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Report No. 1382

December, 1955

FINAL REPORT

ON

INSECTICIDE SPECIAL PROBLEMS

COMPARATIVE LABORATORY EVALUATION OF NIRAN, NIRAN AND BENZIL, AND NIRAN AND AROCLOR 5460 FORMULATIONS (JULY 14 TO OCTOBER 12, 1955)

Job No. 2-02-750.01-2760

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INTRODUCTION

Niran, Monsanto's parathion, is a potent insecticide with a broad spectrum of activity. However, it is also very toxic to man and animals (acute oral LD_{50} averages 3.5 mg./kg., chronic m.l.d. is 25 p.p.m., dangerous level for inunction is 50 mg./kg.). Our research, development and sales personnel would like to develop a formulated product of lower toxic hazard, and are ever watchful for promising leads in this direction. Farbenfabriken Bayer, A.G. markets a "saftened" parathion emulsion concentrate, "Folidol", which contains an emulsifying agent that is claimed to reduce its toxicity via dermal absorption.

Monsanto has been offered a license to a patent claiming a marked reduction in dermal toxicity by the addition of benzil (diphenyl glyoxal) to parathion emulsion concentrates. The inventor also claims that the addition of benzil increases the effectual life of parathion residues by a factor of three times, and that it reduces the phytotoxicity produced by parathion formulations.

This report concerns only the evaluation of the last two claims.

Since we were also interested in evaluating the effectiveness of Arcclor 5460 as an additive to promote the longevity of parathion residues, formulations containing it were compared directly with parathion and parathion and benzil formulations.

SUMMARY

Specifically, answers were required for the following questions: (GFL/LVS, 8/11/55)

"1. Whether formulated combinations of parathion and benzyl maintain their insecticidal potency, compared to parathion formulated alone.

2. Whether the addition of benzil modifies longevity of effectual treatment.

3. Whether the addition of benzil decreases phytotoxicity."

To obtain the answers and to compare with any action produced by Aroclor 5460 several lots of potted, randomly selected Black Valentine bean plants were sprayed with three formulations - twenty five per cent Niran, fifteen per cent Niran and fifteen per cent benzil and fifteen per cent Niran and fifteen per cent Aroclor 5460. The concentration of Niran in the finished spray was one to two thousand on 0.4 pounds per one hundred gallons in each case.

Half of the plants treated with each formulation were held in the greenhouse and half were held under outdoor conditions. Evaluations were made at varying intervals after spraying by infesting plants with either Mexican bean beetle or with Southern armyworm larvae. All testing was done in the greenhouse.

Photographs of a series of sprayed plants were taken to obtain a pictorial record of certain observed manifestations of toxicity.

CONCLUSIONS

1. The incorporation of benzil in a parathion emulsion concentrate did not affect its insecticidal potency as compared to parathion formulated alone. This may be said for Aroclor 5460 also.

2. The addition of benzil neither shortened the longevity of effectual treatment; nor did it prolong effectual treatment as claimed by the inventor. Aroclor 5460 likewise was found to have hardly better than a neutral effect on the duration of effectual treatment.

3. The addition of benzil, contrary to the inventor's claim, appeared to increase rather than decrease the manifestation of phytotoxic symptons induced by paratnion on Black Valentine beans. Plants sprayed with a formulation containing Aroclor 5460 showed less evidence of injury than did those sprayed only with parathion.

RECOMMENDATION

Unless a favorable report is returned on the pharmacological phase of this evaluation, the attempt to obtain a license under this patent should be abandoned.

REFERENCES

Kimball, C. S. Toxicity tests on parathion and parathion-benzil. Memo to E. I. Sudy, July 25, 1955.

Kimball, C. S. Toxicity tests. Letter to F. B. Zienty, July 26, 1955.

Zienty, F. B. Parathion-benzil: toxicity tests. Memo to E. P. Wheeler, July 29, 1955.

Magner, J. M. Parathion safener. Memo to F. B. Zienty, August 1, 1955.

Sherwood, L. V. Parathion safener. Memo to File, August 3, 1955.

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Wheeler, E. P. Toxicity of Niran-benzil formulations. Interim Report to F. B. Zienty, August 3, 1955.

Kimball, C. S. Toxicity test program on parathion-vs-parathion plus benzil. Letter to Monsanto Chemical Company, August 8, 1955.

