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Synergism of Several Synergists of Antii-Juvenile Hormone Analog, 7-Ethoxy Precocene II to Milkweed Bug *Oncopeltus fasciatus* Dallas

7-Ethoxy precocene II 항유약호르몬유사물에 대한 몇가지 협력제의 *Oncopeltus fasciatus* Dallas에 있어서 협력작용

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ABSTRACT The second instar milkweed bugs, *Oncopeltus fasciatus* were exposed to the residue of 6-methoxy-7-ethoxy-2,2-dimethylchromene(or 7-ethoxy precocene II, 7-EP-II) with one, two, or four different kinds of synergist(s) deposited as a residue on 9 cm diameter petri dishes. The anti juvenile hormone analog, 7-EP-II yielded a 50% effectiveness concentration at 1.18 μg/cm^2. The concentration of 7-EP-II and RO20-9747 combined gave 0.084 μg/cm^2, as much 14 times more potent than with 7-EP-II alone. Most of the other synergists, including geraniol, 4-chalcone oxide, isosafrole, and piperonyl butoxide however, showed comparatively low level synergism with 1.0-4.3 times, depending upon the combinations of the synergist(s). The obtained results were considered that RO20-9747, an analog of benzopyran with similar structure to that of 7-EP-II inhibited selectively the insect monooxygenase oxidative defense mechanism, in the body except for corpora allata because general antioxidants of piperonyl butoxide, isosafrole, and 4-phenyl chalcone oxide showed relatively mild synergism.

KEY WORDS Anti juvenile hormone, precocene, synergism, *Oncopeltus fasciatus.*

초 록 몇가지 협력제의 7-ethoxy precocene II와 협력작용을 통한 활성증가와 작용기작을 구명하기 위하여 milkweed bug, *Oncopeltus fasciatus* 2령충을 항유약호르몬유사물과 협력제가 처리된 petri dish에 접종하여 생리활성 정도를 측정하였다. 얻어진 결과는 다음과 같다. 7-Ehtoxy precocene II의 반수활성농도는 1.18μg/cm^{2}이었다. 7-Ethoxy precocene II와 다른 산화저해제인 RO20-9747의 협력비가 14.05로 가장 높았고 그들의 반수활성농도는 0.084μg/cm^{2}이었다. 7-Ethoxy precocene II와 다른 협력제 1종, 2종, 4종을 조합을 달리하여 처리하였을 때 뚜렷한 협력작용 증가효과를 가져오지 못했다. 이상의 결과에서 다른 협력제들은 곤충의 방어기작을 알라타체를 포함하여 비선택적으로 저해하는 것으로 생각되었으나 RO20-9747은 7-ethoxy precocene II가 알라타체로 이행하는데 까지 곤충의 방어적 산화기작인 monooxygenase활성을 선택적으로 저해함으로써 7-ethoxy precocene II가 알라타체로 이행량이 증가하여 활성증가를 가져온 것으로 생각되었다.

검색어 항유약호르몬, Precocene, 협력작용, *Oncopeltus fasciatus.*

The problems and benefits in the use of most of conventional insecticides are achieved by exploiting nervous system common to insect pests and the most other animals. Environmentally friendly and biodegradable insecticides of which insect growth regulators are highly promising in the near future (Bowers 1992) are demanded increasingly because there are no endocrinological counterparts in arthropods to other so called higher animals. As one of these approaches studies on anti juvenile hormone were conducted very actively to find potent chemicals and their biological and chemical mode of actions because juvenile hormone analogs were labile in practical field and worked at a critical immature stage which was destructively feeding in most of agricutural insect pests (Bowers 1985, Staal 1986)

The anti-juvenile hormone analog, 6-methoxy-7-ethoxy-2,2-dimethylchromene or 7-ethoxy precocene II(7-

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EP-II) is a highly potent chemical analog of the natural phytochemical, precocene II, 6,7-dimethoxy-2,2-dimethylchromene (Bowers 1977). The precocenes penetrate cuticle very quickly and are stored mainly in the fat body of *Oncopeltus fasciatus* (Haunerland and Bowers 1985). They are detoxified into 3,4-dihydrodiol of precocene and other minor metabolites(Hamnett *et al.* 1981, Pratt *et al.* 1980, Soderland *et al.* 1980) however a small amounts reaches to the corpora allata(CA). The precocenes are activated by a cytochrome P_{450}-dependent microsomal monooxygenase into a highly reactive epoxide across 3,4-double (Hamnett *et al.* 1981, Pratt *et al.* 1980). The epoxides undergo nucleophilic alkylation (Pratt *et al.* 1980, Aizawa *et al.* 1985) of elements in the parenchymal cells of CA causing very rapid destruction and dissolution of the cell (Unnithan *et al.* 1977), resulting in the cessation of juvenile hormone biosynthesis and secretion. The chemical ablation of CA has hydrolase induced precocious metamorphosis of the immature stages along with reduced feeding and adult sterility (Bowers 1982a).

