	7	0	8	8	6	0	0	10	7	10	0	0	20	6	8	8	0	0
	<u>Salmonella typhimurium</u>	0	8	0	8	10	0	8	12	0	0	0	6	0	10	18	0	0	8	0	12	10	10	12	0	0
Gram-positive	<u>Streptococcus sp.</u>	0	30	28	8	10	20	22	20	7	0	0	8	0	18	14	0	0	10	0	26	16	10	28	0	0
	<u>Staphylococcus aureus</u>	10	16	12	8	8	12	12	26	8	0	12	15	0	16	18	12	16	8	0	12	16	8	28	0	0
	<u>Staphylococcus aureus A</u>	6	12	10	6	10	8	10	8	6	7	8	8	0	10	12	10	10	7	0	8	16	10	14	0	0
	<u>Staphylococcus aureus B</u>	7	12	8	8	15	12	10	10	0	0	10	12	0	12	12	18	18	8	0	6	14	8	18	0	0
	<u>Micrococcus luteus</u>	8	10	8	0	14	8	8	14	8	0	6	12	0	18	12	12	18	8	0	22	12	10	22	0	0
	<u>Bacillus cereus</u>	8	14	8	7	10	10	10	12	8	0	10	12	8	12	14	16	12	9	0	30	10	10	24	0	0
Yeasts	<u>Candida albicans</u>	8	16	8	10	10	10	12	14	8	8	12	0	0	16	16	16	18	10	7	30	22	10	14	0	0
	<u>Saccharomyces cerevisiae</u>	7	12	10	8	12	10	10	10	9	0	10	16	0	14	12	14	12	12	0	36	10	8	16	0	0

Table (3): Effect of 25% concentration of plant oils and extracts on different test organisms

Test Organisms	Diameter of inhibition zone in m.m. related to:																								
	Aniseed	Caraway	Celery	Coriander	Cumin	Dill.	Fennel	Jasmdn.	Chamomile	Drosera.ex.	Geranium	Pelargonium	Onion ex.	Basil	Marjoram	Peppermint	Spearmint	Rosemary	Salvia ex.	Thyme	Pine.	Rose	Clove	Castor	Lemongrass
<u>Escherichia coli</u> <u>Klebsiella aerogenes</u> <u>Klebsiella pneumonia</u> <u>Pseudomonas aeruginosa</u> <u>Pseudomonas fluorescens</u> <u>Salmonella typhimurium</u>	10	8	0	6	0	0	0	8	0	0	0	0	0	0	8	0	0	6	0	0	12	10	0	0	0
	6	10	6	0	8	8	8	8	7	0	10	14	0	10	10	8	8	6	0	0	16	16	0	0	8
	8	8	7	0	8	8	8	12	8	0	8	8	0	10	10	0	0	6	0	0	8	8	14	0	8
	11	8	8	7	8	8	8	14	10	0	10	0	0	8	12	0	12	7	0	0	24	12	20	0	12
	6	8	0	0	0	0	8	6	0	0	6	0	0	0	0	6	8	0	0	0	6	6	0	0	0
<u>Streptococcus sp.</u> <u>Staphylococcus aureus</u> <u>Staphylococcus aureus A</u> <u>Staphylococcus aureus B</u> <u>Micrococcus luteus</u> <u>Bacillus cereus</u>	0	0	0	8	8	0	6	10	0	0	0	0	0	6	12	0	0	0	0	0	15	8	10	0	8
	0	18	10	0	8	12	8	18	6	0	0	6	0	18	13	0	0	7	0	0	20	10	24	0	6
	0	12	8	6	0	8	10	18	8	0	10	12	0	12	14	10	12	7	0	10	10	22	0	10	
	0	8	8	0	7	8	8	6	0	7	6	7	0	8	6	8	8	6	0	6	12	12	0	10	
	6	10	0	6	12	8	8	8	0	0	8	10	0	8	10	10	8	6	0	0	10	16	0	10	
<u>Candida albicans</u> <u>Saccharomyces cerevisiae</u>	0	8	6	0	8	8	0	8	7	0	6	8	0	16	10	10	12	6	0	18	8	18	0	10	
	6	10	8	6	8	10	8	8	8	0	10	12	8	10	10	15	10	6	0	22	10	18	0	12	
	7	10	8	8	8	8	10	10	8	8	10	0	0	8	12	14	12	8	7	28	16	14	0	14	
	6	10	8	7	10	8	8	6	8	0	6	12	0	8	8	10	10	6	0	34	0	14	0	14	

Table (4) : Effect of 12.5% concentration of plant oils and extracts on different test organisms.

Test Organisms		Diameter of inhibition zone in m.m. related to:																								
		Aniseed	Caraway	Celery	Coriander	Cumin	Dill.	Fennel	Jasmin.	Chamomile	Drosera.ex.	Geranium	Pelargonium	Onion ex.	Basil	Marjoram	Peppermint	Spearmint	Rosemary	Salvia ex.	Thyme	Pine.	Rose	Clove	Castor	Lemongrass
Gram-negative	<u>Escherichia coli</u>	10	0	0	0	0	0	0	6	0	0	0	0	0	0	6	0	0	0	0	0	10	0	10	0	0
	<u>Klebsiella aerogenes</u>	0	8	0	0	8	0	0	6	0	8	12	0	0	0	8	6	0	0	0	18	8	0	10	0	0
	<u>Klebsiella pneumonia</u>	6	8	0	0	8	0	8	0	0	6	0	0	0	0	8	0	0	0	0	18	7	0	10	0	8
	<u>Pseudomonas aeruginosa</u>	10	8	7	6	8	7	8	8	8	8	0	0	0	8	10	0	8	6	0	22	10	7	16	0	10
	<u>Pseudomonas fluorescens</u>	6	8	0	0	0	0	6	0	0	6	0	0	0	0	0	0	8	0	0	16	0	6	0	0	0
	<u>Salmonella typhimurium</u>	0	0	0	0	8	0	0	8	0	0	0	0	0	0	8	0	0	0	0	8	0	0	10	0	8
Gram-positive	<u>Streptococcus sp.</u>	0	14	10	0	0	12	14	14	0	0	0	0	0	16	12	0	0	6	0	18	8	6	20	0	0
	<u>Staphylococcus aureus</u>	0	10	8	0	0	7	8	10	7	8	10	0	0	8	12	7	8	0	0	8	8	0	18	0	0
	<u>Staphylococcus aureus A</u>	0	8	6	0	7	6	6	0	0	0	6	0	0	8	0	7	0	0	0	0	12	0	10	6	8
	<u>Staphylococcus aureus B</u>	0	8	0	0	8	8	6	0	0	0	6	8	0	0	8	0	6	0	0	0	8	0	12	0	10
	<u>Micrococcus luteus</u>	0	8	0	0	7	7	0	6	6	0	0	7	0	10	8	8	8	0	0	16	0	0	12	0	0
	<u>Bacillus cereus</u>	6	8	8	0	8	8	8	6	7	0	8	8	8	8	8	10	8	6	0	20	8	6	14	0	10
Yeasts	<u>Candida albicans</u>	6	8	8	7	7	8	8	8	7	0	8	0	0	0	10	10	8	6	7	22	12	7	8	0	8
	<u>Saccharomyces cerevisiae</u>	0	8	7	0	8	7	8	0	7	0	0	8	0	0	6	8	8	0	0	26	0	0	10	0	10

Table (5): Effect of 6.25% concentration of plant oils and extracts on different test organisms.

Test Organisms		Diameter of inhibition zone in m.m. related to:																								
		Aniseed	Caraway	Celery	Coriander	Cumin	Dill.	Fennel	Jasmin.	Chamomile	Prosera.ex.	Geranium	Pelargonium	Onion ex.	Basil	Marjoram	Peppermint	Spearmint	Rosemary	Salvia ex.	Thyme	Pine.	Rose	Clove	Castor	Lemongrass
Gram-negative	<u>Escherichia coli</u>	8	0	0	0	0	0	0	6	0	0	0	0	0	0	9	0	0	0	0	10	8	0	8	0	0
	<u>Klebsiella aerogenes</u>	0	6	0	0	0	0	0	6	0	6	8	0	0	0	9	0	0	0	0	18	6	0	8	0	0
	<u>Klebsiella pneumonia</u>	0	6	0	0	8	0	7	0	0	0	0	0	0	0	9	0	0	0	0	16	6	0	8	0	0
	<u>pseudomonas aeruginosa</u>	8	8	0	6	8	6	7	7	0	0	6	0	0	8	8	0	9	0	0	22	8	6	16	0	8
	<u>pseudomonas fluorescens</u>	6	8	0	0	0	0	0	0	0	6	0	0	0	0	0	0	6	0	0	14	0	0	0	0	0
	<u>salmonella typhimurium</u>	0	0	0	0	8	0	0	8	0	0	0	0	0	0	6	0	0	0	0	6	0	0	8	0	0
Gram-positive	<u>streptococcus sp.</u>	0	8	16	0	0	8	10	12	0	0	0	0	0	8	8	0	0	0	0	16	6	0	16	0	0
	<u>staphylococcus aureus</u>	0	8	7	0	0	6	7	7	0	7	8	0	0	8	8	6	7	0	0	6	6	0	16	0	0
	<u>staphylococcus aureus A</u>	0	6	6	0	6	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	10	0	10	0	8
	<u>staphylococcus aureus B</u>	0	0	0	0	6	6	7	0	0	6	6	0	0	0	6	0	0	0	0	0	0	0	10	0	8
	<u>Micrococcus luteus</u>	0	6	0	0	6	7	0	0	6	0	7	0	0	8	6	7	6	0	0	16	0	0	11	0	0
	<u>Bacillus cereus</u>	0	8	7	0	7	8	8	6	7	0	6	7	8	8	7	8	6	0	0	18	6	6	10	0	8
Yeasts	<u>Candida albicans</u>	6	6	6	6	6	6	8	7	6	0	0	0	0	0	8	8	6	0	7	8	10	6	8	0	0
	<u>Saccharomyces cerevisiae</u>	0	8	6	0	6	6	6	0	6	0	8	0	0	0	6	6	6	0	0	22	0	0	8	0	10

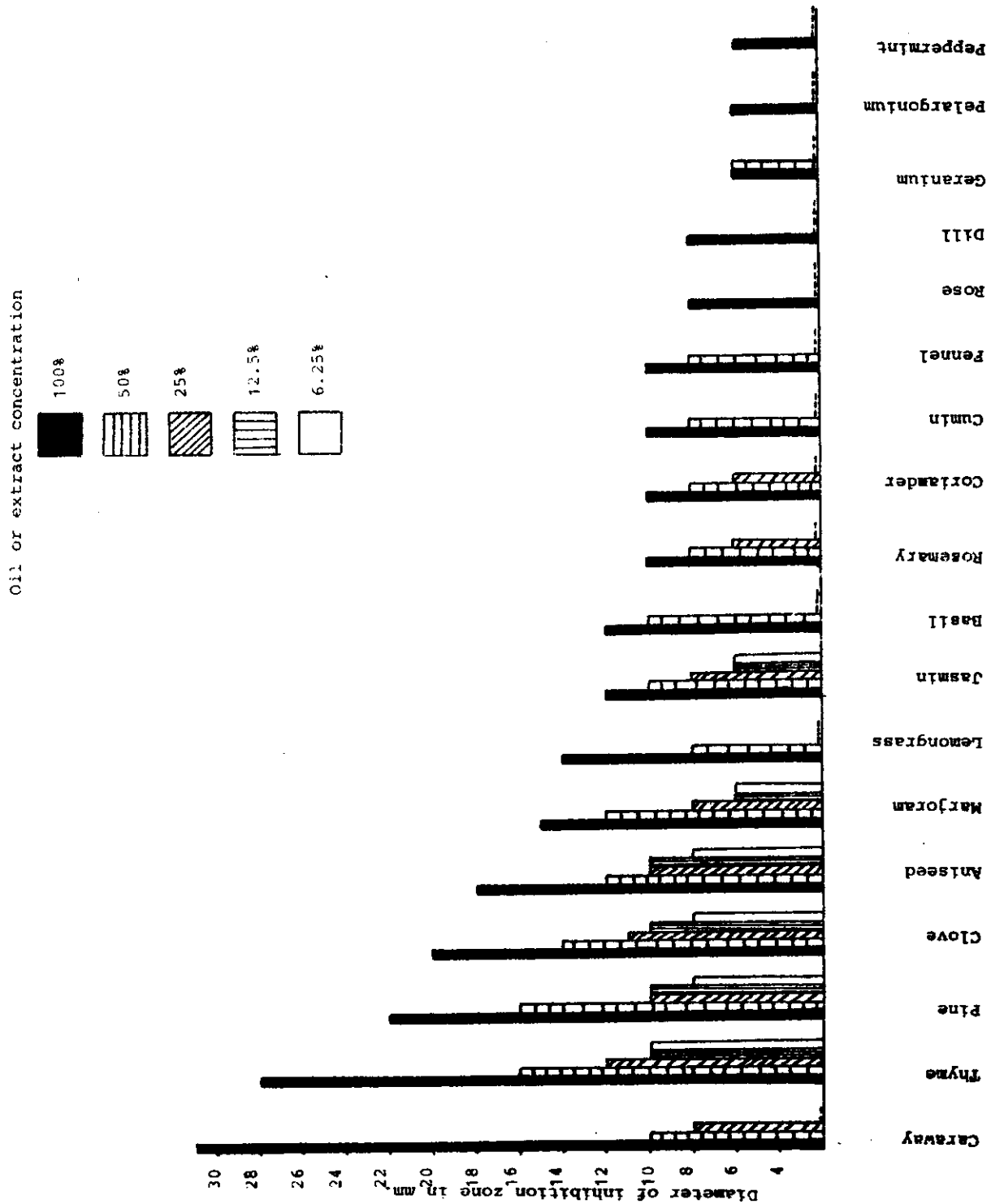


Fig. (1): Effect of plant oils and extracts against *E. coli*.

Concerning the effect of the different dilutions of oils on the test organism, results in Tables (1-5) showed a general trend which indicates the higher the dilution of the oil, the lower the activity of the oil against E.coli. This was true for all investigated oils.

The lowest investigated concentration (6.25%) of the oils caraway, celery, coriander, cumin, dill, fennel , Jasmine, chamomile, geranium, pelargonium, peppermint, spearmint , rosemary, rose, castor, lemongrass, drosera extract, onion extract and salvia extract were found to be non-effective against E.coli.

Only the oils of pine, thyme, aniseed, jasmine, marjoram, and clove were found to be still effective against E.coli at this lowest concentration used (6.25%). The oils which showed activity against the test organism at the lowest concentration used (6.25%) were considered efficient in their antimicrobial activity.

Critical concentrations of the efficient oils against E.coli were calculated. The lower the critical concentration of the oil the higher its efficiency. Efficient oils could be arranged descendingly according to their efficiencies against E.coli as follows: aniseed oil (cr. concentration 1.17%), thyme oil (1.62%) , clove oil (2.24%), jasmine oil (2.45%), marjoram oil (3.09%) and pine oil (3.09%).

2- Effect of different plant oils and extracts against

klebsiella aerogenes:

Data in Tables (1-5) and illustrated in figure (2) show the effect of different concentrations of plant oils and extracts (crude, 50%, 25%, 12.5% and 6.25%) against Kl.aeragenses. The effect of crude oils and plant extracts on Kl.aerogenes could be reported in a descending order as follows: Thyme (36 mm.) pine (24 mm.), clove (22 mm.) marjoram (22 mm.) , jasmine(20 mm.), caraway (18 mm.), pelargonium (16 mm.), peppermint (16 mm.) , basil (16 mm.), geranium (14 mm.), cumin (14 mm.), dill (14 mm.), lemongrass (12 mm.), fennel 12 mm.), spearmint (12 mm.) rose (12 mm.), chamomile (10 mm.), rosemary (10 mm.), celery (10 mm.), coriander (8 mm.), aniseed (8 mm.) and castor (8 mm.). The crude extracts of onion, drosera, and salvia were non-effective against Kl.aerogenes. The organism showed no inhibition zone with the aformentioned extracts.

The effect of different dilutions of the tested oils against Kl.aerogenes showed a general trend of reduction in the activity as the dilution was increased. This was true for all investigated oils except basil oil. The width of the inhibition zone was greater at 50% concentration (18 mm.) than that of crude basil oil (16 mm.). This may be due to the more diffusion of the chemical in the agar at 50% dilution or due to the greater up take of basil oil by Kl.aerogenes at 50% concentration.

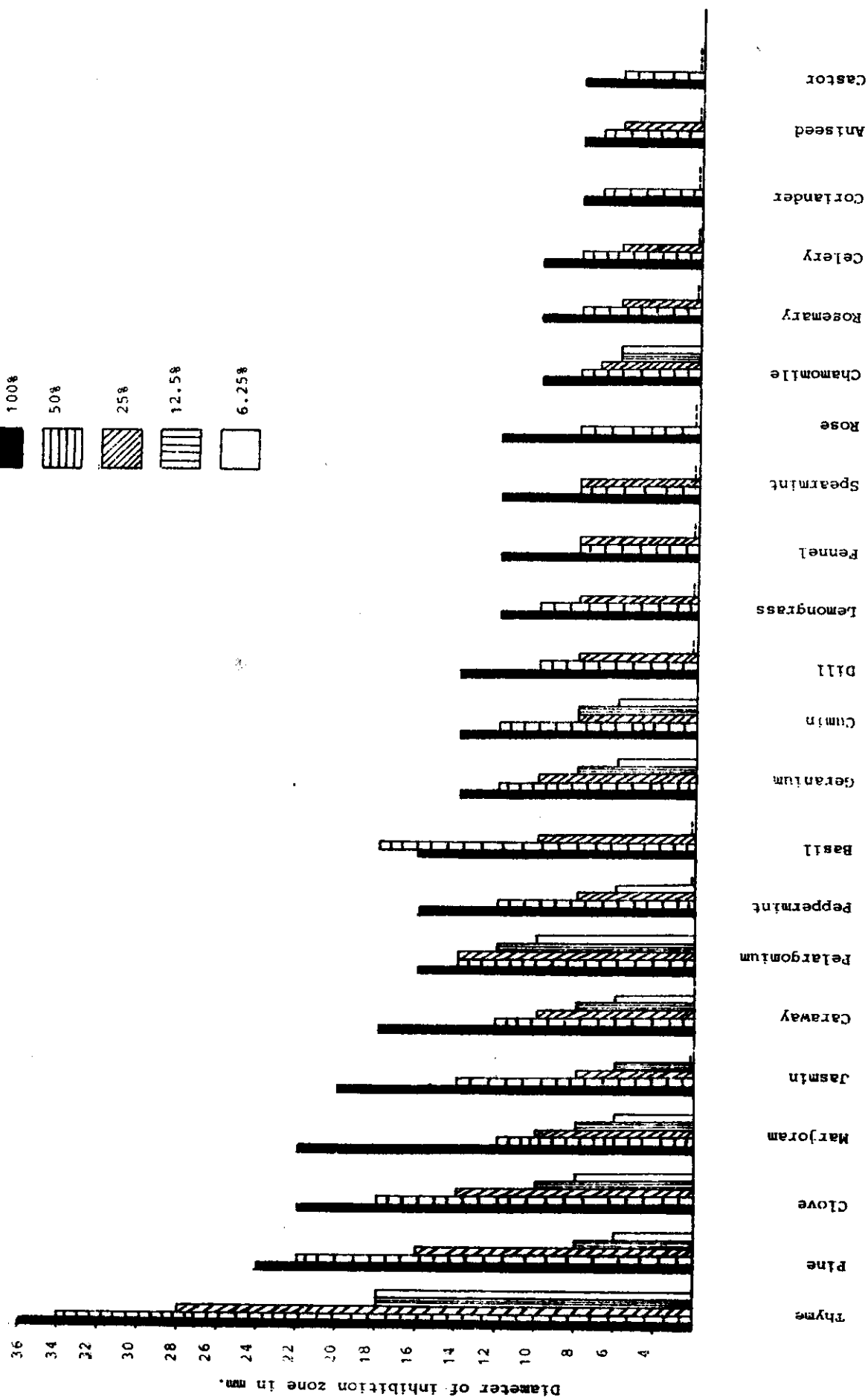
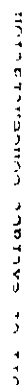


Fig. (2): Effect of different plant oils and extracts against Kl. aerogenes

The oils which showed zones of inhibition against Kl.aerogenes at the lowest concentration used (6.25%) were thyme, clove, pelargonium, marjoram, pine, geranium, chamomile, cumin, caraway. These oils were considered efficient since they remained possessing antimicrobial activity against Kl.aerogenes even at the lowest concentration used. Efficient oils were arranged descendingly according to their efficiencies against Kl.aerogenes as follows: chamomile (Cr.concentration 0.44%), pelargonium (0.79%), thyme (2.45%), clove (2.45%), caraway (3.09%), geranium (3.09%), marjoram (3.09), cumin (4.37%) and pine (5.01).

