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NEW SYNTHETIC UREIDES: EFFECTIVE MOSQUITO LARVICIDES*

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The ovicidal effect of six ureides (A13-29054 (dimilin), A13-63223 (penfluron). A13-63218, A13-63220, A13-63308, and A13-63756 against 6.24 hrs. old eggs of yellow-fever mosquito, *Aedes aegypti* L. was very mild after exposure of 24 hr. These compounds are difluorobenzoyl analogues of dimilin. The hatchability of treated eggs ranged from 65-100% at 0.5-10 ppm doses. The susceptibility of various developmental stages of the test insect varied with test compounds. Pupae were less suceptible as compared to fourth instar larvae. Compound A13-63218 (3,4-dichlorphenyl analogue) proved least effective against pupae.

The substitution of halogen radicals, especially at the para-position of the phenyl ring or at its *para* and *meta* positions, increased the larvicidal potential of these compounds.

The test compounds proved very effective larvicides at concentrations ranging from 0.01 to 5 ppm. The anatomical distortions and the patterns of mortality observed indicate that these compounds mimic the action of various other compounds variably known as juvenoids, insect growth regulators (IGR's) insect growth inhibitors, juvenile hormone analogues JHa and morphogenetic agents.

INTRODUCTION

In recent years the availability of compounds inhibiting chitin synthesis has led to extensive efforts to evaluate their potential as insect control agents. Dimilin, 1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl) urea, is one of the most promising ureide now commercially available for mosquito control [1-5]. Besides mosquitoes, it has been tested against many other species of insects and has produced varying degrees of population control by disrupting the development of their immature stages. This compound is known to keep the larvae of insects literally prisoners in their own outer 'skin' preventing them from molting into next larval stage. Dimilin is also reported to cause inhibition of reproduction and egg hatch in the stable fly *Stomoxys Calcitrans* (L.) and house fly, *Musca domestica* (L.) [6].

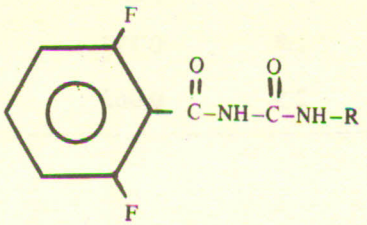
Penfluron, a close analogue of dimilin, has also been thoroughly studied by various authors [7-17]. It is claimed to be superior to dimilin against certain insects. Mulla and Darwazeh [18] and Lacey and Mulla [19] tested various urea-type insect growth regulators against the larvae of *Culex quinquefasciatus, Culiseta incidens* and *Simulium vittatum* in field conditions.

The activity of ureides is known to vary under different experimental conditions, methods of application, stages of development of insect, sex, concentrations, and treatment time, besides the strain specificity.

Keeping these parameters in view, an attempt has been made to evaluate a series of close analogues of dimilin in aqueous suspensions against the various life stages of Laboratory-reared strain of *Aedes aegypti* L.

MATERIALS AND METHODS

The test compounds are difluorobenzoyl analogues of dimilin with the following general structure:



where R represents various substituents. The ARS number of test compounds with substitution at the R position are given below:-

ARS number	R	Common name
A13-29054	(4-Chlorophenyl)	Dimilin

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A13-63223	(4-Trifluoromethy-lphenyl)	Penfluron
A13-63218	(3,4-Dichlorophenyl)	–
A13-63220	(4-Bromophenyl)	–
A13-63308	(5-Chloro-2-pyridinylphenyl)	–
A13-63756	(4-Ethylpheyl)	–

The evaluation work was conducted on eggs, larvae and pupae of *Aedes aegypti* L. reared in the laboratory. The chemicals were dissolved in acetone with traces of triton X-180 (0.02%) and diluted to appropriate concentrations, from which 1 ml was added to 249 ml water, as per standard WHO test for the detection of resistance in mosquito larvae. Twenty eggs, larvae of various instars and early pupae (0-4 hr old) per replicate were exposed to different concentrations of the test compounds for 24 hr. Experiments were replicated thrice.

