EVALUATION OF FUNGICIDAL PROPERTIES OF LIGNIN ACETIC ACID

Part I—Studies on Fungus Organisms in Petri-plates

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DURING the investigation on utilization of lignin from paper mills' waste (black liquor), we were able to synthesise lignin acetic acid which had been said[1] to have fungicidal and insecticidal properties. Our attention was drawn to the fungal diseases of jute plants which is one of the main agricultural crops of East Pakistan. The fungal diseases sometimes cause havoc to the plants in some places, even about 60.0% damage of the crops has so far been reported[2] and this was gradually becoming a headache to the growers.

The following parasitic fungi of jute plants were included in the present study : (a) *Rhizoctonia sp.*—a soil-borne pathogen causing wilting of jute plants in older age and damping off in seedling period ; (b) *Colletotrichum corchori*—a seed-borne pathogen of jute plants causing anthracnose of jute seedlings ultimately killing the affected plants ; (c) *Macrophomina phaseoli* and (d) *Diplodia corchori* (*Botryo-diplodia*)—organisms causing stem rot and black band diseases to jute plants. Along with the jute pathogens *Memoniella echinata*, a highly cellulose destroying organism isolated from mildewed cotton, was also used to find out the fungicidal properties of the above chemical against it.

Considering the above facts, preliminary sets of experiments were arranged with the above mentioned organisms in petri plates. Experiments conducted with lignin acetic acid included (i) the effect upon the growth of the fungus organism in the laboratory medium (ii) the effect of the treatment on jute seeds and (iii) the effect of treating cotton threads against *Memnoniella.*

Preparation of Medium: Czapeck's agar medium was used for the experiments in the petri-plates. The composition of the medium used was as follows :—

Glucose	.. ..	1.00%
Peptone	.. ..	0.50%
KH_2PO_4	.. ..	0.20%
$MgSO_4$	.. ..	0.50%
Agar	.. ..	2.00%

Thus the prepared medium was found to be most suitable one and used in petri-plate experiments.

Toxic Effect

Some experiments were carried out with the sample of lignin acetic acid* sprayed on the surface of 15 ml. sterilized medium in the petri-plate and the respective organisms cultured at the centre to see the growth of the fungus. Some peculiar behaviour has been observed. In some cases the fungus grew outwards uniformly, in some cases there were quite irregular growth and others had very thin layer. These irregularities and lesser growth of fungus compared to the control was a clear indication of toxic effect of lignin acetic acid upon some fungi. The results of a particular case of *Rhizactonia sp.* tried in this manner are shown in Table I.

Modified Procedure

In view of the above results, the work of quantitative evaluation of fungicidal properties of lignin acetic acid was undertaken and the experimental procedure was modified to some extent in the light of previous observation. Lignin acetic acid was quite soluble in ethanol. In 10 ml. ethanol 1.0 g. of the substance could be dissolved and when added to 100 ml. portion of the above mentioned Czapeck's medium, it was found to remain fairly in solution. Different concentrations (*e.g.*, 0.25%, 0.5% and 1.0% etc.) of the substance were prepared in the medium along with the blank Czapeck's medium in a medium in a separate flask for control experiment. These media were sterilized under 15 lb. pressure for 15 minutes. After proper sterilization the media were plated in petri-plates, 15 ml. in each plate. The test organisms were cultured at one edge of the dishes so that the linear growth after each 24 hours could conveniently be measured (radius in mm.) The results were always compared with the control experiments. Moreover, lignin acetic acid was also compared with a standard fungicide Nomersan (I.C.I. product) as available in the market.

*Lignin acetic acid found insoluble in water.

Table I

Extent of Inhibition of the Growth of Fungus Organism

Organism inoculated—*Rhizoctonia sp.*

Lignin acetic acid samples*	Concn. of the sample in 15 ml. medium (%)	Diameter of the ring in cm.	Mean (cm.)	Difference of diameters of the control and test expt. in cm.	Percentage of inhibition† (%)
Sample I ..	0.5	5.5 5.7	5.6	0.8	12.5
	1.0	5.0 4.8	4.9	1.5	23.4
Sample II ..	0.5	5.6 5.8	5.7	0.7	10.9
	1.0	5.1 4.9	5.0	1.4	21.8
Control ..	nil	6.4	6.4	—	—

*Sample I and II were prepared in aqueous and acid medium respectively.

†Calculation :—

$$\text{Percentage of inhibition} = \frac{D_X}{D_C} \times 100$$

where D_X, the difference of diameters of the control and test experiment and D_C, the diameter of the control experiment.

The aforesaid fungus organisms were studied elaborately under modified conditions with different concentrations of lignin acetic acid so as to ascertain the minimum percentage required to check the growth of the test organisms. Concentrations varied between 0.25—5.0% of lignin acetic acid and the organisms were allowed to grow upto 240 hours in some cases. Results are tabulated in Tables II-VI below.