The idea that insect epoxide hydrolase inhibitors may be potential synergists of insect control agents such as juvenile hormones and insecticide chemicals possessing epoxide rings, has been discussed (Brooks 1973, 1973). But Burt *et al* (1978) found that cabbage looper (*Trichoplusia ni* Hübnner) and European corn borer (*Ostrinia nubilalis* Hübnner) were immune to insect epoxide hydrolase inhibitors, although the compounds tested inhibited metabolism of precocene II by mixed function oxidases. This experiment was conducted to inhibit activation defense mechanism in *O. fasciatus* of 7-EP-II by several combinations using anti oxidant synergists.

MATERIALS AND METHODS

1. Chemicals

The anti-juvenile hormone analog, 7-EP-II and the synergist 4-phenyl chalcone oxide(pco) were used as pure chemicals from Bowers' laboratory. RO20-9747(3,4-dihydro-2,5,7,8-tetramethyl-6-hydroxy-2H-1-benzopyrane-2-carboxylic acid) from Hoffmann-La Roche, Inc., geraniol, isosafrole, and piperonyl butoxide(pb) from Aldrich, Milwaukee.

2. Insects

Milkweed bug, *O. fasciatus* was reared at 29±0.5°C with 50% R.H., 16L 8D on sunflower seed and water *ad libitum* within 30cm diameter by 25cm height plastic bucket. A cohort of eggs was collected daily to obtain newly molted second instar nymphs used in the testing.

3. Bioassays

The synergists, 7-EP-II, or synergist(s)+7-EP-II in mixture were applied in 200 μl analytical aceton or chlorform solution to the 9cm diameter petri dishes. To the residue of the test chemicals in the bottom of the dishes, twenty second instar *O. fasciatus* were exposed. To confine the insects to the bottom, the wall of petri dish was coated with Fluon AD-1 (Northern Products, Inc., Woonsocket, R.I.) as a slippery agent. Sunflower seeds and drinking water in dental wicks were supplied 24 hours after treatment to make the bug look for their feed for exposure. The effect anticipated was the appearance of precocious adults which were characterized by the number of tarsi, no wing expansion, disappearance of distinctive two black spot on the dorsal sideor occasionally death from the conbination of chemicals. The responses of the precocious effects were observed on six days after exposure. The data were analyzed with SAS probit analysis program (SAS 1989). When 7-EP-II was mixed with pb for synergistic study, the data for mixtures were analyzed by the method of Sun and Johnson (1960), although the toxic effect of pb was almost negligeable in the each test range.

RESULTS

Table 1 showed the effectiveness of 7-EP-II and several synergists. Fifty percent effective concentration(EC 50) of 7-EP-II was 1.18 $\mu g/cm^2$. Three synergists such as RO20-9747, geraniol, and isosafrol were virtually non-toxic to *O. fasciatus*. 4-Phenyl chalcone oxide showed no significant toxicity up to 1.26 $\mu g/cm^2$.

Table 1. Synergism of 7-ethoxy precocene II with several synergists in milkweed bug, *Oncopeltus fasciatus*

Treatments	Mixture ratio	n	Slope±SE	EC50* (95% fiducial limits)	Synergistic ratio
Anti juvenile hormone analog					
7-Ethoxy precocene II	-	273	1.91±0.28	1.18(0.84-1.79)*	-
Synergists					
RO20-9747	-	119	-	No activity at <400	-
Geraniol	-	175	-	No activity at <20	-
Isosafrole	-	139	-	No activity at <40	-
4-Phenyl chalcone oxide	-	122	-	No activity at <1.26	-
Piperonyl butoxide	-	118	1.66±0.31	1.14(0.67-1.64)*	-
Two way combinations					
7-EP-II+RO20-9747	1 : 5	162	1.90±0.44	0.084(0.026-0.17)	14.05
+Geraniol	〃	96	4.07±1.15	0.56(0.10-1.12)	2.11
+Isosafrole	〃	99	1.63±0.73	0.53(0.04-1.03)	2.23
+pco	〃	89	1.99±0.66	0.34(0.20-2.25)	3.47
+pb	1 : 0.1	108	1.57±0.39	1.19(0.82-2.32)	0.99
Three way combinations					
7-EP-II+RO20-9747+Geraniol	1 : 2.5 : 2.5	139	2.15±0.30	0.28(0.21-0.37)	4.21
〃 + 〃 +Isosafrole	〃	101	2.78±0.54	0.61(0.47-0.77)	1.93
〃 + 〃 +pb	〃	133	2.25±0.50	1.56(0.76-2.82)	1.27
〃 + Geraniol +Isosafrole	〃	106	1.82±0.44	0.72(0.50-1.03)	1.64
〃 + 〃 +pb	〃	196	0.90±0.15	0.76(0.47-1.26)	2.60
〃 + Isosafrole +pb	〃	138	1.89±0.50	0.95(0.32-2.10)	2.08
Five way combinations					
7-EP-II+RO29-9747+Geraniol +Isosafrole+pb	1 : 1.25 : 1.25 : 1.25 : 1.25	218		10% mortality at <2	-