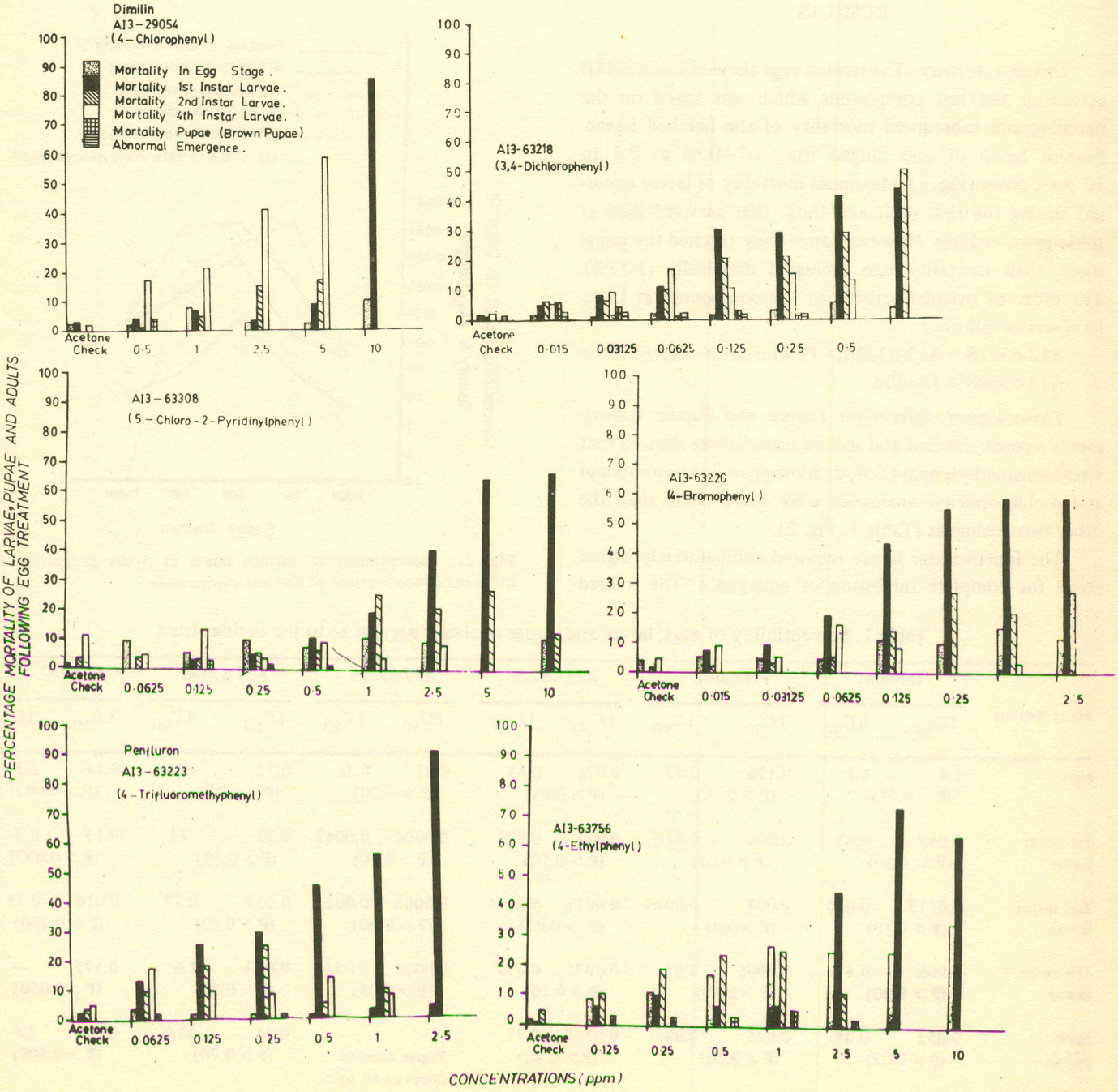


Fig. 1. Pattern of mortality of ureides following egg treatment.

After the exposure period they were transferred to clean water to observe the mortality and morphogenetic effects in larvae and pupae beyond the treated stages at 28 ± 4° and 75 ± 5% R.H. Percent mortalities were corrected by applying Abbott's formula. The ovicidal and larvicidal activities of the compounds were based on LC_{50} and LC_{90} values obtained from the statistical analysis of the data computed from log dose probit regression analysis [20].