Morrill, H. L. Parathion formulations. Letter to C. S. Kimball, August 9, 1955.

Morrill, H. L. Parathion-benzil formulations. Memo to E. P. Wheeler, August 9, 1955.

Sherwood, L. V. Benzil (diphenyl glyoxal) - Biologic tests needed. Memo to G. F. Ludvik, August 11, 1955.

Sherwood, L. V. Benzil (diphenyl glyoxal). Memo to file. August 11, 1955.

Ludvik, G. F. Plan for laboratory evaluation of Niran-benzil formulation. Memo to O. DeGarmo. September 2, 1955.

Ludvik, G. F. Niran formulations. Memo to H. L. Morrill. September 6, 1955.

Magner, J. M. Parathion formulations-phytotoxicity. Memo to G. F. Ludvik. September 8, 1955.

Magner, J. M. Dermal toxicity of benzil. Memo to L. V. Sherwood. September 9, 1955.

Ludvik, G. F. Evaluation of Niran-benzil formulations. Memo to F B Zienty. September 19, 1955.

Ludvik, G. F. Comparative evaluation of Niran and Niran-benzil Formulations. Progress Report 2-02-750.01-2760, No. 2. November 30, 1955.

Hill, A. B., 1937. Principles of medical statistics. Laucet Limited, London vii & 171. (Ref. pp 165-6.)

EXPERIMENTAL WORK

Potted, greenhouse grown, Black Valentine bean plants were used for this project. It was not possible to grow, spray, or hold sufficient plants at one time to satisfy the requirements of this evaluation so it was necessary to make several treatments. All spraying was done outside of the laboratory under as nearly the same conditions as possible. A three gallon Hudson garden sprayer was used to make the applications. Similar volumes of spray were applied to similar numbers of plants on each occasion. Plants were selected randomly for each formulation, and the lots of treated plants were held, infested, and observed

under identical conditions. Three plants were used for each formulation, species and age of residue tested. Forty-hour mortality observations were recorded.

The following three formulations were evaluated:

I - Niran — parathion - 25%
Alrodyne 6104 - 5%
xylene - 70%

II - Niran & benzil — parathion - 15%
benzil - 15%
Alrodyne 6104 - 5%
xylene - 65%

III - Niran & Aroclor 5460 — parathion - 15%
Aroclor 5460 -15%
Alrodyne 6104 - 7.5%
xylene - 62.5%

For the final analysis, calculations of per cent kill were based on the numbers of live larvae recovered from ten applied per plant. The residue ages reported are the means of the ages at the beginning and end of infestation e.g., a one day old residue was freshly dried when infested and was two days old when mortality observations were completed. Similarly, a five day old residue was infested four days and observed six days after spraying, respectively.

Experimental data are recorded on notebook pages A-40394 - 40400 and A-71102-04.

The following statistical procedures were utilized to test for significance.

Standard Deviation:

$$\sigma = \sqrt{\frac{\Sigma d^2}{n-1}}$$

where σ = standard deviation,

d = deviation from the arithmetic mean,

and n = number of replicates.

Standard Error of the Difference Between Two (Arithmetic) Means:

$$S.E. = \sqrt{\frac{\sigma_1^2}{n_1} + \frac{\sigma^2}{n_2}}$$

"Significance" - results will occur by chance -

a) about once in twenty observations when

$$m_1 - m_2 > 2 \text{ (S.E.)},$$

b) about once in eighty observations when

$$m_1 - m_2 > 2.5 \text{ (S.E.)},$$

where m = arithmetic mean
and S.E. = standard error.

Color and black and white photographs were taken to preserve a record of one experiment made to evaluate phytotoxic effects of the active ingredient, the additives, and the solvent as related to emulsifier content of the formulation.

Figures 1,2,3, and 4 show the duration of effectiveness of the residues from the three formulations under interior and exterior conditions against larvae of the Mexican bean beetle and Southern armyworm. The data on which these figures are based are given in the Appendix, Tables 1,2, & 3. Summaries of the statistical analyses are given in the Appendix, also.

The arrows on the figures indicate the standard deviations from the means obtained for the various tests.

DISCUSSION

Since the inventor claimed that the addition of benzil would prolong the effectual life of Niran residues by a factor of three, it was necessary to plan for activity determinations at weekly intervals for a minimum of four weeks. A determination of the effectiveness of freshly applied sprays was also required. For some time we had been interested in similar reported effects for Aroclor 5460 so we had an opportunity to kill two birds with one stone.

