

Fiskeridirektoratets kjemisk-
tekniske forskningsinstitutt

FISKERIDIREKTORATET
BIBLIOTEKET

Eks. 2

NORWEGIAN FISHERIES RESEARCH INSTITUTE

PYRETHRUM TREATMENT FOR THE PROTECTION OF
DRYING FISH FROM BLOW-FLY INFESTATION

BY

NORVALD LOSNEGARD AND KÅRE BAKKEN

R.nr. 130/73

A. h. 59

BERGEN

Pyrethrum treatment for the protection of
drying fish from blow-fly infestation

by

Norvald Losnegard and Kåre Bakken

Introduction

The investigations described in this paper were performed in 1963 and 1964, and reports in Norwegian were prepared the same years.

At the request from the National Codex Alimentarius Committee the reports have been translated to English and contemporarily rewritten into one single report with references to relevant literature up to date.

The blow-fly infestation causing damage to drying fish is a severe problem which has partly been overcome by stopping the production of stockfish in warm time of year. Fish dried in the late spring or in the autumn may nevertheless be spoiled when sudden changes in climate make the conditions favourable for blow-fly activities. A stop in production represents in itself a loss to the fishermen and to the stockfish producers.

From 1958 on the Norwegian Fisheries Research Institute started experiments to find a method for the control of blow-fly infestation. As shown by Sømme and Gjessing¹ 70 to 90 per cent of the blow-flies trapped were specimens of *Calliphora uralensis*. Earlier investigations by Soot-Ryen² showed *Calliphora vicina* and *C. uralensis* to be the most predominant species.

Insecticidal treatments of fish were performed as field experiments in 1960 and 1961 by Sømme and Gjessing¹ where 5 different insecticides were compared. Both pyrethrum, ronnel, and dipterex were found effective as protecting agents.

Pyrethrum was chosen for the further experiments in 1963 and 1964 because of its low toxicity to mammals as reported in the literature.

Investigations on the acute toxicity of pyrethrins by oral, parenteral or topical administration have been reviewed by Jolly and Waterhouse³, stating that the active principles of pyrethrum are rapidly detoxified by hydrolysis in the gastrointestinal tract of the mammal. The metabolism of pyrethrins in mammals has lately been studied by Casida et al.⁴ and Elliott et al.⁵, showing that mammalian systems can quickly and extensively degrade pyrethroids, evidently into less toxic products. The lack of detoxifying metabolic systems renders pyrethrum a selective toxicant to a variety of insect species. Moore⁶ points

308442

R 3886

at the wide margin between the effective dosage on insects and the dosage that might be toxic to man, referring to Dr. A.J. Lehman (Division of Pharmacology, U.S. Food and Drug Administration) who states pyrethrins to be among safest of insecticides.

Materials and methods

Raw material: Fish species and the quantities involved are shown in Table 1.

Table 1. Survey of the experiments.

Experiment No.	Place	Fish specie	Quantity of fish treated kg	Treatment	Pyrethrins in dip or spray %
1	Godøy at Ålesund	tusk	21.400	dip	0.106-0.12
2	Hammerfest	cod haddock	3.600 10.000	dip	0.12
3	Stamsund, Lofoten	cod saihte haddock	21.400 3.100 150	dip	0.12
4	Ersfjordbotn at Tromsø	cod saihte haddock	27.000 9.800 2.800	dip	0.12
5	Gjesvær, Finnmark	cod saihte	6.700 8.000	dip dip or spray	0.12 0.06-0.24

In addition to the figured quantities of fish some 5000 kg untreated fish served as references.

Pyrethrum emulsions: Formulations synergized 1:10, 1:5 or 1:2 by piperonyl butoxide or 1:10 by synepirin were used.