A graphical representation of the above results is shown in figure I. In the case of *Rhizoctonia* and *Colletotrichum* the effect is very pronounced and in the case of *Macrophomina* and *Botryodiplodia* too there are some definite effects. A very interesting phenomenon was observed with regard to the rate of growth of the test organisms. Almost in all cases the growth of the fungi was checked considerably at the initial stages but after certain period of time the growth was quite rapid. The inhibiting power of lignin acetic acid was found diminished with the time which might be due to some pathogenic decomposition of the acid in the medium. This may throw some light on the utilization of lignin acetic acid. This point has not yet been ascertained.

In view of the results in table VI, lignin acetic acid was tried to use as a controlling agent for *Memononiella* causing damage to the textile goods. One experiment with cotton thread was carried out under favourable conditions. The cotton thread was sprayed with 0.1% lignin acetic acid with spore suspension in the solution and kept in a saturated humid chamber. A similar suspension of spores in distilled water was also sprayed in another set of cotton thread under identical conditions for control. A profuse growth of the organism was noticed on the control set after two to three weeks whereas a feeble growth observed on the other set. It was considered a higher concentration might give better results as seen in the petri-plate experiments (*vide* Table VI). Some of the experiments were also done with different concentrations of lignin acetic acid.

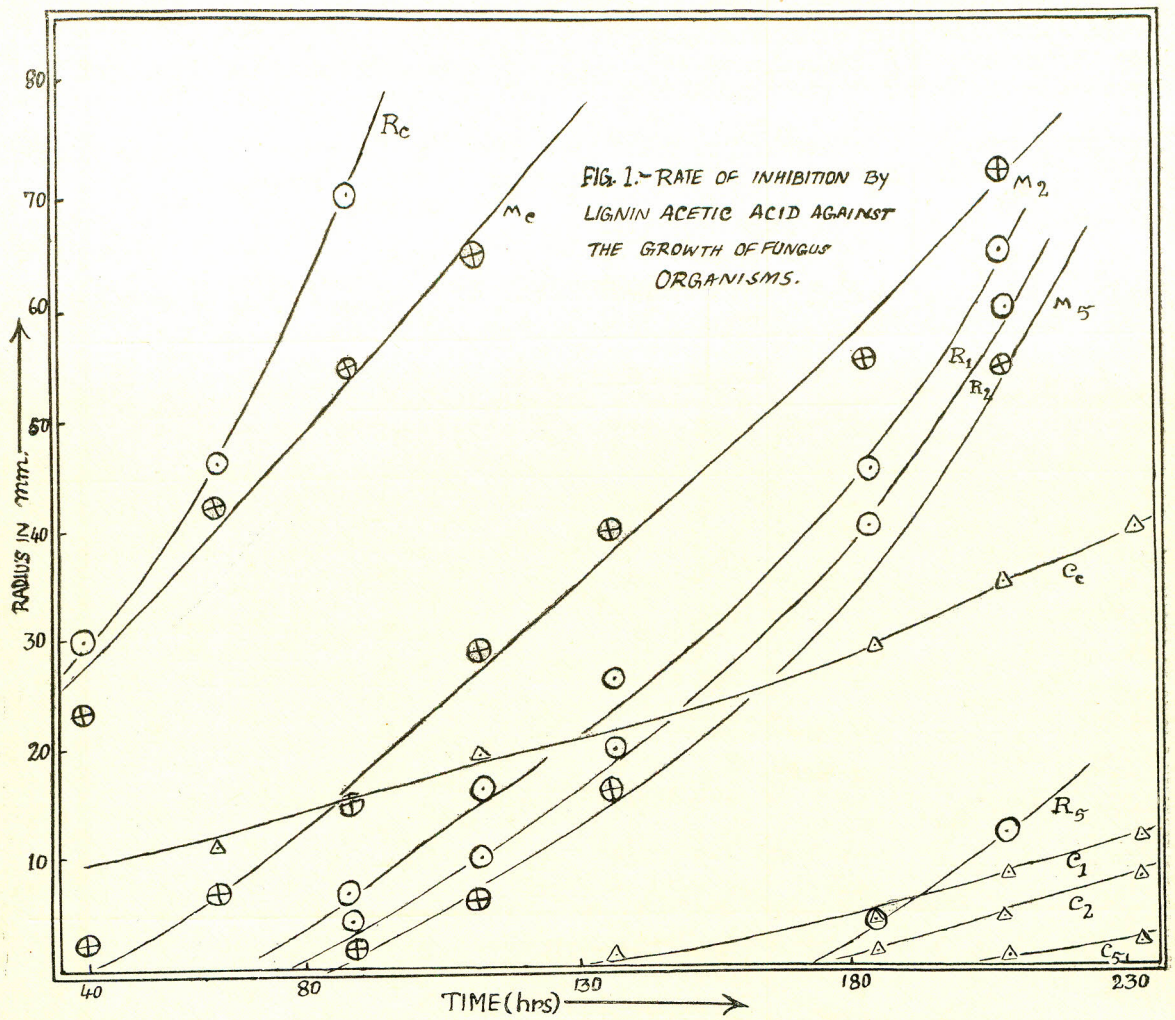

Fig. 1.—Rate of inhibition by lignin acetic acid against the growth of fungus organisms.

