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Laboratory and Field Evaluation of the Molluscicidal Properties of *Phytolacca dodecandra*

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Dried berries of endod (Phytolacca dodecandra) *(known also as soapberry) are widely used in Ethiopia instead of soap for laundering clothes. It was observed that in natural bodies of water where* endod *had been used there was a high mortality of snails. Subsequently, the molluscicidal potencies of various parts of male and female* endod *plants were determined and the berries were found to be the most potent. The potency of* endod *remained stable over a wide range of temperatures and pH values, in the presence of various concentrations of river-bed mud and after ultraviolet irradiation of solutions. The toxicity of* endod *to mammals and plants has been shown to be very low. Its toxicity to snail eggs also is low but it has been shown that this difficulty can be overcome in the field by repeated treatments.* Endod *kills leeches and schistosome cercariae and miracidia at very low concentrations. Comparative tests with* endod *and several standard molluscicides have given encouraging results.*

Being a natural product, endod *could become a cheap and effective means of controlling schistosomiasis in certain areas since, under suitable climatic conditions, the plant grows rapidly and bears fruit twice a year.*

The molluscicidal properties of *Phytolacca dodecandra* were first observed during a study of the distribution and ecology of schistosomiasis-transmitting snails in the town of Adwa in the northern part of Ethiopia about 800 km from Addis Ababa (Lemma, 1965a). It was noticed that in places along rivers or streams where people washed clothes, there were comparatively more dead snails than there were in adjacent areas, whether upstream or downstream from these washing places. Subsequently, it was shown that a preparation of *P. dodecandra*, widely used in Ethiopia instead of soap for laundering clothes, possesses molluscicidal properties.

P. dodecandra (L'Herit) (synonyms: *P. abyssinica* Hoffm., *Pircunia abyssinica* Moq.), a member of the Phytolaccaceae, is known in Ethiopia as *endod* and elsewhere may be referred to as soapberry. The distribution of this plant is East, West, Central and South Africa and parts of South America and Asia (Dalziel, 1963). *Endod* has small berries which when dried, powdered and placed in water, yield a foaming detergent solution. In Ethiopia, *endod* exists as two main varieties, *arabe* with pinkish, and *ahiyo* with greyish, berries. *Arabe* (possibly meaning " from Arabia ") has more powerful detergent properties than *ahiyo* (meaning " donkey " and implying that it is less active than the other type). The plant is a climber with hanging branches; it grows very rapidly, reaching a height of up to 10 m but the average height is 2 m–3 m (Fig. 1). Under favourable climatic conditions in Ethiopia the plant bears fruit twice a year, in January and July.

Although some substances of vegetable origin are known to be lethal to snails, little investigation has been carried out in this field. Mozley (1939, 1952) tested a number of different vegetable substances against schistosome-transmitting snails, and listed the fruits of *Balanites aegyptiaca* (Del.) (Zygophyllaceae), *Sapindus saponaria* (L.) (Sapindaceae), and *Swartzia madagascariensis* (Desv.) (Leguminosae) among the most promising vegetable molluscicides. The active ingredients in the fruits of these plants are known to be saponins. Msangi & Zeller (personal communication, 1965), working in Tanzania, further studied the fruits of *Sapindus saponaria*, confirming Mozley's original observations and recommending the use of this plant for snail control in

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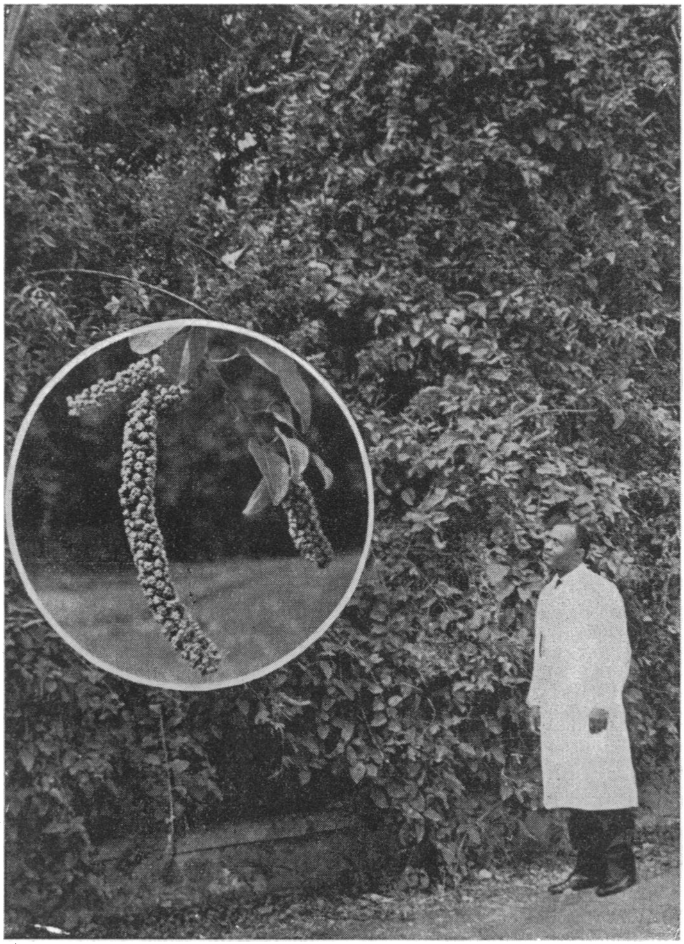
FIG. 1
AN *ENDOD* PLANT (*PHYTOLACCA DODECANDRA*) WITH (INSET) FRUITS AND LEAVES



small bodies of water. In the UAR, Sherif & El-Sawy (1969) reported the possible use of the herb known locally as *damsissa* for snail control. In all these studies, no quantitative approach was made but, in general, large quantities of the plants had to be used to show any molluscicidal effect.[1]

A preliminary report on the molluscicidal properties of *endod* has already been published (Lemma, 1965b). The present report is a summary of studies conducted primarily in the laboratory, but also on a limited scale in the field, on the quantitative evaluation of the molluscicidal properties of *endod.*

[1] In the case of *damsissa*, more than 1000 ppm is needed to produce a molluscicidal effect.

MATERIALS AND METHODS

Except where otherwise stated, standard test procedures (WHO Expert Committee on Bilharziasis, 1960; *Bull. Wld Hlth Org.*, 1965) were followed throughout the study covered in this report.

Source and size of snails used in toxicity tests

The following species and populations of snails were used in toxicity tests:

(1) Freshly collected *Bulinus (B.) truncatus sericinus* (Jickeli) (hereinafter referred to as *B. t. sericinus*) from Lake Hora Abijata, a relatively large crater lake near the town of Debre Zeit about 45 km south of Addis Ababa.

(2) *Biomphalaria pfeifferi ruppellii* (Dunker) from Lake Aba Samuel (about 25 km south of Addis Ababa) and from the irrigation canals at the HVA Sugar Estate in Wonji-Shoa (about 110 km south of Addis Ababa).

(3) *Lymnaea natalensis* (Krauss) obtained from Debre Zeit.

