

NOTE ON EXPERIMENTS MADE WITH THE OBJECT OF
FINDING AN EFFICIENT AND ECONOMICAL INSECTI-
CIDE SPRAY FOR THE DESTRUCTION OF MOSQUITOES
AND FLIES.

BY CAPTAIN E. BABER,

Hygiene Officer, South African Medical Corps.

THE primary object of these experiments was an endeavour to find an insecticide for the destruction of mosquitoes and flies which would comply with the standards of efficiency and economy laid down by the Union Department of Public Health.

In the preliminary tests, flies (*Musca domestica*) were used as a basis for experiments as it was thought that a spray effective in the destruction of flies would be equally or more so against mosquitoes. Flies were specially bred out from larvæ obtained by means of the fly-larval-trap manure midden, as described in THE JOURNAL OF THE ROYAL ARMY MEDICAL CORPS, December, 1925. The flies were suitably fed for four days, and only active, healthy insects were used. Later, large numbers of mosquitoes (*Culicinae*) were bred from larvæ collected in the surrounding district and these were fed on fruit for three days prior to testing. Altogether many hundreds of tests were carried out by the writer.

At the outset standard advertised proprietary preparations were purchased from local stores and these were submitted to test with the object of obtaining a basis upon which to work and to give some idea of what results might reasonably be expected. This was followed by tests with a number of reagents, published formulæ, and particularly with liquid extracts of pyrethrum. Tests were also made with naphthaline, dichlorbenzol, formalin, thymol, betanaphthol, cresol, creosote, methyl salicylate, carbon tetrachloride, sodium fluoride, oil of sassafras, oil of citronella, pyrethrum powder, turpentine, varnoline (turpentine substitute), petrol and paraffin oil.

As the object of the experiments was to find an efficient but inexpensive insecticide, more particularly for the destruction of mosquitoes, many formulæ containing one or more of the above ingredients are not detailed in this article, the chief reasons for their rejection being one or other of the following: (a) recovery rate high, resulting in a low index figure of insects killed; (b) killing power satisfactory but too costly for general use; (c) pungent, causing either conjunctival or nasal irritation; (d) use limited owing to inflammable qualities.

As the tests proceeded, the sprays giving the best results when employed against flies were noted for further test against mosquitoes, unsatisfactory reagents and formulæ being discarded.

Household insecticides may be subdivided in their composition as follows: (a) the reagent, containing the principal killing properties; (b)

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the perfume (usually essential oils), which may also act to some extent as a killer or as a repellent; (c) the vehicle, which acts as a carrier of the active killing ingredients.

TECHNIQUE IN CARRYING OUT TESTS.

The insects were passed into spherical shaped wire-gauze cages, twenty insects per cage; for testing purposes each cage was placed beneath a metal container having a capacity of 0.65 cubic feet.

The solution to be tested was atomized by means of a small glass nebulizer, as used for throat sprays, the nebulizer being placed beneath the slightly tilted metal cover as far removed from the cage as possible, and a specified number of pressures made upon the bulb of the nebulizer. After a lapse of thirty minutes the cover was removed and the results observed. The cages were now given free ventilation and further observations were made at the end of one hour and twelve hours. The number of insects lying apparently dead at the bottom of the cages was recorded at each observation. The test in the case of each solution was frequently repeated with the object of counteracting any possible source of error likely to give inconsistent results and most of the solutions were submitted to ten or more separate tests.

After carrying out some initial experiments it was decided to submit flies to two series of tests, viz., ten pressures and thirty pressures upon the bulb of the nebulizer. The latter test gave an index figure of sixty-five per cent killed in the case of the most effective proprietary spray tested and, therefore, was considered to be a fair basis upon which to work. In the case of mosquitoes it was found that one pressure and ten pressures upon the bulb were sufficient. Those sprays giving the best results in the killing of flies were later tested against mosquitoes.

