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Effects of Mode of Testing on Susceptibility to Insect Growth Regulators of Various Stages of Development of *Musca domestica*

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ABSTRACT

In the study of the effects of Insect Growth Regulators (IGR) in relation to various stages of larval development, two susceptible strains and one DDT-resistant strain of Musca domestica were tested by three methods (oral feeding with IGR treated food, IGR in Brain Heart Infusion Agar and IGR treated cotton wool) against ZR-515, PH 60-40, PH 60-38 and Mos-0585.

The LC_{50} for all tested compounds varied irrespective of the methods of exposure and the developing stages of the flies. However, ZR-515 showed the immense effect especially on the early third instar larva. The LC_{50} by treated cotton wool was about 8 times of those by treated in Brain Heart Infusion Agar and about 2 times of those by treated food. On the whole, exposure to IGR in Brain Heart Infusion Agar was the best method and early third instar larvae was the most effective stage for applying the IGR. Based on these specific criteria, the LC_{50} values of ZR-515, PH 60-40, PH 60-38 and Mons-0585 in WHO standard strain were 0.11, 0.65, 28 and 1,000 ppm respectively.

In comparison of the toxic effect of the compounds, ZR-515 was the most potent. It produced very high pupal-adult intermediate mortalities, whereas PH 60-38 inhibited chitin synthesis and produced main effect in pupa. Mons-0585 was the least potent compound and the lethal effect occurred in both pupa and prepupal stages, but it tends to be more in the latter one.

Considering the involvement in DDT-resistance, the DDT-resistant strain showed cross resistance to three IGR with resistant factors 40, 38 and 10 tiems for ZR-515, PH 60-40 and PH 60-38 respectively.

INTRODUCTION

Control of housefly, *Musca domestica*, by Insect Growth Regulator (IGR) was the most interesting method among alternative methods since the discovery of Juvenile Hormone Mimics as insecticide[1]. Numerous attempts have been initiated to find the effects of IGR on treated adult and pupa of housefly[2-4].

Although in recent years, IGR have shown promise in controlling a lot of insects[5], but investigation and bioassay for comparison with activities of Insect Growth Regulators on housefly larvae were scarecely reported. Recently, Gingrich and Hopkins[6] reported that when the 3rd instars of *Haematobia irritans* were exposed to methoprene in cow manure, emergence of adult was inhibited. However, there was no effect when the 1st or 2nd instar or pupae were exposed to the same treatment. Since the modes of administration of IGR are known to affect the stage of development of the fly and also assessments of their relative potencies, it was considered desirable to investigate these factors and to develop a rapid practical bioassay procedure and to compare the activity of four IGRs on housefly larvae.

MATERIALS AND METHODS

1. The housefly colonies

1.1 S_1 The standard susceptible strain was obtained initially from WHO collaborating centre for standardized Houseflies, Pavia, Italy. A sub-colony was started at the Insecticide Research Unit, Faculty of Tropical Medicine, Bangkok, Thailand in 1975.

1.2 S_1 This susceptible strain was originated from Hadyai and was colonized in the laboratory of Insecticide Research Unit in 1975. Among the strains collected from various places in Thailand, this is the most DDT-susceptible strain.

1.3 R A highly DDT-resistant strain which was collected from Samsennai area, Bangkok and maintained in the laboratory from 1975. Selections were made for this study to raise the resistance to DDT.

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2. The chemicals

The following compounds were used.

2.1 Juvenoids (juvenile hormone mimics)

Methoprene, isopropyl (±)-(E, E)-11-methoxy, 3, 7, 11-trimethyldodeca-2, 4-dienoate. "Altosid", "ZR-515", Zoecon Co.

2.2 Other insect growth regulators

Mons-0585, 2, 6-di-tert-butyl-4 (αα-dimethylbenzyl) phenol [Monsanto Co.] Dimilin, 1-(4-chlorophenyl)-3-(2, 6-difluorobenzoyl) urea ["Diflubenzuron", "PH 60 : 40", Philips-Duphar N.V.] PH 60 : 38, 1-(4-chlorophenyl)-3-(2, 6-dichlorobenzoyl) urea [Philips-Duphar].