Reference to Figures 1,2,3, and 4 shows that neither of the additives, benzil and Aroclor 5460, depressed the activity of Niran formulations against either the Mexican bean beetle or the Southern armyworm.

Aroclor 5460 may have been of some benefit in prolonging the effectual residual life of Niran outdoors against the Southern armyworm as shown by Figure 4. Against both species, effectual residues of all formulations disappeared from plants held outdoors for seven days after spraying. This was observed in two separate series of tests. The inventor's claim that the incorporation of benzil would prolong the effectual life of Niran residues could not be substantiated as shown by the data given in Figures 3 & 4.

Effectual residues of all formulations on plants held in the greenhouse definitely disappeared between seven and eleven days after treatment as shown by the reactions of the test species. In one set of tests held in the greenhouse for seven days, the benzil formulation appeared to be superior against Mexican bean beetle larvae, but no difference was shown among the three formulations in a second series of similar tests. The same situation occurred with Southern armyworm larvae, but in this case the second series of tests appeared to favor benzil. If such results had occurred for both species in the same test series, it might have been necessary to acknowledge that benzil does add a small plus valve under greenhouse conditions. Even if this actually were the case, the inventor's claim would still be much too extravagant.

Early in this work, evidences of phytotoxicity were observed. There appeared to be little difference between the Niran and Niran and benzil formulations in this respect, but was noticeably less apparent from the Niran and Aroclor 5460 formulation. The injury was initially attributed principally to solvent toxicity resulting from poor wetting properties of the formulations. This thesis was supported by the fact that the Niran and Aroclor 5460 formulation contained 2.5 per cent more emulsifier than did the other two. Subsequently, twelve formulations containing differing amounts of emulsifier were prepared and sprays were applied to Black Valentine bean plants. The formulations were:

1. Niran - 15%
   Alrodyne 6104 - 10, 7.5 & 5%
   Xylene - to make 100%

2. Niran - 15%
   Benzil - 15%
   Alrodyne 6104 - 10, 7.5 & 5%
   Xylene - to make 100%

3. Niran - 15%
   Aroclor 5460 15%
   Alrodyne 6104 - 10, 7.5 & 5%
   Xylene - to make 100%

Three plants were sprayed at one part formulation to three hundred parts of water (Niran: 1/2000) with each formulation and were held in the (plastic) greenhouse six days. Color and black and white photographs were taken to record the results. Injury decreased in all cases as the amount of emulsifier was increased from five to ten per cent. The least amount of injury was observed in the series containing Aroclor 5460 and the most was observed on the plants treated with the Niran and benzil formulation. This observation negates another of the inventor's claims.

G. F. Ludvik

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Appendix No. 1

Table 1. Mortality Data for Mexican Bean Beetle Larvae on Residues Maintained in the Greenhouse

Residue Age, Days	Formulation	Per Cent Kill			Ave.	σ
1	Niran	100	100	100	100 ±	0.0
	Niran + Benzil	100	100	100	100 ±	0.0
	Niran + Aroclor 5460	100	100	90	96.7 ±	5.7
5	Niran	100	100	100	100 ±	0.0
	Niran + Benzil	100	100	100	100 ±	0.0
	Niran + Aroclor 5460	100	100	100	100 ±	0.0
8	Niran	70	60	40	56.7 ±	15.3
	Niran + Benzil	80	100	100	93.3 ±	11.5
	Niran + Aroclor 5460	0	0	0	0 ±	0.0
8	Niran	100	100	100	100 ±	0.0
	Niran + Benzil	100	100	100	100 ±	0.0
	Niran + Aroclor 5460	100	90	100	96.7 ±	5.7
12	Niran	40	80	70	63.3 ±	20.8
	Niran + Benzil	30	20	0	16.7 ±	15.3
	Niran + Aroclor 5460	20	20	0	13.3 ±	11.5
15	Niran	20	10	0	10.0 ±	10.0
	Niran + Benzil	0	0	10	5.3 ±	5.7
	Niran + Aroclor 5460	0	0	0	0 ±	0.0
15	Niran	0	0	0	0 ±	0.0
	Niran + Benzil	40	0	0	13.3 ±	23.1
	Niran + Aroclor 5460	0	40	20	20 ±	20.0