RESULTS

Ovicidal Activity. The treated eggs showed low ovicidal action of the test compounds which was based on the hatching and subsequent mortality of the hatched larvae. Percent hatch of eggs ranged from 65-100% at 0.5 to 10 ppm doses (Fig. 1) Maximum mortality of larvae occurred during the first molt and those that survived died at subsequent ecdyses. However, once they reached the pupal stage, their mortality rate decreased drastically (1-13%). The order of ovicidal activity of the compounds at LC_{50} level was as follows:

A13-63218 = A13-63220 > Penfluron > A13-63756 = A13-63308 > Dimilin

Toxicological Effects on Larvae and Pupae. Experiments against the first and second instar larvae showed that 4-trifluoromethylphenyl,3,4,-dichlorophenyl,4-bromopheyl and 4-chlorophenyl analogues were more toxic than the other two analogues (Table 1, Fig. 2).

The fourth instar larvae required comparatively higher doses for complete inhibition of emergence. The treated larvae and pupae showed various morphological changes before death (Fig. 3). The number of adults that emerged incompletely from the pupal skin increased with increasing doses. Penfluron, 3,4-dichlorophenyl, 4-bromophenyl and 4-chlorophenyl analogues proved to be very effective larvicides at comparable doses. The parent compound dimilin showed high activity against the fourth instar larvae as compared to younger once at comparable doses. However, the maximum mortality occurred during the larval stages of the mosquito showing that these com-