Treatment procedures: Batchwise dipping of fish for 30 seconds was performed in a 200 l dip usually containing 0.12 per cent of pyrethrins. As for Experiment 3, a portion of 53 g pyrethrins was added to the dip for every 5000 kg of fish treated in order to compensate an expected loss. Treated as well as untreated fish were hung on wooden frames in the open air for drying. Spray treatment with emulsions containing 0.06, 0.12 or 0.24 per cent pyrethrins was carried out after the fish had been hung.

Meteorological data as observed by the Norwegian Meteorological Institute at the nearest stations to the experimental places are given in Table 2.

Table 2. Meteorological data.

Experiment No.	Month	Mean temperature °C	Mean rel. humidity %	Mean wind velocity Beaufort	Monthly rain-fall mm
1	May	9.4	77	2.7	40
	June	11.7	82	3.1	18
	July	11.5	80	3.1	72
2	June	7.1	74	2.7	39
	July	9.4	77	2.3	37
	August	11.3	75	2.3	47
3	July	12.4	70	2.9	156
	August	12.1	80	3.0	123
	September	7.9	84	3.4	226
4	July	12.2	78	1.7	76
	August	10.2	85	1.4	60
	September	5.8	86	1.7	104
5	August	9.6	93	2.6	55
	September	6.4	91	3.0	49
	October	4.9	92	2.4	90

Judgement of the dried fish: Treated and untreated fish were judged by trained public sorters with respect to the degree of blow-fly damage.

Analyses: The determination of residual pyrethrins was undertaken by Avebury Research Laboratories Ltd., England, using a bio-assay method developed by Baker⁷. To prepare a sample, usually 4 fishes were ground together and well mixed. Extraction of samples before bio-assay was performed by Dr. G. Ferguson, London. Piperonyl butoxide residues were determined by Cooper McDougall & Robertson, England, using thin layer chromatography.

Results and discussion

It was repeatedly observed that the blow-flies avoided the treated fish. Casually there was not a single blow-fly to be seen on batches of treated fish, whereas neighbouring batches of untreated fish were overcrowded. Similar observations have been reported by other investigators^{1, 8, 9, 10}.

The repellent effect lasted for several days after treatment, but as it faded away, egg deposits also occurred on treated fish. The further development to maggots will largely depend on weather. When drying conditions are good the initial 3 weeks from hanging of fish are the most critical regarding the risk of blow-fly damage. As the fish gradually dries up and the surface grows hard the fish becomes less attractive to the flies, and deposited eggs may not develop to maggots for lack of moisture. In calm weather, low tempera-

ture, rain, and high relative humidity it may take twice the time before the fish is safe with respect to blow-fly damage.

The meteorological data given as mean values in Table 2 do not reflect the variations in weather conditions the first critical period, so some comments have to be added for the understanding of the results in Table 3.

Regarding Experiment 2 the weather conditions were unfit for blow-fly activities, and untreated as well as treated fish were unexposed to any infestation. Most of the fish from Experiment 1 was hung at a time of heavy rainfall, and in the subsequent period there was some rain. It is therefore to be assumed that pyrethrum deposits on the fish to some extent were washed away. During the initial drying periods of the experiments 3 and 4, weather was changeful with respect both to rain, temperature, and wind velocity. As regards Experiment 5 weather must be characterized as calm with high relative humidity.

Some other variable factors that may have influenced the results ought to be mentioned, as for instance fish quality at start, individual fish weight, and drying facilities. As observed in Experiment 1 drying facilities placed above sea proved advantageous to those placed above soil.

So, when studying Table 3, a horizontal rather than a vertical comparison should be made.

Table 3. Blow-fly damage among treated and untreated fish.