(4) *Physa acuta* (Drapavnaud) from the irrigation canals in Wonji-Shoa.

As far as possible, snails of uniform size were used. The average shell diameter of the *B. p. ruppellii* was 7.0 mm, the average height of shell for *B. t. sericinus* was 6.7 mm, for *L. natalensis* 11.5 mm and for *P. acuta*, 6.5 mm.

Sources of endod

Most of the *endod* used in the present study was bought from the market at an average cost of US $0.12 (Eth. $0.30) per kg. This price was, however, highly variable, depending upon the place and season, and ranged from US $0.06 to US $0.20 per kg. Most of the material used in the present study was collected in, or bought from areas, at or above 2000 m above sea level.

Preparation of endod *solutions for molluscicidal testing*

The ripe berries of *endod* were harvested and allowed to dry in the sun for 3 or 4 days. The dried fruits were ground to a fine powder manually or with an electric grinder. The same procedure was used also to prepare powders of different parts of the *endod* plant. Weighed amounts of powdered *endod* were then added to different volumes of standard reference water (prepared as described by Freeman, 1953) to make up the desired weight/volume concentrations, expressed in terms of parts per million (ppm).

Other molluscicides

Niclosamide ethanolamine salt (Bayluscide, 70% wettable powder), copper sulfate crystals, sodium pentachlorophenate (NaPCP) pellets, and *N*-tritylmorpholine (18.2% w/v emulsifiable concentrate) were used in the comparative study.

Number of replicates, determination of lethal concentrations and exposure and recovery periods in each test.

The number of replicates of each test was 4 and the average number of snails per test was 10. The average of the 4 replicates was taken as the final result.

The lethal concentrations of molluscicides and their 95% confidence limits, where quoted below, were determined according to the method of Litchfield & Wilcoxon (1949).

Except when otherwise stated, 24-hour exposure and recovery periods were used in all the tests.

RESULTS

Comparison of the molluscicidal properties of the different parts of male and female endod *plants*

Since only the *endod* berries were originally seen to have molluscicidal properties, it was thought desirable to make a comparative evaluation of the potencies of various parts of the plant so that the most active part could be identified. Therefore, comparisons of the molluscicidal properties of different parts of the female and male plants, after they had been dried and reduced to powder, were made. For this, the following parts of the plants were used: ripe pinkish berries, unripe greenish berries, flowers, leaves, stem, bark and root.

The results (Table 1) show that the ripe berries are the most active part of the plant. Comparison of different parts of male and female plants reveal that while the potency of the flowers is about the same, the leaves, stem and bark of the male plant seem to have higher molluscicidal activity than those of the female plant.

In order to determine if any seasonal variation occurs in the concentration of the active principles in the *endod* plant, samples of the bark, leaves, stem, roots, flowers and berries were taken from the same plants every month for a 12-month period and the molluscicidal potency of each sample was determined. The results showed no differences due to seasonal variation.

Since the berries were found to be the most active part of the *endod* plant, the possibility that berries from plants growing at various altitudes and under different climatic conditions might show differences in their molluscicidal potencies was studied. *Endod* berries collected from 10 different parts of Ethiopia were used for this purpose. The results (Table 2)

TABLE 1

COMPARISON OF THE MOLLUSCICIDAL POTENCIES[a] OF DIFFERENT PARTS OF FEMALE AND MALE *ENDOD* PLANTS

Parts of *endod* plant tested	Percentage mortality of *B. t. sericinus* at following concentrations (ppm) of *endod* from female (F) and male (M) plants														
	1 000		500		250		100		50		25		15		0 (control)
	F	M	F	M	F	M	F	M	F	M	F	M	F	M	
Ripe berries	100	—	100	—	100	—	100	—	100	—	100	—	25	—	0
Green berries	100	—	100	—	100	—	100	—	80	—	40	—	—	—	0
Flowers	100	100	100	100	100	100	100	95	45	55	0	0	—	—	0
Leaves	30	100	0	100	0	100	0	80	0	40	0	0	—	—	0
Stem	0	80	0	60	0	5	0	0	0	0	0	0	—	—	0
Bark	0	80	0	60	0	0	0	0	0	0	0	0	—	—	0
Root	0	0	0	0	0	0	0	0	0	0	0	0	—	—	0

TABLE 2

COMPARATIVE MOLLUSCICIDAL POTENCIES OF *ENDOD* FROM DIFFERENT PARTS OF ETHIOPIA

Origin of *endod*		Percentage mortality of *B. t. sericinus* at following concentrations (ppm) of *endod*							
Town	Distance and direction from Addis Ababa, and altitude (m)	40	30	25	20	15	10	5	0 (control)
Adwa	800 km N; 1800 m	100	100	100	80	60	10	0	0
Dessie	400 km NE; 2703 m	100	100	90	60	20	0	0	0
Holeta I	40 km W; 2500 m	100	100	100	90	60	10	0	0
Holeta II	45 km W; 2450 m	100	100	100	70	10	0	0	0
Addis Ababa (Merkato v)	2355 m	100	95	80	70	30	10	0	0
Harar[a]	500 km E; 1800 m	100	100	*100*	*80*	*40*	*10*	0	0
Menagesh	30 km W; 2520 m	100	100	80	50	30	10	0	0
Debre Libanos	80 km N; 2300 m	100	100	100	100	85	35	20	0
Ginchi	90 km W; 2500 m	100	100	100	70	50	10	0	0
Entoto	6 km N; 2600 m	100	100	100	90	60	40	10	0

[a] LC_{50} with 95 % confidence limits = 14.3 (17.6–11.6); LC_{90} with 95 % confidence limits = 21.6 (29.2–16.0).

show no significant differences in their potencies. A comparison of the molluscicidal potencies of the *arabe* and *ahiyo* varieties of *endod* also showed no differences.

Comparative susceptibility of different species of snails to the molluscicidal action of endod *and some other molluscicides*

Since it is known that different species of snails vary in their susceptibility to different molluscicides, it was necessary to determine their comparative susceptibility to the action of the berries of *endod*. From the results presented in Table 3, it appears that there are some significant differences in the susceptibility of the snails tested to several molluscicides during various exposure times. For example, *P. acuta* was killed by *endod* at a concentration of 26 ppm whereas 100 ppm were required to kill *B. p. ruppellii* during 6-hour exposures. However, when the exposure period was increased to 24 hours,