3- Effect of different plant oils and extracts against
Klebsiella pneumonia:

The effect of different plant oils and extracts against Kl.pneumonia is reported in Tables (1-5) and illustrated in figure (3). Data in Table (1) and figure (3) show that Kl.pneumonia was sensitive to the crude oils of thyme (24 mm.), caraway (20 mm.), clove (20 mm.), lemongrass (18 mm.), marjoram (18 mm.) basil (17 mm.) , dill (16 mm.) jasmine (16 mm.), fennel (12 mm.) geranium (12 mm.) pelargonium (12 mm.) coriander (12 mm.), cumin (12 mm.), rosemary (11 mm.) aniseed (10 mm.), celery (10 mm.), spearmint (10 mm.), pine (10 mm.), chamomile (10 mm.), and rose (10 mm.) On the other hand, Kl.pneumonia showed no sensitivity to the crude extracts of onion, drosera and salvia in addition to the crude oils of castor and peppermint.

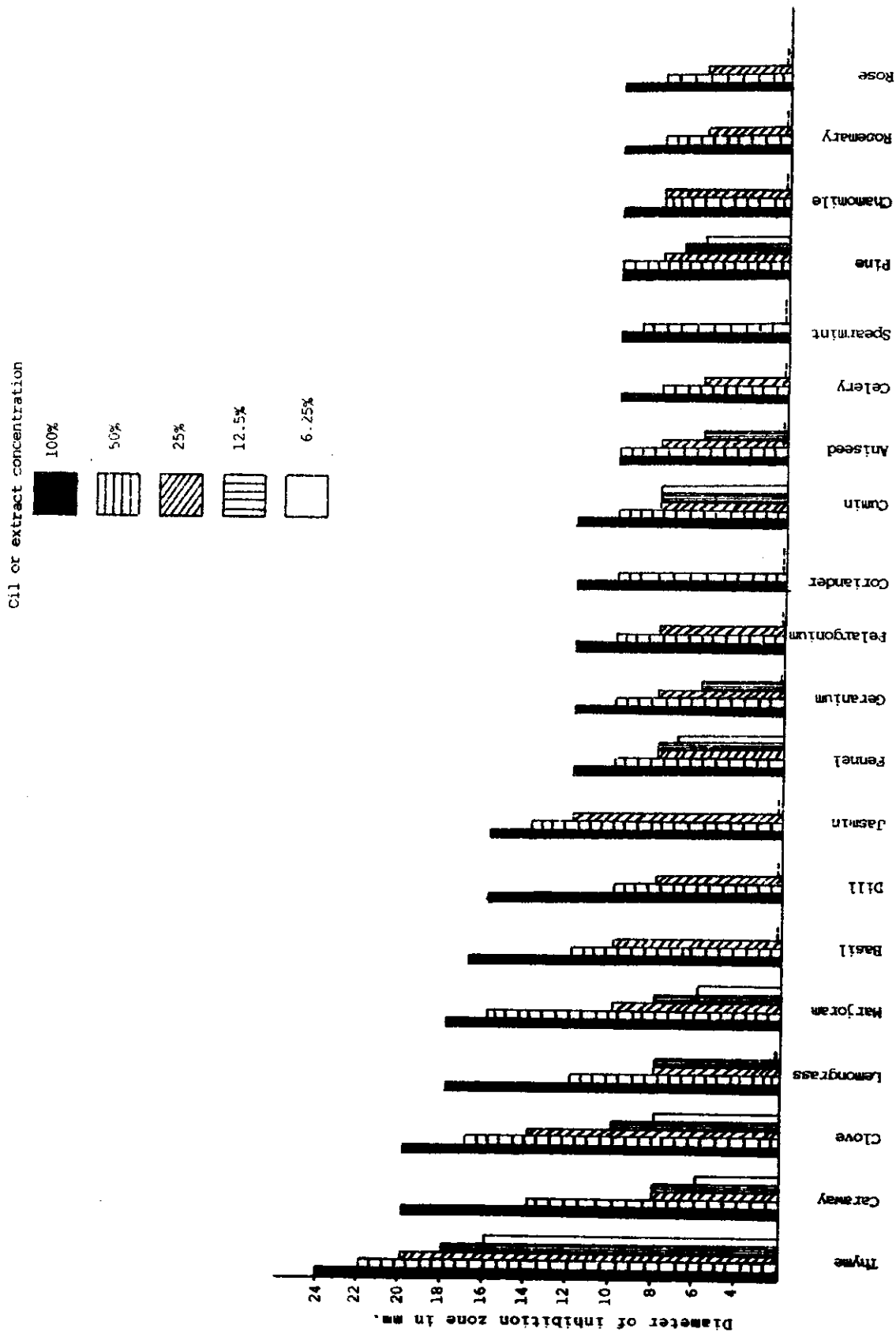


Fig. (3): Effect of different plant oils and extracts against Klebsiella pneumoniae

The plant oils and extracts which showed inhibition zones against Kl.pneumonia at the lowest concentration used (6.25%) were considered efficient in their germicidal effect against Kl. pneumonia.

The efficient oils were thyme, clove, cumin, fennel, caraway, marjoram and pine.

Critical concentrations of the efficient oils against Klebsiella pneumonia were calculated. The efficient oils were arranged descendingly according to their efficiencies against Kl.pneumonia as follows: thyme (cr.concentration 0.66%), Fennel (0.85%), pine (1.29%), cumin (2.29%), carway (3.89%), clove (4.07%) and marjoram (4.4%).

4- Effect of different plant oils and extracts against Pseudomonas aeruginosa :

Data in Table (1-5) and demonstrated by Figure (4) indicate the variation in the effectiveness of different plant oils and extracts against Pseudomonas aeruginosa . It could be noticed, in figure (4), that all the investigated oils in the curde state (100% concentration) showed different responses against this organism which ranged from 40 mm. inhibition zone in case of thyme oil to 7 mm. inhibition zone for pelargonium oil. On the other hand, the extracts of drosera and salvia were completely inactive against Ps.aeruginosa.

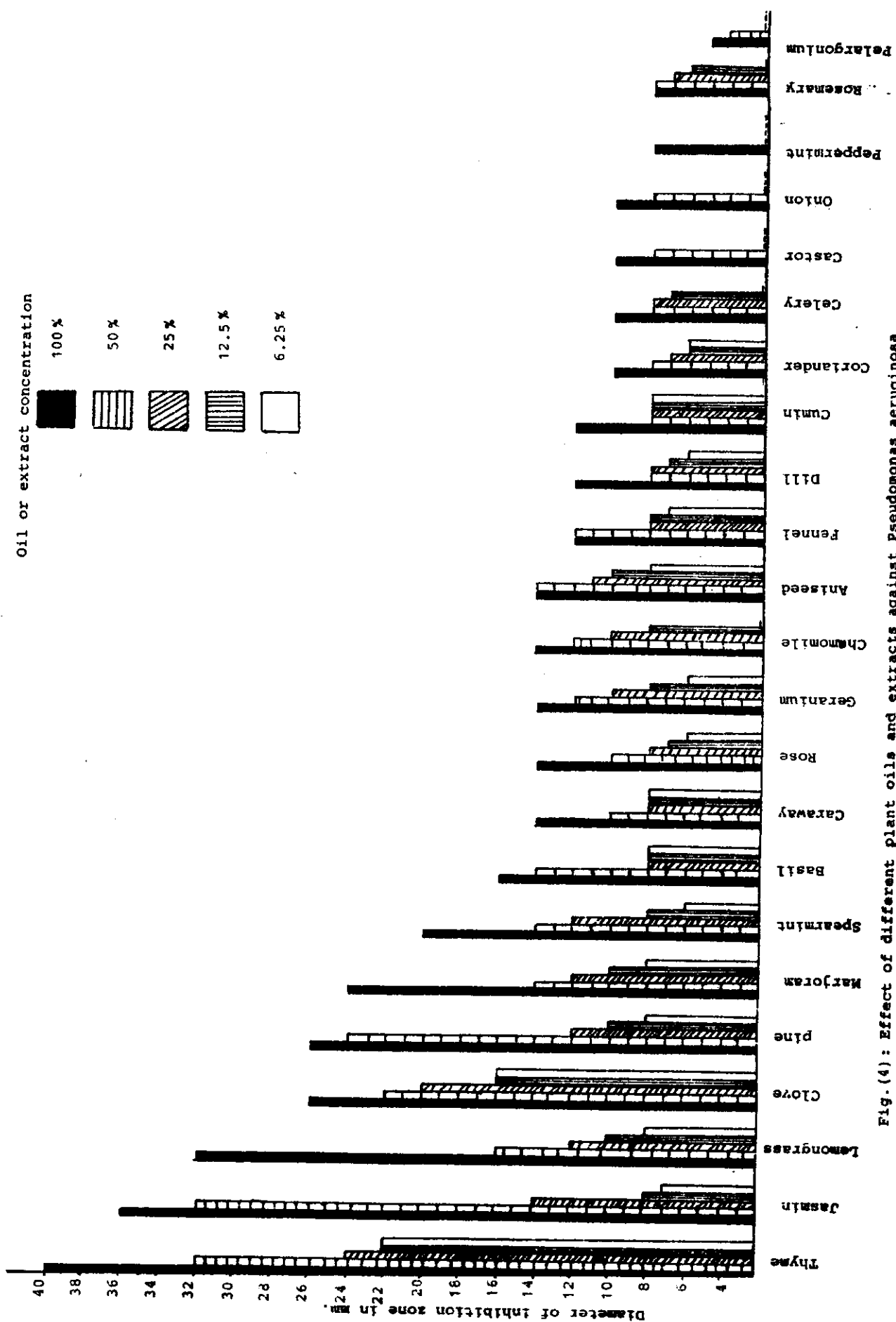


Fig. (4) : Effect of different plant oils and extracts against *Pseudomonas aeruginosa*

The different dilutions of the oils showed a general trend of reduction in the antimicrobial activity of the oil as dilution was increased. This was true for all the investigated oils against Ps. aeruginosa.

The oils which proved to be efficient in their antimicrobial activity against Ps. aeruginosa showed clear zone of inhibition at the lowest concentration used (6.25%). The efficient oils were thyme, clove, lemongrass, jasmine, pine, marjoram, spearmint, basil, caraway, rose, geranium, aniseed, fennel, dill, cumin and coriander.

The critical concentrations of the aforementioned efficient oils against Pseudomonas aeruginosa were calculated. Then efficient oils were arranged descendingly according to their efficiencies as follows: Caraway (cr. conen. 0.15), coriander (0.42%), dill (0.42%), thyme (0.95%), fennel (1.58%), aniseed (1.60%), marjoram (2.24%), cumin (2.29%), geranium (3.09%), spearmint (3.09%), rose (3.09%), lemongrass (3.09%), Basil (3.86%), pine (4.57%), clove (4.57%), and jasmine (5.5%).

5- Effect of different plant oils and extracts against
Pseudomonas fluorescens.

The effect of different concentrations of plant oils and extracts against Pseudomonas fluorescens is reported in Tables (1-5) and demonstrated in figure (5).

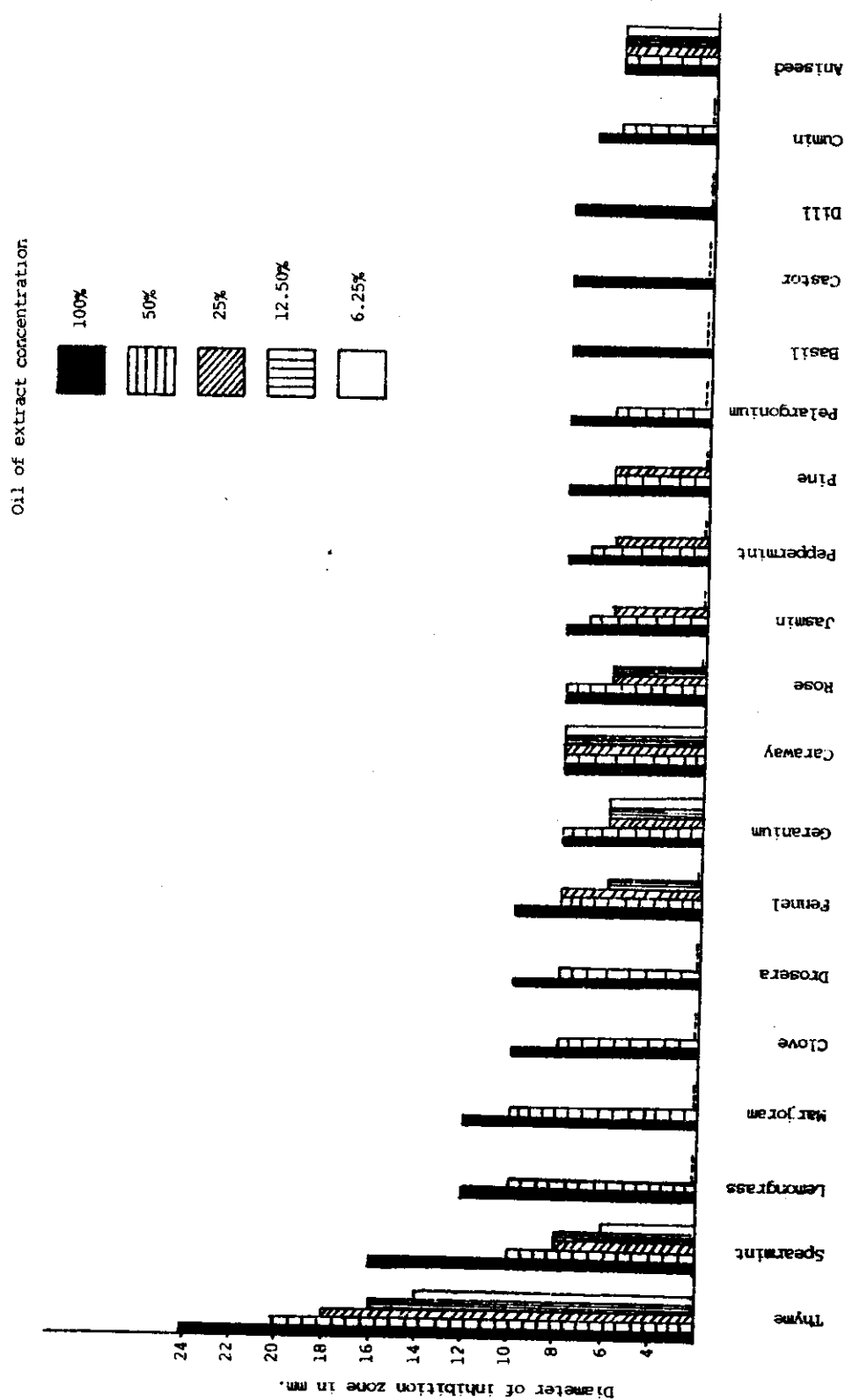


Fig.(5): Effect of plant oils and extracts against *Pseudomonas fluorescens*.

Ps.fluorescens showed no sensitivity towards the crude oils of rosemary, chamomil, coriander and celery in addition to the crude extracts of onion and salvia. This organism was sensitive to the crude oils of thyme (24 mm.), spearmint (16 mm.) marjoram (12 mm.) lemongrass (12 mm.) fennel (10 mm.) , clove (10 mm.) drosera crude extract 10 mm.), caraway (8 mm.) dill (8 mm.) jasmine (8 mm.), geranium (8 mm.) pelargonium (8 mm.) basil (8 mm.), peppermint (8 mm.), rose (8 mm.), castor (8 mm.) cumin (7 mm.) and aniseed (6 mm.).

The efficient oils against this organism showed clear zone of inhibition at the lowest concentration used of the oil (6.25%). These efficient oils were thyme, spearmint, geranium, caraway and aniseed.

Concerning the degree of efficiency of the efficient oils, caraway and aniseed oils seemed to be the most efficient oils against Ps.fluorescens because the successive dilutions did not affect their efficiency against this organism. Their critical concentrations could not be calculated. Concerning other oils their efficiencies could be descendingly arranged as follows: geranium (Cr. concen. 0.17%), thyme (0.85%), and spearmint (1.91%). The lower the critical concentration the more efficiency of the oil.

6- Effect of different plant oils and extracts against *Salmonella typhimurium* :

Results in Tables (1-5) and Fig. (6) show that *Salmonella typhimurium* completely resisted the antimicrobial action of onion, drosera and salvia crude extracts. This organism also resisted the effect of dill, geranium, castor, spearmint, chamomile, aniseed and celery at their 100% concentration or crude state. It was also found that oils of caraway, rosemary, pelargonium and rose could not affect the growth of *Salmonella typhimurium* at concentrations less than 50%. Peppermint oil was only effective at the crude state.

The oils of thyme, marjoram, jasmine, clove and cumin were considered efficient against *Salm. typhimurium* because they showed clear zones of inhibition at the lowest concentration used (6.25%). Efficient oils against *Salm. typhimurium* were arranged descendingly, according to their efficiency as follows: Jasmine (cr.concn. 1.17%), clove (1.17%), thyme (3.09%), cumin (4.47%) and marjoram (4.79%).

7- Effect of plant oils and extracts against *Streptococcus* sp.

Data in Tables(1-5) and Figure (7) show the effect of different plant oils and extracts against the test organism *Streptococcus* sp.