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Appendix No 1

Table 2. Mortality Data for Southern Armyworm Larvae on Residues Maintained in the Greenhouse

Residue Age, Days	Formulation	Per Cent Kill			Ave		σ
1	Niran	100	100	100	100	±	0.0
	Niran + Benzil	100	100	100	100	±	0.0
	Niran + Aroclor 5460	70	100	100	90	±	17.3
5	Niran	100	90	90	93.3	±	5.7
	Niran + Benzil	100	100	80	93.3	±	11.5
	Niran + Aroclor 5460	100	90	80	90	±	10.0
8	Niran	60	70	80	70	±	10.0
	Niran + Benzil	40	80	100	73.3	±	30.6
	Niran + Aroclor 5460	60	80	70	70	±	10.0
8	Niran	50	50	90	63.3	±	23.1
	Niran + Benzil	100	90	90	93.3	±	6.7
	Niran + Aroclor 5460	50	70	40	53.3	±	12.9
12	Niran	20	0	10	10	±	10.0
	Niran + Benzil	0	20	40	20	±	20.0
	Niran + Aroclor 5460	30	0	0	10	±	17.3
15	Niran	50	40	0	30	±	26.5
	Niran + Benzil	50	10	10	23.3	±	20.4
	Niran + Aroclor 5460	40	40	90	56.7	±	28.9
15	Niran	20	10	20	16.7	±	5.7
	Niran + Benzil	30	30	30	30	±	0.0
	Niran + Aroclor 5460	10	20	30	20	±	10.0

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Appendix No. I

Mortality Data for Residues Maintained Outdoors

Table 3. Mexican Bean Beetle Larvae

Residue Age, Days	Formulation	Per Cent Kill			Ave.	σ
5	Niran	100	100	100	100 ±	0.0
	Niran + Benzil	100	100	100	100 ±	0.0
	Niran + Aroclor 5460	100	100	100	100 ±	0.0
8	Niran	90	0	0	30 ±	41.0
	Niran + Benzil	50	80	10	46.7 ±	35.2
	Niran + Aroclor 5460	0	70	60	43.3 ±	37.8
8	Niran	10	20	10	13.3 ±	5.8
	Niran + Benzil	0	10	0	3.3 ±	5.8
	Niran + Aroclor 5460	40	30	60	43.3 ±	15.3
12	Niran	0	0	10	3.3 ±	5.8
	Niran + Benzil	0	0	0	0 ±	0.0
	Niran + Aroclor 5460	0	0	0	0 ±	0.0

Table 4. Southern Armyworm Larvae

Residue Age, Days	Formulation	Per Cent Kill			Ave.	σ
5	Niran	70	50	20	46.7 ±	25.2
	Niran + Benzil	80	80	20	60 ±	34.6
	Niran + Aroclor 5460	100	100	100	100 ±	0.0
8	Niran	40	50	30	40 ±	10.0
	Niran + Benzil	50	60	30	46.7 ±	15.3
	Niran + Aroclor 5460	50	70	60	60 ±	10.0
8	Niran	10	0	10	6.7 ±	5.8
	Niran + Benzil	0	10	0	3.3 ±	5.8
	Niran + Aroclor 5460	20	10	20	16.7 ±	5.8
12	Niran	30	40	0	23.3 ±	20.8
	Niran + Benzil	0	0	10	3.3 ±	5.8
	Niran + Aroclor 5460	20	10	0	10 ±	10.0

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Fig. 1. Effectiveness against the Mexican Bean Beetle of Residues Maintained in the Greenhouse