TABLE 3

COMPARATIVE SUSCEPTIBILITY OF DIFFERENT SPECIES OF SNAILS TO VARIOUS MOLLUSCICIDES

Molluscicide	Exposure period (hours)	LC_{90} (ppm) with 95 % confidence limits for following species of snails			
		B. p. ruppellii	*B. t. sericinus*	*L. natalensis*	*P. acuta*
Endod	6	102.7 (140–75)	56 (94–34)	33.4 (40–28)	26.0 (44–17)
	24	25.0 (32–20)	17.6 (22–14)	28.9 (75–11)	13.8 (18–10)
N-tritylmorpholine	6	—	0.091 (0.167–0.051)	—	25.8 (31–23)
	24	—	0.078 (0.13–0.046)	—	4.73 (8–3)
Copper sulfate	6	29.9 (40–22)	11.2 (20–6)	—	—
	24	4.9 (8–3)	1.5 (2.8–0.8)	—	—
Niclosamide ethanolamine salt	6	0.23 (0.5–0.1)	0.59 (1.3–0.3)	—	0.55 (0.9–0.3)
	24	0.053 (0.1–0.03)	0.072 (0.1–0.05)	—	0.199 (0.3–0.1)

each species died with an LC_{90} below 30 ppm and no significant difference in the susceptibility of the four different species of snails to the action of *endod* was observed. *P. acuta*, on the other hand, was about 260 times more resistant than *B. t. sericinus* to *N*-tritylmorpholine during a 6-hour exposure, and about 60 times more so during a 24-hour exposure. *B. p. ruppellii* was about 3 times more resistant than *B. t. sericinus* to copper sulfate during both 6- and 24-hour exposures. *B. t. sericinus* and *P. acuta* were both about twice as resistant as *B. p. ruppellii* to niclosamide ethanolamine salt during a 6-hour exposure, but during a 24-hour exposure, *B. t. sericinus* and *B. p. ruppellii* died in about the same concentration whereas about twice that amount was required to obtain the same kill of *P. acuta.*

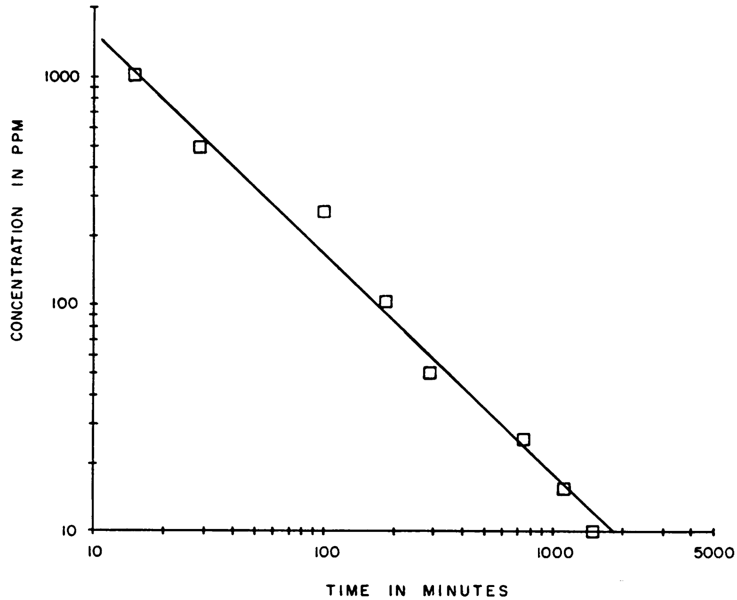
Time-concentration relationships of the molluscicidal activity of endod

In order to investigate further the molluscicidal action of *endod* and also to gather more information on the length of time that applications of *endod* would require to be continued in the field, time–concentration relationships of *endod* on *B. t. ruppellii* were studied (for discussions of the methods, see World Health Organization, 1965). In these experiments, batches of snails were exposed to various concentrations of *endod* and the mean time of death was plotted against the concentration. From the results presented in Fig. 2, it appears that snails take up, and react to, the active principle of *endod* at a constant rate. The steep slope of the curve may be indicative of the fact that the longer is the exposure of snails to *endod*, the more sensitive the snails become to its molluscicidal action.

Comparative toxicities of endod, N-*tritylmorpholine and niclosamide ethanolamine salt for the eggs of* B. t. sericinus

Batches of 10–20 eggs of *B. t. sericinus* at different stages of development were exposed for 24 hours to different concentrations of *endod*, *N*-tritylmorpholine and niclosamide ethanolamine salt. The results showed that whereas the LC_{90} of *endod* against adult snails is about 18 ppm, for eggs it is about 500 ppm. *N*-tritylmorpholine had an LC_{90} of 0.078 ppm for adults and over 15 ppm for eggs. Niclosamide ethanolamine salt, a well-known molluscicide with ovicidal properties, killed both adults and eggs at a concentration of 0.75 ppm. It is

FIG. 2
TIME-CONCENTRATION RELATIONSHIPS [a] OF THE MOLLUSCICIDAL EFFECT OF *ENDOD* ON *B. T. SERICINUS*



[a] Average time to death of batches of *B. t. sericinus* in various concentrations of *endod.*

apparent that *endod*, like *N*-tritylmorpholine, has a very low ovicidal potency by comparison with the toxicity for adults.

Susceptibility of B. p. ruppellii *of various ages to the molluscicidal action of* endod

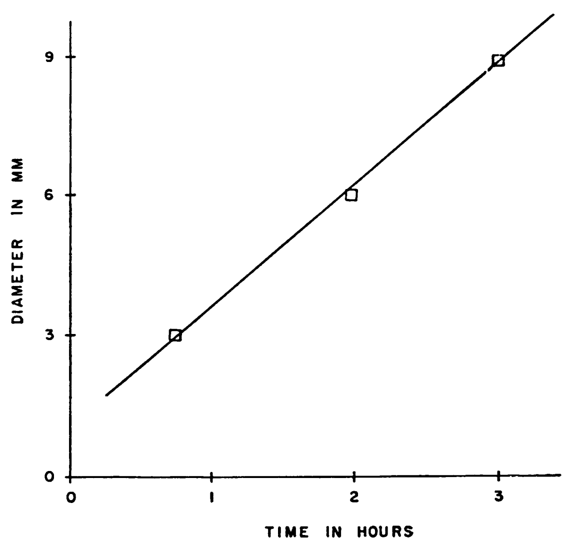
Newly hatched (3 mm diameter), juvenile (6 mm) and adult (9 mm or larger) *B. p. ruppellii* were exposed to 100 ppm of *endod* solution. Newly hatched snails died within 45 minutes, juveniles within 2 hours and adults within 3 hours of exposure (Fig. 3).

Effect of temperature on the molluscicidal potency of endod

Three main points were studied. The first was the effect of water temperature on the molluscicidal action of *endod.* The second and third aspects were the effect of heat on *endod* as powder and in solution.