Experiment No.	Fish specie	Treatment	Pyrethrins in dip or spray %	Pyrethrins: pip.butoxide ratio	% damage among treated fish	untreated fish
1	tusk	dip	0.106	1:10	15.0	30.0
	tusk		0.12		21.8	55.5
2	cod	dip	0.12	1:10	0.0	0.0
	haddock				0.0	0.0
3	saithe	dip	0.12	1:10	13.9	38.6
	cod				14.8	52.4
	cod				23.3	100.0
4	haddock	dip	0.12) 1:5	5.4	10.1
	cod			6.5	28.1	
	saithe			1:2	0.5) 30.9
	saithe			1:10	0.6	
5	saithe) spray	0.24) 1:10	2.8) 59.2
	saithe		0.12		3.6	
	saithe) dip	0.06	1:10	10.2	
	saithe		1:10	0.2		
	saithe		1:10 by synepirin	1.7		

The figures demonstrate that the per cent damage in every single case is lower in treated than in the corresponding reference of untreated fish. Treatment has, however, not implied a complete protection, but the effect is pronounced in most cases. This is in good correlation with the findings of Sømme and Gjessing¹ and McLellan⁸ regarding pyrethrum treatment of drying, unsalted fish. Morris and Andrews⁹, on the other hand, found a 0.125 per cent pyrethrum emulsion to be completely effective in preventing blow-fly infestations of light-salted fish. A pyrethrum concentration as low as about 0.02 per cent proved satisfactory when treating dried fish against beetle infestations as reported by McLellan¹¹ and Green¹². The latter found 0.16 per cent pyrethrins in the dip to be efficient in preventing blow-fly infestations, which were also markedly reduced at lower concentrations of pyrethrins.

Using the pyrethrins concentrations 0.12 per cent in dip, 0.24, 0.12, or 0.06 in spray the respective figures for blow-fly damage were 0.2, 2.8, 3.6, and 10.2 per cent, compared with 59.2 per cent for the reference (Table 3). The interpretation of these results should be that dipping is the more effective treatment method. On the other hand, spraying is the more rapid one, and handling can be done whenever needed after the fish has been hung for drying. Later experience has, however, shown that spraying must be very carefully executed if good results are to be obtained. Furthermore it is to be concluded that 0.12 per cent pyrethrins is an adequate concentration in dip or spray.

As has also been observed by McLellan¹¹, a reduction of the piperonyl butoxide:pyrethrins ratio from 10:1 to 2:1, caused no apparent loss of effectiveness (Table 3, Experiment 4).

Table 4. Blow-fly damage among fish treated in freshly prepared and in "exhausted" dip.

Dip	No. of control fish	No. of fish infested	% damage
Freshly prepared	166	20	12.0
"Exhausted"	159	43	27.0

It was assumed that successive dipping of fish would render the dip exhausted, and apparently this is the case according to Table 4. The findings in Table 7 point in the same direction, omitting Experiment 3 where portions of pyrethrum were added at intervals in order to refresh the dip.

"Residues" in untreated fish, determined as pyrethrins varied from 0.0 to 3.6 ppm as shown in Table 5. It should be noticed that sample number 5 contains 1.2-2.7 ppm "pyrethrins" but has got no piperonyl butoxide. Obviously

the bio-assay employed does not distinguish between pyrethrins and other substances acting as toxicants upon the test organism (Aedes aegypti larvae).

Table 5. Residue analysis of pyrethrins ^{a)} and piperonyl butoxide in untreated fish.

Experiment No.	Sample No.	Weeks from start experiment to analysis	Fish specie	Residues, ppm	
				"pyrethrins"	pip.butoxide
1	1	10	tusk	0.00	0
	2			0.00	
	3			0.00	
2	4	12	haddock	0.00	0
	5		cod	1.20-2.70	
	6	16	sample No.5 reanal.	0.85	
	7		cod	0.70	
3	8	16	cod	1.10-3.60	
	9		saithe	0.50	
4	10	15	haddock	0.03	
5	11	14	saithe	0.20	

a) i.e. recorded toxic effect on bio-assay corresponding to the quoted figures of pyrethrins.

Pyrethrins residues in treated fish varied from 0.10 to 11.3 ppm (Table 6). The figures represent pyrethrins plus other biological active substances. When the established blank values in Table 8 are taken into account, 31 analyses out of 45 show contents of "true" pyrethrins below the Norwegian statutory limit of 3 ppm, whereas 3 analyses exceed 5 ppm.