3. Experimental methods

3.1 Test by exposure to insecticide treated food

The food mixture were prepared by using rice bran and fish powder in the ratio of 1 : 1. About 20 g of this food was mixed with 15 ml of water. Graded dilutions of insecticide in food mixture were made in a plastic cup (10 cm in diameter and 12.5 cm in height). Then each batch of 50 early third instar larvae was introduced into different plastic cups to be continuously exposed until they developed to pupae, then were transfered into a clean plastic cup and were maintained in the laboratory to assure that all adults emerged.

The first and second stage larvae were tested in the same method of exposure as those for the early third stage larvae.

3.2 Test by exposure to insecticide in Brain Heart Infusion Agar

Graded dilutions of insecticide in Brain Heart Infusion Agar medium (10 g of Brain Heart Infusion Agar in 500 ml of water) were prepared in a flask. A batch of 100 early third instar larvae was transferred into the different flask to have continuous exposure until they developed to pupae which were maintained to observe the adult emergences.

The same procedure was used for the second and late third stage larvae.

3.3 Tests by exposure to insecticide treated cotton wool

These were based essentially on the graded dilution method, deseribed by Du Toit and Fiedler[7] and by Shaw and Blockman[8]. Five ml each of different concentrations was pipetted into an individual 4 x 9 cm flat-bottomed vial which contained 0.5 g of absorbent cotton wool. A batch of 50 early third instar larvae was then continuously exposed in each vial with serum serving as food supply and suspending agent for the insecticide. The larvae were left in the test medium for pupation.

In all cases the chemicals were dissolved in acetone and diluted to appropriate concentrations. Observations were counted of the mortalty in different instars until all were dead or had emerged.

RESULTS

Three series of experiments were undertaken in this study to investigate the susceptibility of the various stages of larvae to ZR-515, PH 60-40, PH 60-38 and Mons-0585. The results were summerized in Table 1 and were considered as follows:

1. Tests by exposure to insecticide treated food

1.1 Tests with first stage larvae

The first stage larvae of both susceptible S_1 and S_2 strains were tested with 4 concentrations of each IGR (100, 10, 1 and 0.1 ppm).

ZR-515 inhibited development at pupal-adult intermediate stage. Most of emerged adults were normal. The abnormal adults died within 24 hours after emergence.

PH 60-40 and PH 60-38 gave similar effects. The development of larvae was affected at pupal stage. The puparium was soft with vesicular fluid inside and a bubble appeared in the anterior notum beneath the cuticle. The cuticle at this point seemed transparent and thinner and it could easily be broken by placing a dissecting needle on the deformed area.

Mons-0585 showed most of the effects in the pupal stage especially at the high concentrations.

LC_{50} values recorded from the tests showed that ZR-515 was the most toxic of all the tested compounds. PH 60-38 was comparatively inferior to PH 60-40 and Mons-0585 was the least potent. The potencies of each tested compounds were in the same order in both of S_1 and S_2 strains.