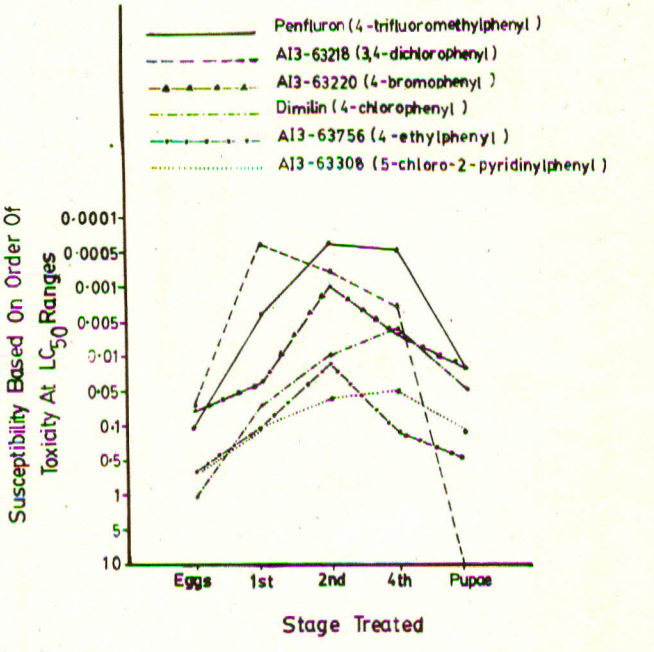
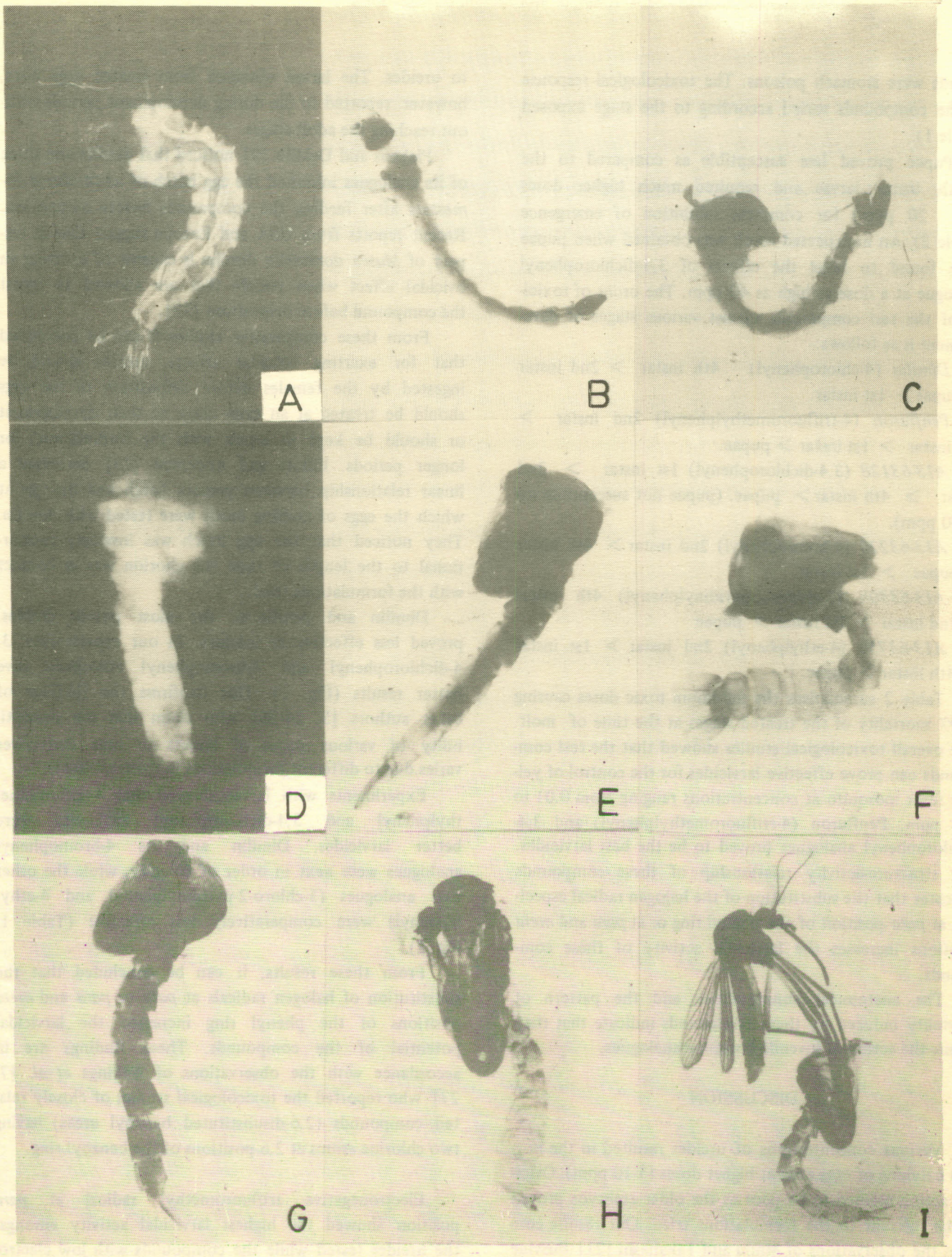


Fig. 2. Susceptibility of various stages of *Aedes aegypti* L. to different concentrations of the test compounds.

Table 1. Susceptibility of eggs, larvae and pupae of *Aedes aegypti* L. to the ureides tried

Stage treated	Dimilin		Penfluron		A13-63220		A13-63218		A13-63308		A13-63756	
	LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}
eggs	1.4	6.5	0.126	0.72	0.076	0.45	0.07	0.56	0.72	4.3	0.71	2.3
	(P > 0.01)		(P > 0.10)		(P > 0.01)		(P > 0.20)		(P > 0.01)		(P > 0.002)	
1st instar larvae	0.069	0.83	0.004	0.017	0.035	0.078	0.0004	0.0047	0.12	14	0.13	0.3
	(P > 0.30)		(P > 0.05)		(P > 0.50)		(P > 0.50)		(P > 0.08)		(P > 0.030)	
2nd instar larvae	0.0113	0.035	0.004	0.0019	0.0011	0.0045	0.0008	0.0027	0.057	0.77	0.016	0.25
	(P > 0.20)		(P > 0.05)		(P > 0.03)		(P > 0.30)		(P > 0.20)		(P > 0.200)	
4th instar larvae	0.006	0.4	0.0005	0.02	0.0075	0.072	0.0031	0.034	0.047	1.6	0.175	—
	(P > 0.20)		(P > 0.10)		(P > 0.10)		(P > 0.05)		(P > 0.20)		(P > 0.050)	
Early pupae	0.052	0.25	0.025	0.05	0.0275	0.77	–	–	0.21	32.0	0.53	3.8
	(P > 0.02)		(P > 0.30)		(P > 0.20)		Pupae resisted doses as 40 ppm.		(P > 0.20)		(P > 0.500)	

P, represents level of significance calculated by probit analysis.