—⊙— for Rhizoctonia sq. (vide Table II).

R1	,,	,,	against 1.0 % lignin acetic acid.
R2	,,	,,	,, 2.0 % ,,
R5	,,	,,	,, 5.0 % ,,
Rc	,,	,,	control

—△— for Colletotrichum (Vide Table III).

C1	,,	,,	against 1.0 % lignin acetic acid.
C2	,,	,,	,, 2.0 % ,,
C5	,,	,,	,, 5.0 % ,,
Cc	,,	,,	control

—⊕— for Macrophomina (Vide Table IV).

M2	,,	,,	against 2.0 % lignin acetic acid.
M5	,,	,,	,, 5.0 % ,,
Mc	,,	,,	control

As a controlling agent the acid proved quite promising but there is a little handicap in using this material for it imparts a somewhat brown colour to the cotton and hence further investigation in this line was abandoned.

From close observation of the results in Table II-V it was clearly indicated that the acid

Effect of Different Concentrations of Lignin Acetic Acid Upon the Growth of Fungi (Radius Measured in mm.) for a Different Length of Time Hrs.)

Table II

Organisam—*Rhizoctonia sp.*

Concentrations (%)	Length of time in hours							Remarks
	40	64	88	112	136	184	208	
0.25 ..	8	18	30	45	60	over 70	—	
0.50 ..	5	15	25	40	53	70	over 70	
1.00 ..	nil	nil	6.5	16	26	45	65	Rapid growth with uniform inhibition.
2.00 ..	nil	nil	4.5	10	20	40	60	
5.00 ..	nil	nil	nil	nil	nil	4	12	
Nomersan (0.6%) ..	nil	nil	nil	nil	nil	nil	nil	
Control ..	30	46	70	80	over 90	—	—	

Table III

Organism—*Collectotrichum*

Concentration (%)	Length of time in hours								Remarks
	40	64	88	112	136	184	208	232	
0.25 ..	nil	nil	2	5	9	15	20	25	
0.5 ..	nil	nil	nil	1	3	6	10	14	
1.0 ..	nil	nil	nil	nil	1	4	8	11	Normal
2.0 ..	nil	nil	nil	nil	nil	1	4	8	
5.0 ..	nil	nil	nil	nil	nil	nil	0.5	2	
Nomersan (0.6%)	nil	nil	nil	nil	nil	nil	nil	nil	
Control ..	6	10.5	15	20	25	29	35	40	

TABLE IV

Organism—*Macrophomina*

Concentration (%)	Length of time in hours							Remarks
	40	64	88	112	136	84	208	
1.0 ..	3	12	25	41	58	75	over 80	
2.0 ..	2	6.5	15	29	40	55	72	
5.0 ..	nil	nil	2	5	16	30	55	Rapid growth
Nomersan (0.6%) ..	nil	nil	nil	nil	nil	nil	nil	
Control ..	23	42	55	65	78	over 80	—	

TABLE V

Organism—*Botryodiplodia* (*Diplodia corchori*)

Concentration (%)	Length of time in hours				Remarks
	48	72	120	144	
1.0 ..	20	58	over 80	—	
2.0 ..	15	48	75	over 80	
5.0 ..	10	35	65	80	Irregular growth
Nomersan (0.6%) ..	nil	nil	nil	nil	
Control ..	29	75	over 80	—	

might have its use as a controlling agent for diseases of jute plants. Elaborate studies of this possibility have subsequently been made with jute seedlings and will be published later.

For the suitable utilization of lignin acetic acid, its fungicidal properties were studied on a quantitative basis against five pathogens *viz. Rhizactonia sp.*, *Collectotrichum*, *Macrophomina*, *Botryodiplodia* and *Memnoniella*. The experiments were carried out in the petri-plates in Czapeck's agar medium. Lignin acetic acid was proved a promising controlling agent against the above fungi especially *Rhizoctonia sp.* and *Collectotrichum*. A graphcial repretation of the above results is also given.

Acknowledgement

The investigation was carried out under a research scheme on "Studies on Lignin", financed by the Pakistan Council of Scientific and Industrial Research and the paper is being published with the permission of the Council.

A part of the work was done in the Mycology Section of the Department of Botany and our

TABLE VI

Organism—*Memnoniella*

Concentration (%)	Length of time in hours							Remarks
	48	72	120	168	192	216	240	
0.25 ..	nil	nil	nil	nil	1	2.5	5	
0.5 ..	nil	nil	nil	nil	nil	1	3	
1.0 ..	nil	nil	nil	nil	nil	nil	1	Very slow growth.
Nomersan (0.6%) ..	nil	nil	nil	nil	nil	nil	nil	
Control ..	1	2	3	4	5	7	9	

thanks are due to Prof. M. Ahmad for the courtesy.

Our thanks are also due to Karnaphuli Paper Mills Ltd. for the supply of the black liquor.