Table 1　The potencies of IGR in relation to various stages, strains and methods

Tested compound	Strain	LC_{50} of IGR in larval test (ppm)						
		Exposed to IGR treated food			Exposed to IGR in BHIA			Treated cotton wool
		L_1	L_2	eL_3	L_2	eL_3	IL_3	eL_3
ZR-515	S_1	0.13	0.75	0.48	0.90	0.11	2.20	0.80
	S_2	0.55	1.00	0.60	2.00	0.46	5.50	1.40
	R	–	–	–	–	4.40	62.50	4.60
	Rf	–	–	–	–	44	28.41	5.75
PH 60-40	S_1	18.00	18.00	8.00	10.00	6.50	24.00	12.00
	S_2	22.00	24.00	11.00	15.00	7.50	40.00	11.00
	R	–	–	–	–	250.00	320.00	500.00
	Rf	–	–	–	–	38.46	13.33	41.67
PH 60-38	S_1	30.00	90.00	30.00	36.00	28.00	50.00	38.00
	S_2	46.00	48.00	44.00	80.00	42.00	140.00	44.00
	R	–	–	–	–	290.00	400.00	600.00
	Rf	–	–	–	–	10.36	8.00	15.79
Mons-0585	S_1	1000	1300	1100	1100	1000	1400	1150
	S_2	3000	2400	1300	3400	1200	2000	2000
	R	–	–	–	–	1800	2000	2200
	Rf	–	–	–	–	1.80	1.43	1.91

L_1 = first stage larvae　　IL_3 = late third stage larvae
L_2 = second stage larvae　　BHIA = Brain Heart Infusion Agar
eL_3 = early third stage larvae　　Rf = Resistant factor (compared with S_1)

The S_1 strain was more susceptible to all compounds than the S_2 strain as can be seen from the following LC_{50} values, for S_1 were 0.13, 0.18, 30, 1100 while S_2 were 0.55, 22, 46, 3,000 for ZR-515, PH 60-40, PH 60-38 and Mons-0585 respectively.

1.2　Tests with second stage larvae

The tests were performed with S_1 and S_2 strains. The over all results were almost the same as the test with first stage. ZR-515 showed the main effects at pupal-adult intermediate stage, while PH 60-40 and PH 60-38 produced their main effects rather early in pupal stage. The effect of Mons-0585 was not clear. However, it seemed to be more active in early metamorphosis from larvae to pupa.

Among the four insect growth regulators, evidence of most promising potency was obtained for ZR-515 with LC_{50} values 0.75 and 1.0 for S_1 and S_2 stains. The remaining PH 60-40 had 18 and 24 for S_1 and S_2 respectively. The figures of PH 60-38 were 90 for S_1 and 48 for S_2. The Mons-0585 had very low potency, that is the highest concentration of 100 ppm only produced less than 50% kill.

1.3　Tests with early third stage larvae

The results showed that all compounds still produced their main effects as in previous tests. Mons-0585 gave more specific effects in the pupal-adult intermediate stage especially at the 100 ppm concentration.

Although the larval mortalities were also high as the previous experiment, the percentages of inhibition of adult celosion were highly increased notably with Mons-0585. The LC_{50} values of ZR-515, PH 60-40, PH 60-38 and Mons-0585 were 0.48, 8, 30 and 1100 ppm. The S_2 strain was more tolerant than S_1 and the figures of LC_{50} were in the order of 0.6, 11, 44 and 1300 ppm.

2. Tests by exposure to IGR in Brain Heart Infusion Agar

Because of the dull results and disadvantages obtained from the previous tests, so this method was tried out in the second, early third and late third instar larvae of S_1, S_2 and R strains.

2.1 Tests with second stage larvae

The effects of all IGR on the second stage larvae were not promising. The larval mortality rates in each control and test groups of S_1 and S_2 strains were still high although they were slightly less than the previous methods.

ZR-515 showed noticeable effects in pupal-adult intermediate stage. The effects of PH 60-40 and PH 60-38 were the same and were particularly evident in the pupal stage Mons-0585 did not show any special effect. It seemed to produce no effect on the abnormal adult.

In consequence of dose and response curves, the LC_{50} values of the four IGRs in the preceding order were 0.90, 10, 36, 1100 for S_1 strain and 2, 12, 80, 1800 ppm for the S_2 strain.

PH 60-40 and PH 60-38 were more effective than in the pervious tests. The other two compounds were less potent than expected. The S_1 strain was more susceptible than the S_2 strain.