In using *endod* for snail control, the effect of variations in water temperature during daytime and at night must be considered. Therefore, the mortality rate of snails exposed to different concentrations of *endod* and incubated at temperatures ranging from 5°C to 35°C in 5-degree stages was determined. Exposure periods of 6 hours and recovery periods

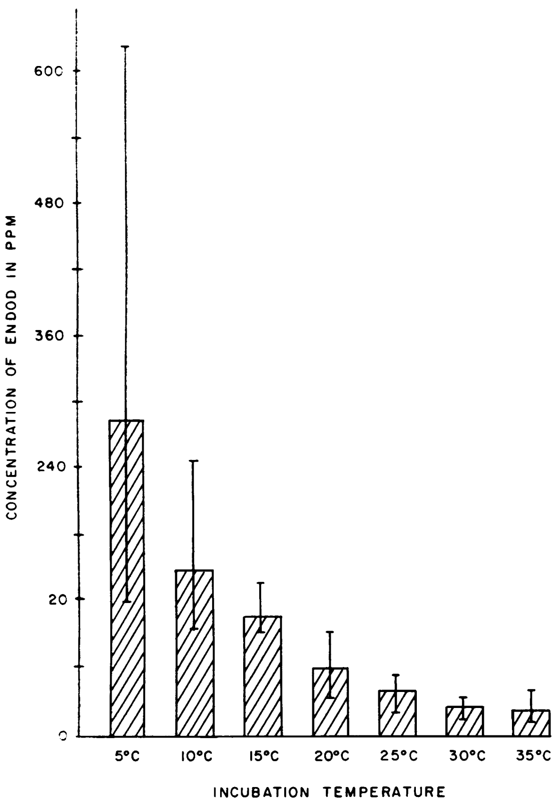
FIG. 3
SUSCEPTIBILITY OF *B. P. RUPPELLII* OF VARIOUS SHELL DIAMETERS (AGES) TO THE MOLLUSCICIDAL EFFECT OF *ENDOD*



[a] Each small square represents the average 100 % mortality time of 30 snails exposed to a 100-ppm solution of *endod.*

FIG. 4

EFFECT OF VARIATION IN INCUBATION TEMPERATURE ON THE MOLLUSCICIDAL POTENCY OF *ENDOD* ON *B. T. SERICINUS* [a]



[a] LC_{90} with 95 % confidence limits.

of 24 hours were used. From the results presented in Fig. 4, it is evident that the molluscicidal action of *endod* is temperature-dependent. At higher water temperatures (30°C–35°C) less than 20 ppm is needed to kill all snails within a 6-hour exposure period, whereas about 3 times that concentration (60 ppm) is needed to produce the same effect at room temperature (20°C±2°C) and about 6 times as much (ca. 250 ppm) at 5°C.

In an attempt to discover more about the nature of the active principle in *endod*, powdered berries were incubated for 24 hours in drying ovens which were regulated to different temperatures ranging from 50°C to 105°C. The molluscicidal potencies of the incubated berries were then determined at room temperature (22°C) using the regular procedure. The results (Table 4) show that the exposure

TABLE 4

MOLLUSCICIDAL POTENCIES OF POWDERED *ENDOD* NCUBATED FOR 24 HOURS AT VARIOUS TEMPERATURES

Incubation temperatures (°C)	Percentage mortality in 40 specimens of *B. t. sericinus* exposed for 6 hours [a] to following concentrations of *endod* (ppm)				
	100	75	50	40	0 (control)
105	0	0	0	0	0
100	70	15	0	0	0
90	90	35	21	0	0
80	100	90	40	10	0
70	100	100	63	34	0
60	100	100	83	83	0
50	100	100	100	46	0
22 (room temperature; control)	100	100	100	52	0

[a] Recovery period of 24 hours.

of berries to temperatures of up to, and including, 50°C for a 24-hour period has no effect on the molluscicidal potency of *endod.* However, when incubated at 60°C and above, the powder progressively loses its molluscicidal potency until at 105°C it is completely inactivated.

In another approach, solutions of *endod* were boiled for different lengths of time and their molluscicidal potencies were then determined. An *endod* solution was also evaporated to dryness by boiling, then reconstituted, and the molluscicidal potency determined. It was found that a reconstituted *endod* solution has the same molluscicidal potency as one prepared from fresh berries.

Effect of pH on the molluscicidal potency of endod

According to Strufe & Gönnert (1962), the pH of the water used for preparing test solutions of various molluscicides influences their activity. It was therefore necessary to determine the pH values at which such effects on the molluscicidal activity of *endod* could be detected. For this purpose, different concentrations of *endod* were prepared in standard reference water previously adjusted (with $NaHCO_3$, NaOH or HCl) to pH values of 3, 4, 5, 6, 7, 8, 9 and 10. Tests using 6-hour exposure and 24-hour recovery periods were made, and the mortality of

TABLE 5

EFFECT OF pH ON THE MOLLUSCICIDAL POTENCY OF *ENDOD*

pH values of standard and adjusted reference water	Percentage mortality of *B. t. sericinus* exposed for 6 hours [a] to following concentrations of *endod* (ppm)					LC$_{50}$ of *endod* (ppm) for *B. t. sericinus* with 95 % confidence limits in standard reference water (pH 7.2)
	100	75	50	40	0 (control)	
3.1	20	15	10	—	0	
4.0	100	100	70	20	0	
5.2	100	100	85	65	0	
6.1	100	100	97	62	0	
7.2 [b]	*100*	*100*	*95*	*57*	*0*	*56.0 (94–34)*
8.1	100	100	72	50	0	
9.1	100	100	75	40	0	
10.1	65	15	0	0	0	

[a] Recovery period of 24 hours.
[b] Standard reference water.

snails was determined in each test. The pH measurements were made only once in this experiment, namely, before the *endod* solution was added; no follow-up measurements were made during the exposure period since they would not be made in field trials of *endod*. The results of this experiment are given in Table 5. Molluscicidal activity was not significantly affected by pH in the range 4–9; at pH 3 and pH 10, however, much of the activity was lost.

Effect of river-bed mud containing organic and inorganic matter on the molluscicidal potency of endod

It is well known that some molluscicides become absorbed into or adsorbed on to organic and inorganic matter in the water to which they are applied (Dobrovolny & Barbosa, 1953; Paulini, 1956). In such situations, it would be necessary to use larger quantities of the molluscicide to make up for the amounts lost by absorption or adsorption. Experiments were therefore designed to determine whether or not the molluscicidal potency of *endod* is affected by the presence of different concentrations of river-bed mud.

Under natural conditions, flowing water has sufficient turbulence to cause rapid mixing of particles in the water. In an attempt to simulate this condition in the laboratory, solutions of *endod* were prepared with water containing different concentrations of river-bed mud. A number of *B. t. sericinus* were then put into beakers containing different concentrations of *endod* and river-bed mud and the beakers were continuously shaken on an electric shaker for 6 hours. For comparison, the effect of river-bed mud on the molluscicidal action of copper sulfate was also determined in the same way (Table 6). The concentration of *endod* required to kill all snails in the presence of 10 000 ppm of river-bed mud was 100 ppm compared with 60 ppm in the absence of mud. Using copper sulfate, 80 ppm were required in the presence of 10 000 ppm of mud in contrast to only 20 ppm without the mud.

Effect of ultraviolet irradiation on the molluscicidal potency of endod

Some molluscicides such as NaPCP are known to be partially inactivated by the ultraviolet portion of sunlight (Hiatt, Haskins & Olivier, 1960). As the stability of molluscicides is very important in field trials, the effect of ultraviolet radiation on the molluscicidal potency of *endod* was determined. Solutions containing different concentrations (40 ppm–100 ppm) of *endod* were exposed to ultraviolet irradiation[1] for periods of 4 and 8 hours at a distance of about 30 cm from the light source. For comparative purposes, the effects of ultraviolet irradiation on the molluscicidal potency of NaPCP were also determined in the same manner (Fig. 5). Ultraviolet irradiation for 4 and 8 hours does not have any

[1] From a Hanovia 30-W ultraviolet lamp, model CH.1.