The effect of the crude oils and plant extracts on *Streptococcus* sp., as estimated by the width of the inhibition

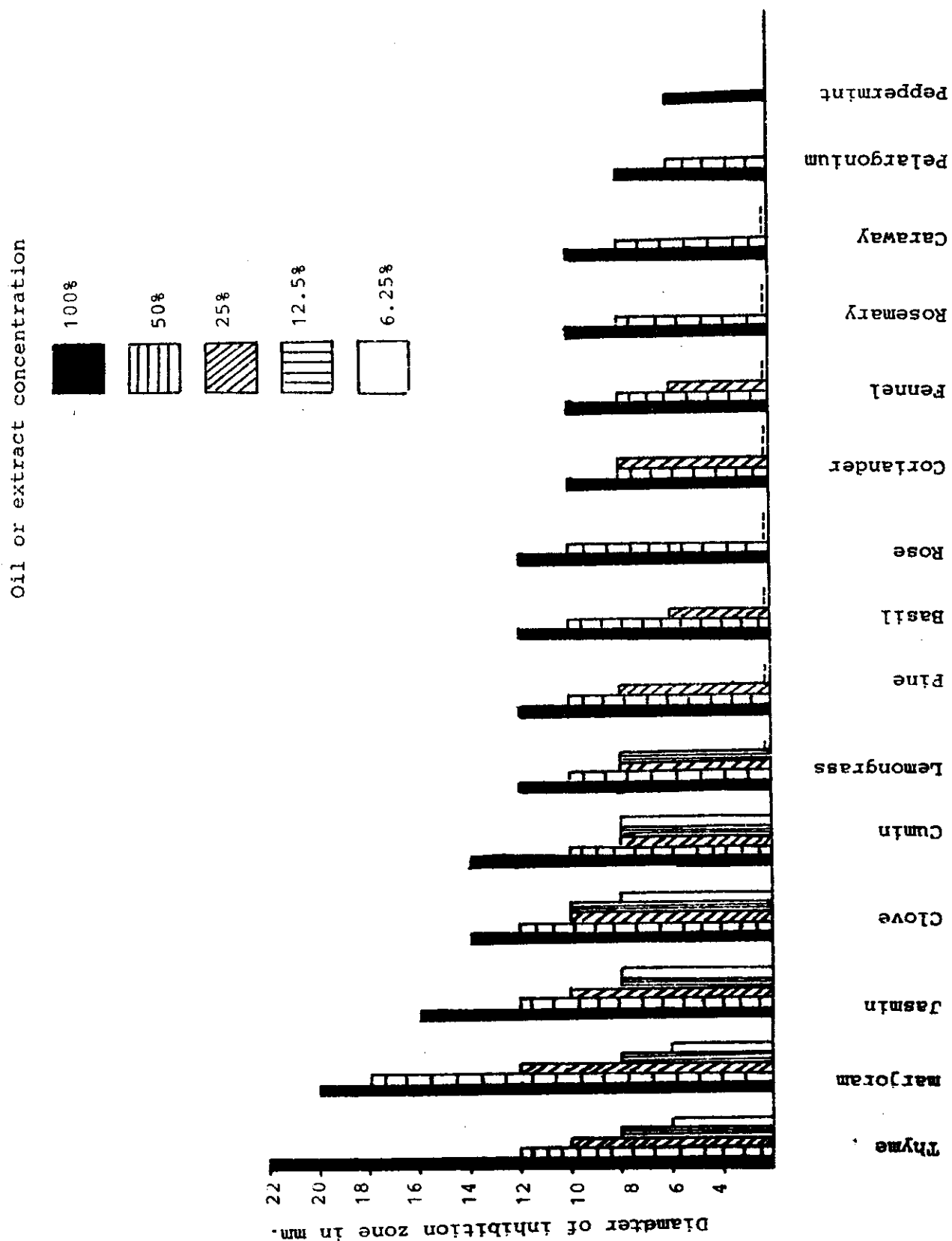


Fig. (6): Effect of plant oils and extracts against Salmonella typhimurium.

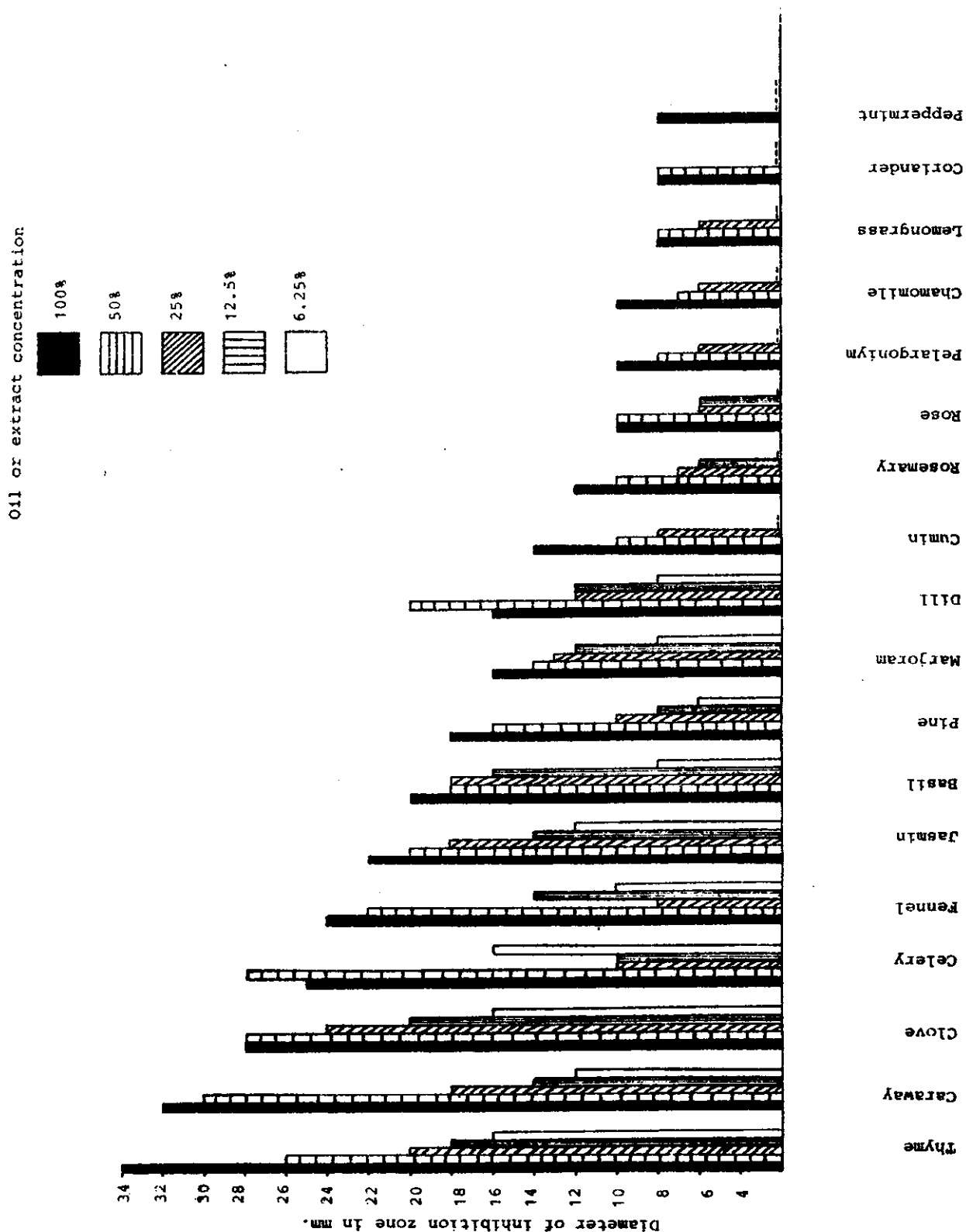


Fig. (7) Effect of plant oils and extracts against Streptococcus Sp.

zone could be arranged in a descending orders as follows:

Thyme (34 mm.), caraway (32 mm.) clove (28 mm.), celery (25 mm.), fennel (24 mm.), jasmine (22 mm.) basil (20 mm.) , pine (18 mm.) marjoram (16 mm.), dill (16 mm.), cumin (14 mm.), rosemary (12 mm.) rose (10 mm.), pelargonium (10 mm.), chamomil (10 mm.) lemongrass (8 mm.) coriander (8 mm.) and peppermint (8 mm.). The crude extracts of onion, drosera and salvia showed no biological activity against Streptococcus sp. The crude oils of spearmint, geranium, castor, and aniseed were also inactive(inert) against this test organism.

The oils which could be considered efficient, showed antimicrobial activity at the lowest concentration used.

The oils which proved to be efficient against Streptococcus sp. were thyme, caraway, clove, celery, fennel, jasmine, basil, pine, marjoram and dill.

There was a general trend of decrease in the width of the inhibition zone as the dilution of the oil was increased. This was true for all the investigated oils except celery oil and dill oil which showed greater inhibition zones at 50% concentration than that of their crude oils. This may be due to that the uptake of the chemical (S) by this organism is greater at 50% concentration than that of the crude oil.

The critical concentrations of the efficient oils against Streptococcus sp. were calculated. The efficient oils could be arranged descendingly according to their efficiencies as follows: thyme (cr. concn. 1.74%), marjoram (2.5%), dill (2.5%), jasmine (3.24%), fennel (3.47%), clove (3.47%), basil (3.60%), pine (4.37%), caraway (5.01%) and celery (10%).

8- Effect of different plant oils and extracts against Staphylococcus aureus:

Data in Tables (1-5) and Figure (8) show the effect of different dilutions of plant oils and extracts against Staph.aureus.

Jasmine oil in full strength (crude) caused a wide inhibition zone of 38 mm. against Staph.aureus. Clove oil came next 36 mm. . The least inhibition zone for the effective crude oils was that of aniseed (8 mm.) as well as that of the extract of onion (8 mm.)

All the investigated oils were found to be effective (show a clear zone of inhibition) in the crude state except drosera and salvia extracts in addition to castor oil.

The efficient oils were those who showed clear zones of inhibition at the lowest concentration used. These efficient oils were clove, jasmine, morjoram, basil, celery,

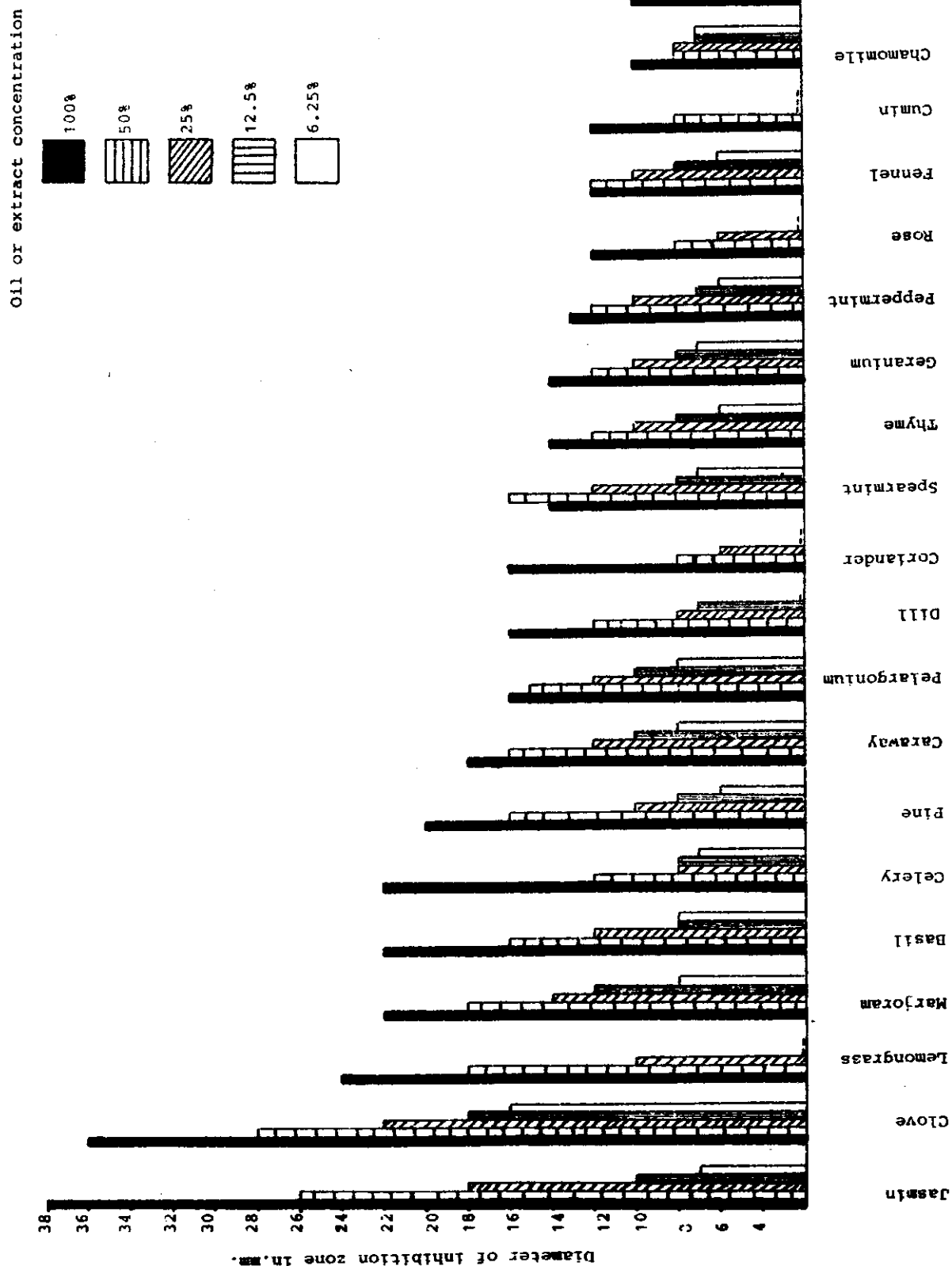


Fig (8): Effect of plant oils and extracts against *Staph. aureus*

pine, caraway, pelargonium, spearmint, thyme, geranium, peppermint, fennel and chamomile.

It was found that the inhibition zone increased by increasing the concentration of the oil except spearmint and aniseed oils. The 50% concentrations of spearmint and aniseed oils showed wider inhibition zones than those of their respective crude oils.

The critical concentrations of the efficient oils against Staph.aureus were calculated. Then, efficient oils were arranged descendingly according to their efficiencies as follows: chamomile (cr. concn. 1.66%), geranium (1.66%), pelargonium (1.82%), celery (2.14%), fennel (2.45%), spearmint (2.5%), thyme (3.09%), basil (3.09%), caraway (3.24%), clove, (3.47%), marjoram (3.71%), pine (4.37%), peppermint (5.25 %) and jasmine (5.25%).

9- Effect of different plant oils and extracts against Staphylococcus aureus A (enterotoxin A producer):

Data in Tables (1-5) and Figure (9) indicated that Staphylococcus aureus A was not sensitive to the crude extracts of onion and salvia. On the other hand, the organism was sensitive to the crude oils of clove (width of inhibition zone 20 mm.) lemongrass (20 mm.) pine (18 mm.) caraway (16 mm.) marjoram (16 mm.), dill (14 mm.) , celery (14 mm.),

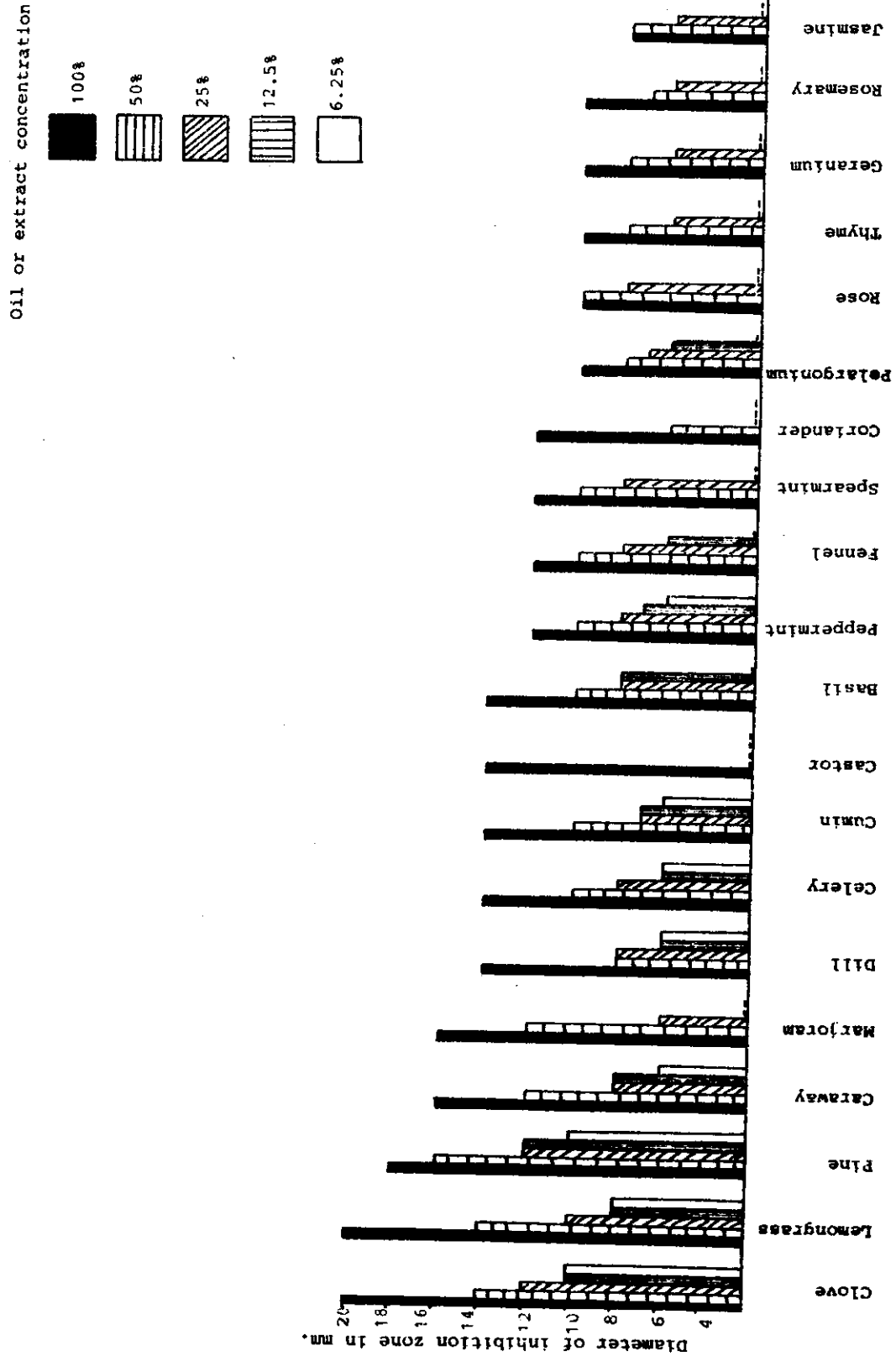


Fig. (9) Effect of plant oils and extracts against *Staph. aureus A*

cumin (14 mm.) castor (14 mm.) peppermint (13 mm.), Fennel (12 mm.), spearmint (12 mm.), coriander (12 mm.), pelargonium (10 mm.) rose (10 mm.) thyme (10 mm.), gernium (10 mm.), rosemary (10 mm.) jasmine (8 mm.), drosera extract (7 mm.), aniseed (7 mm.) and chamomile (7 mm.).

Of the interesting results, is that of the effect of drosera extract against this organism, which showed the same zone of inhibition (7 mm.) when different concentrations (100%, 50% and 25%) of the extract were used. This may be due to the interaction between many factors such as the rate of diffusion of the chemical in the agar and the uptake of the chemical by the organism.

The efficient oils which showed clear zones of inhibition against this organism at the lowest concentration used were clove, lemongrass, pine, caraway, dill, celery, cumin and peppermint. Critical concentrations were calculated for the efficient oils. The efficient oils were arranged descendingly according to their efficiencies against Staph. aureus A., as follows: dill (cr. concn. 0.43%), pine (1.62 %), celery (1.91%), cumin (1.91%), peppermint (1.91%), lemongrass (2.24%), clove (2.45%) and caraway (3.09%).

10- Effect of different plant oils and extracts against

Staphylococcus aureus B (enterotoxin B producer)

The antimicrobial activity of the tested oils

and extracts against Staph. aureus B is given in Tables (1-5) and illustrated by Fig. (10).

Results showed that salvia and drosera extracts as well as chamomil oil showed no antimicrobial activity against this organism. The crude oil of clove and spearmint showed wide inhibition zones which reached 26 mm., in both cases. They were followed by peppermint (24 mm.) , caraway (18 mm.), lemongrass (18 mm.), cumin (16 mm.), dill (16 mm.), pelargonium (16 mm.), marjoram (16 mm.), pine (16 mm.), coriander (14 mm.). fennel (12 mm.), jasmine (12 mm.) geranium (12 mm.), basil (12 mm.), celery (10 mm.), rosemary (10 mm.), rose (10 mm.) aniseed (8 mm.), onion extract (8 mm)), thyme (8 mm.) castor (8 mm.).

The efficient oils against Staph. aureus B. were clove oil, dill oil, marjoram oil, cumin oil, pelargonium oil, fennel oil and geranium oil. showed clear zones of inhibition at the lowest concentration used.