2.2 Test with early third stage larvae

Early third stage larvae of S_1, S_2 and R strains were treated with IGR mixed with in Brain Heart Infusion Agr. It was clear that all IGR produced their evident effects almost in one category especially at the high concentration. Treatments with ZR-515 gave results in pupal-adult intermediate category. The larvae treated with PH 60-40 and PH 60-38 died mostly in the puapl stage. Mons-0585 tends to be more active in pupal-adult intermediate stage than in pupal stage. All compounds showed little toxicity against the larvae stage.

Based on the LC_{50} values, ZR-515 was the most potent of all IGR, followed by PH 60-40, PH 60-38 and Mons-0585. The S_1 strain was the most susceptible and S_2 was slightly tolerant. There was some corss-resistance in the R strain which is the DDT-resistant strain.

2.3 Test with late third stage larvae

From the data presented here it was apparent that ZR-515 most yielded the pupal-adult intermediate effects. The PH 60-40 and PH 60-38 gave the effects in pupal stage. Finally, Mons-0585 seemed to produce nearly the same number of two main effects, pupal and pupal-adult intermediate. It was interesting to note that there were very little or no effect on the larvae especially with the Mons-0585 treatments of all strains.

Again, the susceptibility of the strains were in the order of S_1, S_2 and R strains. High resistant factors were shown in the R strain.

3. Tests by exposure to IGR treated cotton wool

To gain futher insight into the factors concerned with route of application of IGR, tests were undertaken with the early third stage larvae of S_1, S_2 and R strains. The larvae were exposed to four concentrations of IGR treated cotton wool. The results of this investigation were set out in Table 1.

ZR-515, PH 60-49 and PH 60-38 produced their main effects as previous results, but Mons-0585 showed some more particular evidence in the pupal stage. There was no effect on the larvae and the number of abnormal adults were very small.

Comparing with LC_{50} value of all IGR in each dorsage mortality curve of the experiments, it was clearly found that ZR-515 was the most potent. PH 60-40 and PH 60-38 were moderate and Mons-0585 was the least potent. There was evidence of some degree of resistance in the R strain.

The over all results that had been described leaded to the following conclusions :–

1. Method

Among the larval tests in various types of medium, exposure to IGR in Brain Heart Infusion Agar was the best method as it gave the lowest LC_{50} values expecially with the early third stage larvae (Figure 1). Similar results were obtained when using various stages of larvae and strains.

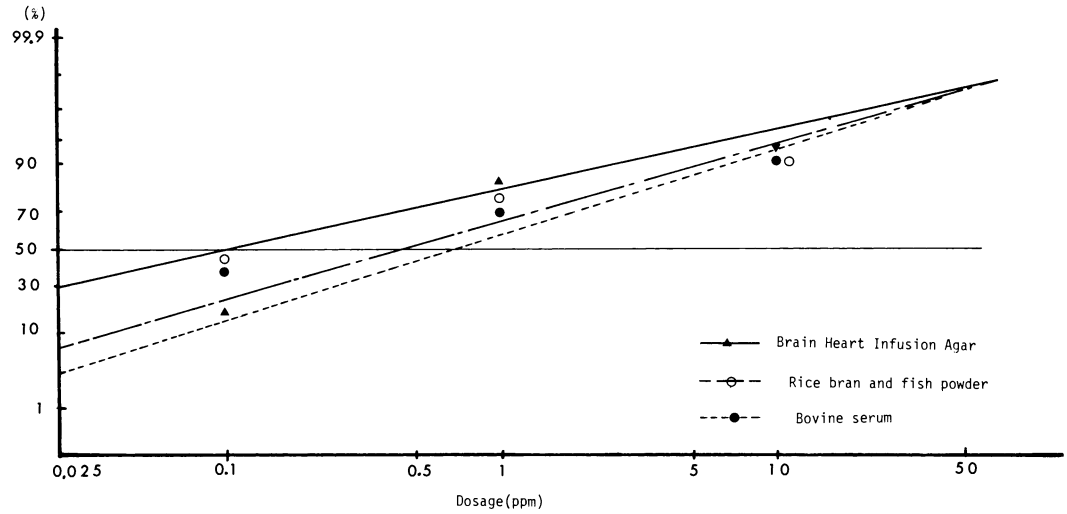

Figure 1. Comparative potencies of ZR-515 against early third stage larvae of house fly S_1 strain by exposure to IGR in various types of medium