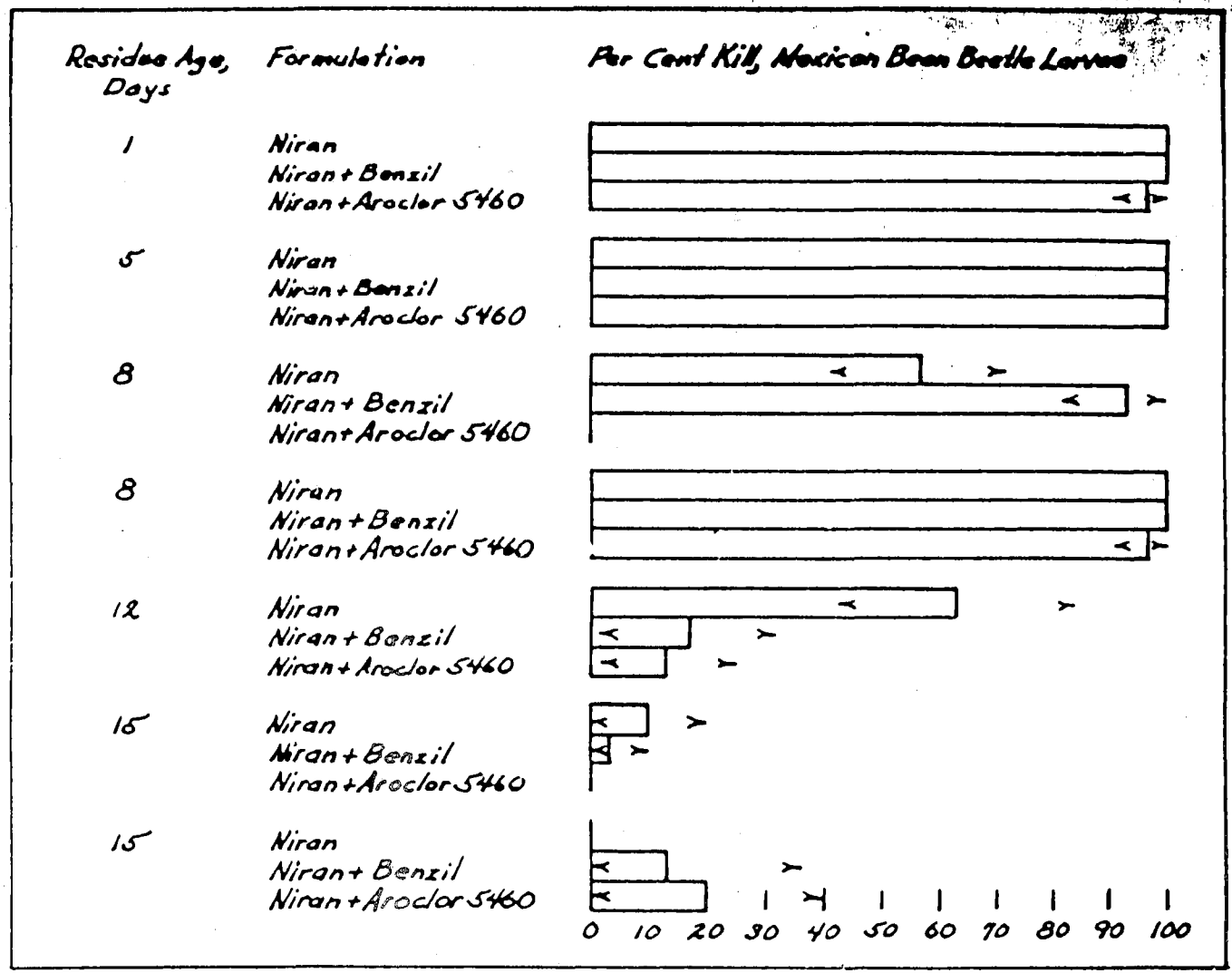

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Fig. 2. Effectiveness against the Southern Armyworm of Residues Maintained in the Greenhouse