*Fifty percent effective concentration in μg/cm^2

Three of five synergists showed no activity to the concentration ranges tested for synergistic mixture. However, pb showed similar effectiveness in mortality to 7-EP-II, without showing hormonal disturbances at the levels tested.

Synergism was shown in the mixtures of 7-EP-II with synergists by two, three, or five ways of combinations against *O. fasciatus*. In two way combinations of 7-EP-II and synergists at 1:5 mixture except for pb, the mixture of 7-EP-II and RO20-9747 showed the highest synergistic ratio of 14.0. Other synergists such as geraniol, isosafrole, and pco also showed mild synergism with ratios of 2.1 to 3.5. When pb was mixed with 7-EP-II at the ratio of 1:0.1 to reduce intrinsic toxicity of piperonyl butoxide, the synergistic ratio was 0.99 with quite independent action.

In three way combinations of 7-EP-II and two synergists at 1:2.5:2.5, the synergistic ratio including RO 20-9747 showed rather decreased synergistic ratio compared with the combination of 7-EP-II plus RO 20-9747. This trend of reduced synergism was a common inclination except for the mixture of 7-EP-II+ geraniol+pb.

In five way combinations of 7-EP-II and four synergists at 1:1.25:1.25:1.25:1.25 the results were far from optimistic, showing less than 10% mortality at nearly 2 μg/cm^3 of 7-EP-II that had 1.18 μg/cm^3 in EC50 value.

DISCUSSION

The concentration for inducing 100% precocious metamorphosis of 7-EP-II was 0.4 μg/cm^3 when

second instar *O. fasciatus* nymphs were exposed in a 9cm dish. This level of 7-EP-II activity was approximately 20 times higher than the naturally occurring chromenes of precocene I and II which yielded 7.8 and 1.9 μg/cm^2, respectively (Soderlund *et al.* 1981). The EC50 of 7-EP-II in this experiment showed 1.18 μg/cm^2 with slightly lower level compared with Soderlund *et al* (1981), because the figure was obtained six days after treatment and found to show some acute toxicity as well as anti-juvenile hormone activity.

In two way combinations 7-EP-II and the analog of chromene compound, RO20-9747 showed 0.084 μg/cm^2 which was much as 14 as times potent as the 7-EP-II alone with 1.18 μg/cm^2. The synergistic ratio indicated that 7-EP-II arrived successfully at the CA to cause suicidal atrophy *O. fasciatus*, while RO20-9747 inhibited the microsomal monooxygenase system throughout all of the insect organs except for CA preventing metabolism of the 7-EP-II like RO20-9747. Such a synergism with RO20-9747 would be synergistic even in insensitive holometabolous insects because the CA from holometabolous insectsare also susceptible in tissue implantation experiments (Bowers and Feldlaufer 1982). On these aspects these results will be highly valuable as supporting evidence of practical insect control means with structurally similar synergists, anticipating skillful formulation.

When the methylendioxyphenyl synergists such as isosafrole or pb were mixed with 7-EP-II, each combination revealed mild synergism and similar acitvity with synergistic ratio of 2.23 and 0.99. Pb alone showed some toxicity in this experiment. This seemed like *O. fasciatus* have been exposed to the excessive dose of to juvenile hormonally active pb (Bowers 1991) or the insect might have been exceptionally susceptible to pb.

Since methylenedioxy funtional groups were responsible for the inhibition of cytochrome P450 monooxygenase system these results coincide with those of Brooks *et al* (1979). They showed that methylenedioxy precocene was inactive because inhibition of the enzyme, which would have activated precocene, prohibited self-catalyzed suicidal destruction of the CA. The other antioxidants, geraniol and pco also showed mild synergism. These general antioxidants were considered less effective synergists for the potentiation of 7-EP-II because these are apparently non selective inhibitors of the insect defense system.

In three way combinations the synergistic ratios were more or less similar to the two way combinations. When RO20-9747 was incorporated as a component, the mixture showed rather decreased synergism. And the five way combinations showed the more decreased effectiveness. Since increasing the number of synergists in the combinations with 7-EP-II showed sabstantially no increase in synergistic ratio, these results, therefore, demonstrate that the synergists did not work to inhibit the insect defense mechanisms in a stratal way but they indicated the barrier in a competition with or counteraction against each synergist.

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