Generally, the three tested species of Staphylococcus aureus indicated that a test microbial strain can have its own pattern of sensitivity towards the drastic effect of the plant oil. In case of Staph. aureus (non-toxin producer) and Staph. aureus B (enterotoxin B producer), they completely resisted drosera and salvia extracts. On the other hand , Staph aureus A (enterotoxin A producer), showed higher

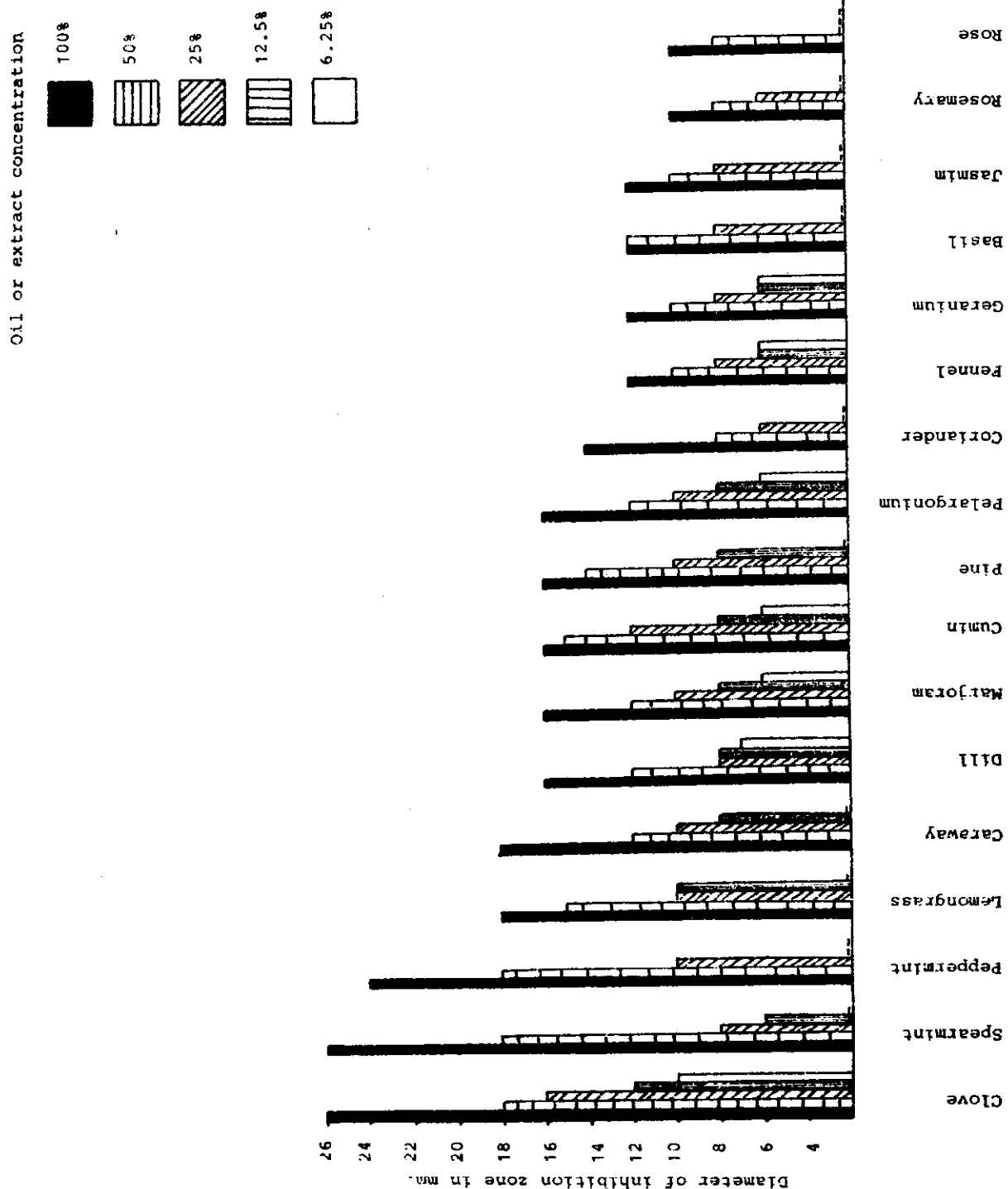


Fig. (10) Effect of plant oils and extracts against Staph. aureus B

sensitivity towards drosera at a concentration of 25%, while showed no sensitivity towards the crude extract of onion.

Critical concentrations of the efficient oils against Staph.aureus B were calculated. Then, these efficient oils were arranged descendingly according to their efficiencies against Staph aureus B. as follows: geranium (cr.concn.1.9%), dill (2.14%), clove (2.45%), marjoram (3.09%), Pelargonium (3.09%), cumin (3.89%), and Fennel (6.17%).

11- Effect of plant oils and extracts against Micrococcus luteus:

Different plant oils and extracts showed different effects against M. luteus (fig.11)

The crude oils which were effective against M.luteus showed clear zones of inhibition. They could be arranged descendingly according to the width of the inhibition zone as follows:

Spearmint (26 mm.) , thyme (24 mm.) , clove (18 mm.)
peppermint (18 mm.), basil (18 mm.), cumin (18 mm.), marjoram (16 mm.), jasmine (16 mm.), lemongrass (14 mm.), rose (14 mm.), pelargonium (14 mm.), caraway (14 mm.), pine (12 mm.), celery (12 mm.), rosemary (10 mm.), chamomile (10 mm.), dill (10 mm.),

Oil or extract concentration

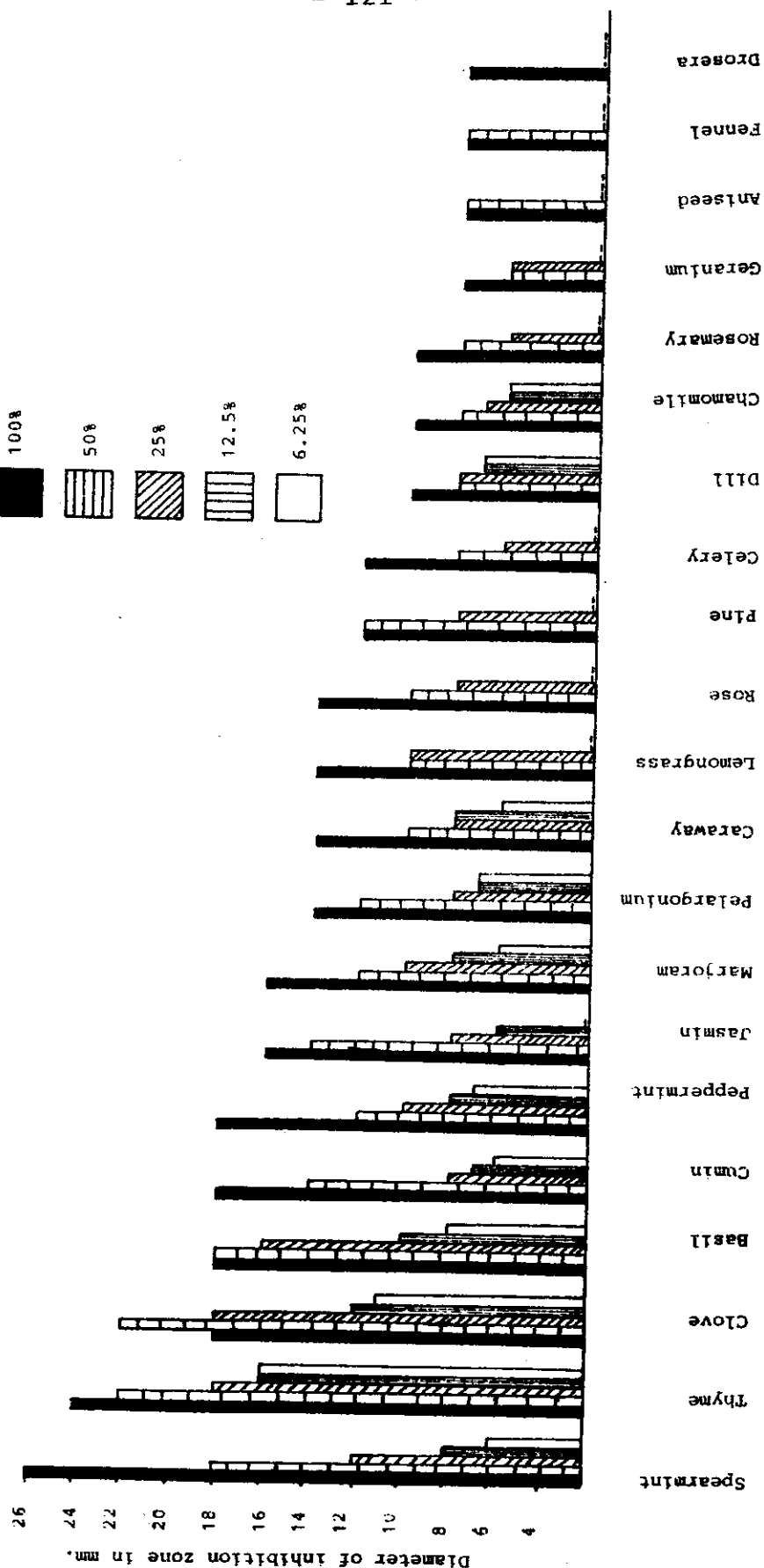
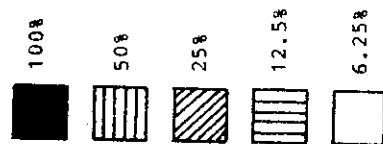


Fig. (11) Effect of plant oils and extracts against *Micrococcus luteus*

geranium (8 mm.), drosera extract (8 mm.) fennel (8 mm.), aniseed (8 mm.). The oils coriander and castor as well as the extracts of onion and salvia were found to be inert against M. luteus.

The efficient oils in their antimicrobial activities against M. luteus were thyme, clove, spearmint, basil, cumin, peppermint, marjoram, pelargonium, caraway, dill, and chamomile. They showed clear zones of inhibition at the lowest concentration used.

According to their efficiencies, efficient oils against M. luteus were arranged descendingly as follows: pelargonium (cr. concn. 0.01%), dill (1.29%), caraway (1.91), peppermint (2.14%), thyme (2.29%), marjoram (3.09%), clove (3.09%), basil (3.09%), chamomile (3.80%), cumin (3.89%) and spearmint, (4.79%).

12- Effect of plant oils and extracts against Bacillus cereus:

Figure (12) shows the effect of different concentrations of oils and extracts against B. cereus.

The crude oils and extracts which were active against B. cereus were thyme, clove, lemongrass, basil, peppermint, jasmine, caraway, spearmint, celery, fennel, cumin, dill, geranium, pine, rose, rosemary, aniseed, chamomile, coriander, onion extract and castor.

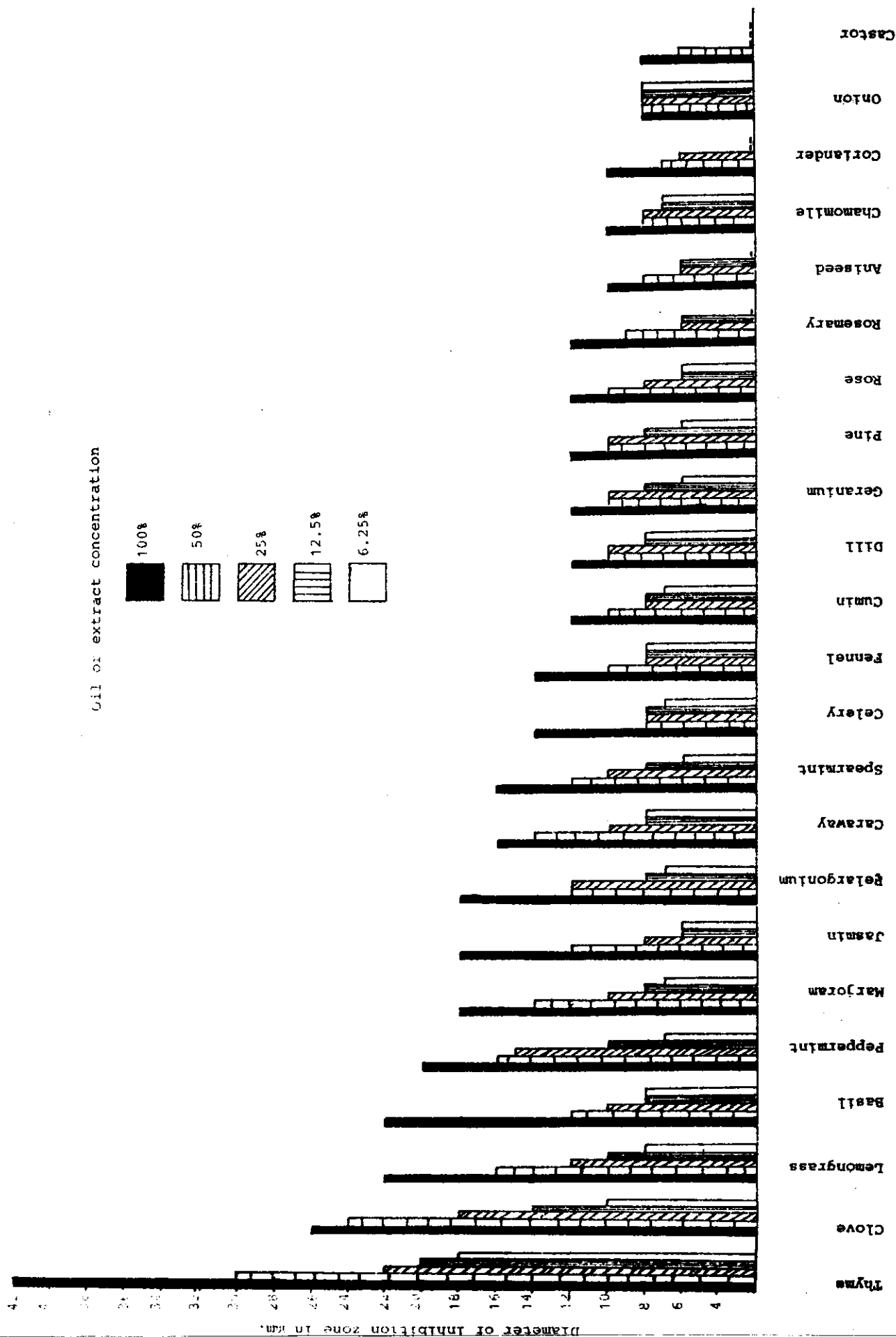


Fig. (12) Effect of plant oils and extracts against *Bacillus cereus*

The oils which proved to be efficient against Bacillus cereus were thyme, clove, lemongrass, basil, peppermint, marjoram, jasmine, pelargonium, caraway, spearmint, celery, fennel, cumin, dill, geranium, pine, rose, chamomile and onion extract. This organism seemed to be very sensitive to onion extract because the lowest concentration used of the onion extract (6.25%) gave the same width of the inhibition zone as that of the crude extract. So, onion extract seemed to be the most efficient against B.cereus. Other efficient oils could be arranged descendingly according to their efficiencies against B.cereus as follows: Dill (cr. concn. 0.15%), chamomile (0.42%), cumin (0.85%), caraway (2.07%), basil (1.17%), thyme (1.91%), pelargonium (2.14%), geranium (2.45%), pine (2.51%), rose (2.51%), marjoram (3.09%), peppermint (3.09%), spearmint (3.09%), jasmine (3.09%), clove (3.98%), celery (7.08) and lemongrass (7.08).

13- Effect of different plant oils and extracts against
Candida albicans:

The antimicrobial effects of different oils and extracts against Candida albicans are demonstrated in Fig. (13).

Results showed that all the investigated oils and extracts were effective against this organism. The only exception was onion extract which proved to be non-effective

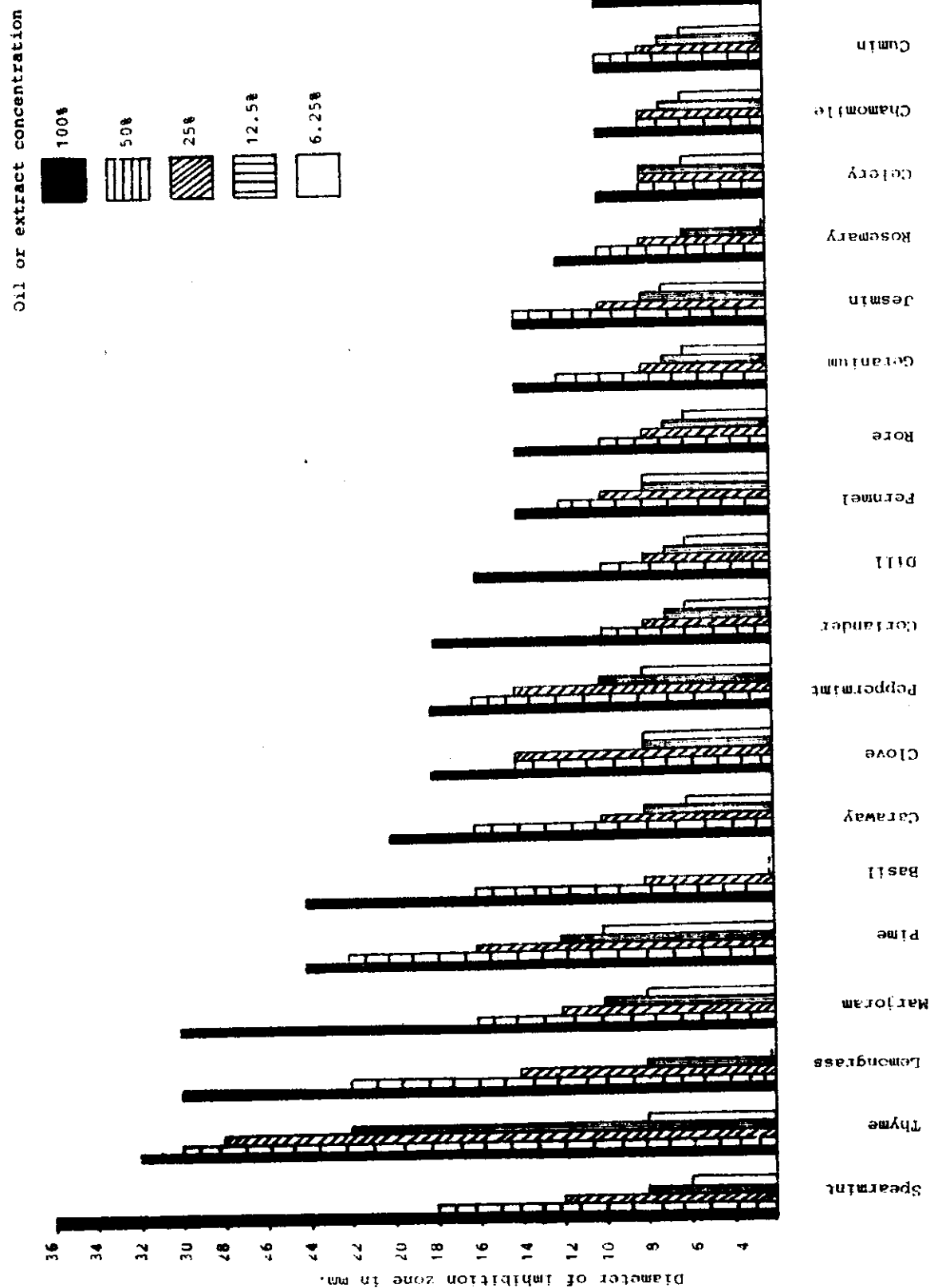


Fig (13) Effect of plant oils and extracts against *Candida albicans*

against Candida albicans.

The efficient oils against this yeast showed inhibition zones at the lowest concentration used (6.25%).

These efficient oils were spearmint, thyme, marjoram, pine, caraway, clove, peppermint, coriander, dill, fennel, rose, geranium, jasmine, celery, chamomile, cumin, aniseed and salvia.