CATEGORIES OF LETHAL ACTION ON MOSQUITO LARVAE TREATED AT VARIOUS STAGES (EGGS, LARVAE, PUPAE)

Fig. 3. A. Death as larva, B. Larval cuticle with pupa inside. C. Partially emerged pupa. D. White pupa. E. Brown pupa. F. Adult visible inside pupal cuticle. G. Death as pupa. H. Partial emergence. I. Feeble adult.

pounds were stomach poisons. The toxicological response of the compounds varied according to the stage exposed (Table 1).

Pupae proved less susceptible as compared to the fourth instar larvae and required much higher doses (> 20 ppm) for complete inhibition of emergence (Table 2). An unexpected result was obtained when pupae were found to resist the effects of 3,4-dichlorophenyl analogue at a dose as high as 40 ppm. The order of toxicity of the test compounds against various stages of development is as follows:-

Dimilin (4-chlorophenyl) 4th instar > 2nd instar > pupae > 1st instar.

Penfluron (4-trifluoromethylphenyl) 2nd instar > 4th instar > 1st instar > pupae.

A13-63128 (3,4-dichlorophenyl) 1st instar > 2nd instar > 4th instar > pupae. (pupae not susceptible up to 40 ppm).

A13-63220 (4-bromophenyl) 2nd instar > 4th instar > pupae > 1st instar

A13-63308 (5-chloro-2-pyridinylphenyl) 4th instar > 2nd instar > 1st instar > pupae

A13-63756 (4-ethylphenyl) 2nd instar > 1st instar > 4th instar > pupae.

Table 2 summarises the minimum toxic doses causing 100% mortality of the treated stages at the time of molt. The overall toxicological studies showed that the test compounds can prove effective larvicides for the control of yellow fever mosquito at concentrations ranging from 0.01 to 5.0 ppm. Penfluron (4-trifluoromethylphenyl) and 3,4-dichlorophenyl analogues proved to be the best larvicides. The structure-activity relationship of these compounds indicates that the substitution of the halogen radical especially at *para* position of the phenyl ring or at *para* and *meta* positions increases the larvicidal activity of these compounds.

The anatomical abnormalities and the pattern of mortality induced by these compounds indicate that they mimic the action of juvenile hormone analogues.

DISCUSSION

Various concentrations of ureides resulted in the normal hatching of eggs even at higher doses (5-10 ppm). Ovicidal action seemed to be poor as the older embryos proved to be tolerant to the formulations tried. Our results confirm the observations of Miura and Takahashi [21] Busvine *et al* [5]. who exposed eggs of various species of mosquitoes to SIR-8514, dimilin and pH 60-38. According to them the eggs with older embryos showed low sensitivity to ureides. The larvae obtained from treated eggs were, however, reported to die during development periods without reaching the adult stages.

Pickens and DeMilo [9] noticed that dimilin and three of its analogues inhibited the egg hatch of adult *Musca domestica* after feeding the compounds before oviposition. Recent reports from USA and Europe suggest that in respect of *Musca domestica* dimilin is capable of exerting an ovicidal effect when female flies are allowed to ingest the compound before oviposition [22].

From these comparative studies it can be concluded that for exerting ovicidal effects, ureides should be ingested by the females before oviposition or the eggs should be treated at an early stage of their development or should be kept in touch with the formulations for longer periods. Elliott and Anderson [23] described a linear relationship between percent hatch and the age at which the eggs of codling moth were tested with dimilin. They noticed that the egg hatch was inversely proportional to the length of time the chorion was in contact with the formulations tried.