TABLE 6

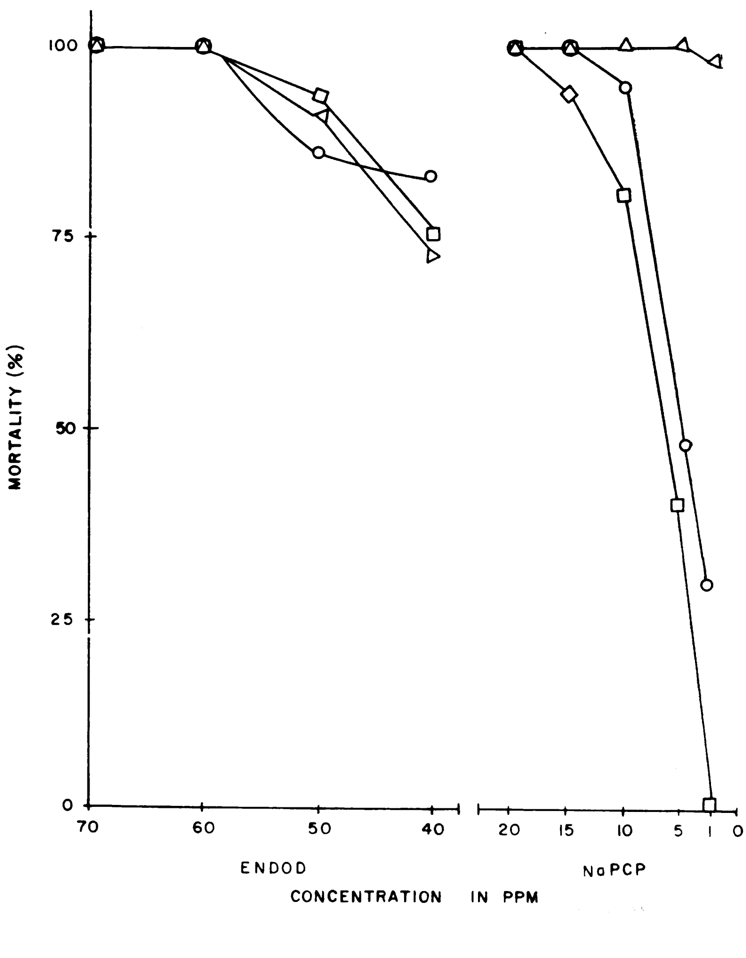
COMPARATIVE EFFECTS OF DIFFERENT CONCENTRATIONS OF RIVER-BED MUD ON THE MOLLUSCICIDAL POTENCIES OF *ENDOD* AND COPPER SULFATE ON *B. T. SERICINUS* FOR 6-HOUR EXPOSURE AND 48-HOUR RECOVERY PERIODS

Concentration of molluscicide (ppm)	Percentage mortality of snails in *endod* with following concentrations of river-bed mud (ppm)[a]				Percentage mortality of snails in copper sulfate with following concentrations of river-bed mud (ppm)[a]			
	10 000	5 000	1 000	0 (control)	10 000	5 000	1 000	0 (control)
100	*100*	100	100	100	100	100	100	100
80	90	*100*	100	100	*100*	100	100	100
60	75	90	*100*	*100*	80	*100*	100	100
50	40	82	65	90	80	92	*100*	100
40	30	57	60	45	87	85	92	100
20	—	—	—	—	52	77	79	*100*
10	—	—	—	—	35	57	52	90
7	—	—	—	—	—	—	—	80

[a] Values in italics indicate lowest concentration of molluscicide to produce 100 % mortality.

FIG. 5

COMPARATIVE EFFECTS OF ULTRAVIOLET IRRADIATION ON THE MOLLUSCICIDAL POTENCIES OF *ENDOD* AND NaPCP



□ Irradiation for 8 hours

○ Irradiation for 4 hours

△ Control

apparent effect on the molluscicidal potency of *endod*. However, there was a progressive and significant reduction on the molluscicidal potency of NaPCP with increased exposure to ultraviolet irradiation.

Storage stability of the molluscicidal property of endod

An important property of a good molluscicide is its stability in storage under different environmental conditions. Therefore, the stability of *endod* in storage at working dilutions (10 ppm–100 ppm), and as whole and powdered berries, was studied.

When *endod* at working dilutions is left at room temperature (22°C), its molluscicidal potency deteriorates over a period of 3 days. However, if such a solution is kept in a refrigerator at 4°C, or if it is sterilized by boiling and then kept at room temperature, its molluscicidal potency remains constant for more than a month.

In order to determine the stability of the molluscicidal potency of *endod* berries after prolonged storage, whole and powdered berries, kept at room temperature (22°C) for 4 years, were tested at 6-monthly intervals. It was found that their molluscicidal potency had not changed.

Solubility of endod *in water*

An experiment was conducted to find the rate at which the active principle from whole berries, crushed berries and finely powdered berries of *endod* is released in water. This was determined under different conditions simulating those in rivers or lakes, with and without filtration (Table 7).

In tests with whole berries, 1-g quantities of dried berries were put into each of 2 flasks containing 1 litre of standard reference water. One of the flasks was left undisturbed and the other was put on an electric shaker revolving at about 130 rev/min. Samples of the solutions were taken at regular intervals and filtered to remove the berries. Serial dilutions of the filtrates were then made and the molluscicidal potency in 6-hour exposures determined. For shaken whole berries it took 48 hours for enough of the active ingredients to dissolve to cause 100% mortality at a concentration of 50 ppm but for unshaken berries 60 hours soaking was necessary. Thus, as would be expected, shaking leads to quicker solution of the active principle.

To determine the rate of diffusion of the active ingredient from crushed berries, 1-g quantities of mortar-crushed berries were put in each of 2 flasks containing 1 litre of standard reference water. One of the flasks was left undisturbed while the contents

TABLE 7

COMPARATIVE DIFFUSION RATES OF THE ACTIVE PRINCIPLE FROM WHOLE BERRIES, CRUSHED BERRIES AND FINELY POWDERED *ENDOD*

Concentration of *endod* (ppm)	Percentage mortality of *B. t. sericinus* exposed for 6 hours [a] to filtered *endod* solutions in which whole berries were soaked or shaken for the following periods (hours)						Percentage mortality of *B. t. sericinus* exposed for 6 hours [a] to filtered and unfiltered solutions of crushed *endod* berries after following extraction periods (min)			Percentage mortality of *B. t. sericinus* exposed for 6 hours [a] to unfiltered solutions of finely powdered *endod* extracted for 30 seconds
	Soaked			Shaken			Filtered		Unfiltered	
	24	36	48	36	48	60	1	5	5	
1 000	60	100	100	70	100	100	100	100	100	100
500	30	100	100	40	100	100	100	100	100	100
250	0	100	100	0	100	100	100	100	100	100
125	0	100	100	0	100	100	100	100	100	100
100	0	100	100	0	100	100	55	100	100	100
75	0	70	100	0	30	100	50	100	100	100
50	0	0	100	0	0	90	42	95	90	100
0	0	0	0	0	0	0	0	0	0	0

[a] Recovery period of 24 hours.

of the other were immediately filtered through No. 1 filter-paper. Serial dilutions of the filtrate and of the unfiltered solution were made at different intervals after adding the crushed berries to the water. Determination of percentage mortality of snails placed in the two solutions made after the first minute of mixing showed that the unfiltered solution was more potent than the filtered one. This seems to be have been due to the removal of the crushed berries from the filtered solution before the active principle had dissolved completely. When samples of the same solution were taken 5 minutes later and filtered, the filtrates had the same potency as the unfiltered solution. The active ingredient appears to dissolve completely in water within 5 minutes of mixing and the suspended fragments of crushed berries have no molluscicidal properties after the first 5 minutes or so.