This yeast seemed to be very sensitive to salvia extract since the highest dilution of salvia extract showed the same width of inhibition as that of the crude salvia extract. Other efficient oils against C.albicans could be arranged descendingly according to their efficiencies as follows: aniseed (cr. concn. 0.43%), celery (0.43%), chamomile (1.29%), rose (1.29%), cumin (1.91%), clove (2.29%), jasmine (2.69%), geranium (3.09%), marjoram (3.09%), pine (3.63%), caraway (4.37%), thyme, (4.57%), spearmint (4.79%), and fennel (5.75%). The lower the critical concentration of the oil, the higher the efficiency of the oil.

14- Effect of different plant oils and extracts against
Saccharomyces cerevisiae:

Figure (14) showed that 23 crude oils were effective against Saccharomyces cerevisiae. The crude extracts of

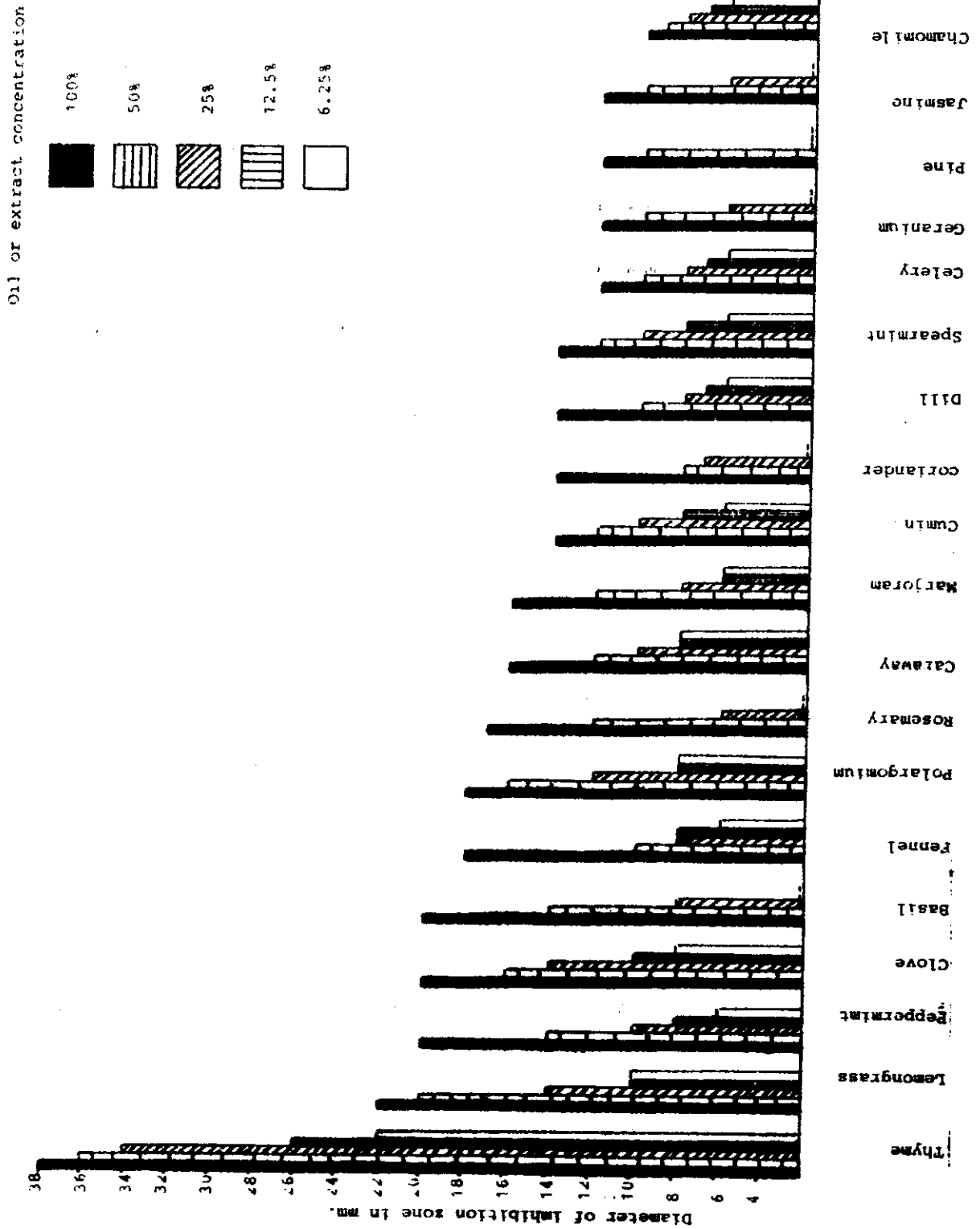


Fig. (14) Effect of plant oils and extracts against *Saccharomyces cerevisiae*

onion and salvia were not effective against this organism. Drosera extract was only effective at crude state (100%).

Oils of thyme, chamomile, pelargonium, peppermint, cumin, celery, spearmint, marjoram, caraway, lemongrass, clove, fennel, and dill were effective at any dilution down to the lowest concentration used (6.25%), So, these oils are considered efficient against Sacch.cerevisiae.

Critical concentrations of the effecient oils against Saccharomyces cerevisiae were calculated. The lower the critical concentration of the oil, the higher its efficiency. Efficient oils against Saccharomyces cerevisiae could be arranged descendingly as follows: caraway (cr. concn.1.17%), chamomile (1.17%), pine (1.78%), celery (1.91%), dill (1.91%), fennel (1.91%), pelargonium (3.09%), marjoram (3.09%), spearmint (3.09%), clove (3.09%), lemongrass (3.09%) and peppermint (3.89%).

Effect of plant oils and extracts against fungi:

For the determination of the antimicrobial activity of some plant oils and extracts against fungi, five different fungal species were used as test organisms. These species were Microsporium gypseum, Trichophyton rubrum, Epidermophyton Floccosum, Fusarium exysporum and Aspergillus niger.

Most of these species are of medical interest as, Microsporium gypseum, Trichophyton rubrum and Epidermophyton floccosum.

Of the most interesting results obtained during this investigation is the high sensitivity of all the examined species towards the vapors of all the tested crude oils. It was noticed that the growth of any of the tested fungi was completely inhibited in the presence of any crude oil. Mostly, this inhibition effect may be due to oil vapours inside the petri plates. On the other hand, all the tested fungi, showed no sensitivity towards 10% or lower concentrations of the following oils and extracts: rose , pine, thyme, geranium, castor, coriander, aniseed, jasmine, spearmint, marjoram, caraway, ether extract of onion, drosera liquid extract, salvia extract, fennel and dill oils. No data could be recorded for the aforementioned oils and extracts because they showed no zones of inhibition.

Some oils, at concentrations 10^{-1} - 10^{-5} showed moderate effects against different fungal species as, basil, lemongrass, chamomile, clove, cumin, celery, peppermint and pelargonium. Nevertheless, Fusarium oxysporum completely resisted all the above oils except clove which showed active antimicrobial action against this species.

Furthermore, Aspergillus niger tolerated the effects of all the above oils except in the case of celery oil. This oil showed no definite inhibition zone to be measured, but at concentrations 10^{-1} and 10^{-2} , it stopped fungal sporulation up to five days incubation at 28°C.

Table (6) indicates the inhibitory effects of the active oils at different concentrations, against the sensitive tested fungal species.

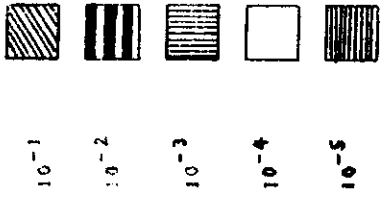
These effects are presented in figure (15).

Results in table (6) and fig. (15) showed a general trend of reduction in the inhibition zone as the concentration of the oil decreased. This was almost true with the majority of the effective examined oils. However, some oils showed greater inhibition zone at lower concentrations than that of their respective higher concentrations. This may be due to differences in the diffusion and the uptake of these oils at such concentrations.

Table : (6) The inhibitory effects of some plant oils and their active concentrations against different fungal species (zone of inhibition in mm)

Test organisms	oil sources	Oil concentrations.				
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
<u>Microsporum gypseum.</u>	Clove	13	16	12	12	0
	Chamomile	16	14	8	0	0
	Peppermint	14	0	0	0	0
	Pelargonium	18	16	0	0	0
	Lemongrass	17	14	12	10	0
	Basil	20	12	0	0	0
	Cumin.	34	22	12	12	0
	Celery	26	0	0	0	0
<u>Trichophyton rubrum</u>	Clove	12	20	17	0	0
	Chamomile	18	16	14	12	0
	Peppermint	15	0	0	0	0
	Pelargonium	12	10	0	0	0
	Lemongrass	12	14	16	12	0
	Basil	28	22	14	0	0
	Cumin	26	22	24	0	0
	Celery	21	10	0	0	0
<u>Epidermophyton Flocosum.</u>	Clove	8	10	8	0	0
	Chamomile	30	16	12	0	0
	Peppermint	22	8	10	10	10
	Pelargonium	15	12	0	0	0
	Lemongrass	43	28	15	12	0
	Basil	Faint	0	0	0	0
	Cumin.	17	0	0	0	0
	Celery.	24	10	0	0	0
<u>Asarium oxysporum</u>	Clove	10	8	7	7	6
	Cumin.	14	0	0	0	0
<u>Aspergillus niger.</u>	Celery	Faint	Faint	0	0	0
		without sporulation				

CONCENTRATION



T. rubrum

M. gypseum

E. floccosum

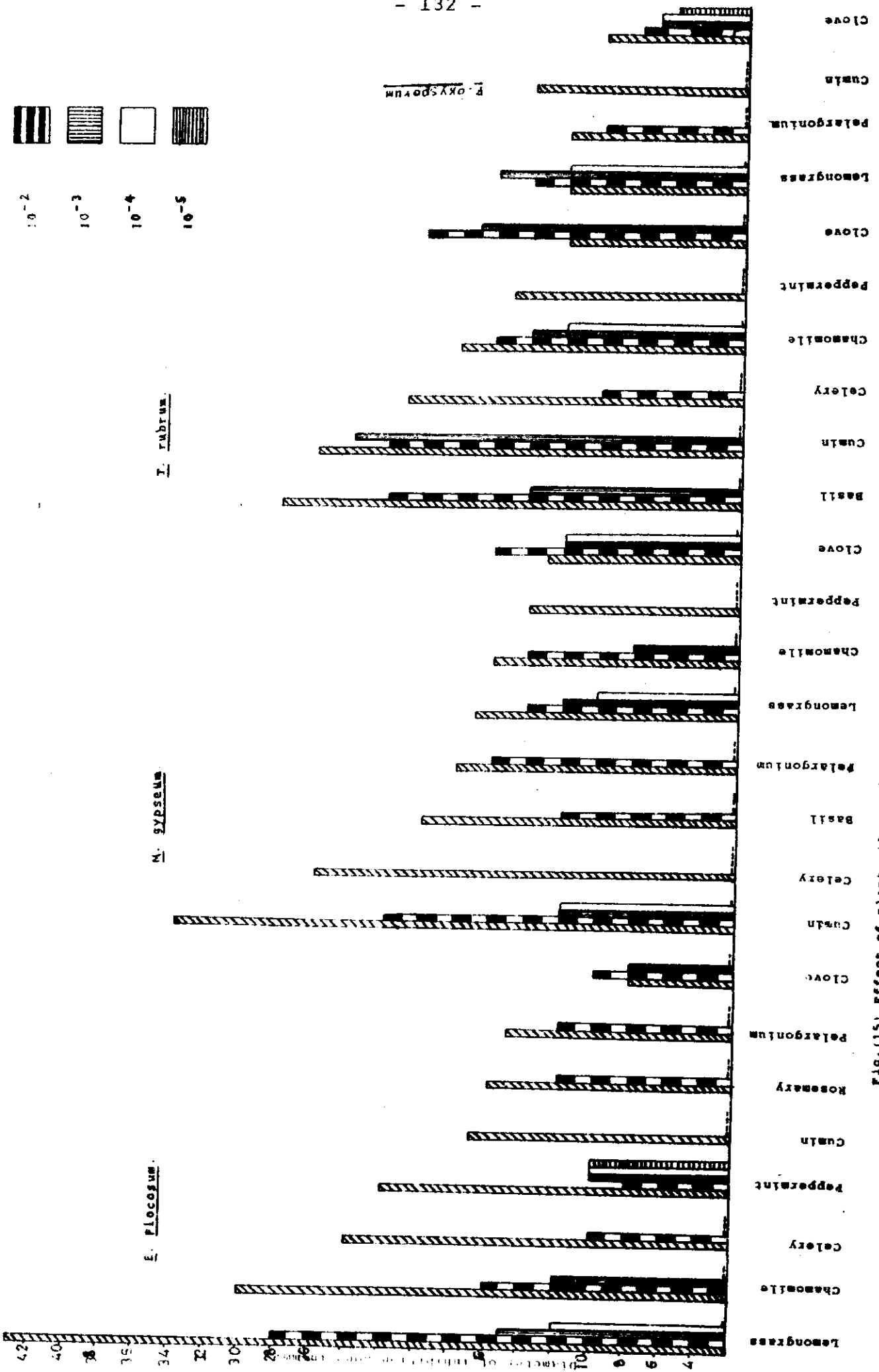


Fig. (15) Effect of plant oils against some fungal species

Part II. Effect of addition of some oils to the seeded plates on the plate count of the test organism:

In the aforementioned part of this study, the effect of the oils on the microorganisms was estimated as inhibition zone by the agar diffusion method. Effective oils showed clear zones of inhibition and non-effective oils did not show any inhibition distance with the crude oils. The effective oils which showed clear zones of inhibition at the lowest concentration used of the oil (6.25%) were considered efficient in their antimicrobial activity.

In this part (second part of the study, the effect of the oils; marjoram, clove leaves, thyme, clove buds, cedar, jasmine and rose on some test organisms namely, E.coli, M.luteus, Staph.aureus, Kl.aerogenes, Candida albicans and Salm. typhimurium was investigated. This was carried out by incorporating the oil in the seeded medium and after incubation the plates were counted. Mixing the oil with the seeded agar medium, to be in continuous contact, at certain concentration, with the growing organism eliminated the problems of agar diffusion method. In the agar diffusion method, other factors than the biological activity may affect the width of the inhibition zone. These factors include the amount of agar in the medium, the incubation temperature and the size of the molecules of the chemical.

So, in this part of the study the different dilutions of the oils were added directly to the seeded medium, and the plates were counted after incubation.

Data of the microbial counts of control plates and those of plates having different concentrations (dilutions) of oils were recorded. Also, the percentages of surviving organisms as compared to control were estimated and recorded. The percentages of reduction in the microbial counts were also calculated for every treatment. Finally the reduction % /survivors% were also estimated.

Therefore, four basis for demonstrating the results were recorded in each table.

Effect of marjoram oil against the tested microorganisms:

The effect of addition of different concentrations of marjoram oil to the medium on the plate count of E.coli, M.luteus, Staph.aureus, Kl.aerogenes, Candida albicans and Salm. typhimurium was reported in table (7) and illustrated in figure (16).

Data in Table (7) showed that the counts of the test organism decreased as the concentration of the added marjoram oil increased. This was true for all the investigated test organisms.

Table (7): Microbial counts of six microbial species at different treatments of marjeram oil.

Essential oil concn (v/v)		Microbial species						Average
		<u>E.coli</u>	<u>M.luteus</u>	<u>Staph. aureus</u>	<u>Kl. aerogenes</u>	<u>C.albicans</u>	<u>Salm. typhimurium</u>	
I. Microbial counts :								
10^{-1}		32	0	0	0	0	0	5.33
10^{-2}		48	32	80	0	40	0	33.33
10^{-3}		64	48	120	30	40	0	50.33
10^{-4}		84	128	200	40	80	40	95.33
10^{-5}		92	144	280	240	120	80	159.33
(Control)		97	156	680	680	600	160	395.50
II. Survivors %:								
10^{-1}		33	0	0	0	0	0	5.50
10^{-2}		49.50	20.51	11.76	0	6.67	0	14.74
10^{-3}		66	30.77	17.60	5.88	6.67	0	21.15
10^{-4}		86.60	82.05	29.41	5.88	13.33	25	40.38
10^{-5}		94.85	92.31	41.18	35.29	20	50	55.61
Average.		65.99	45.13	19.99	9.41	9.33	15	27.476
III. Reduction in counts %:								
10^{-1}		67	100	100	100	100	100	94.50
10^{-2}		50.50	79.49	88.24	100	93.33	100	85.26
10^{-3}		34	69.23	82.40	94.12	93.33	100	78.85
10^{-4}		13.40	17.95	70.59	94.12	86.67	75	59.62
10^{-5}		5.15	7.69	58.82	64.71	80	50	44.40
Average		34.01	54.87	80.01	90.59	90.67	85	72.53
		F	E	D	B	A	C	
IV. Reduction in counts %								
<u>Survivors %</u>								
10^{-1}		2.03	100	100	100	100	100	
10^{-2}		1.02	3.88	7.50	100	13.99	100	
10^{-3}		0.52	2.25	4.68	16.01	13.99	100	
10^{-4}		0.15	0.22	2.40	16.01	6.50	3.00	
10^{-5}		0.05	0.08	1.43	1.83	4.00	1.00	

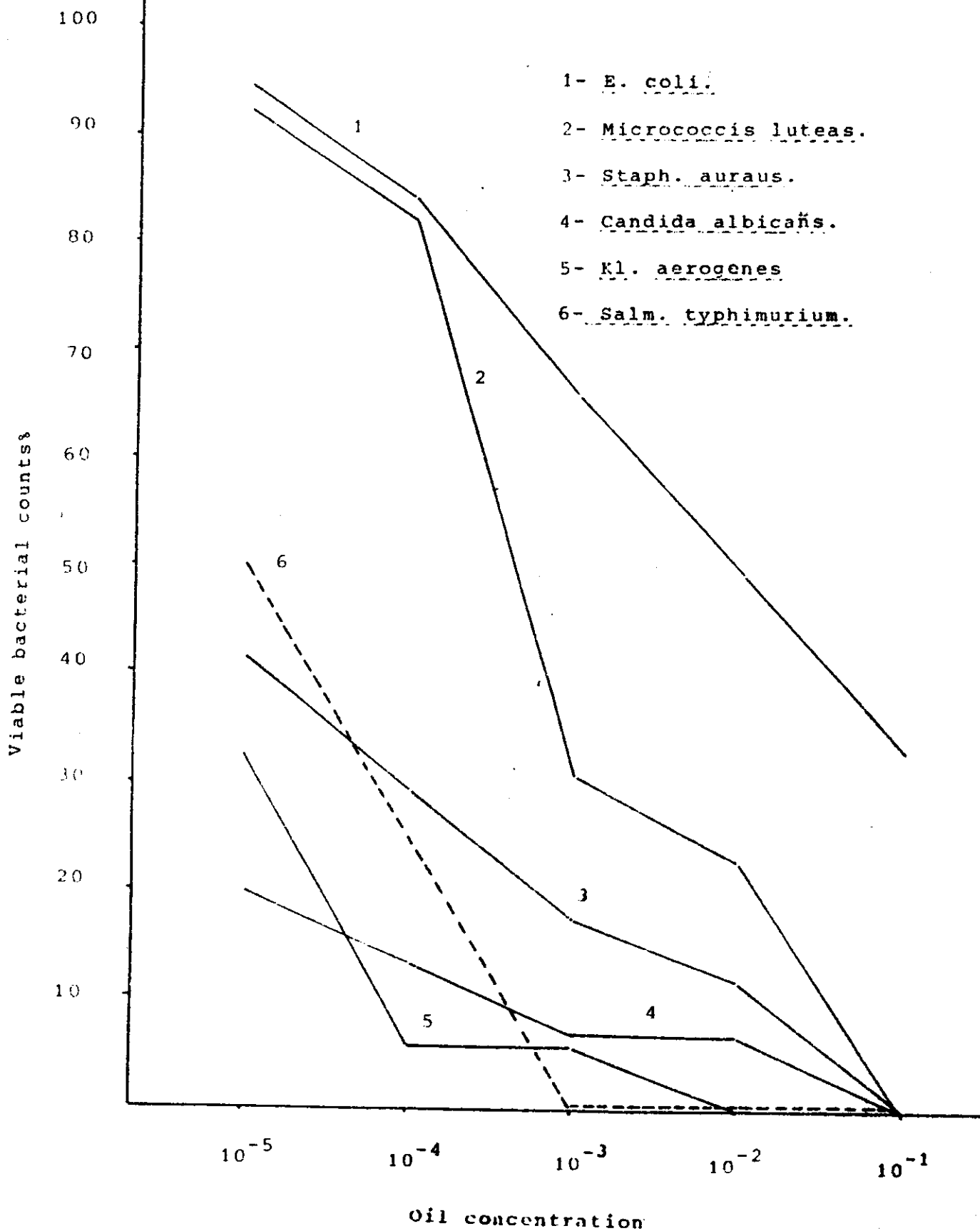


Fig. (16) Reduction in some bacterial species counts with Marjoram oil

The addition of marjoram oil to the agar medium to produce the following concentrations: 10^{-5} , 10^{-4} , 10^{-3} , 10^{-2} and 10^{-1} caused the following reduction percentages in microbial counts 44.4%, 59.62%, 78.85%, 85.26% and 94.5% respectively. Concerning the sensitivity of the tested organisms towards marjoram oil, they could be arranged descendingly as follows: (A) C.albicans (reduction percentage 90.67%), (B) Kl.aerogenes (90.59%), (C) Salm.typhimurium (85%), (D) Staph.aureus (80.01%), (E) M.luteus (54.87%) and (F) E.coli (34.01%).