Table 6. Pyrethrins and piperonyl butoxide residues in treated fish.

Experiment No.	Sample No.	Weeks from treatment to analysis	Fish specie	Treat-ment	Pyrethrins in dip or spray %	Pyrethrins: pip.but. ratio	Residues, ppm				
							pyrethrins	pip.but.			
1	1	10	tusk	dip	0.106	1:10	1.75				
	2						1.31				
	3						1.85				
	4						3.18	30-50			
	5						3.20	30-50			
	6						1.68	30-50			
	7	0.12			2.60						
	8				2.32						
	9				1.75						
	10	33			0.106		1.58				
	11						1.38				
	12						2.78				
	13						1.80				
14	0.90		about 50								
2	15	12	cod			0.40	about 50				
	16				1.55	about 50					
	17		haddock		1.50	about 50					
3	18	28				3.35					
	19	16	saithe	dip	0.12	1:10	5.00				
	20						4.40				
	21						6.40				
	22		cod				3.80				
	23						6.40				
	24						5.60				
	25		15	saithe			dip	0.12	1:10	3.90	
26	2.90										
27	4.80										
28	3.70										
29	6.10										
30	2.70										
31	3.10										
32	4.60										
4	33	14	cod	dip	0.12	1:10	0.10				
	34						0.10				
	35	15	haddock				1:5	0.50			
	36							2.80			
	37							8.00			
	38	14	saithe				spray	0.06	1:10	0.80	
	39						0.12	0.60			
	40						0.24	1.50			
	41						1:10 by synepirin			1.10	
	42							3.70			
43	dip			0.12	11.30						
44	1:10			2.60							
45	13	cod		2.10							

Table 7. Pyrethrins residues in fish from the first and the second half of treated fish parcel.

Experiment No.	Pyrethrins residues, ppm	
	Fish from the first half of treated parcel	Fish from the second half of treated parcel
1	2.51	1.76
2	1.23	0.95
3	5.27	5.27
4	3.68	3.34

Table 8. Pyrethrins residues in relation to fish species.

Fish specie	Mean pyrethrins residues, ppm		
	Treated fish	Untreated fish	Net value
Tusk	2.09	0.00	2.09
Haddock	2.95	0.02	2.93
Saithe	3.62	0.35	3.27
Cod	3.16	1.46	1.70

On the basis of the Tables 5 and 6 mean pyrethrins residues in the different fish species are estimated in Table 8.

Few data concerning residues of pyrethrins have been published. According to McLellan⁸ "the general acceptance of its safety has led residue analysts to by-pass pyrethrum."

The figures quoted in the Tables 6 and 9 vary over a wide range. As one reason for this should be mentioned that the fish involved is not equally exposed to sunlight and rain, which cause degradation and loss of pyrethrins.

Table 9. Data on pyrethrins residues as reported in literature.

Pyrethrins in dip %	Range of residues ppm	Referance	Method
0.02	1	Sömme and Gjessing ¹	Chemical method ^{a)}
0.04	1-6		
0.12	1-8		
0.24	20-30		
0.125	0.23-0.27	Morris and Andrews ⁹	Bio-assay (Baker ⁷)
0.125	0.7-1.2		Gas chromatography (Head ¹⁴)
0.25	4.34-4.87		Bio-assay (Baker ⁷)
0.25	2.5-5.8		Gas chromatography (Head ¹⁴)
0.0625	0.056-0.0661	Moore ¹³	Gas chromatography (Bruce ^{b)})

a) Details are lacking.

b) Unpublished until April 1972.

The results may furthermore depend on the method used for determination. The chemical method is regarded as unreliable when micrograms of pyrethrins are to be analysed¹. Bio-assay does not give the true content of pyrethrins as positive values (up to 3.6 ppm) were found in untreated fish (Table 5). Head¹⁴ says about his gas chromatographic method: "The determination of residues of "pyrethrins" on crops and foodstuffs has to date only been partially successful, due to the lack of a suitable quantitative clean up procedure." We do not know the progress that might have been achieved regarding gas chromatography of pyrethrins since then. The quoted figures of Moore¹³ (Table 5) are extremely low and can hardly be considered representative for pyrethrum residues in treated fish as they are based on 4 replicates only.