References

1. Device U.S.P. 2,503, 297.
2. K.M. Badruddoza, Krishi-Katha **14**, No. 2, 120, (1954).

PESTICIDAL ACTION OF MAKEROL, SHARIGOL AND JHIMPIROL

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IN a process worked out by Siddiqui et al., [1,2] for the desulphurisation of coal and the simultaneous recovery of depolymerised coal resin through the superheated steam treatment of pulverised coal at sub-carbonization temperatures, steam volatile sulphur containing liquids were obtained as by-products. These liquids which have been provisionally named as Makerol, Sharigol and Jhimpirol according to the places of origin of coals, have a sulphur content of about three per cent and a distillation range corresponding to that of terpenes and sesqueterpenes.

The isolation, chemical composition and constitution of the various fractions constituting these liquids are being carried out in the Chemical Division of the Central Laboratories.

On the suggestion of S. Siddiqui, to study the pesticidal properties of these products, preliminary work was carried out on their insecticidal action under a scheme financed by the Pakistan Council of Scientific and Industrial Research, at the Department of Zoology, University of Karachi, Siddiqui et al., [3,4] Ashrafi.[5]

The present paper deals with studies carried out in this direction at the Central Laboratories, initially with the object of finding out :

1. the immediate knock down effect of sprays ;
2. the effect of oral feeding, application on cuticle and injection into the body cavity of insects ;

3. the fumigant action on stored grain pests;
4. the effect of vapours of these liquids on flying insects like houseflies and mosquitoes ;
5. the repellent action on anopheline mosquitoes and houseflies ;
6. the synergistic action of Makerol and Sharigol on insecticides in common use ;
7. the effect on the germination of seeds after fumigation and soaking in Makerol and Sharigol.

For testing the immediate knock down effect, five to ten per cent solutions of Makerol and Sharigol in kerosene and in water with Teepol [sodium secondary alkyl (C10-C18) sulphate] as emulgant were used.

The results were compared with a solution of Pyretherum in kerosene corresponding to 0 1% content of pyretherins.

Evaluation of Toxicity

To evaluate the toxicity of Sharigol by oral feeding in cockroaches (*Periplaneta americana*), they were allowed to lick desired amounts of Sharigol through a syringe needle and were then kept under observation for 24 hours. Table I summarizes the results obtained.

The values when plotted cumulatively on a dosage mortality graph give an asymmetrical sigmoid curve. In order to calculate LD_{50} from these data the mortality percentages have been transformed into probability units and then plotted against log dosage, Bliss, [6,7] figure I.

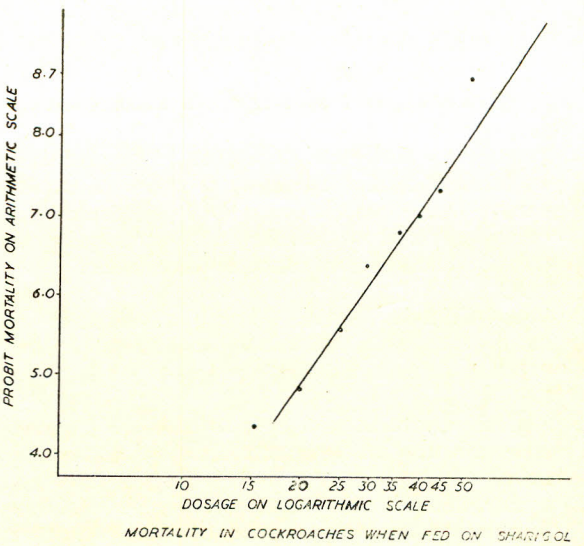

Fig. 1.

Sharigol was also applied on the tarsi cuticle and the neck of cockroaches *P. americana*. When 50 mg. per kg. of the body weight of Sharigol was applied to cockroach integument, death occurred within 24 hours.

Five per cent aqueous emulsion of Sharigol was also injected by means of a microsyringe in the body cavity of cockroaches. Table II below summarizes the results.

TABLE I

TOXICITY OF SHARIGOL TO COCKROACHES BY ORAL FEEDING

Concentration mg./Kg. of body weight	Percent mortality in 24 hours	LD_{50}	LD_{90}
15	27		
20	45	20.1	
25	68		
30	91		
35	95		
40	98		30
45	98		
50	100		

TABLE II

SHOWING THE TOXICITY OF SHARIGOL TO COCKROACHES BY INJECTION

mg./kg. of body weight of Sharigol injected	Percent mortality in 24 hours.	LD_{50}	LD_{90}
5	30		
6	38		
7	52	6.2	
8	83		
9	92		
10	98		
11	98		8.8
12	99		

Controls were run with all the above experiments and the results have been corrected for deaths in controls using Abbott's formula $\frac{(x-y)}{x} \times 100$, where x is the per cent survival in the untreated control, and y is the per cent survival in the treated experimental sample. [8]

Makerol and Sharigol as Fumigants

Makerol and Sharigol appear to be promising as fumigants and therefore a series of experiments have been carried out to assess their effectiveness in this respect, against stored grain pests. The following insects were used :

1. *Calandra oryzae* adult ; 2. *Bruchus sp.* adult ; 3. *Tribolium castaneum* adult ; 4. *Rhizopertha dominica* adult.