Data in table (7) showed that the concentrations of marjoram oil in the medium required to cause LD₅₀ (50% reduction in the microbial count) were 10^{-5} or lower concentrations for the sensitive organisms (C.albicans, Kl.aerogenes, Salm.typhimurium and Staph.aureus) but the resistant organisms required the concentrations 10^{-2} (E.coli) and 10^{-4} - 10^{-3} (M.luteus) to cause LD₅₀ reduction.

Concerning the effect of different concentrations of marjoram oil against the different test organisms, results showed that the addition of marjoram oil to the medium caused 72.53% reduction in the microbial counts.

Effect of clove leaves oil against the tested microorganisms:

Table (8) and Fig. (17) report the effect of clove leaves oil against the test organisms E.coli, M.luteus,

Table (8) : Microbial counts of six microbial species at different treatments of clove leaves oil.

Essential oil concn(v/v)	Microbial species						Average	
	<u>E. coli</u>	<u>M. luteus</u>	<u>Staph. aureus</u>	<u>Kl. aerogenes</u>	<u>C. albicans</u>	<u>Salm. typhimurium</u>		
I. Microbial counts :								
10 ⁻¹	8	0	0	0	0	0	1.33	
10 ⁻²	12	4	120	120	0	40	49.33	
10 ⁻³	16	56	160	200	0	40	78.67	
10 ⁻⁴	32	64	200	240	80	80	116.00	
10 ⁻⁵	52	72	400	360	320	120	220.67	
(Control)	97	156	680	680	600	160	395.50	
II. Survivors %:								
10 ⁻¹	8.25	0	0	0	0	0	1.38	
10 ⁻²	12.37	2.56	17.65	17.65	0	25	12.54	
10 ⁻³	16.49	35.90	23.53	29.41	0	25	21.72	
10 ⁻⁴	32.99	41.03	29.41	35.29	13.33	50	33.68	
10 ⁻⁵	53.61	46.15	58.82	52.94	53.33	75	56.64	
Average.	24.74	25.13	25.88	27.06	13.33	35	25.19	
III. Reduction in counts%:								
10 ⁻¹	91.75	100	100	100	100	100	98.63	
10 ⁻²	87.63	97.44	82.35	82.35	100	75	87.46	
10 ⁻³	83.51	64.10	76.47	70.59	100	75	78.28	
10 ⁻⁴	67.01	58.97	70.59	64.71	86.67	50	66.33	
10 ⁻⁵	46.39	53.85	41.18	47.06	46.67	25	43.36	
Average.	75.26	74.87	74.12	72.94	86.67	65	74.81	
IV. Reduction in counts %:								
10 ⁻¹	Survivors %	11.12	100	100	100	100	100	
10 ⁻²		7.08	38.06	4.67	4.67	100	3.00	
10 ⁻³		5.06	1.79	3.25	2.40	100	3.00	
10 ⁻⁴		2.03	1.44	2.40	1.83	6.50	1.00	
10 ⁻⁵		0.87	1.17	0.70	0.89	0.88	0.33	

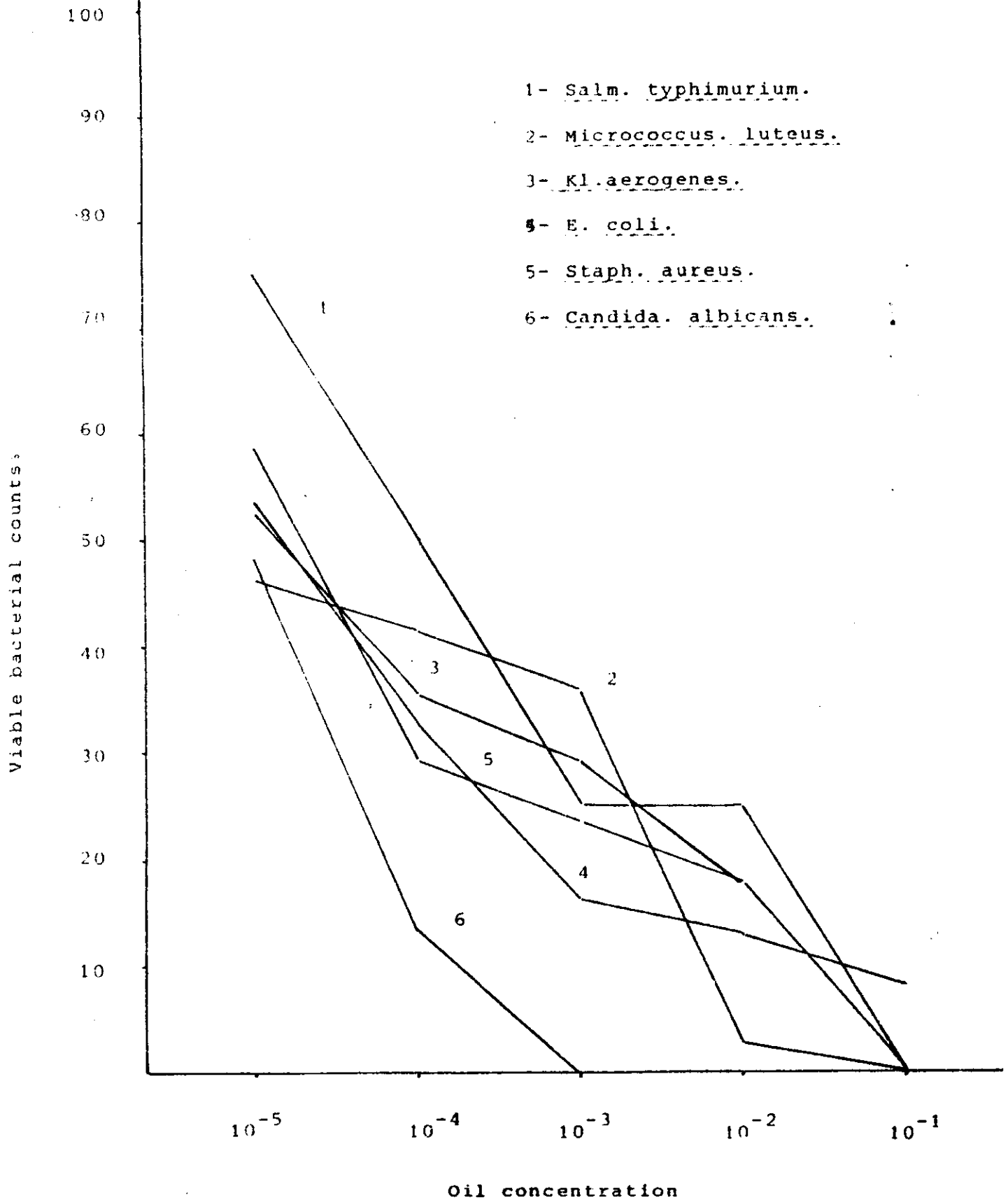


Fig. (17) : Reduction in some bacterial species counts with Clove leaves oil.

Staph.aureus, Kl.aerogenes, Candida albicans and Salm. typhimurium.

The addition of clove leaves oil to the medium at concentrations 10^{-5} , 10^{-4} , 10^{-3} , 10^{-2} , 10^{-1} caused 43.36%, 66.33%, 78.28%, 87.46% and 98.63% reduction in microbial counts, respectively.

Concerning the sensitivity of the test organisms towards clove leaves oil, they could be arranged descendingly as follows:

(A) C.albicans (reduction percentage 86.67%), (B) E.coli (75.26%), (C) M.luteus (74.87%) (D) Staph.aureus (74.12%), (E) Kl.aerogenes (72.94%) and (F) Salm. typhimurium (65%).

The concentration of clove leaves oil which caused LD_{50} to almost all investigated organisms was 10^{-5} . Only Salm.typhimurium required 10^{-4} concentration to cause LD_{50} to this organism.

The effect of different concentrations of clove leaves oil against different test organisms was estimated from the data in Table (8).

It was found that clove leaves oil caused (74.8%) reduction in the microbial counts.

Effect of clove buds oil against tested microorganisms:

Table (9) and Fig. (18) reported and illustrated the germicidal effect of clove buds oil against the tested microorganisms.

Data in Table (9) indicated that successive dilutions of clove buds oil up to 10^{-4} did not reduce the germicidal activity of the oil towards Salm.typhimurium and Kl.aerogenes. The oil reserved its complete efficiency against C.albicans up to 10^{-3} concentration.

The dilutions of the oils at any level from 10^{-1} to 10^{-5} reduced its activity against E.coli, M.luteus and Staph.aureus. The higher the dilution of the oil, the greater reduction in its activity occurred. The addition of this oil to the medium caused reduction in counts, of Kl.aerogenes, C.albicans, Salm.typhimurium, M.luteus and E.coli which averaged 98.82%, 96%, 95% , 76.92%, 76.47 and 75.26% , respectively, The dilutions of clove buds oil required to cause LD_{50} for M.luteus was 10^{-5} - 10^{-4} and those for E.coli and Staph.aureus were approximately 10^{-5} . The organisms Salm.typhimurium, C.albicans and Kl.aerogenes were very sensitive and their LD_{50} (S) were higher than the highest dilution used in the experiment. In view of the sensitivity of the microbial species towards clove buds oil, they could be arranged descendingly as follows: (A) Kl.aerogenes ,

Table (9): Microbial counts of six microrbial species at different treatments of thyme oil.

Essential oil concn(v/v)	Microbial species						Average	
	<u>E. coli</u>	<u>M. luteus</u>	<u>Staph. aureus</u>	<u>Kl. aerogenes</u>	<u>C. albicans</u>	<u>Salm. typhimurium</u>		
I. <u>Microbial counts</u> :								
10 ⁻¹	0	0	0	0	0	0	0	
10 ⁻²	12	28	120	0	0	0	26.67	
10 ⁻³	20	44	200	0	0	0	44.00	
10 ⁻⁴	24	52	280	40	120	40	92.67	
10 ⁻⁵	40	68	320	80	120	80	118.00	
(Control)	97	156	680	680	600	160	395.50	
II. <u>Survivors %</u> :								
10 ⁻¹	0	0	0	0	0	0	0	
10 ⁻²	12.37	17.95	28.21	0	0	0	9.76	
10 ⁻³	20.62	28.21	29.41	0	0	0	13.04	
10 ⁻⁴	24.74	33.33	41.18	5.88	20	25	25.02	
10 ⁻⁵	41.24	43.59	47.06	11.76	20	50	35.61	
Average.	19.79	24.62	29.17	3.53	8	15	16.69	
III. <u>Reduction in counts%</u> :								
10 ⁻¹	100	100	100	100	100	100	100	
10 ⁻²	87.63	82.05	71.79	100	100	100	90.25	
10 ⁻³	79.38	71.79	70.59	100	100	100	86.96	
10 ⁻⁴	75.26	66.67	58.82	94.12	80	75	74.98	
10 ⁻⁵	58.76	56.41	52.94	88.24	80	50	64.39	
Average.	80.21	75.38	70.83	96.47	92	85	83.315	
IV. <u>Reduction in counts %</u> :								
10 ⁻¹	Survivors %	100	100	100	100	100	100	
10 ⁻²		7.08	4.57	2.54	100	100	100	
10 ⁻³		3.85	2.54	2.40	100	100	100	
10 ⁻⁴		3.04	2.00	1.43	16.01	4.00	3.00	
10 ⁻⁵		1.42	1.29	1.12	7.50	4.00	1.00	

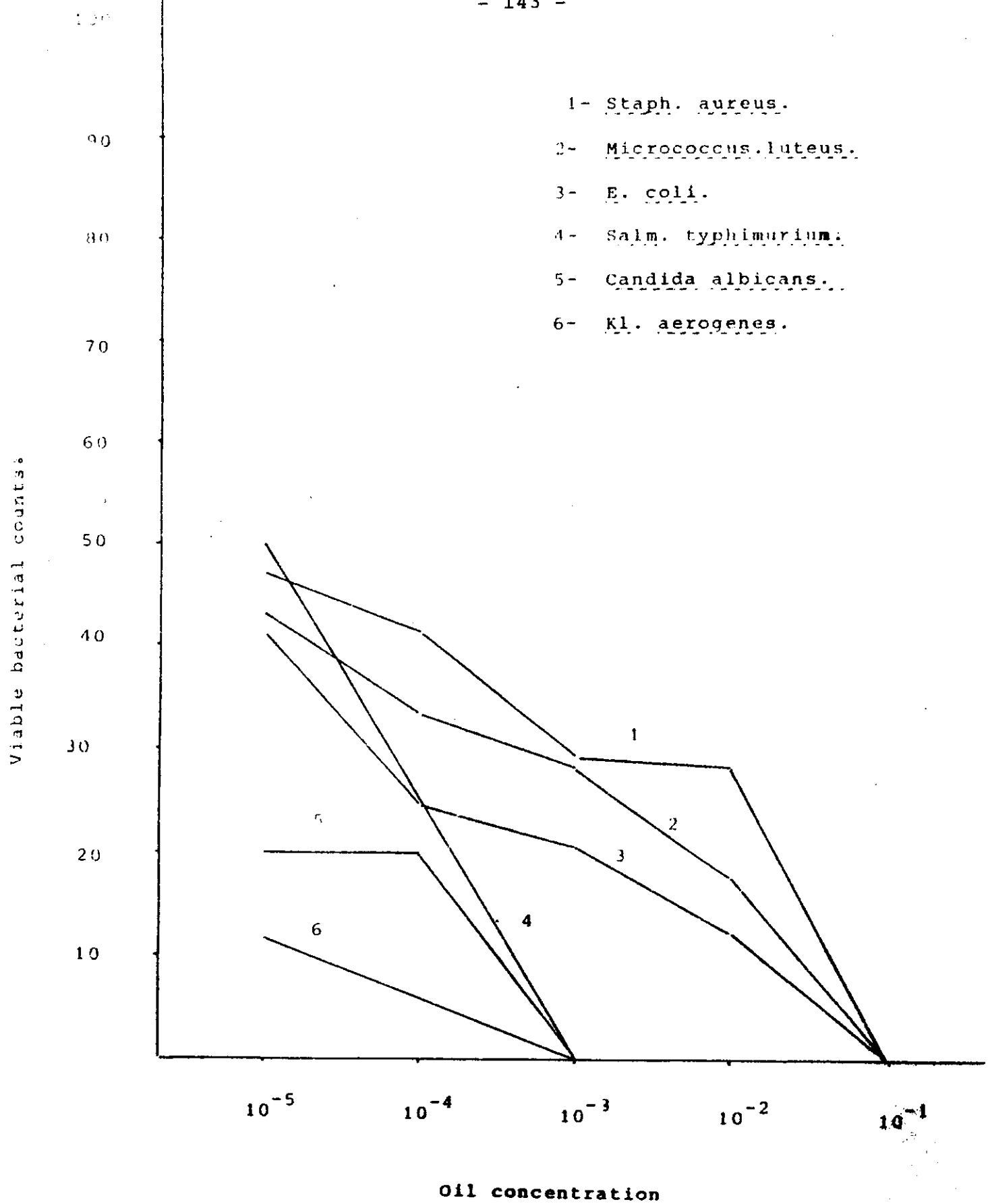


Fig. (18): Reduction in some bacterial species counts with Thyme oil.

(B) C.albicans, (C) Salm.typhimurium, (D) M.luteus, Staph.aureus and E.coli.

Clove buds oil caused 86.41% reduction in the microbial counts.

Effect of thyme oil against tested microorganisms:

The effect of different concentrations of thyme oil against E.coli, M.luteus, Staph.aureus, Kl.aerogenes, C.albicans and Salm.typhimurium is reported in Table (10) and illustrated in Fig. (19).

Results showed that successive dilutions of thyme oil up to 10^{-3} did not reduce the germicidal effect of thyme oil against Kl.aerogenes, C.albicans and Salm.typhimurium. Concerning the other tested organisms, the germicidal effect of the oil decreased as the dilution increased.

The addition of thyme oil to the medium at concentrations 10^{-5} , 10^{-4} , 10^{-3} , 10^{-2} and 10^{-1} caused reduction in microbial counts which averaged 64.39%, 74.98%, 86.96%, 90.25% and 100%, respectively.

The addition of different dilutions of thyme oil to the medium of the test organism caused the following reduction in their counts which averaged as follows:

Kl.aerogenes (96.47%), C.albicans (92%), Salm.typhimurium (85%), E.coli (80.21%), M.luteus (75.38%) and Staph.aureus (70.83%).

Table (10): Microbial counts of six microbial species at different treatments of clove buds oil.