An endeavour was made to maintain the conditions as to temperature and humidity as evenly as possible throughout the tests, but detailed figures in this connection were not recorded.

It will be noted that fluids were finely atomized and that they were not sprayed immediately upon the insects, the intention being that the insects should be affected by the fumes only. It is thought that if a spray is to be of any value in the destruction of mosquitoes it must kill those insects which might be concealed beneath beds, behind and below furniture, and among the folds of curtains, as visible and accessible insects hardly require spraying, an ordinary hand swatter being all that is required. Further, to direct a heavy spray immediately on insects provides little proof of the efficacy of an insecticide, for even ordinary paraffin oil will kill most insects if actually coming in contact with them.

RECORDING RESULTS.

The percentage of insects found to be dead after a lapse of twelve hours was recorded after each test and the result given by each formula or solution

over the full series of tests was averaged; this figure was termed the Index Figure of Insects Killed.

The results were tabulated as follows:—

| <i>Average Percentage Dead 12 hours after Spraying.</i> | | | | |
|---|----|--------------------|----|--------------------|
| <i>Flies</i> | 10 | pressures on bulb. | 30 | pressures on bulb. |
| <i>Mosquitoes</i> | 1 | pressure | 10 | " " " " |

Generally it may be said that the effect of insecticides is first to stupefy and paralyse the insects and subsequently, depending upon the degree of efficiency of the spray, a proportion of the insects die, otherwise they recover.

None of the solutions employed, whether proprietary or otherwise, was 100 per cent efficient, and most of them gave an amazingly high percentage of recoveries, and although insects were stupefied for an hour or more recoveries in periods of from three to twelve hours were frequently as high as 100 per cent. Most recoveries occurred after a lapse of two or three hours, and insects which in the early stages appeared to be dead were found to recover in such large numbers as to render valueless some formulæ which at first seemed to be very promising.

The tests clearly demonstrated the futility of judging the killing powers of an insecticide by the number of insects apparently dead after the lapse of a period of three hours or less. Such a method of assessing the effective killing powers of a spray has been shown by these experiments to be almost valueless.

SUSCEPTIBILITY OF MOSQUITOES.

As a result of the tests it was observed that mosquitoes are infinitely more susceptible to the action of insecticide sprays than flies, and that an indifferent fly-killing fluid might be a fairly efficient mosquito destroyer, whilst an insecticide of only moderate efficiency when employed against flies may be 100 per cent efficient against mosquitoes. An examination of the figures given in the table demonstrates the fact that a higher kill results with mosquitoes than with flies even though but one-thirtieth the quantity of the insecticide be used in the case of the former. It was further observed that mosquitoes appear to be remarkably susceptible to insecticides prepared from good quality extract of pyrethrum.

The mosquitoes used in these experiments were principally Culicini. Doubtless it would have been better to have used Anophelini, but these were not obtainable in sufficient numbers for the purpose; however, there appears to be no reason to suppose that they have a higher degree of resistance to the action of insecticides than Culicini, Anophelini being considered even more delicate than culicinæ.

PROPRIETARY SPRAYS.

There is a large number of proprietary sprays on the market. Many of these are made in the United States, some are made in the Union and, with few exceptions, they all appear to employ pyrethrum as the killing agent.

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As indicating the enormous public demand for fly sprays it is stated that 25,000,000 dollars worth of insecticides were sold in the United States in 1931.

Proprietary-prepared fly spray solutions vary considerably in killing powers, although each manufacturer claims his particular spray to be 100 per cent. efficient, which is far from being the real position. In the case of one manufacturing firm various grades of fly sprays are made up and sold under different titles, the cheapest being specially prepared for the multiple bazaar business; the cheaper fly sprays probably contain a smaller proportion of the somewhat expensive killing agent, but a larger proportion of mineral oil, and for this reason a cheap spray may be almost worthless.

One advantage of proprietary sprays is the fact that they are carefully packed in special containers for distribution and sale, but these containers add considerably to the cost of the fluid, particularly in the case of the smaller size receptacles.