Glass containers of one to 20 litre capacity were taken. In these containers 50 laboratory bred adults between five to eight days old were released. A green filter paper No. 405 was hung in the middle of the container. By means of a microsyringe a measured quantity of the fumigant was dropped on the paper. The container was then tightly corked and kept closed for a desired length of time, after which the insects were transferred to fresh containers and observed for 24 hours.

After some experimentation, concentrations were selected to determine the ranges of mortality at high to low levels of concentration. Several replications at one concentration were done and their mean taken. Table III below gives the mean values at each concentration for the replicates.

The values with respect to *Calandra Oryzae* in Table III are plotted cumulatively on a dosage mortality graph, fig. 2. The resulting curves in the case of carbon bisulphide and Sharigol are more or less typical of fumigants but the curve of Makerol tends to become very much asymmetrical as the percentage kill exceeds 85 per cent. This shows that a very high dose of Makerol is required to obtain kills of more than 85 per cent.

In figure 3 the above curves have been transformed into straight lines by transforming percentages into probability units, probits, and then plotting them against log dosages, Bliss.[7] LD_{50} and LD_{90} have been calculated from figure 3.

From figure 3 it would be seen that in lower dosages, both Makerol and Sharigol are

TABLE III

FUMIGATION OF STORED GRAIN PESTS

Fumigant	Insect	Concentration of the fumigant in parts per million	Percent Mortality in 24 hours	LD_{50}	LD_{90}
Sharigol (a)	*Calandra oryzae*	4	42	5.7	
		7	52		
		8	67		
		10	85		
		12	92		
		13	97		
		14	98	9.7	
		16	99		
		17	100		
Makerol	*Calandra oryzae*	4	40		
		7	48	7.6	
		8	58		
		10	74		
		11	82		
		12	85		
		14	88		
		16	90		13.3
Makerol (a)		20	95		
		24	100		
Carbon-bisulphide	*Calandra oryzae*	4	0		
		6	38		
		8	44		

TABLE III—contd.

Fumigant	Insect	Concentration of the fumigant in parts per million	Percent Mortality in 24 hours	LD_{50}	LD_{90}
		10	80	7.9	11.8
		12	95		
		14	99		
		16	100		
Sharigol (b)	*B.phaseoli*	4	25		
		6	37		
		7	60	5.5	7.4
		8	95		
		11	00		
Makerol	*B.phaseoli*	8	16		
		12	84		
		16	90	10.3	17.8
		24	94		
		32	98		
		40	100		
Carbon-bisulphide (b)	*B.phaseoli*	8	4		
		12	50		
		14	96	10.7	12.7
		16	100		

superior to carbon bisulphide. LD_{50} for Sharigol is 5.7 parts per million, for Makerol 7.6 p.p.m. for CS_2 7.9 p.p.m. At a dose of about 9.3 p.p.m. Makerol and carbon-bisulphide are equally effective giving about 65 per cent kill. At higher doses, however, Makerol becomes less effective. Upto a dose of 11 p.p.m. Sharigol is more effective than carbon bisulphide at 11 p.p.m. ; both are equally effective giving a mortality of about 88 per cent, but at higher doses carbon bisulphide excels Sharigol. LD_{90} for the three fumigants are as follows :—

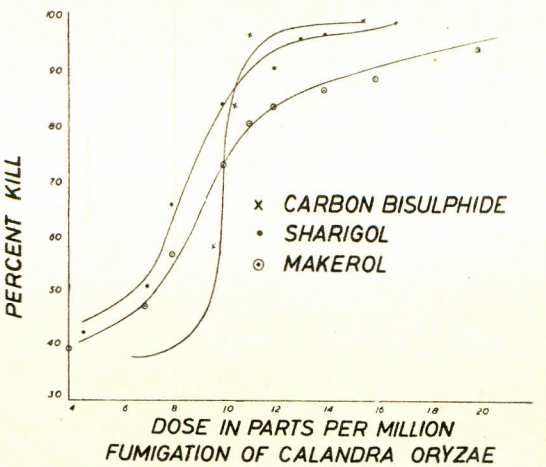

Fig. 2

Makerol	..	13.3 p.p.m.
Sharigol	..	9.7
Carbon bisulphide	..	11.8

Giving better kill at lower dosage is a desirable feature of Sharigol and Makerol, and, used in conjunction with other fumigants, they might, on further work which is in progress, prove very valuable.

Results with *Tribolium castaneum* and *Rhizopertha dominica* were similar to those obtained with *Calandra oryzae*.

Experiments on the amount of fumigant absorbed by grains and on fumigation of infested grains are underway and will be reported in a separate paper.