Essential oil concn(v/v)	Microbial species						Average
	<u>E. coli</u>	<u>M. luteus</u>	<u>Staph. aureus</u>	<u>Kl. aerogenes</u>	<u>C. albicans</u>	<u>Salm. typhimurium</u>	
I. Microbial counts :							
10 ⁻¹	0	0	0	0	0	0	0
10 ⁻²	12	8	80	0	0	0	16.67
10 ⁻³	20	20	120	0	0	0	26.67
10 ⁻⁴	40	52	280	0	40	0	68.67
10 ⁻⁵	48	100	320	40	80	40	104.67
(Control)	97	156	680	680	600	160	395.50
II. Survivors %:							
10 ⁻¹	0	0	0	0	0	0	0
10 ⁻²	12.37	5.13	11.76	0	0	0	4.88
10 ⁻³	20.62	12.82	17.65	0	0	0	8.52
10 ⁻⁴	41.24	33.33	41.18	0	6.67	0	20.40
10 ⁻⁵	49.48	64.10	47.06	5.88	13.33	25	34.14
Average.	24.74	23.08	23.53	1.18	4.00	5.00	13.59
III. Reduction in counts%:							
10 ⁻¹	100	100	100	100	100	100	100
10 ⁻²	87.63	94.87	88.24	100	100	100	95.12
10 ⁻³	79.38	87.18	82.35	100	100	100	91.49
10 ⁻⁴	58.76	66.67	58.82	100	93.33	100	79.59
10 ⁻⁵	50.52	35.90	52.94	94.12	86.67	75	65.85
Average.	75.26	76.92	76.47	98.82	96.00	95.00	86.41
	D	D	D	A	B	C	
IV. Reduction in counts %:							
10 ⁻¹ Survivors %	100	100	100	100	100	100	
10 ⁻²	7.08	18.49	7.50	100	100	100	
10 ⁻³	3.85	6.80	4.67	100	100	100	
10 ⁻⁴	1.42	2.00	1.43	100	13.99	100	
10 ⁻⁵	1.02	0.56	1.13	16.01	6.50	3.00	

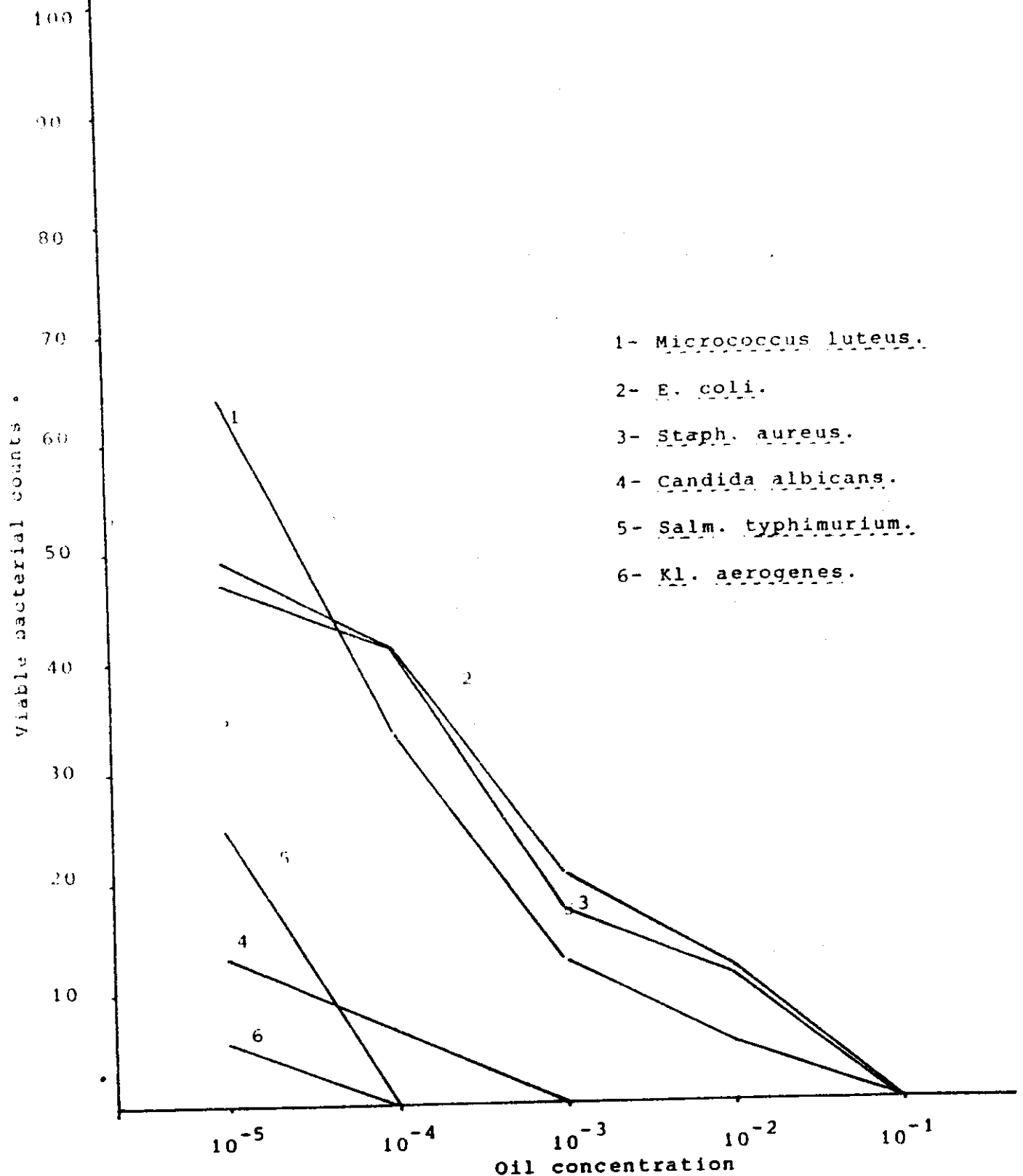


Fig. (19): Reduction in some bacterial species counts with clove buds oil.

Results also showed that the dilution 10^{-5} of thyme oil caused LD_{50} for Salm.typhimurium while higher dilutions were required for the LD_{50} of the other tested organisms.

Thyme oil caused 83.315% reduction in the microbial counts.

Effect of cedar oil on the plate count of the tested microorganisms:

Table (11) and Fig. (20) showed that the concentration 10^{-1} of cedar oil completely destroyed E.coli, M.luteus and Staph.aureus, while the concentration 10^{-1} was sufficient for the complete destruction of Kl.aerogenes and C.albicans. Salmonella typhimurium was very sensitive to cedar oil and the concentration 10^{-4} showed no colonies in the plates of counting.

The addition of cedar oil to the medium at concentrations 10^{-5} , 10^{-4} , 10^{-3} , 10^{-2} , and 10^{-1} caused reduction in microbial counts which averaged 55.25%, 70.71%, 79.82%, 89.94% and 100%, respectively.

Concerning the sensitivity of the test organisms towards cedar oil, they could be descendingly arranged as follows:

Table (11): Microbial counts of six microbial species at different treatments of cider oil.

Essential oil concn(v/v)	Microbial species						Average
	<u>E. coli</u>	<u>M. luteus</u>	<u>Staph. aureus</u>	<u>Kl. aerogenes</u>	<u>C. albicans</u>	<u>Salm. typhimurium</u>	
I. Microbial counts :							
10 ⁻¹	0	0	0	0	0	0	0
10 ⁻²	28	40	40	0	0	0	18.00
10 ⁻³	32	72	120	120	40	0	64.00
10 ⁻⁴	40	96	200	160	120	0	102.67
10 ⁻⁵	48	128	360	200	160	40	156.00
(Control)	97	156	680	680	600	160	395.50
II. Survivors %:							
10 ⁻¹	0	0	0	0	0	0	0
10 ⁻²	28.87	25.64	5.88	0	0	0	10.07
10 ⁻³	32.99	46.15	17.65	17.65	6.67	0	20.19
10 ⁻⁴	41.24	61.54	29.41	23.53	20	0	29.29
10 ⁻⁵	49.48	82.05	55.88	29.41	26.67	25	44.75
Average.	30.52	43.08	21.76	14.12	10.67	5.00	20.86
III. Reduction in counts%:							
10 ⁻¹	100	100	100	100	100	100	100
10 ⁻²	71.13	74.36	94.12	100	100	100	89.94
10 ⁻³	67.01	53.85	82.35	82.35	93.33	100	79.82
10 ⁻⁴	58.76	38.46	70.59	76.47	80.00	100	70.71
10 ⁻⁵	50.52	17.95	44.12	70.59	73.33	75	55.25
Average.	69.48	56.92	78.24	85.88	89.33	95.00	79.14
IV. Reduction in counts %:							
10 ⁻¹	Survivors %						
10 ⁻²	100	100	100	100	100	100	
10 ⁻³	2.46	2.90	16.01	100	100	100	
10 ⁻⁴	2.03	1.17	4.67	4.67	13.99	100	
10 ⁻⁵	1.42	0.62	2.40	3.25	4.00	100	
10 ⁻⁵	1.02	0.22	0.79	2.40	2.75	3.00	

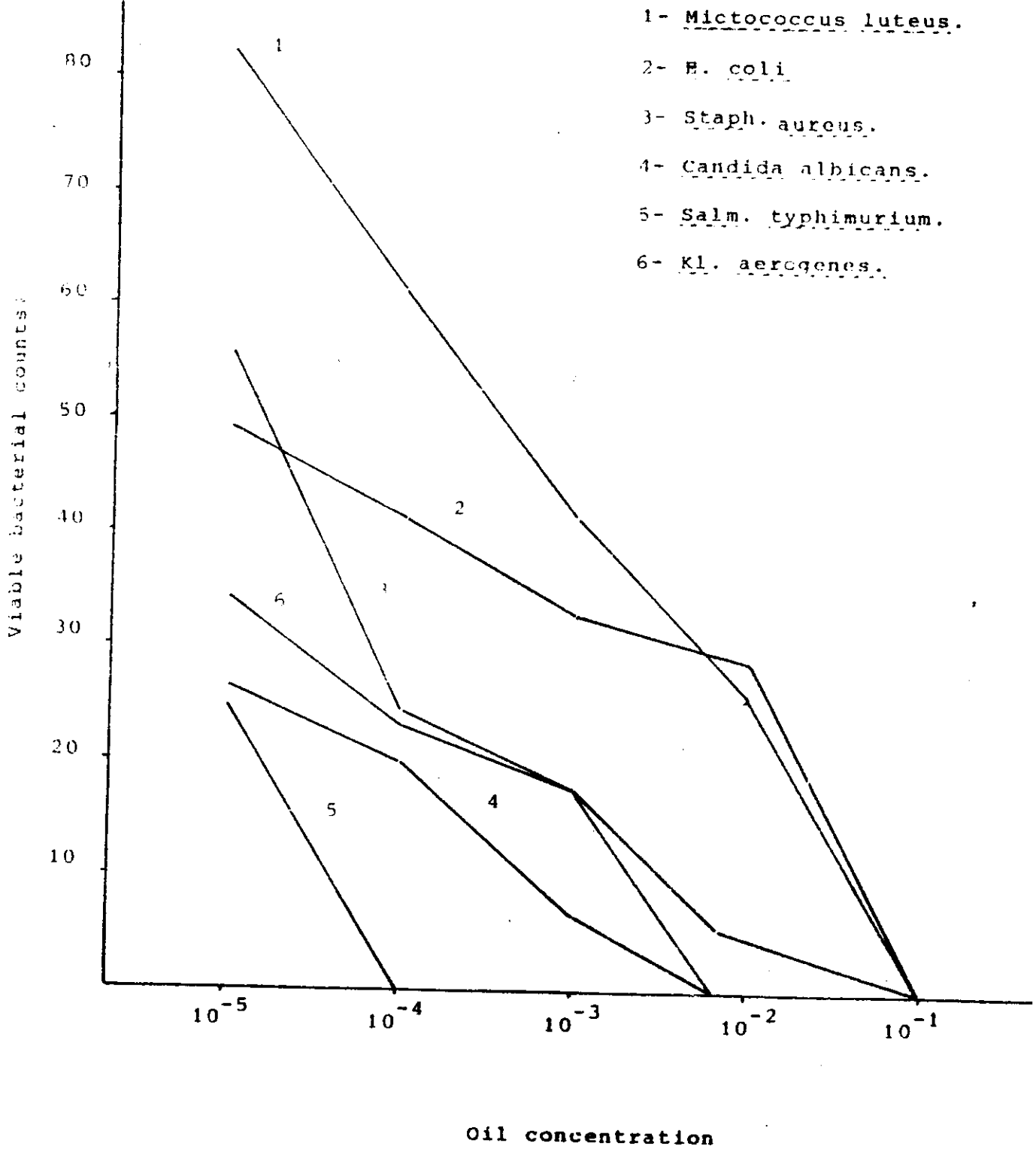


Fig.(20) Reduction in some bacterial species counts with cedar oil.

Salm. typhimurium (average of reduction percentages for different concentrations 95%), C.albicans (89.33%), Kl. aerogenes (85.88%), Staph.aureus (78.24%), E.coli (69.48%) and M.luteus (56.92%).

The dilution which caused LD₅₀ for E.coli was 10^{-5} and LD₅₀ for Staph.aureus was (10^{-4} - 10^{-5}), but higher dilutions than that used in the experiment were required for LD₅₀ of the other tested organisms.

Cedar oil caused 79.14% reduction in the microbial counts.

Effect of jasmine oil on the plate count of the tested microorganisms:

Data presented in Table (12) and illustrated by Fig. (21) showed that no growth could be detected for E.coli and M.luteus at the concentration 10^{-1} of jasmine oil. The concentration 10^{-2} was sufficient to prevent the growth of any colonies of Staph. aureus, Kl.aerogenes and C.albicans. Salmonella typhimurium was more sensitive to jasmine oil than other investigated microorganisms.

The different dilutions of jasmine oil namely 10^{-5} , 10^{-4} , 10^{-3} , 10^{-2} and 10^{-1} caused reduction in the microbial

Table (12): Microbial counts of six microbial species at different treatments of Jasmin oil.

Essential oil concn(v/v)	Microbial species						Average	
	<u>E. coli</u>	<u>M. luteus</u>	<u>Staph. aureus</u>	<u>Kl. aerogenes</u>	<u>C. albicans</u>	<u>Salm. typhimurium</u>		
I. Microbial counts :								
10 ⁻¹	0	0	0	0	0	0	0	
10 ⁻²	16	20	0	0	0	0	0	6
10 ⁻³	24	32	320	80	40	0	0	82.67
10 ⁻⁴	32	40	400	200	200	40	40	152.00
10 ⁻⁵	36	68	520	360	440	80	80	250.67
(Control)	97	156	680	680	600	160	160	395.50
II. Survivors %:								
10 ⁻¹	0	0	0	0	0	0	0	0
10 ⁻²	16.49	12.82	0	0	0	0	0	4.89
10 ⁻³	24.74	20.51	47.06	11.76	6.67	0	0	18.46
10 ⁻⁴	32.99	25.64	58.82	29.41	33.33	25	25	34.20
10 ⁻⁵	37.11	43.59	76.47	52.94	73.34	50	50	55.58
Average.	22.27	20.51	36.47	18.82	22.67	15	15	22.62
III. Reduction in counts%:								
10 ⁻¹	100	100	100	100	100	100	100	100
10 ⁻²	83.51	87.18	100	100	100	100	100	95.12
10 ⁻³	75.26	79.49	52.94	88.24	93.33	100	100	81.54
10 ⁻⁴	67.01	74.36	41.18	70.59	66.67	75	75	65.80
10 ⁻⁵	62.89	56.41	23.53	47.06	26.66	50	50	44.43
Average.	77.73 C	79.49 C	63.53 D	81.18 B	77.33 C	85 A	85	77.38
IV. Reduction in counts %:								
10 ⁻¹	Survivors %	100	100	100	100	100	100	100
10 ⁻²		5.06	6.80	100	100	100	100	100
10 ⁻³		3.04	3.88	1.12	7.50	13.99	100	100
10 ⁻⁴		2.03	2.90	0.70	2.40	2.00	3.00	3.00
10 ⁻⁵		1.69	1.29	0.31	0.89	0.36	1.00	1.00

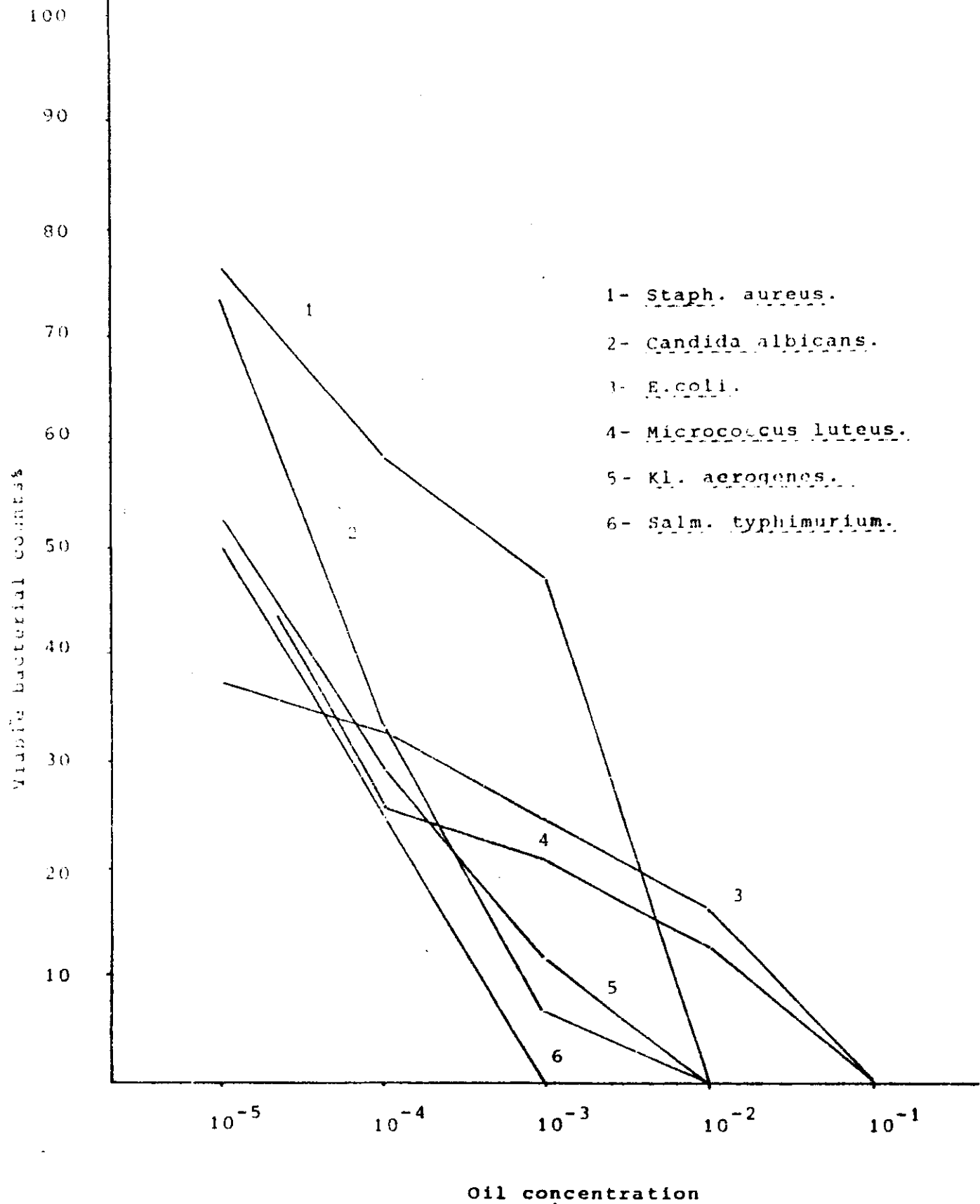


Fig.(21): Reduction in some bacterial species counts with Jasmine oil.

counts which averaged 44.43%, 65.8%, 81.54%, 95.12% and 100%, respectively.

The test organisms were arranged descendingly according to their sensitivity to jasmine oil as follows: Salm.typhimurium (85%), Kl.aerogenes (81.18%), M.luteus (79.49%), E.coli (77.73%), C.albicans (77.33%) and Staph.aureus (63.53%). LD₅₀ (S) of jasmine oil were 10⁻⁵ for Salm. typhimurium 10⁻⁵, 10⁻⁴ for C.albicans and Kl.aerogenes; 10⁻³ for Staph.aureus and LD₅₀ (S) for E.coli and M.luteus were at lower concentrations than 10⁻⁵. Jasmine oil caused 44.43% reduction in the microbial counts.

Effect of rose oil on the plate count of the tested micro-organisms:

The effect of rose oil on E.coli, M.luteus, Staph.aureus, Kl.aerogenes, C.albicans and Salm. typhimurium is presented in Table (13) and illustrated in Fig. (22).

Results showed that the concentration 10⁻¹ of rose oil prevented the growth of E.coli , M.luteus, Staph.aureus and Salm.typhimurium. In case of C.albicans and Kl.aerogenes the concentration 10⁻² of rose oil was sufficient to prevent their growth.

Data showed that the microbial counts decreased as the concentration of rose oil added to the medium was

Table (13): Microbial counts of six microbial species at different treatments of rose oil.

