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Interaction of Plumbagin with Hormones in the Cotton Stainer, *Dysdercus koenigii* Fabricius

G T GUJAR

Division of Entomology, Indian Agricultural Research Institute
New Delhi 110 012

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Plumbagin, 2-methyl-5-hydroxy-1, 4-naphthoquinone, a naturally occurring insect growth regulator isolated from *Plumbago* spp. a medicinal plant, was investigated for its interaction with hormones in the last instar nymphs of *Dysdercus koenigii*. Last instar nymphs of *D. koenigii* showed high morphogenetic response to plumbagin as age progressed upto 96-120 hr, after which it decreased till they moulted to adult stage. Pretreatment of nymphs with plumbagin was antagonistic to the action of a juvenile hormone analogue, hydroprene, while pretreatment of nymphs with hydroprene synergised morphogenetic effects with plumbagin. The synergistic interaction of these two insect growth regulators was achieved on treatment of 0-24 hr old nymphs with a sublethal dose of hydroprene followed by plumbagin treatment 4 days later. Ecdysterone was successful in rescuing the morphogenetic effects of plumbagin in 4 day old last instar nymphs. The possible effect of plumbagin through an interaction with hormone pools in last instar nymphs of *D. koenigii* with regard to its mode of action is discussed.

Key Words: Plumbagin, Cotton stainer, *Dysdercus koenigii*, Interaction, Insect growth regulator

Introduction

Plumbagin, 2-methyl-5-hydroxy-1, 4-naphthoquinone, isolated from *Plumbago* spp., a medicinal plant, has been recently reviewed for its pesticidal and pharmacological activities (Gujar 1990). It was found to be an insect antifeedant and a chitin synthesis inhibitor in a number of lepidopterous insect pests (Kubo et al. 1983). Biological activity of plumbagin was reported against the cotton stainer, *Dysdercus koenigii* and *Dysdercus cingulatus* (Joshi et al. 1988). Gujar and Mehrotra (1988) reported its toxicity and morphogenetic activity against *D. koenigii* and hypothesized that plumbagin might be acting on neuro-endocrine system and its integration with moulting processes. Joshi et al. (1988) had earlier reported dark colouration of prothoracic glands and pericardial cells in *D. cingulatus* nymphs treated with plumbagin. Later studies by Joshi and Sehnal (1989) reported *in vitro* and *in vivo* inhibition of ecdysteroid (makisterone A) synthesis in the last instar nymphs of *D. cingulatus* on plumbagin treatment.It is likely that plumbagin also affects the other hormones under *in vivo* conditions. The present communication reports the results of our investigations on interaction of plumbagin with juvenile and moulting hormones in *D. koenigii*.

Materials and Methods

Last instar nymphs used in the present investigations were obtained from the laboratory colony of *D. koenigii* maintained at 27°C and 50-70% RH as per Gujar and Mehrotra (1988). Plumbagin was a

gift from Bhabha Atomic Research Centre, Bombay.

Age-dependent Response of Last Instar Nymphs to Plumbagin

Last instar nymphs of different age-groups viz., 0-24, 48-72, 96-120, 144-168 and 168-192 hr were individually weighed and then topically treated with an uniform dose of plumbagin (5 μg/25 mg body weight). Control insects received 5 μl of acetone per insect. A minimum of 30 insects were treated in each age group. Observations were recorded on nymphal mortality daily and on morphogenetic effects at the end of moulting of all surviving nymphs to the adult stage.

Interaction of Plumbagin with a Juvenile Hormone Analogue in the Last Instar Nymphs of ***D. koenigii***

Pretreatment of nymphs with plumbagin: Last instar nymphs, 0-24 hr old, were treated with plumbagin at 1.25 μg/insect. After 6 hr, these were again treated topically with hydroprene-ethyl (2*E*, 4*E*)-3, 7, 11-trimethyl-2-dodecadienoate (Altozar, ZR 512, a gift from Zoecon Corporation; presently, Sandoz Crop Research Institute, Palo Alto, California) at different doses ranging from 0.03125 to 1.0 μg/insect. A minimum of 15 insects were used in each treatment. A group of insects were first treated individually with 1 μl of acetone each and 6 hr later, with hydroprene at different doses similar to those in cotreatment with plumbagin. A minimum of 15 insects were used for each treatment in this set of experiment.

Pretreatment of nymphs with hydroprene: In another set of experiments, 0-24 hr old last instar nymphs were treated with hydroprene at a dose of 0.03125 μg/insect and then 6 hr later with plumbagin at different doses ranging from 1.25 to 20.0 μg/insect. Control insects were treated with acetone and then with hydroprene at doses ranging from 0.03125 to 1.0 μg/insect. A minimum of 10 insects were used in each treatment in this set of experiments.

In another set of experiments, 0-24 hr old last instar nymphs were treated with 0.016 μg hydroprene/insect followed by plumbagin treatment at 5 μg/insect 4 days later, at the age of 96-120 hr. In control, insects, 0-24 hr old, were individually treated with 0.016 μg hydroprene each while in another set, insects, 0-24 hr, were pretreated with 1 μl of acetone then followed with plumbagin 5 μg each, 4 days later. A minimum of 15 insects were used for each treatment.