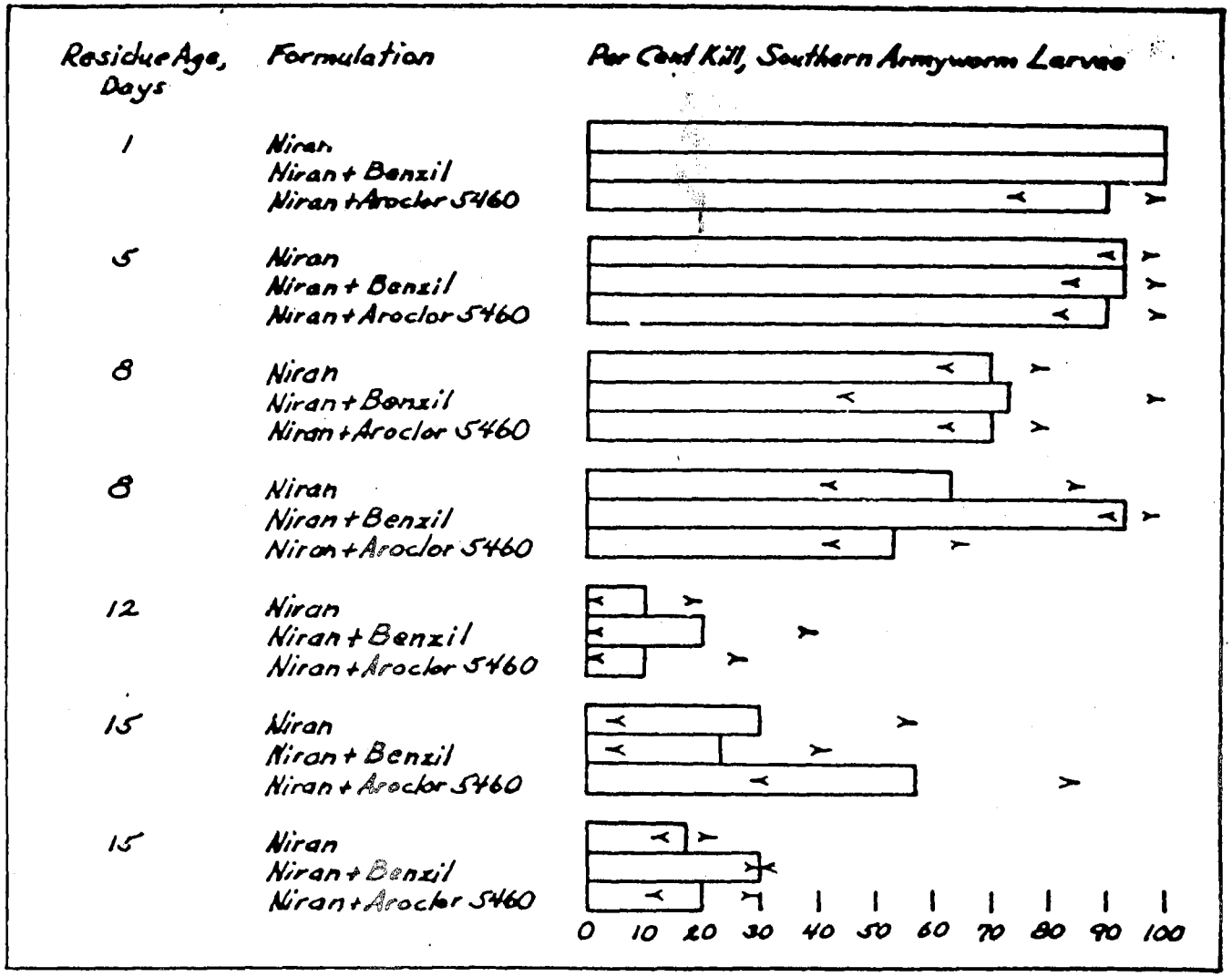

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Fig. 3. Effectiveness against the Mexican Bean Beetle of Residues Maintained Outdoors