The toxicity of the vapours of Makerol and Sharigol to flying insects like houseflies and mosquitoes was also tested to explore the possibility of their utilization in the shape of insecticide joss sticks and for controlling insects in enclosed spaces or in places like thick bushes etc., where it is impossible to obtain immediate control through the use of contact insecticides. A field where this may be effective would be

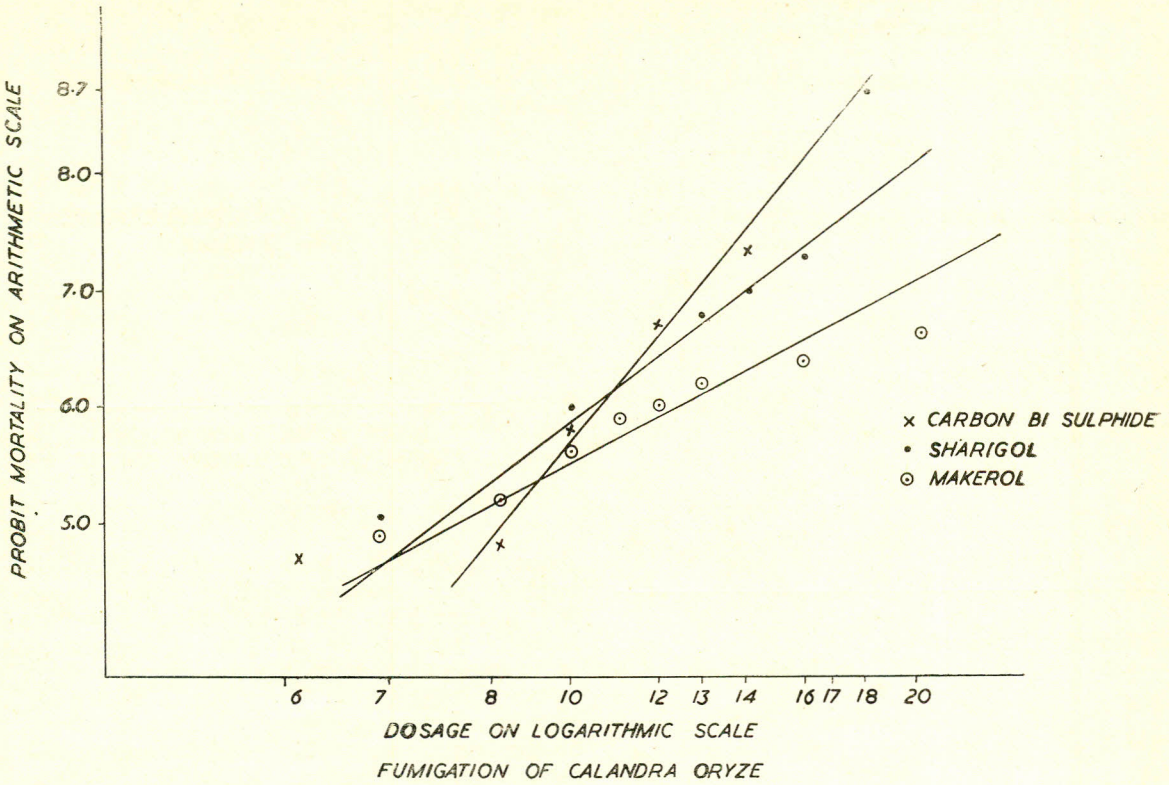

Fig. 3.

the control of Tsetse flies in Africa. For their control Buxton[9] has recommended DDT "smoke". Sharigol and Makerol used in conjunction with DDT "Smoke" will have the added advantage of deep penetration and give immediate knock down.

Sharigol and Makerol by virtue of being fumigants as well as contact poisons and being almost non-toxic to man will also fine a good use in the immediate control of cockroaches, bed bugs, fleas etc. in houses.

To test the knock down action of these vapours laboratory reared house flies (*Musca domestica*) 3 days old were exposed to vapours in enclosed spaces of 10 and 20 litres. An arbitrary effective limit of 100% knock down in 20 minutes time with not more than one per cent revival in 24 hours was chosen and different concentrations which gave 100% knock down in less than 20 minutes with not more than one per cent revival were tried and the times of first and subsequent knock downs were noted. After an exposure of 10 or 20 minutes as given in table IV flies were removed to a fresh cage and were kept under observation for 24 hours. Revivals, if any, were noted. Several replicates were done and the mean for each knock down was taken. Table IV summarizes the results of the first 50% and the last knock down as well as revivals at different concentrations.

Figures 4 a, b and c also show percentage mortality at different exposure times in minutes plotted on a probit log time scale taking probit as an independent variable.

A comparison has also been made of the relative knock down properties of Makerol and Sharigol at median effective time *i.e.*, the time required for 50% knock down. Table V below gives the relative effectiveness :—

TABLE V

SHOWING COMPARATIVE EFFECTIVENESS OF MAKEROL AND SHARIGOL AT DIFFERENT CONCENTRATIONS

Concentration in parts per million	Insecticide	Median effective time (time of 50% K.D.)	Makerol/ Sharigol
16	Makerol	18.3	1.25
	Sharigol	14.6	
32	Makerol	8.5	1.32
	Sharigol		
48	Makerol	5.6	2
	Sharigol	2.8	