2. Stage of application

Based on the above best method, the early third stage was the most effective stage since the LC_{50} of ZR-515 were 0.90, 0.11 and 2.20 ppm for the second stage, early and late third instar larvae respectively (Figure 2). Comparagive potencies of other tested compounds and other methods also showed similar results. Therefore, the early third stage was the most appropriate applying the IGR.

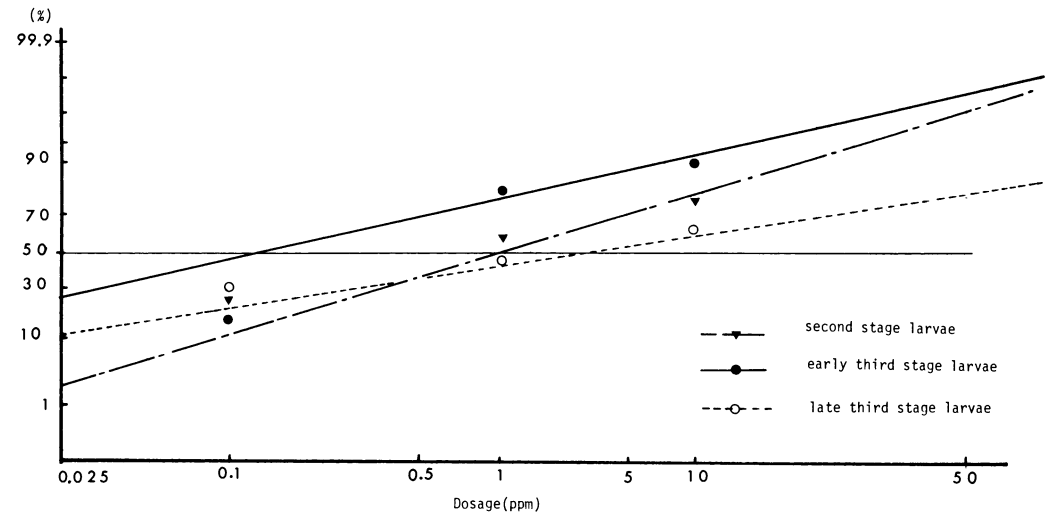

Figure 2. Comparative potencies of ZR-515 against various stages of house fly S_1 strain Test by exposure to IGR in Brain Heart Infusion Agar

3. Efficiency of the test compounds

According to the best method and stage for application as previously mentioned, it was revealed that ZR-515 was the most potent compound. PH 60-40 and PH 60-38 was moderately effective and Mons-0585 gave the least effect. (Figure 3).