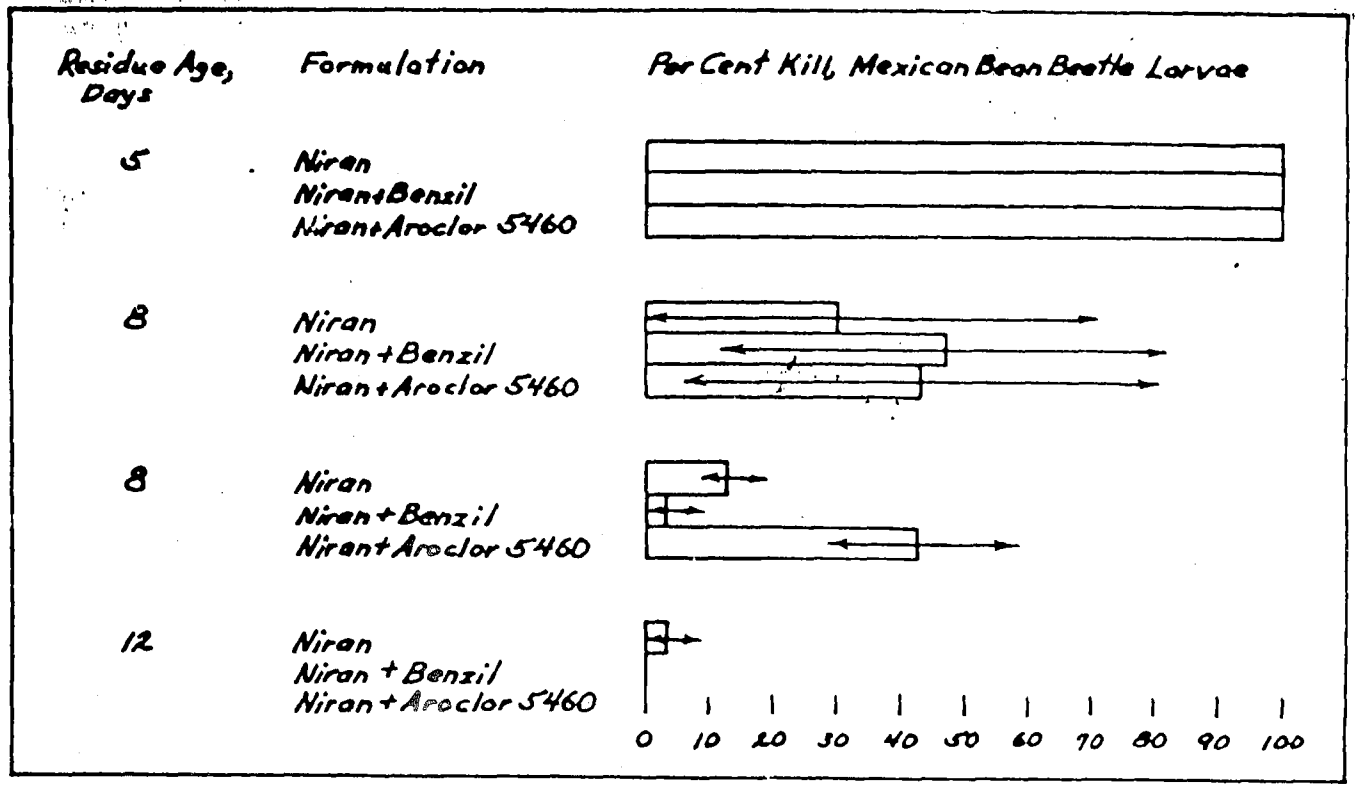

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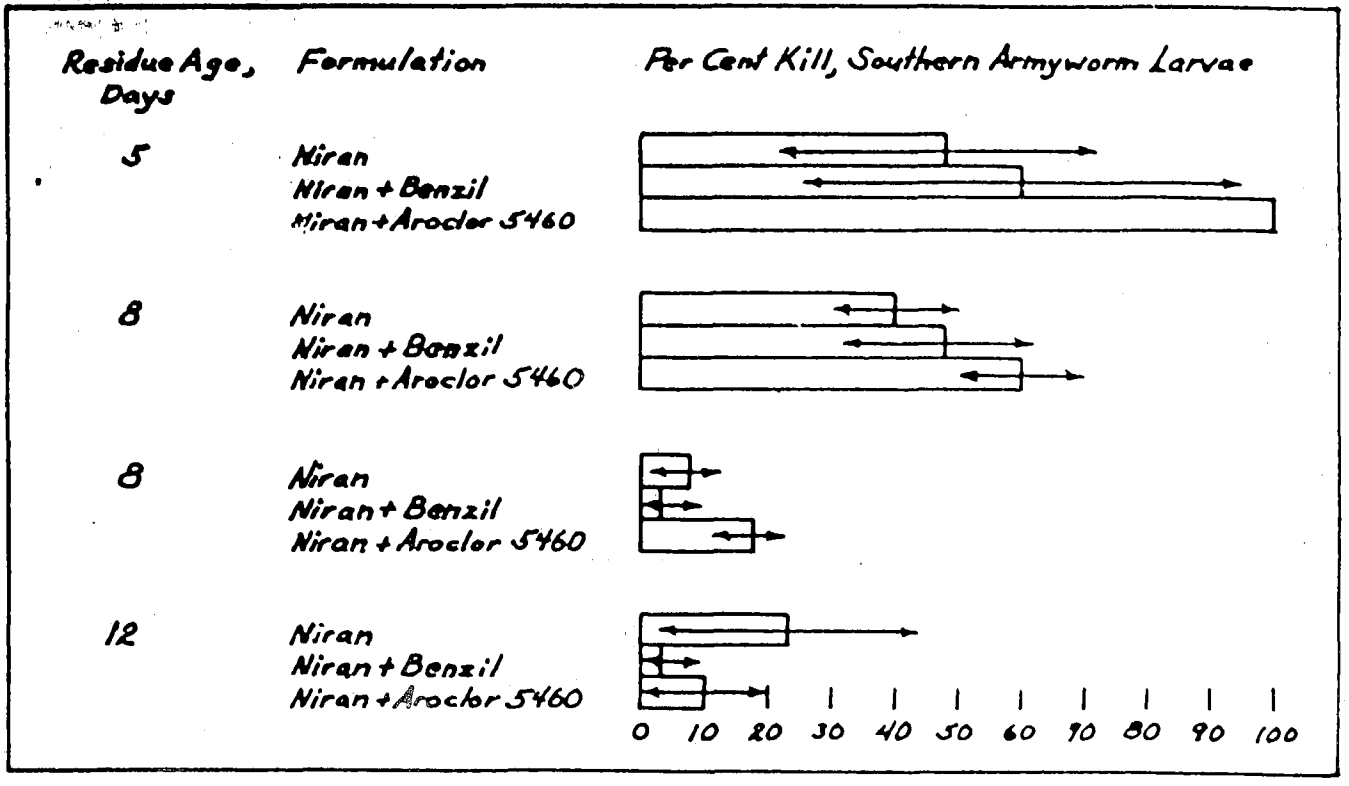
Fig. 4. Effectiveness against the Southern Armyworm of Residues Maintained Outdoors