TABLE IV

SHOWING FIRST MEDIAN AND LAST KNOCK DOWN TIMES AT DIFFERENT CONCENTRATIONS OF MAKEROL AND SHARIGOL

Insecticide	Concentration in parts per million	1st K.D. time	Median K.D. time	Last K.D. time	Exposure time in minutes	Revival percentage
Sharigol	16	7.83	14.6	16.66	20	nil.
,,	32	3.71	6.5	8.78	10	—do—
	48	1.59	2.8	5.29	10	—do—
Makerol	16	8.45	18.3	19.80	20	0.01
	32	4.61	8.5	10.44	10	nil.
	48	2.83	5.6	8.73	10	—do—

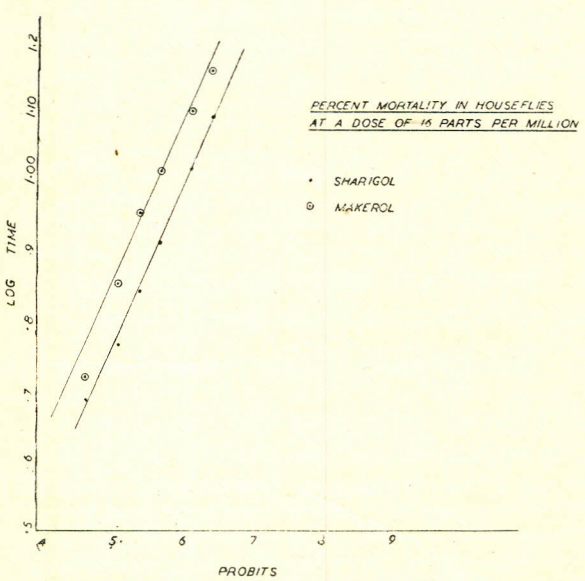

Fig. 4 (a).

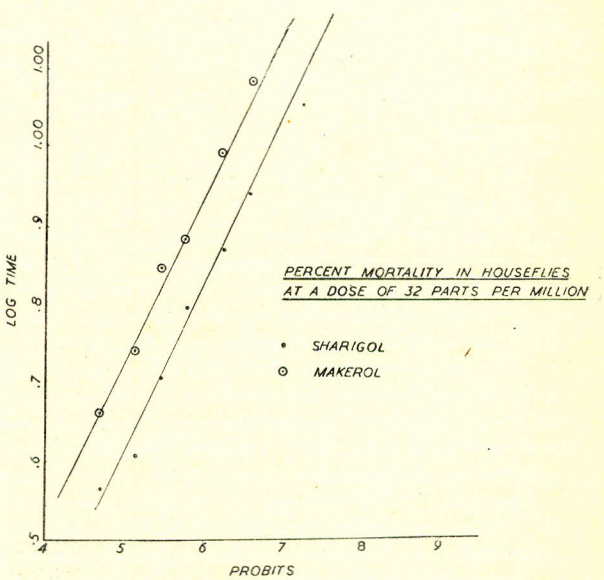

Fig. 4 (b).

At all the levels Sharigol gives quicker median effective (50%) knock down time than Makerol. At lower concentration *i.e.*, at 16 p.p.m. the difference is small *i.e.*, of the order of 125:100 but as the dose increases Sharigol becomes relatively more and more effective. At 48 p.p.m. Sharigol is twice as effective as Makerol.

Insect Repellence

Sharigol and Jhimpirol are good anopheline repellents. Experiments were conducted with *Anopheles stephensi*, the vector of malaria in Karachi.

The technique used was a modification of

that developed by Granett (1940)[10] and Morton et al.[11]

Two-tenth ml. of the substance under trial was distributed evenly over a hand and part of a forearm which were then exposed to about 1,000 female mosquitoes, *A. stephensi*, in a one ft. cube cage for 10 minutes at 30 minutes intervals. The time between the application and first bite was taken as the maximum effective repellent time.

The following repellents were used in the comparative study : oil of citronella, dimethyl phthalate, Makerol, Sharigol, Jhimpirol and a commercial liquid repellent. Table VI below summarises the results.

TABLE VI.—SHOWING THE PROTECTION TIME OF DIFFERENT REPELLENTS

Repellent	*Protection time*
Dimethyl phthalate ..	.. 3 hr. 11 min.
Sharigol ..	.. 2 hr. 48 min.
Jhimpirol ..	.. 2 hr. 42 min.
Commercial repellent	.. 2 hr. 32 min.
Makerol ..	.. 1 hr. 23 min.
Oil of citronella ..	.. 0 hr. 44 min.

As a further test of mosquito repellence the following experiment was conducted. A hand and part of an arm, treated with 0.2 ml. of the compound to be tested, were exposed for 10 minutes at 30 minutes intervals in a 1 ft. cube cage containing 1,000 hungry *A. stephensi* mosquitoes. The number of mosquitoes alighting on the treated hand and arm during the ten minute period was noted. The mosquitoes were not allowed to take a blood meal. Controls were run under similar conditions with each experiment. The percentage repellence was calculated by using the formula $\frac{(x-y)}{x} \times 100$ where x is the number of mosquitoes alighting on the hand in a ten-minute period in the control, y the number of mosquitoes alighting on the hand in the experiment.

The same compounds were used in this experiment as in the previous one. Table VII below summarises the results :

Figure 5 expresses these results on a graph for easy comparison.