None of the published non-proprietary formulæ which were submitted to test gave killing results equal to the better grades of proprietary-prepared fly-spraying solutions. Doubtless the reputable manufacturer of highly advertised products takes considerable pains to obtain a high grade pyrethrum extract, as otherwise the product will ultimately fall into disfavour. Instances of fairly well known but inefficient insecticide sprays falling entirely out of the market have occurred.

In view of the great variation in the efficiency of prepared proprietary insecticide sprays, it would appear to be desirable that the public and reputable manufacturers should be protected by a law requiring insecticides to comply with some biological standard of efficiency and that they be graded accordingly.

Naphthalene.

Naphthalene has shown some promise as a killing ingredient in fly sprays and its low cost is an important factor.

Crude naphthalene is a hydrocarbon occurring in coal tar.

Naphthalene is soluble in 1 in 25 alcohol, 1 in 1½ chloroform, 1 in 3 ether, 1 in 7½ turpentine.

The naphthalene available was in the form of ordinary commercial naphthalene balls and flaked naphthalene; it is quite possible these were not of the most suitable type.

In the application of naphthalene as an insecticide for the destruction of lice, Bacot recommended the use of crude, drained, unwhizzed naphthalene from modern coke ovens, that obtained from gas plants being far less efficient.

Naphthalene was experimented with in various admixtures, the solvents employed being ordinary paraffin oil burning fluid, chloroform and methylated spirit; additions were made of carbon tetrachloride, dichlorbenzol,

and oil of sassafras, but these additions made no very appreciable improvement in the killing powers of the resultant solution.

It was generally found that within thirty minutes of spraying practically all insects were stupefied and lying still on the bottom of the cages, but when these insects were exposed to free ventilation they recovered so rapidly that at one hour a considerable proportion had recovered, whilst after a lapse of twelve hours the recoveries reached 40 per cent. in the case of thirty pressures on the bulb, and 70 per cent. in the case of ten pressures.

A simple saturated solution of naphthalene in ordinary paraffin oil when tested against flies gave an index figure of insects killed of 60 per cent. This may be regarded as a moderately effective fly spray and has the advantage of very low cost, though its use in a closed room causes the operator slight nasal and conjunctival irritation. Mosquitoes proved to be relatively less susceptible to its influence than flies.

Pyrethrum Extracts.

The tests revealed the definite superiority of spraying solutions containing pyrethrum extracts, and mosquitoes proved to be peculiarly susceptible to insecticides prepared from a good grade of liquid extract of pyrethrum.

The preparation of sprays from ordinary commercial pyrethrum powder by the somewhat rough and ready means of soaking the pyrethrum powder in oil for twenty-four hours and subsequently filtering did not give satisfactory results, and no better results were obtained when pyrethrum powder was percolated with chloroform or methylated spirit.

Liquid extract of pyrethrum, as prepared by manufacturing chemists, having proved in the experiments to be so potent in the preparation of sprays for the destruction of mosquitoes, a few remarks thereon may not be out of place.

Pyrethrin, the active principle of the pyrethrum plant, whilst highly toxic to insects, is harmless to man and warm-blooded animals. It is manufactured from pyrethrum flowers, and these must be of selected quality. The best are considered to be the half opened flowers. These flowers are cultivated principally in England, Japan and Dalmatia, and are specially cultivated to produce the greatest possible percentage of pyrethrin. The activity of different samples of flowers varies considerably and it is stated that in some instances the disparity between them may be as much as 300 per cent.

To produce a pyrethrum extract of uniform killing power it is necessary to standardize the preparation according to the activity of the flower rather than the quantity used, the quality of the extract being finally confirmed by biological tests.

Ready prepared pyrethrum extracts of very high potency are now obtainable from manufacturing chemists and provide a ready and easy means of making up a useful insecticide, having a killing power equal or

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superior to the advertised proprietary household insecticides, which are very much more costly.