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Appendix No. 2

Table 5. Statistical Appraisal of Results from Residues Maintained in the Greenhouse

Residue Age, Days	Formulations	Mexican Bean Beetle Larvae Test Means		Significant Difference?	Southern Armyworm Larvae Test Means		Significant Difference?
1	Niran vs Benzil	100	100	No	100	100	No
	Niran vs 5460	100	96.7	No	100	190	No
	Benzil vs 5460	100	96.7	No	100	90	No
5	Niran vs Benzil	100	100	No	93.3	93.3	No
	Niran vs 5460	100	90	No	93.3	90	No
	Benzil vs 5460	100	90	No	93.3	90	No
8	Niran vs Benzil	56.7	93.3	Yes	70	73.3	NO
	Niran vs 5460	56.7	0	Yes	70	70	No
	Benzil vs 5460	93.3	0	Yes	73.3	70	No.
8	Niran vs Benzil	100	100	No	63.3	93.3	Yes
	Niran vs 5460	100	96.7	No	63.3	53.3	No
	Benzil vs 5460	100	96.7	No	93.3	53.3	Yes
12	Niran vs Benzil	63.3	16.7	Yes	10	20	No
	Niran vs 5460	63.3	13.3	Yes	10	10	No
	Benzil vs 5460	16.7	13.3	No	20	10	No
15	Niran vs Benzil	10	3.3	No	30	23.3	No
	Niran vs 5460	10	0	No	30	56.7	No
	Benzil vs 5460	3.3	0	No	23.3	56.7	No
15	Niran vs Benzil	0	13.3	No	16.7	30	Yes
	Niran vs 5460	0	20	No	16.7	20	No
	Benzil vs 5460	13.3	20	No	30	20	No

Appendix No. 2

Table 6. Statistical Appraisal of Results from Residues Maintained Outdoors

Residue Age, Days	Formulations	Mexican Bean Beetle Larvae			Southern Armyworm Larvae		
		Test Means		Significant Difference?	Test Means		Significant Difference?
5	Niran vs Benzil	100	100	No	46.7	60	No
	Niran vs 5460	100	100	No	46.7	100	Yes
	Benzil vs 5460	100	100	No	60	100	Yes
8	Niran vs Benzil	30	46.7	No	40	46.7	No
	Niran vs 5460	30	43.3	No	40	60	Yes
	Benzil vs 5460	46.7	43.3	No	46.7	60	No
8	Niran vs Benzil	13.3	3.3	No	6.7	3.3	No
	Niran vs 5460	13.3	43.3	Yes	6.7	16.7	Yes
	Benzil vs 5460	3.3	43.3	Yes	3.3	16.7	Yes
12	Niran vs Benzil	3.3	0	No	23.3	3.3	No
	Niran vs 5460	3.3	0	No	23.3	10	No
	Benzil vs 5460	0	0	No	3.3	10	No