Essential oil concn(v/v)	Microbial species						Average	
	<u>E. coli</u>	<u>M. luteus</u>	<u>Staph. aureus</u>	<u>Kl. aerogenes</u>	<u>C. albicans</u>	<u>Salm. typhimurium</u>		
I. Microbial counts :								
10 ⁻¹	0	0	0	0	0	0	0	
10 ⁻²	12	36	40	0	0	40	21.33	
10 ⁻³	20	60	120	40	280	80	100	
10 ⁻⁴	24	88	200	80	320	120	138.67	
10 ⁻⁵	28	120	360	200	360	160	204.67	
(Control)	97	156	680	680	600	160	395.50	
II. Survivors %:								
10 ⁻¹	0	0	0	0	0	0	0	
10 ⁻²	12.37	23.08	5.88	0	0	25	11.06	
10 ⁻³	20.62	38.46	17.65	5.88	46.67	50	29.88	
10 ⁻⁴	24.74	56.41	29.41	11.77	53.34	75	41.78	
10 ⁻⁵	28.87	76.92	52.94	29.41	60	100	58.02	
Average.	17.32	38.97	21.18	9.41	32.0	50	28.15	
III. Reduction in counts%:								
10 ⁻¹	100	100	100	100	100	100	100	
10 ⁻²	87.63	76.92	94.12	100	100	75	88.95	
10 ⁻³	79.38	61.54	82.35	94.12	53.33	50	70.12	
10 ⁻⁴	75.26	43.59	70.59	88.23	46.66	25	58.22	
10 ⁻⁵	71.13	23.08	47.06	70.59	40	0	41.98	
Average.	82.68	61.03	78.82	90.59	68.00	50	71.85	
	B	E	C	A	D	F		
IV. Reduction in counts %:								
10 ⁻¹ Survivors %	100	100	100	100	100	100		
10 ⁻²	7.08	3.33	16.01	100	100	3.00		
10 ⁻³	3.85	1.60	4.67	16.01	1.14	1.00		
10 ⁻⁴	3.04	0.77	2.40	7.50	0.87	0.33		
10 ⁻⁵	2.46	0.30	0.89	2.40	0.67	0.00		

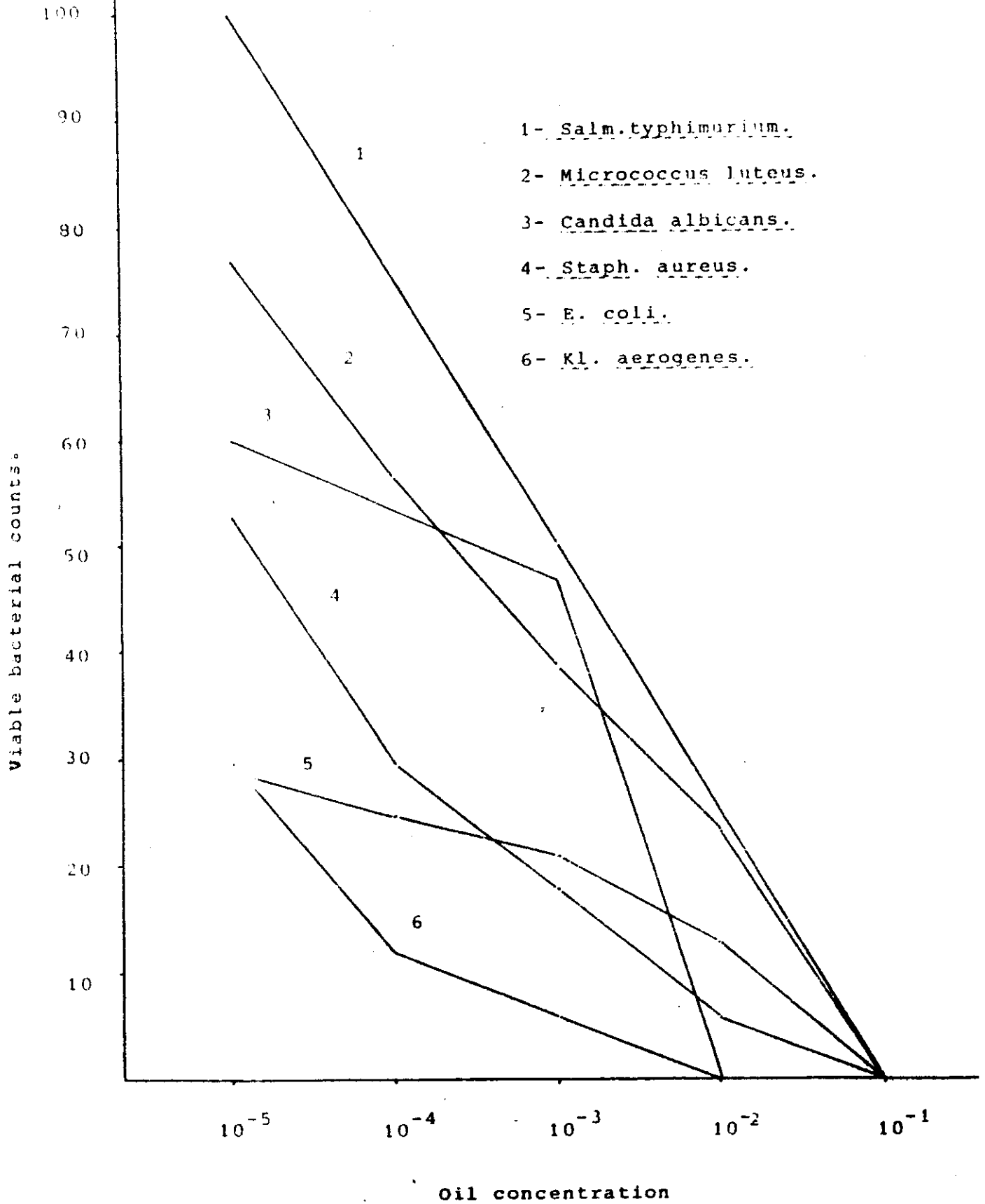


Fig (22); Reduction in some bacterial species counts with Rose oil.

increased. The concentrations 10^{-5} , 10^{-4} , 10^{-3} , 10^{-2} and 10^{-1} of rose oil in the medium caused reduction in microbial counts which averaged 41.98%, 58.22%, 70.12%, 88.95% and 100%, respectively.

In view of the sensitivity of the test organisms towards rose oil, they could be arranged descendingly as follows: Kl.aerogenes (90.59%), E.coli (82.68%), Staph.aureus (78.82%), C.albicans (68 %), M.luteus (61.03%) and Salm.typhimurium (50%).

The concentration of rose oil which caused LD_{50} for the tested organisms were 10^{-3} for Salm.typhimurium , 10^{-4} - 10^{-3} for Candida albicans and M.luteus, 10^{-5} - 10^{-4} for Staph.aureus; but LD_{50} (S) for E.coli and Kl.aerogenes were at lower concentrations than the lowest concentration used in the experiment.

Rose oil caused 71.85% reduction in microbial counts.

Part III: Effect of some popular drinks and water extracts
of some spices on the intestinal flora:

Effect of some popular drinks as roselle, fenugreek, caraway and aqua cinnamomi as well as seasoning materials as garlic juice, cumin and pepper on the intestinal normal flora of human gut were tested. Aqua aurantifloris and aqua rosae were also investigated by the addition to some fresh cold drinks or water.

Table (14) presents the results of application of watery extract of each material to the natural mixture of human intestinal flora for different periods of time, then surviving organisms were counted.

Results are expressed as total viable bacterial counts per/ml of the faecal mixture after different periods of contact time with any of the aforementioned plant extracts. The periods of contact were 15 minutes, 60 minutes, six hours and twenty four hours. The percentages of reduction in microbial counts (removal) due to the application of these extracts were calculated after estimating the survivors and control counts.

Of the interesting noted results is the highly drastic effects of the tested nine materials against the natural intestinal flora of the human gut within the 15 minutes of mixing.

Table (14): Viable bacterial counts per 1 ml. of faecal mixture after treatment with popular drinks and water extracts of spices.

Extracts.	Contact time			
	After addition (15 minutes)	One hour	Six hour	24 hour.
<u>Hibiscus sabdariffa</u> (Roselle).	80×10^6	80×10^6	84×10^5	80×10^3
Reduction in counts.	97.6%	97.6%	99.7%	99.9%
<u>Aqua aurantifloris</u>	160×10^6	60×10^6	280×10^4	352×10^4
Reduction in counts	95.2%	98.2%	99.9%	99.9%
<u>Aqua rosae</u>	168×10^7	76×10^5	104×10^5	154.8×10^5
Reduction in counts.	49.4%	97.7%	99.7%	99.5%
<u>Trigonella foenum-Greacum</u> (Fenugreek)	92×10^7	188×10^6	64×10^5	120×10^4
Reduction in counts	72.3%	94.3 %	99.8%	99.9%
<u>Carum carvi</u> (caraway)	144×10^7	52×10^6	40×10^5	316×10^4
Reduction in counts.	56.6%	98.4%	99.8%	99.9%
Control.	332×10^7	172×10^7	372×10^7	429.6×10^7
 <u>Allium sativum</u> (garlic juice).	40×10^6	40×10^5	20×10^5	209×10^5
Reduction in counts	99.5%	99.9%	99.9%	99.7%
<u>Aqua cinnamomi</u>	80×10^6	50×10^6	280×10^5	216.8×10^5
Reduction in counts	98.9%	99.3%	99.6%	99.7%
<u>Cuminum ciaminum</u> (cumin).	80×10^6	52×10^6	260×10^5	85.6×10^5
Reduction in counts.	98.9%	99.3%	99.6%	99.9%
<u>Capsicum frutescens</u> (pepper)	160×10^6	80×10^5	280×10^4	$244. \times 10^5$
Reduction in counts.	97.9%	99.8%	99.9%	99.7%
Control.	79.6×10^8	50.6×10^8	85.44×10^2	91.6×10^8

Reduction % = reduction in microbial counts as compared to control.

After 15 minutes of contact of the faecal organisms with roselle, aqua aurantifloris, garlic juice, aqua cinnamomi, cumin and pepper, the percentages of removal (reduction in counts) reached more than 95% of total number of original flora. Aqua rosae, caraway, and fenugreek water extracts showed reduction percentages of 49.4%, 56.6% and 72.3% respectively.

After one hour of mixing the faecal flora with any of the aforementioned plant extracts, the rate of reduction percentages in microbial counts were more than 97%, except fenugreek (94.3%). The responses of the normal intestinal mixed flora of human gut towards the first shock of contact between different materials as expressed by reduction percentages in microbial counts were quite different according the added extract. Garlic showed a sharp drop in counts (99.5%) within 15 minutes. Aqua rosae was the least effective, after 15 minutes of contact (49.4%), but showed a sharp drop therefore, to cause reduction percentage of 97.7% after one hour of contact.

After six hours, more than 99% of the faecal organisms were removed by the drastic effects of the tested extracts including the fenugreek extract.

After 24 hours of contact time for the faecal organisms with the plant extracts, the viable organisms were

determined again. Two different effects of activity of extracts against the faecal flora were obtained. The extracts of what is known as popular drinks which included caraway, fenugreek, roselle, and cinnamon, continued their inhibitory action against the remaining organisms which survived the aforementioned shorter periods of contact namely 15, 60 minutes and 6 hours. They did not only stop their propagation but also reduced their counts (figure , 23). The second picture of behaviour is what was noticed with mixtures of spices or seasoning materials as garlic, pepper, aqua rosae and aqua aurantifloris. In this case, the counts of the mixed flora were reduced at the short periods of contact (15 minutes, 1 hour and 6 hours) but the surviving organisms adapted themselves and began to increase in counts after 24 hours contact with these extracts, (Fig. 24).

The study proved that the popular drinks and extracts of spices affected the microbial population of the intestine. So, further research is recommended which includes the effect of these substances on the specific groups of the normal intestinal flora, such as coliform group, streptococci, lactobacilli, clostridia etc. as well as the enteric pathogens. The effect of these substances on the counts of non-pathogenic and pathogenic bacteria affects of microbial equilibrium in the intestine which enhances or depresses the occurrence of the disease. Further studies should also include the absorption of the active ingredients in the blood stream and their antimicrobial activity against the pathogenes in the presence of blood constituents.

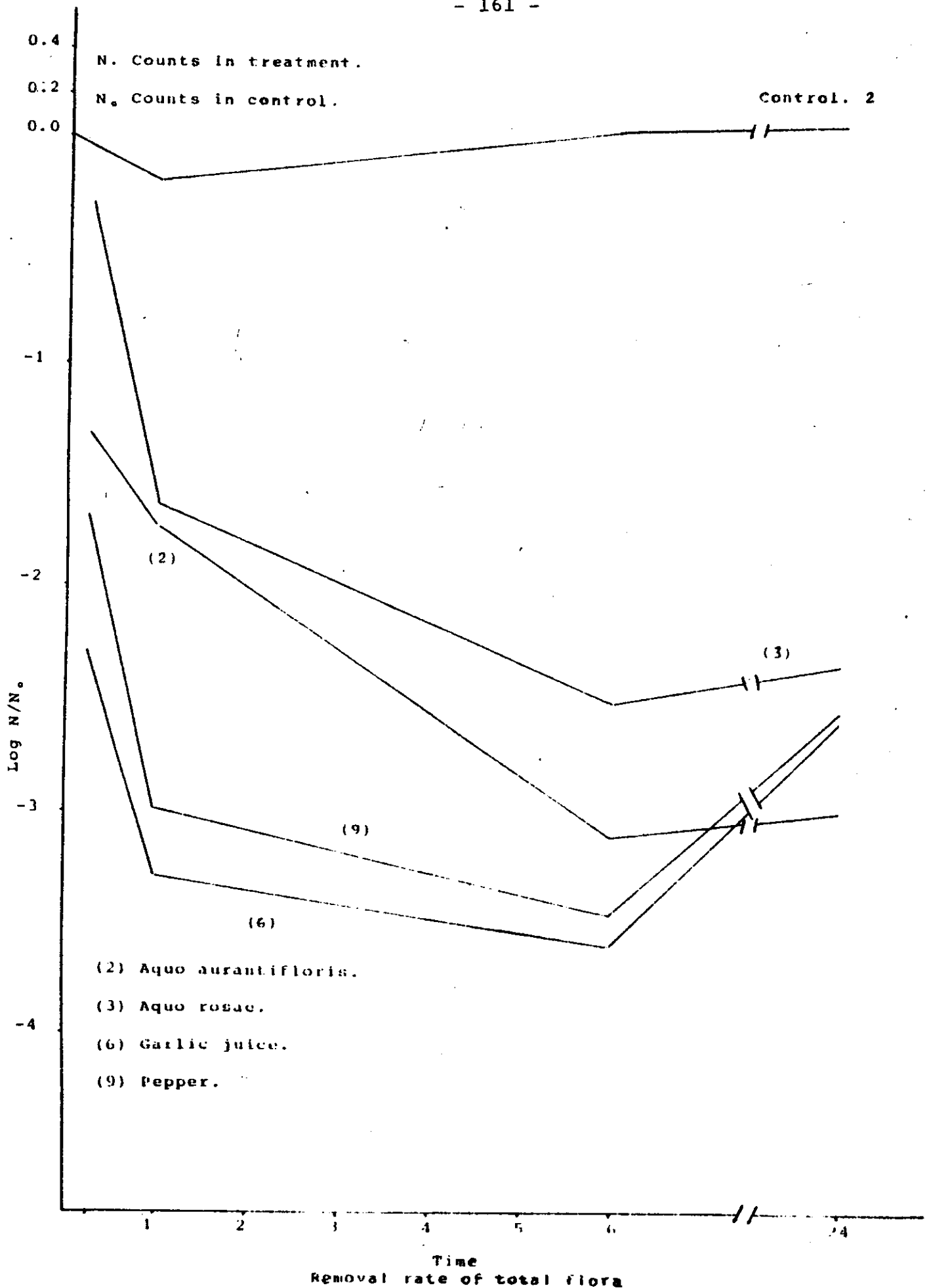


Fig.(23):Effect of some popular drinks and water extracts of some spices on the intestinal flora.

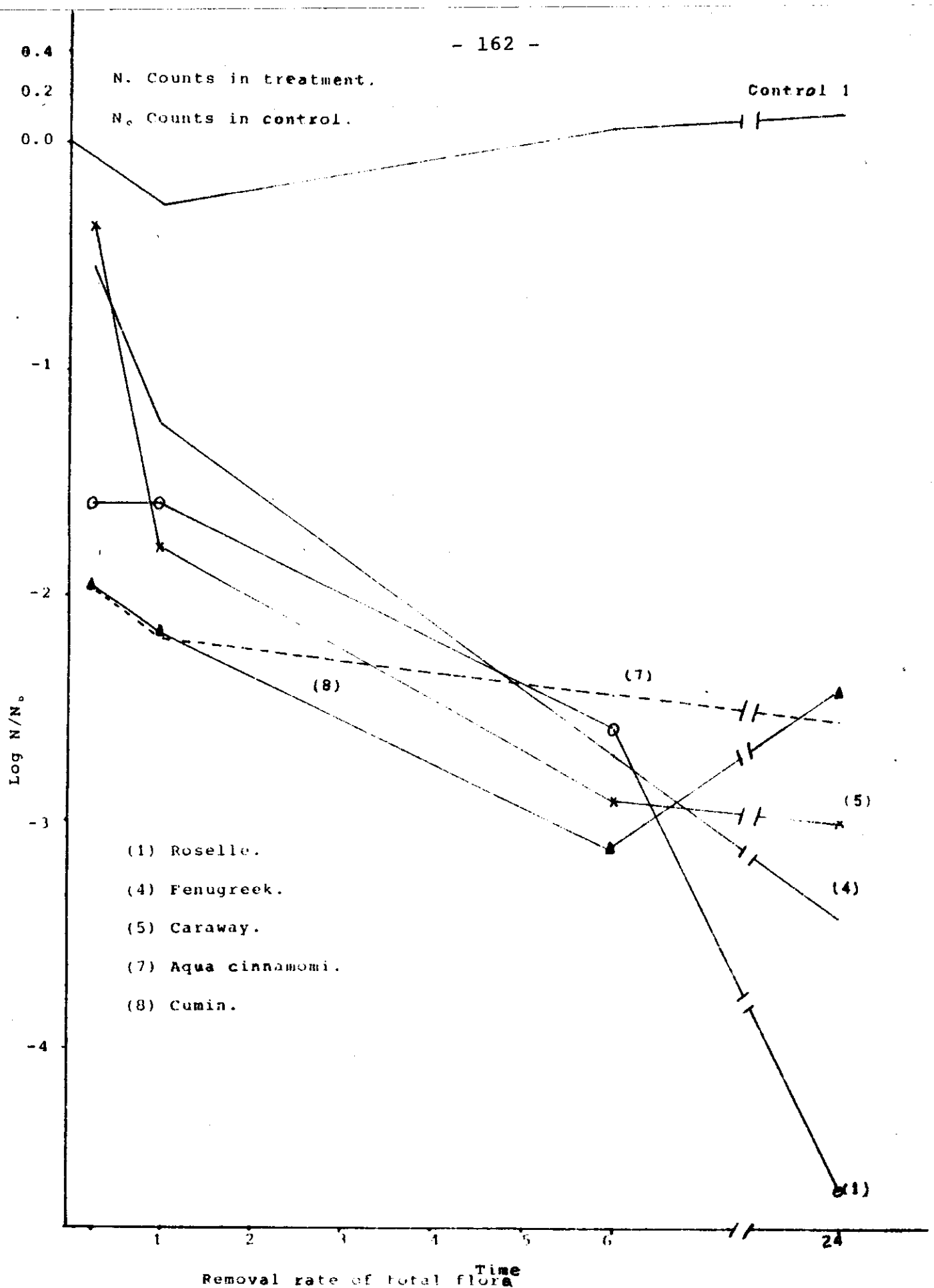


Fig.(24):Effect of some popular drinks and water extracts of some spices on the intestinal flora.