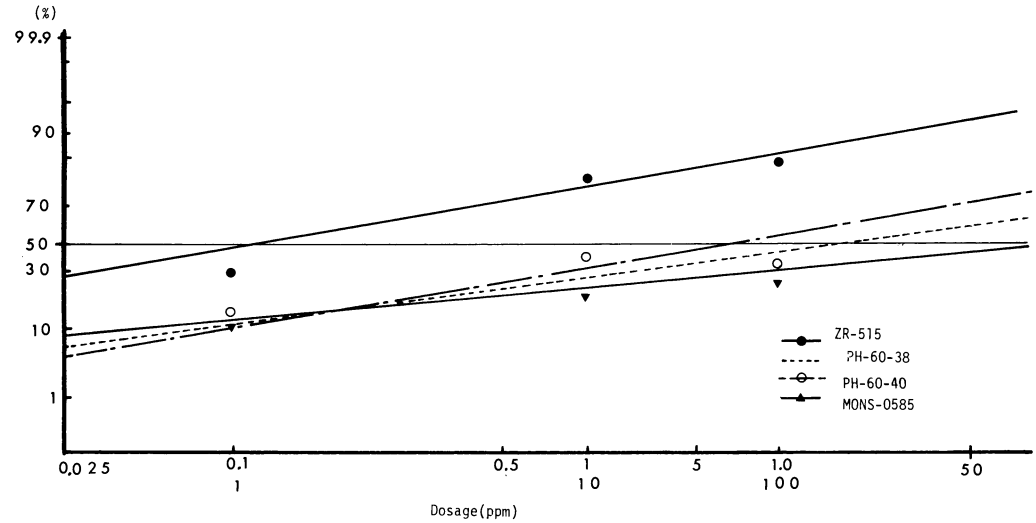

Figure 3. Comparative potencies of IGR against early third stage lavae of house fly S_1 strain Test by exposure to IGR in Brain Heart Infusion Agar

4. Involvement in resistance

In the tests with DDT-resistant strain, it gave 44 times resistance with ZR-515 and 38 times resistance in test with PH 60-40. The resistant factor with PH 60-38 was not resistant. There was no cross resistance to Mons-0585.

DISCUSSION

As ZR-515 was the most effective compound against housefly, this result agreed with the result of Sehnal and Zdarek[9]. It inhibited the development at pupal-adult transformation and gave 50% reduction progeny of emerged adult. Similar result was reported by Jakob[10]. He revealed that after seeding newly hatched larvae with 100 ppm ZR-515, it gave 99% or complete inhibition of adult eclosion in contrast with PH 60-40 which killed housefly at period of pupa formation. Both inhibit chitin synthesis which results in bubble appearance beneath the cuticle. This result confirmed that of Wright[11]. The disruption of chitin synthesis of Yu and Terriere[12] reported that PH 60-40 was more effective than PH 60-38, which agreed with this result. PH 60-40 and PH 60-38 not only inhibit chitin synthesis, but reproductive inhibition was also found with the exposure to both IGR in Brain Heart Infusion Agar. This result agreed with the work of Wright et al.,[15] as they reported that PH 60-40 was able to reduce hatchability of egg to 23.1% and only 10% of them developed to normal adult.

Mons-0585 was not effective enough for the control even in larval test, it inhibited melanization of mosquitoes and killed them at a period of pupa transformation[16]. But from this investigation, Mons-0585 could not inhibit melanization of housefly pupae and gave only 12% inhibition adult eclosion. This result was similar to the work of Jakob[10], who reported that even at the highest dose, 250 ppm, it gave only 10% inhibition adult eclosion.

On the whole, the IGR although quite potent against the third instar larvae of housefly showed, reduced effects on the first and second instar larvae too. The moulting disturbance compounds (PH 60-40 and PH 60-38), although structurally unrelated to Juvenile Hormone analogue (ZR-515), showed effects largely similar to those of the latter, with the possible exception of Mons-0585, which was rather ineffective against the housefly.

So for as the results obtained by the methods concern, exposure in Brain Heart Infusion Agar was the best method and the early third stage of larvae was the most effective stage. Recently, it has been shown that methoprene inhibited the adult emergence when applied to the third stage larvae of H. irritans in cow manure, but there was no effect at all with the other instars or pupae[6]. It was evident that these have to be related to such factors as the stage of the fly tested, the method of exposure, and the type of insecticide used for each test. It is in the

light of these factors that such other considerations as differential rates of contact penetration (or absorption), metabolism and detoxification of the test compounds by the different insect forms must be viewed.

Thus the fact that the toxicity of many insecticides does vary with the methods and circumstance of their application is not surprising, although Sun and Jonson[17] did observe that the exact nature of such variation is often difficult to explain. For their effectiveness, virtually all the insecticides used in the present study depend primarily on reaching and interferring with the metamorphosis of the exposed flies. The significant difference noted between the tolerable dose of insecticides administered orally and by dermally to the flies underscores the importatance of not only the physical barriers posed by the fly integment (i.e. the suticle and body wall on the one hand, and the gut wall on the other) in terms of penetration, but also the physiological barrier posed by the insect metabolic system in terms of detoxification and excretion.