To determine the rate at which the active principle in finely powdered *endod* berries would dissolve, 1 g of powder was placed in 1 litre of water and within 30 seconds serial dilutions were made. The snail mortality record after a 6-hour exposure showed that even 50 ppm of powdered *endod* berries were enough to kill 100% of the snails.

From these results it appears that the active principle of *endod* is highly water-soluble; powdered or crushed *endod* berries yield an active solution almost immediately after the powder is put in water. Intact whole berries, however, release the active ingredient slowly and this may be an advantage when this molluscicide is used in the field.

Preliminary studies on toxicity of endod

The toxicity of *endod* to mammals, birds, fish, leeches, insects, plants, and to cercariae and miracidia of schistosomes, was determined.

Toxicity of endod *to birds and mammals.* The acute oral toxicity of *endod* to standard laboratory mice and rats, domestic chickens, domestic sheep and rhesus monkeys was quantitatively determined (Table 8). The results show that all these animals have very high tolerance to *endod* and that the concentrations of *endod* used in the field (about 100 ppm) should have no harmful effects on them. A mouse, for example, would have to drink more than 26 000 litres of water treated with 100 ppm of *endod* to obtain an LC_{50} dose. The possible chronic effects of *endod* have, however, yet to be studied.

Skin-irritation tests were performed with a paste made from *endod* placed in large quantities on human volunteers and on the shaved skin of guinea-pigs. No harmful effects were detected. This finding is, of course, supported by the circumstantial evidence that the many people who use high concentrations of *endod* for washing clothes appear to suffer no skin irritation, not even on the hands.

Preliminary phytotoxicity studies. A molluscicide should have no serious effect on crops; the toxicity of *endod* to different kinds of economically important plants was therefore determined. Barley, corn, wheat, millet, beans and peas were grown in pots and treated with different concentrations (100 ppm, 500 ppm and 1000 ppm) of *endod* at different stages of their development. The results showed that *endod* had no effect on the germination and growth rates, or on the apparent physiomorphological condition of these plants. Further tests on the possible chronic effects of *endod* are presently being carried out both in the laboratory and under natural conditions in the field.

Fish toxicity studies. Fish constitutes a major part of the diet in many developing countries and in

TABLE 8
ACUTE ORAL TOXICITY OF ENDOD TO ANIMALS

Test animals	Total no. of animals used	Highest concentration of *endod* tolerated by test animals (g/kg of body-weight)	Lowest concentration of *endod* needed to kill test animals (g/kg of body-weight)	LC_{50} of *endod* (g/kg of body-weight)
Mice	48	2.0	3.25	2.6
Rats	18	1.8	2.5	2.2
Chickens	10	2.2	2.5	—
Sheep	6	6.5	7.8	—
Monkeys	2	2	3	—

TABLE 9

COMPARATIVE TOXICITIES TO FRESHWATER FISHES[a] OF *ENDOD* AND NICLOSAMIDE ETHANOLAMINE SALT

Exposure period (hours)	Percentage mortality of *Tilapia* sp. after exposure to following concentrations of *endod* (ppm)						Percentage mortality of *Tilapia* sp. after exposure to following concentrations of niclosamide (ppm)				
	150	100	75	50	25	0 (control)	1	0.75	0.5	0.25	0 (control)
6	100	100	100	0	0	0	100	100	0	0	0
24	100	100	100	100	70	0	100	100	70	30	0

[a] Using 20 specimens of *Tilapia* sp. in 4 replicates of each test.

some it is also of major importance in the national economy. Thus, from both the public health and the economic point of view, it is important that fishes should not be adversely affected by molluscicidal treatments. Since fishes and snails have, to some extent, a common habitat, the choice of a molluscicide which spares fishes is obvious. Almost all the currently used molluscicides are known to be toxic to fishes.

With this limitation in view, the susceptibility of certain important freshwater fishes (*Tilapia* sp.) to the presence of *endod* was investigated. For comparative purposes, the susceptibility of the same species to niclosamide ethanolamine salt was also determined (Table 9). It was found that the concentrations of both *endod* and niclosamide which kill *Tilapia* sp. are about the same as those needed to kill snails.

Effects on insects. Ethiopians who have traditionally used *endod* for washing clothes believe that clothes so washed will have no lice or ticks remaining on them (Baldrati, 1946). Since this belief has not been proved experimentally, appropriate experiments were performed on lice and mosquito larvae. Clothes containing containing large numbers of lice were thoroughly washed with an emulsion of *endod*, rinsed and dried. Also, mosquito larvae belonging to the species *Culex pipiens fatigans* were suspended in solutions containing different amounts of *endod*, and the number of larvae affected after different periods of exposure was determined. The results showed that clothes washed with *endod* still contained live and active lice and that even concentrations of *endod* as high as 2000 ppm had no effect on mosquito larvae.

Effect of endod *on cercariae and miracidia of schistosomes.* The possibility that *endod* applied to natural bodies of water in concentrations sufficient to kill snails would also kill cercariae and miracidia, thereby contributing to the control of schistosomiasis, was considered. Results of an experiment revealed that all cercariae and miracidia of *Schistosoma haematobium* die within 10 minutes in concentrations of 1000 ppm of *endod* in standard reference water, within 1 hour at 100 ppm and within 2 hours at 50 ppm.

Toxicity of endod *to aquatic leeches.* Aquatic leeches are of tremendous economic importance in Ethiopia and many other parts of the world. In Ethiopia, the species *Lymnotis nilotica* Savigny is a serious nuisance to both cattle and man. Since the habitat of these leeches is commonly the same as that of snails, their susceptibility to *endod*, *N*-tritylmorpholine and niclosamide ethanolamine salt was determined.

The results of the tests (Table 10) show that a concentration of *endod* as low as 4 ppm (compared with about 60 ppm for snails) is sufficient to kill all leeches within 6 hours. Using *N*-tritylmorpholine, 15 ppm were required (compared with less than 0.05 ppm for snails) and the concentration of niclosamide needed to kill both leeches and snails was less than 1 ppm.