Prepared pyrethrum extracts at first glance may seem to be very costly; but when it is considered that some are used in a proportion of 1:65 when mixed with oil in the preparation of sprays, the cost will be such as to permit the preparation of an insecticide and its extensive use, an important point for consideration in antimalaria work.

When comparing the costs of liquid extracts of pyrethrum it is necessary to note the concentration required when mixed for use, as this varies with the different makes and thus considerably affects the cost of the final product.

The following are some details with regard to those pyrethrum extracts which were available in the Union and which were submitted to tests, the results of which are indicated in the table.

The pyrethrum extract which gave the best result was that of Messrs. Stafford Allen and Sons, Limited, Manufacturing Chemists, England. This firm appears to specialize in pyrethrum extracts and cultivates the pyrethrum flowers upon their own farms in Suffolk. The extract is very highly concentrated and is stated by the manufacturers to be biologically standardized; they prescribe its use in a proportion of 1:64 with paraffin oil. The tests showed that even if used in double this dilution it gave an insecticide with high killing powers when employed for the destruction of mosquitoes.

A pyrethrum extract which gave good results was that of Messrs. Gale and Co. Limited, Wholesale Chemists, London. It is also used in a proportion of 1:65 with paraffin oil.

Pyroicide No. 20 is another liquid extract of pyrethrum and can be obtained from the Standard Oil Co. in South Africa; it is used in a dilution of 1:20 with paraffin oil.

Pyagra Concentrate, another pyrethrum extract, is made in South Africa by the Creek Chemical Works, Durban; usable in a dilution of 1:12 with paraffin oil.

The water soluble preparations of extract of pyrethrum, so largely used against agricultural pests, were found unsuitable for the preparation of insecticides for the destruction of flies and mosquitoes.

Liquid extract of pyrethrum was submitted to tests in varying dilutions and with the addition of different classes of essential oils.

Oil of citronella being widely used for its repellent effect against mosquitoes, and being one of the cheapest of the essential oils, was tested out in combination with liquid extract of pyrethrum and when added in a proportion of 5 per cent. was found to cause a very definite increase in the index figure of insects killed, more than sufficient to justify the additional cost of its inclusion.

Of the liquids tested for suitability as vehicles for the preparation of insecticide sprays, ordinary standard quality household paraffin-oil-burning

fluid was found to answer satisfactorily, and it was decided to increase its volatility by adding 15 per cent of petrol. With reasonable care this mixture should not be of such an inflammable nature as to render its general use dangerous. When used with a volatiliser pump it does not stain fabrics or injure furniture.

The formula finally resolved upon as providing a highly efficient insecticide at a low cost was the following, 1 : 64 Stafford Allen's liquid extract of pyrethrum + 5 per cent oil of citronella + 15 per cent petrol + paraffin oil (ordinary burning fluid).

This formula was found to give the highest index figure of insects killed of any formula submitted to test. An insecticide made up according to this formula has recently been prepared under the joint direction of the Union Department of Public Health and the Department of Defence and is distributed under the name of "Hysec 350," at a little above cost price for use of Government Departments and Local Authorities in the Union. It has been well reported upon by users and arrangements have been made for the supply of large quantities during the approaching warm season 1933-1934.

The question of the type of spray pump which should be used has not been dealt with in this report, but a well-constructed pressure pump is recommended, which should provide good atomization so that the insecticide is thoroughly distributed.

SUMMARY.

(1) The tests made of insecticide sprays indicated a high percentage of recoveries in periods of from three to twelve hours and demonstrated the futility of judging the killing power of an insecticide by the number of insects apparently dead after the lapse of three hours or less.

(2) The efficiency of prepared proprietary insecticides was found to vary considerably. The fixing of some specified biological standard of efficiency for insecticide sprays would appear to be desirable.

(3) A simple saturated solution of naphthalene in paraffin oil was found to provide a moderately effective insecticide spray. Its low cost is an important consideration.