The problem of choosing an acceptable laboratory method for assaying insecticides used in the control of specific insects calls for consideration of a variety of factors, the more important being under these headings: (1) the sensitivity and reliability of the test method, (2) its simplicity and convenience, (3) its suitable to field conditions.

In the test with four insect growth regulators, the R strain showed corss-resistance to three compounds. This could perhaps by explained by enhanced microsomal oxidation. These observations agree with those by Dyte[18] and Plapp and Vinson[19] who have respectively reported some measures of cross-resistance between juvenile hormone mimics and different organophosphorus compounds in studies with flour beetle (Tribolium castaneum) and house flies. Cerf and Georghiou[20], while noting varied levels of cross-resistance to ZR-515 in pupae of house flies resistant to parathion, chlorthion, fenthion and dimethoate, reported DDT-lindane resistant strains as entirely susceptible to juvenile hormone analogue.

Furthermore, Plapp and Vinson[19] have noted experimentally induced cross-resistance to various juvenile hormone analogues, implying that even higher levels of resistance may develop when large populations of the insects are expos3d to the hormone in pest control programmes.

Although the physiological and other activity of juvenile and moulting hormones in insect metamorphosis have been widely studied[21], the activity of the new group of compounds used in this study, calls for closer attention. These new compounds, comprising two Duphar products (PH 60-40 and PH 60-38) and the Monsanto product (Mons-0585) produced effects very similar to the juvenile hormone analogue (ZR-515) in the house fly although they are known to differ structurally from the latter.

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Effects of Mode of Testing on Susceptibility to Insect Growth Regulators of Various Stages of Development of Musca

イエバエの各発育ステージにおけるIGRに対する感受性の試験法の影響

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従来の殺虫剤と全く作用機構の異る新しい殺虫剤としてInsect Growth Regulators（IGR，昆虫成長抑制剤）が開発された。

昨今，主要な害虫のひとつであるイエバエMusca domestica Linne, 1758が各種殺虫剤に対し，強い抵抗性を持つ傾向にあることが明確にされた。その対策として，新しい殺虫剤の開発研究がなされ，そのひとつとしてIGRが検討されている。

しかし，IGRの効力を評価するための検定法に関する基礎的な研究がすくなく，未解決の面がすくなくない。

著者らはIGRの効力を適切に評価するため，実験方法および供試令期などについて実験を行い，若干の知見を得たので報告する。

実験方法は餌料摂食法，B.H.I寒天培地法，棉培地法の3方法を用いた。また，供試虫は幼虫を用い，使用令期はI令，II令，III令とした。

実験の結果，実験方法により，IGRの効力順位に若干の相違あることが明かになった。また，IGRの力価を厳しく評価する方法としてはB.H.I寒天培地法が適切であることがわかった。

また，いずれの方法での供試虫の供試令期が結果に大きく影響することが明らかになり，全般的にみてIII令期が供試適期であることがわかった。

なお，本実験に使用したIGRのうち，最も効果的であったのはZR-515 (Juvenoids)で，PH-60：40，PH60：38 (Diflubenzuron)がこれにつぐものであった。Mons-0585の効果は最も劣った。ことに，ZR-515は致死効果が高く，PH-60-38はキチン合成阻害効果に優れ，蛹期に影響がみられた。Mons-0585は羽化阻害作用がみられ，他のものと悄々作用機構の違いがみられた。

また，DDT抵抗性系統を用いた実験では若干感受性の低下がみられ，ZR-515で44倍，PH60-40で38倍，PH60-38で10倍の抵抗性比が認められた。しかし，Mons-0585では交差抵抗性の発達が認められなかった。