Summary

Some 114000 kg of tusk, saithe, haddock, and cod have been treated with pyrethrum emulsions for the protection from blow-fly infestation. In addition about 5000 kg of untreated fish served as references.

Though a complete protection was not obtained the blow-fly damage was markedly reduced in treated fish as compared with untreated fish.

An exhaustion of the dip was shown to take place as demonstrated by increasing blow-fly damage and decreasing pyrethrum residues regarding fish from the second half of the treated fish parcel.

As has also been observed by McLellan¹¹ a reduction of the piperonyl butoxide:pyrethrins ratio from 10:1 to 2:1 caused no apparent loss of effectiveness.

Dipping proved slightly more effective than spraying. On the other hand spraying is the less laborious procedure, but has to be carefully executed.

Regarding residues of "true" pyrethrins in treated fish 31 analyses out of 45 showed contents below the Norwegian statutory limit of 3 ppm, whereas 3 analyses exceeded 5 ppm.

On the basis of the effect achieved and the levels of pyrethrum residues 0.12 per cent pyrethrins in dip or spray has been found an adequate concentration.

Bergen, April 1973

References

1. Sømme L. and Gjessing E.T.: Insecticides for protection against blow-flies in the stockfish industry. *Pyrethrum Post* 7 (1), 3-7, 1963.
2. Soot-Ryen T.: Makkflueundersøkelsene. Aarsber. vedk. Norges Fiskerier, hefte 1, 1925 (in Norwegian).
3. Jolly D.W. and Waterhouse C.E.: The mammalian toxicity of pyrethrum. Report issued by the Huntingdon Research Centre, Huntingdon, England, 1962.
4. Casida J.E., Kimmel E.C., Elliott M., and Janes N.F.: Oxidative metabolism of pyrethrins in mammals. *Pyrethrum Post* 11 (2), 58-62, 1971.
5. Elliott, M., Janes N.F., Casida J.E., and Kimmel E.C.: Mammalian metabolism of pyrethroids. *Pyrethrums Post* 11 (3), 94-103, 1972.
6. Moore J.B.: The Ribicoff Committee. *Pyrethrum Post* 7 (4), 15-17, 1964.
7. Baker A.H.: A biological method for the quantitative determination of pyrethrum residues in dried fish. *Pyrethrum Post* 7 (3), 34-40, 1964.
8. McLellan R.H.: The use of a pyrethrum dip as protection for drying fish in Uganda. *Pyrethrum Post* 7 (1), 8-10, 1963.
9. Morris R.F., and Andrews D.: Investigations into the use of pyrethrum and other insecticides for the control of the blowfly, *Calliphora terraenovae* (Macq.), infesting light-salted cod fish in Newfoundland. *Pyrethrum Post* 9 (4), 9-12, 1968.
10. Proctor D.L.: The protection of smoke-dried fresh-water fish from insect damage during storage in Zambia. *Pyrethrum Post* 11 (4), 144-151, 1972.
11. McLellan R.H.; A pyrethrum dipping treatment to protect dried fish from beetle infestation. *Pyrethrum Post* 7 (3), 30-33, 1964.
12. Green A.A.: The protection of dried sea-fish from infestation by *Dermestes frischii*. *Pyrethrum Post* 9 (2), 24-33, 1967.
13. Moore J.B.: Terminal residues of pyrethrin-type insecticides and their synergists in foodstuffs. *Pyrethrum Post* 11 (3), 106-110, 1972.
14. Head S.W.: The quantitative determination of pyrethrins by gas-liquid chromatography, Part I: Detection by electron capture. *Pyrethrum Post* 8 (4), 3-7, 1966.