Preliminary field trials with endod

After promising results were obtained in laboratory experiments, some limited field trials were conducted. These were carried out in 3 different situations: in the Assam, the small river flowing through the town of Adwa, in Lake Hora Abijata, and in the irrigation canals at the Wonji-Shoa sugar plantation. In the Wonji-Shoa irrigation canals, 2 experiments were performed. The first was to determine the total length of canal which could be made free of snails by

TABLE 10

COMPARATIVE TOXICITIES TO LEECHES[a] OF *ENDOD*, *N*-TRITYLMORPHOLINE AND NICLOSAMIDE ETHANOLAMINE SALT

Concentration of molluscicide (ppm)	Percentage mortality of *L. nilotica* exposed for 6 hours to following molluscicides[b]		
	Endod	*N*-trityl-morpholine	Niclosamide
15	100	*100*	100
12	100	55	100
10	100	0	100
5	100	0	100
4	*100*	0	100
3	55	—	100
2	30	—	100
1	14	—	*100*
0.5	—	—	62
0.25	—	—	30
0 (control)	0	0	0

[a] Using 300 specimens of *Lymnotis nilotica* in 10 replicate tests.

[b] Values in italics indicate lowest concentrations of molluscicide to produce 100 % mortality.

the application of a single treatment of *endod* from a single site. The second was to study the effect of 2 treatments, the first to kill adult snails and the second to kill the young ones hatching from eggs unaffected by the first treatment, on the long-range repopulation rate (due to snails being introduced from other areas).

In all instances, powdered *endod* berries were used. Measured amounts in the form of paste or as highly concentrated (10 000 ppm–100 000 ppm) solutions were dropped into the body of water at regular intervals of time to maintain a concentration of 100 ppm in the entire body of water. In the River Assam, and in a 5-km-long irrigation canal in Wonji-Shoa, this concentration was maintained for 6 hours; in another irrigation canal at Wonji-Shoa a concentration of 50 ppm was maintained for 6 hours. In Lake Hora Abijata, a concentration of 50 ppm was tested over a period of 3 hours. To establish the required concentration in the lake, the approximate volume of water in the first 5 m from the shore along a 200-m test strip was determined. The rate of mixing and diffusion caused by wind action and convection currents in the water was estimated by adding a fluorescent dye to the water and taking samples over a 3-hour period. In this way the initial amount of *endod* required to maintain a concentration of 100 ppm for 3 hours could then be calculated.

Appropriate pretreatment and post-treatment counts of snails were made and the reduction in the snail population due to the molluscicidal action of *endod* was determined. In addition to the natural population of snails, in all except one of the areas where field trials were made (the small irrigation canal in Wonji-Shoa), caged snails were used; appropriate numbers of snails (usually 5 of each species) being placed in pieces of cheesecloth (ca 20 cm × 20 cm) which were firmly tied with string, dropped into the water at various places, and anchored to the shore with string.

The results showed that the performance of *endod* in the River Assam, in Lake Hora Abijata and in the irrigation canals in Wonji-Shoa was very satisfactory. The 5-km-long River Assam, the 200-m strip along the shore of Lake Hora Abijata and the 5-km- and 500-m-long irrigation canals in Wonji-Shoa were all freed of snails by the application of 50 ppm–100 ppm of *endod* during an exposure period of 3–6 hours.

DISCUSSION

For a crude natural product such as *endod*, an LC_{90} of about 20 ppm is noteworthy. It has been shown that this potency remains stable over a wide range of pH values and temperatures, under ultraviolet irradiation and in various concentrations of river-bed mud. The stability of dried *endod* berries in prolonged storage and the slow solution in water of the active principle from the whole berries are also important properties. The main disadvantage of *endod* is that it is not ovicidal at the concentrations which kill adult snails. However, this difficulty can be overcome by repeated treatments, as has been demonstrated in the field.

It has been shown that the acute oral toxicity of *endod* to mammals, birds and plants is very low, and there should, therefore, be no risk to them from the use of *endod* in molluscicidal concentrations in the field. On the other hand, *endod* is lethal to fishes as are all the other presently available molluscicides. From the small number of experiments conducted so far, *endod* does not seem to possess any insecticidal property but is exceedingly effective against leeches. Since leeches are pests of some economic and public health importance, it may be possible to use this natural product in their control. *Endod* is also

TABLE 11

SUMMARY OF FIELD TRIALS WITH *ENDOD*

	River Assam, Adwa	Lake Hora Abijata, Debre Zeit	Irrigation canals at Wonji-Shoa sugar plantation	
			KWR3-KWR8 canal	Drainage canal near O camp on Plot 215
Length of test area	4.5 km	200 m along the shore	5 km	500 m
Approximate flow rate of water or volume treated	25 l/sec	Total volume of water treated was 500 m³	150 l/sec	Total volume of water treated was 260 m³
Pre-treatment estimates of natural populations of snails	Very abundant throughout the test area. In some places more than 200 snails were counted in 5 minutes	Very abundant throughout the test area. In some places over 100 snails were counted in 5 minutes	Very abundant throughout the test area. Average of 20 snails per standard WHO scoop	Very abundant throughout the test area. Average of 20 snails per standard WHO scoop
Species of snails present	*B. p. ruppellii*, *B. t. sericinus*, *Lymnaea* sp.	*B. t. sericinus*	*B. t. ruppellii*, *B. t. sericinus*, *Lymnaea* sp., *Physa* sp.	*B. p. ruppellii*, *B. t. sericinus*, *Lymnaea* sp., *Physa* sp.
Caged snails used	5 of each of the above per cage placed at 100-m intervals	5 *B. t. sericinus* placed at 10-m intervals	5 of each of the above per cage at 100-m intervals	None used
Concentration of *endod* used	100 ppm for 6 hours	100 ppm for 3 hours	70 ppm for 6 hours	50 ppm for 6 hours
Total amount of *endod* used (kg)	54	50	227	13
Climatic conditions during tests	October–November 1966: no rain, average temperature about 23°C, clear water	February–March 1966: no rain, average temperature 20°C, clear water	January–February 1966: no rain, average temperature 22°C, muddy water	October–February 1967–68: no rain, average temperature 22°C, muddy water
Post-treatment counts of snail populations	18 hours after treatment, only the snails in the first 2½ km were dead; 7 days later there were no snails in the entire 4.5 km of test area	24 hours after treatment no live snails or leeches were found	24 hours after treatment no live snails were found in the entire area	24 hours after treatment all except a few *B. p. ruppellii* were dead; the few live snails found were killed on the spot by an application of highly concentrated *endod* (100 000 ppm in paste form)
Comments on other aquatic animals and plants affected by *endod*	Many small fishes and tadpoles were found dead; no water insects were affected; there was no apparent effect on vegetation	No fishes were found dead; all leeches in the treated area were found dead; there was no apparent damage to the vegetation	Some small fishes and tadpoles were dead; neither the weeds in the canal nor the sugar-cane irrigated with the treated water were affected	No fishes present; no apparent effect on the vegetation
Summary of post-treatment examination	About *1 month* later many small snails were observed; they were presumed to have hatched from eggs not affected by the treatment	*6 weeks* later abundant numbers of very small *B. t. sericinus* were observed	*4 weeks* later many small and also a few adult snails were present (presumably the adults were carried down from other parts of the estate)	*4 weeks* later many small snails of different species were present
Second treatment	Not done	Not done	Not done	Done 4 weeks after the first treatment
Duration of absence of snails after second treatment				5 months; after this time, adult snails presumably carried down from upstream were seen establishing colonies

lethal to schistosome cercariae and miracidia in much lower concentrations than those used for the control of snails.