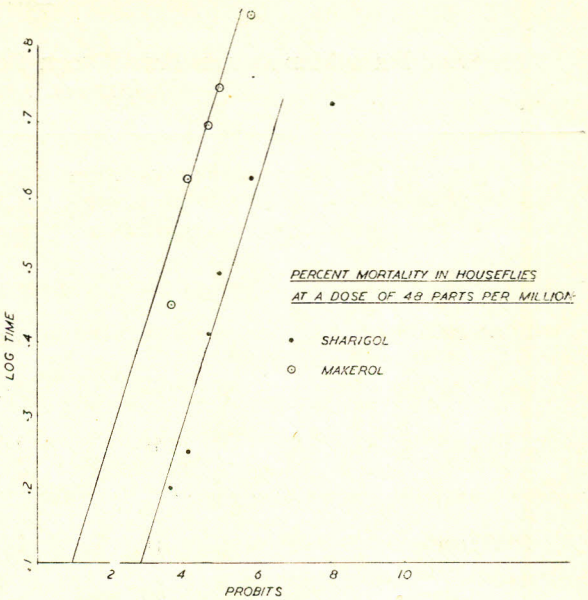

Fig. 4 (c)

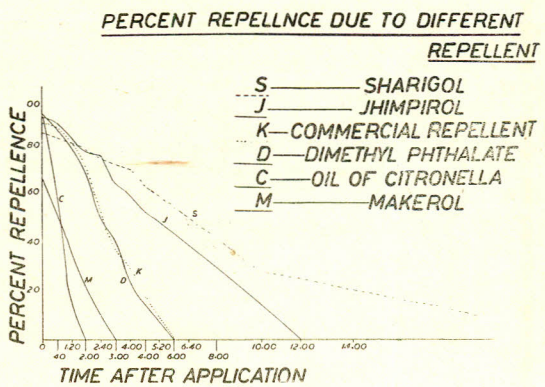

Fig. 5

After application of the repellent, as given in the experiment above, hands were washed with ordinary toilet soap and water and then exposed to the mosquitoes as above. The percentage protection after each washing was calculated as in the above experiment. Table VIII summarizes the results obtained.

There is also some experimental evidence to show that Sharigol has a slight a mild synergistic action on some known insecticides. The results of the work in this direction will be reported in a separate paper.

The effect of fumigation and soaking of seeds in Makerol and Sharigol was also studied. Mung (*Phaseolus mungs*) seeds were chosen for

TABLE VII

SHOWING PERCENT REPELLENCE DUE TO DIFFERENT REPELLENTS

Time in hours after application	Sharigol	Jhimpirol	Commercial repellent	Dimethyl phthalate	Makerol	Oil of citronella
Immediately after application	86	93	90	93	68	93
0 hr. 40 min.	84	90	89	88	52	64
1 hr. 20 min.	82	84	83	82	35	16
2 hr. 0 min.	79	78	70	70	23	0
2 hr. 40 min.	75	77	55	53	11	
3 hr. 20 min.	73	65	38	42	0	
4 hr. 0 min.	71	60	30	20		
4 hr. 40 min.	64	53	19	11		
5 hr. 2 min.	50	49	0	0		
6 hr. 0 min.	48	48				
10 hr. 0 min.	29	27				
18 hr.	15					

TABLE VIII

SHOWING PERCENT REPELLENCE AFTER WASHING WITH SOAP AND WATER

	After one washing with soap and water	After two washings with soap and water
Jhimpirol	63	35
Sharigol	58	29
Commercial repellent	55	21
Oil of citronella	42	10
Makerol	31	10
Dimethyl phthalate	10	0

the experiment. These seeds were fumigated with high doses of Makerol and Sharigol for 24 to 48 hours. They were also soaked in Makerol and Sharigol for 24 to 96 hours. Each lot of the fumigated and soaked seeds after treatment was divided into two equal batches, one was kept for germination between moist cotton pads immediately, and the other was

allowed to desorb for 24 hours and then kept for germination. Controls were run with each batch. The results obtained show that fumigation of seeds by Makerol and Sharigol has a beneficial effect on germination. With the increase in fumigation dose there is an increase in percentage of germination of seeds.

Soaking of seeds in Makerol or Sharigol also does not seem to harm the seeds much, if the seeds are allowed to desorb for 24 hours after soaking. But if the seeds are not allowed to desorb and kept for germination immediately, germination is greatly hampered.

The higher percentage of germination among fumigated seeds is probably due to the killing of fungus spores. On the basis of preliminary studies in the fungicidal properties of Makerol and Sharigol, it appears that these products have a marked fungicidal action.

Acknowledgements

The authors are grateful to Dr. S. Siddiqui for his direction and valuable suggestions through the progress of this work. The authors wish to thank Dr. Shujat-ul-Akbar and Mr. N. H. Naqvi who were associated with this work during its earlier stages. The authors also tender their thanks to Mr. Sadiq Husain and Mr. M.S. Anwar of the Department of Plant Protection and to Dr. Maqsood Nasir of the Ministry of Food for making some stored grain pests available for starting cultures for our tests.

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