Dimilin and penfluron, the most potent ureides, proved less effective as ovicides. In our experiments, 3, 4-dichlorophenyl and 4-bromophenyl analogues gave better results (Fig. 1). This confirms the findings of other authors [5, 24-26] who claim that the susceptibility of various stages of insects to urea derivatives varies due to different formulations in the treatments.

Experiments with larvae showed that 4-trifluoromethylphenyl and 3,4-dichlorophenyl analogues were better larvicides. Dimilin and its 4-bromophenyl analogues were next in order of toxicity, while the other two analogues (5-chloro-2-pyridinylphenyl and 4-ethylephenyl) were comparatively less effective (Table 1, Fig. 2).

From these results, it can be concluded that the substitution of halogen radicals at *para* or *para* and *meta* positions of the phenyl ring increased the larvicidal potential of the compounds. These findings are in accordance with the observations of Wellinga *et al.* [7, 27] who reported the toxicological studies of closely related compounds (2,6-disubstituted benzoyl ureas) having two chlorine atoms at 2,6-positions of the benzoyl ring.

Electronegative trifluoromethyl radical at *para* position showed the highest larvicidal activity amongst the ureides tested while the compounds with low electronegative radical (ethyl radical) at this position was last in the order of effectiveness. From these observations it can be concluded that the effectiveness of the compounds

increased with high electronegative radical at *para* position. These findings are close to the observations of Hajjar and Casida [11] who described the structure-activity relationship of 24 benzoylphenyl ureas against milkweed bugs by treating the abdominal cuticle *in vitro*. According to them the activity of the compounds increased with the increasing electronegativity of the substituents at *para* position.

The maximum mortality of larvae occurred during their developmental periods when the ingestion of food is known to be maximum. Busvine *et al.* [5] reported similar results in other mosquitoes. Many other authors [28-34] have noticed this type of action of ureides in larval stages of various insects. They have also designated these compounds as stomach poisons.

The toxicological pattern and the morphogenetic effects observed are very much similar to other compounds variably known as insect growth regulators, [29], insect development inhibitors [2], morphogenetic agents [31]

Table 2. Minimum toxic doses (ppm) causing 100 % mortality of the treated stages at the time of molt

Compounds	"STAGES TREATED"					Total % inhibition of emergence of adults
	Eggs (cumulative mortality up to 1st instar larvae	1st instar larvae	2nd instar larvae	4th instar larvae	Early pupae	
Dimilin	10	–	–	–	–	100
Dimilin	10	–	–	–	–	100
	–	2.5	–	–	–	100
	–	–	0.06	–	–	100
	–	–	–	1.0	–	100
	–	–	–	–	> 10.00	100
Penfluron	1	–	–	–	–	100
	–	0.125	–	–	–	100
	–	–	0.003	–	–	100
	–	–	–	0.1	–	100
	–	–	–	–	0.1	100
A13-63218	1	–	–	–	–	100
	–	0.003	–	–	–	100
	–	–	0.003	–	–	100
	–	–	–	K 0.125	–	100
	–	–	–	–	Pupae resisted a dose as high as 40 ppm.	
A13-63220	1	–	–	–	–	100
	–	0.25	–	–	–	100
	–	–	0.01	–	–	100
	–	–	–	0.125	–	100
	–	–	–	–	> 20.00	100
A13-63308	5	–	–	–	–	100
	–	2.5	–	–	–	100
	–	–	1.0	–	–	100
	–	–	–	> 2.5	–	100
	–	–	–	–	> 20.00	100
A13-63756	5	–	–	–	–	100
	–	0.5	–	–	–	100
	–	0.5	–	–	–	100
	–	–	0.5	–	–	100
	–	–	–	> 5.0	–	100
	–	–	–	–	> 20.00	100

juvenoides [5] and juveile hormone mimics [24].

The activity of ureides is known to vary from species to species and from stage to stage of the insect treated [2, 10, 29]. These observations so often encountered in our experiments are well documented in literature [24, 25, 35].

It can be concluded that insect control prospects by ureides appear to be more promising in such areas as animal health, public health and stored products. However, only a few areas in plant protection appeared to be suited for the use of these agents because of their delayed effects, problems of critical timing of application, their short persistence in the environment and sure chances of reinfestation of crop again. The limiting factor for their use, as with the JHa, is the time of their application.