(4) Mosquitoes proved to be infinitely more susceptible to the action of insecticide sprays than flies. A higher kill resulted with mosquitoes than with flies even with one-thirtieth the quantity of insecticide spray.

Mosquitoes were found to be peculiarly susceptible to the action of insecticides prepared from liquid extract of pyrethrum.

(5) Prepared extract of pyrethrum proved to have very high killing powers and to provide a ready means for the preparation of a useful insecticide. The addition of oil of citronella resulted in a definite increase in the killing powers of pyrethrum extracts.

(6) A formula which included prepared liquid extract of pyrethrum, oil

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of citronella, petrol and paraffin oil was found to provide a very efficient and economical insecticide for the destruction of flies and mosquitoes and gave results superior to all others tested.

In conclusion I would thank Colonel Sir Edward Thornton, K.B.E., Director of Medical Services, for his interest in these tests and for permission to forward these notes for publication.

TABLE.
RESULT OF TESTS OF SOLUTIONS GIVING INDEX FIGURE OF INSECTS KILLED.

| Solutions tested | MOSQUITOES | | FLIES | |
|---|-------------------|-----|-------------------|--------|
| | Pressures on bulb | | Pressures on bulb | |
| | One | Ten | Ten | Thirty |
| Paraffin oil (burning fluid) | 0 | 0 | 0 | 0 |
| Petrol | 0 | 0 | 0 | 0 |
| Turpentine | — | — | 5 | 15 |
| Varnolene (turpentine substitute) | — | — | 20 | 25 |
| 10 per cent citronella oil in paraffin oil | — | — | 7 | 16 |
| 10 per cent sassafras oil in paraffin oil | — | — | 10 | 15 |
| 10 per cent methyl sal. in paraffin oil | — | — | 7 | 18 |
| Pyrethrum powder, 1 lb. per gal. in paraffin oil | 10 | 70 | 20 | 26 |
| do. do. + 5 per cent citronella oil | — | — | 17 | 48 |
| Pyrethrum powder in chloroform + paraffin oil | — | — | 10 | 25 |
| 1/64 Stafford Allen's ext. of pyrethrum + 5 per cent citronella oil + 15 per cent petrol + paraffin oil | 78 | 100 | 48 | 73 |
| do. do. but diluted with equal parts of paraffin oil | 40 | 90 | — | — |
| 1/64 Stafford Allen's ext. of pyrethrum + paraffin oil | 49 | 100 | 48 | 61 |
| do. do. + varnolene | — | — | 47 | 69 |
| do. do. + 15 per cent petrol + paraffin oil | — | — | 49 | 64 |
| 1/12 pyagra ext. of pyrethrum + paraffin oil | 62 | 100 | 43 | 58 |
| 1/20 do. do. do. + do | 34 | 90 | 25 | 28 |
| 1/65 Gale's ext. of pyrethrum + 15 per cent petrol + paraffin oil | 38 | 100 | 38 | 63 |
| 1/20 pyroicide ext. of pyrethrum + paraffin oil | — | 100 | 36 | 44 |
| do. do. do. + 5 per cent methyl sal. + 15 per cent petrol + paraffin oil | — | — | 43 | 53 |
| 1/13 pyroicide ext. of pyrethrum + 15 per cent petrol + paraffin oil | — | — | 22 | 46 |
| Naphthalene, 10 per cent in paraffin oil | 7 | 60 | 30 | 60 |
| <i>Prepared Proprietary Sprays.</i> | | | | |
| Proprietary spray A | 71 | — | 37 | 65 |
| do. do. B | 13 | — | 34 | 60 |
| do. do. C | 32 | — | 25 | 57 |
| do. do. D | 28 | — | 35 | 41 |
| do. do. E | 12 | — | 7 | 38 |
| do. do. F | 40 | — | — | — |
| do. do. G | 17 | — | — | — |

N.B.—A dash placed in columns indicates no test carried out.