The comparative molluscicidal activity of *endod* against different species of snails in 24-hour exposure periods shows that it has approximately equal potency against all species tested; in a 6-hour exposure, however, *B. p. ruppellii* were more resistant than the other species, *P. acuta* being the most susceptible. In this study, a special attempt was made to use snails of the same size since younger snails are known to be more susceptible to the action of *endod* than the adults are. The differences in susceptibility between different species of snails to various molluscicides, and differences in sensitivity associated with the duration of exposure must be considered when molluscicides are used in field trials.

The information obtained from a study of the time–concentration relationship is valuable since it provides essential information which allows progress from laboratory experiments to field trials. The volume and rate of flow of the body of water are two important factors which determine the quantity of molluscicide to be used. Depending on various local conditions such as length of the working-day, availability of automatic dispensers or other mechanical means of applying molluscicide, an appropriate exposure time can be chosen. Once such a time is selected, the amount of *endod* to be used can be extrapolated from Fig. 2. The preliminary field trials reported in this article were consistent with the laboratory trials.

Investigations into the nature of the active principle in *endod* have been carried out at the suggestion of the World Health Organization and the present author in several laboratories in different parts of the world. Professor W. J. Horton, University of Utah, USA, was the first to attempt such a study; he concluded that the active part of *endod* was a saponigen and traced it to oleanolic acid (W. J. Horton, personal communication, 1966). Studies made by Woldeab Isaac (personal communication, 1967) under the supervision of Professor J. H. Robertson, University College of Dar es Salaam, Tanzania, are in agreement with Professor Horton's conclusions and chemists in the Stanford Research Institute, Calif., USA, have also confirmed them. A more detailed study carried out by Dr K. Jewers, Tropical Products Institute, London, has shown that a freeze-dried aqueous extract was actively mollusci cidal at concentrations between 1.25 ppm and 2.5 ppm (K. Jewers, personal communication, 1968) Extractions of *endod* with other common organic solvents have not yet yielded such an active product but the work is still in progress.

The active principle in *endod* may be a relatively simple molecule that can be easily extracted but if, on the contrary, the extraction procedure proves to be complicated and costly, it may not be economically worth while to extract *endod* berries. In any case, the ultimate goal is to use *endod* in the most convenient and cheapest way so that it is available not only to governmental and private organizations but also to rural communities. *Endod*, being a natural product and easily grown in suitable climatic conditions, could play a very important role in the rural economy of some developing countries. At the same time, farmers and other members of rural communities may be taught to use *endod* for the control of schistosomiasis in their areas.

Both intestinal and urinary schistosomiasis are widely distributed in small foci all over Ethiopia. *S. mansoni* is particularly frequent in the northern highlands of Ethiopia whereas *S. haematobium* is at present limited to low, warm areas such as the Awash and Wabi Shebelle valleys. In some parts of these valleys large-scale agricultural development projects are being undertaken and the prevalence of schistosomiasis is increasing very rapidly. Therefore, the introduction of control measures is essential and the possibility of using *endod* for this purpose is now being explored.

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RÉSUMÉ

ÉVALUATION, AU LABORATOIRE ET SUR LE TERRAIN, DES PROPRIÉTÉS MOLLUSCICIDES DE *PHYTOLACCA DODECANDRA*

En Ethiopie, on constate une mortalité plus élevée parmi les mollusques dont les habitats sont proches des endroits où les riverains lavent le linge en utilisant en guise de savon une poudre de baies de *Phytolacca dodecandra*, plante de la famille des Phytolaccacées, connue dans le pays sous le nom d'*endod*. Le fait a incité l'auteur à étudier quantitativement, au laboratoire et par des essais limités sur le terrain, les propriétés molluscicides de ce végétal.

De toutes les parties de l'*endod*, les baies font preuve de l'activité molluscicide la plus forte: la CL_{90}, exprimée en parties par million, atteint en moyenne 13,8 pour *Physa acuta*, 17,6 pour *Bulinus (B.) truncatus sericinus*, 25,0 pour *Biomphalaria pfeifferi ruppellii* et 28,9 pour *Lymnaea natalensis*, après 24 heures d'exposition et 48 heures de récupération. On ne note aucune variation de la toxicité en fonction de l'origine géographique des fruits et du moment de la récolte.

Les propriétés molluscicides de l'*endod*, de la *N*-tritylmorpholine, du sulfate de cuivre et du sel d'éthanolamine de la niclosamide ont été comparées par exposition de diverses espèces de mollusques pendant 6 et 24 heures. Exposés à l'*endod* pendant 24 heures, tous les vecteurs étudiés sont affectés dans une mesure sensiblement égale et tués par des concentrations inférieures à 30 parties par million. L'activité molluscicide de l'*endod* s'est maintenue à un niveau stable au cours d'une série d'essais pratiqués dans des conditions très diverses de température de l'eau, de pH, de turbidité, ainsi qu'après traitement des solutions du produit par le rayonnement ultraviolet. La conservation pendant 4 ans de l'*endod* sous forme de baies ou de poudre n'altère pas ses propriétés molluscicides et sa solubilité est très satisfaisante.

D'études préliminaires de la toxicité de l'*endod* à l'égard des mammifères et des oiseaux, il ressort que la souris, le rat, le poulet, le mouton et le singe rhésus y sont très peu sensibles, la CL_{50} étant dans tous les cas supérieure à 2 g/kg de poids corporel. Les premiers résultats des tests de phytotoxicité indiquent que l'*endod* n'exerce aucune influence nocive sur la germination, le rythme de croissance et les caractéristiques morphologiques des végétaux. L'*endod* est par ailleurs dépourvu d'activité insecticide et larvicide et sa toxicité pour les poissons est du même ordre que celle du sel d'éthanolamine de la niclosamide. A la concentration de 4 parties par million, il tue les sangsues aquatiques en 6 heures. Au cours d'une expérience, les cercaires et les miracidiums de *Schistosoma haematobium* ont été tués en 10 minutes par 1000 parties par million, en 1 heure par 100 parties par million et en 2 heures par 50 parties par million du produit. Par contre, l'*endod* ne possède qu'un très faible pouvoir ovicide, la CL_{90} en ce qui concerne les œufs de *B. t. sericinus* étant de l'ordre de 500 parties par million.

On a procédé à des essais limités sur le terrain au moyen de pâte ou de solutions très concentrées d'*endod*. Ces préparations ont été déversées dans un cours d'eau, un lac et des canaux d'irrigation en quantités assurant une concentration de 50-100 parties par million pendant 3 à 6 heures. Les premiers résultats ont été très satisfaisants, l'élimination des mollusques ayant été obtenue dans tous les cas.

L'*endod*, produit naturel, facile à cultiver et de croissance rapide, pourrait représenter un moyen efficace et peu coûteux de lutte contre la schistosomiase dans certaines régions.

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