REFERENCES

1. W.L. Jacob, J. Med. Entomol., **10**, 452 (1973).
2. M.Y.G. Hsieh and C.D. Steelman, Mosquito News **34**, 278 (1974).
3. M.S. Mulla, H.A. Darwazeh and R.N Norland, J. Econ. Entomol., **67**, 329 (1974).
4. C.H. Schaefer, W.H. Wilder and F.S. Mullingan, J. Econ. Entomol., **68**, 183 (1975).
5. J.R. Busvine, Y. Rongsriyam and David Bruno, Pesticide Sci., 7, 153 (1976).
6. J.E. Wright and G.E. Spates, J. Econ. Entomol., **69**, 365 (1976).
7. K. Wellinga, R. Mulder and J.J. Van Daalen, J. Agr. Food Chem., **21**, 993 (1973).
8. J.E. Oliver, A.B. DeMilo, R.T. Brown and D.G. McHaffey, J. Econ. Entomol., **70**, 286 (1977).
9. L.G. Pickens and A.B. DeMilo, J. Econ. Entomol., **70**, 595 (1977).
10. H.M. Flint, R.L. Smith, J.M. Noble, D. Shaw, A.B. DeMilo and F. Khalil, J. Econ. Entomol., **71**, 616 (1978).
11. N.P Hajjar and J.E. Casida, Science., **200**, 1499 (1978).
12. S.C. Chang, J. Econ. Entomol., **72**, 479 (1979).
13. S.C. Chang, and C.W. Woods, J. Econ. Entomol., **72**, 482 (1979).
14. A.M. Jordan and M.A. Trewern, Bull. Ent. Res., **69**, 55 (1979).
15. S.C. Chang and A.B. Borkovec, J. Econ. Entomol., **73**, 285 (1980).
16. S.A. Qureshi and S. Mohiuddin, Proc. Ent. Soc. Karachi., 9-10, 53 (1980).
17. R.E. Redfern, A.B DeMilo and A.B. Borkovec, J. Econ. Entomol., **73**, 682 (1980).
18. M.S. Mulla and H.A. Darwazeh, Mosquito News., **39**, 746 (1979).
19. L.A. Lacey and M.S. Mulla, Mosquito News, **39**., 86 (1979).
20. D.J. Finney, *Probit Analysis*, (Cambridge University Press (1952), 2nd ed., p. 318.
21. T. Miura and R.M. Takahashi, J. Econ. Entomol., **72**, 692 (1979).
22. Anonymous, *A New Insecticide Intefering with Chitin Deposition*, Technical Information, (Philips-Duphar B.V., 1980), 7th ed., p. 31.
23. R.H. Elliott and D.W. Anderson, Can. Ent., **114**, 259 (1982)
24. R.E. Redfern, A.B. DeMilo and L.E. Oliver, Botyu-Kagaku., **42**, 89 (1977).
25. G. Tamaki and J.E. Turner, J. Econ. Entomol., **67**, 697 (1976).
26. K.R.S. Ascher and N E. Nemny, Pesticide Sci., **7**, 1 (1976).
27. K. Wellinga, R. Mulder and J.J Van Daalen, J. Agri. Food Chem., **21**, 348 (1973).
28. J.W. Neal Jr., J. Econ. Entomol., **67**, 300 (1974).
29. J.E. Wright, J. Econ. Entomol., **67**, 746 (1974)
30. R.W. Miller, J. Econ. Entomol., **67** 697 (1974).
31. J.J. Menn and F.M. Pallos, Environ., letters **8**, 91 (1975).
32. H.E. McGregor and K.J. Kramer, J. Econ. Entomol., **69**, 479 (1976).
33. H.M. Flint and R.L. Smith, J. Econ. Entomol., **70**, 51 (1977).
34. A B. DeMilo, D.M. Ostromecky, S.C. Chang, R.E. Redfern and R.L. Fye, J. Agr. Food Chem., **26**, 164 (1978).
35. K R.S. Ascher and N.E. Nemny, Pesticide Sci., 7, 447 (1976).