Interaction of Plumbagin with Ecdysterone in ***D. koenigii***

Last instar nymphs, 4 day old (96-120 hr old), were treated topically with plumbagin at a dose of 5 μg/insect. These insects were then topically treated with ecdysterone at dose of 1 μg/insect after 0 hr, 1, 2 and 3 days of plumbagin treatment. In a control group, 4-day old nymphs were treated with ecdysterone at 1 μg/insect and in another set with plumbagin at 5 μg/insect. A minimum of 15 insects were used for each treatment.

In all experiments, insects were topically treated on the abdomen with the help of electrically operated microapplicator (ISCO 1025, Lincoln, Nebr. USA) fixed with 1 ml syringe-27 gauge needle; standardized for its delivery of solution. Insects were allowed to feed on soaked cotton seeds. Routine hygienic conditions were maintained throughout the duration of experiments. Observations were recorded on nymphal mortality daily and on morphogenetic effects at the end of moulting of all surviving nymphs to the adult stage. The morphogenetic effects were scored depending upon intensity of symptoms over 4-5 point scale and a

mean morphogenetic score was calculated for each treatment after modification of method of Williams and Sláma (1966).

Results

Age-dependent Response of Last Instar Nymphs of ***D. koenigii*** *to Plumbagin*

The weight of last instar nymph of *D. koenigii* showed a linear increase from 24.87 mg for 0-24 hr, 48.25 mg for 48-72 hr, 56.17 mg for 96-120 hr, 85.73 mg for 144-168 hr and 98.11 mg for 168-192 hr. Last instar nymphs showed age-dependent response to the action of plumbagin. It was observed that nymphs of 144-168 hr age group were highly susceptible. They died turning melanized before moulting to adult stage while nymphs of 96-120 hr age group showed high degree of morphogenetic effects viz., adultoids about 33.3%; nymphal-adult intermediates 43.3%; highly abnormal adults 6.7% and remaining black dead nymphs (table 1). The mean morphogenetic score was found to increase upto age of 96-120 hr of nymphs treated, then decreasing sharply in nymphs of 168-172 hr age group. Further studies carried out on nymphs of 144-168 hr age showed dose dependent response in terms of morphogenetic effects and melanization of nymphs which died within 24 hr.

Table 1 *Age-dependent response of the last instar nymphs of the cotton stainer,* ***Dysdercus koenigii*** F. *to plumbagin (5 μg/25 mg body weight) treatment*

Age (hr)	No. of insects treated	% toxicity and morphogenetic response
0-24	30	3.3[a], 23.3[e], 23.3[f], 50.1[g] (0.7)
48-72	30	43.3[f], 56.7[g] (0.4)
96-120	30	16.7[a], 43.3[b], 33.3[c], 6.7[d] (4.5)
144-168	30	86.7[a], 3.3[b], 10.0[f] (2.0)
168-192	30	10.0[b], 3.3[e], 13.4[f], 73.3[g] (0.7)

[a]nymphs dead; [b]nymphal-adult intermediates; dead, exuviae sticking up legs, body bent as a result of sticking together of mouthparts and legs; wings highly deformed; [c]adultoids, alive highly nymphal like body, integument untanned, wings short and deformed; [d]adults with highly deformed wings; [e]normal looking adults with moderately deformed wings; [f]normal looking adults with slightly deformed wings; [g]normal adults (0).

Figures in parenthesis refer to morphogenetic score over 5 point scale. All insects in control treatment were normal adults.

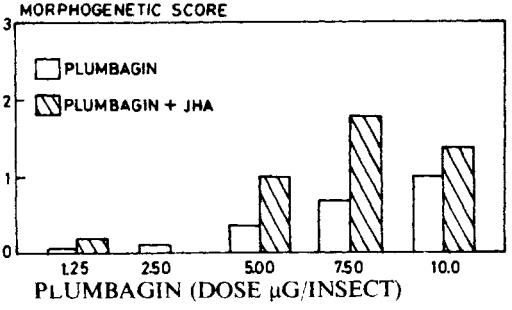

Figure 1 Interaction of plumbagin with hydroprene treated last instar nymphs of *Dysdercus koenigii*. Insects, 0-24 hr old, were pretreated with hydroprene at 0.03125 μg each

Interaction of Plumbagin with Juvenile Hormone Analogue in ***D. koenigii***

Pretreatment of last instar nymphs with hydroprene was found to enhance morphogenetic activity of plumbagin from the mean morphogenetic score of 0.2 for 1.25 μg plumbagin, a lowest treatment of 1.375 for 10 μg plumbagin treatment (figure 1). This cotreatment of nymphs with hydroprene and plumbagin led to the higher morphogenetic score than plumbagin treatment alone. Treatment of nymphs with hydroprene alone caused morphogenetic score of 1.21 suggesting that plumbagin treatment 6 hr later of nymphs pretreated with hydroprene antagonized effect of juvenile hormone analogue up to 5.0 μg/insect dose but later at higher doses, synergized the morphogenetic activity.

Pretreatment of nymphs with plumbagin at a sublethal dose of 1.25 μg/insect led to the increase in morphogenetic activity of hydroprene from 0.625 for 0.03125 μg

to 3.8 for 1.0 μg hydroprene treatment (figure 2). However, morphogenetic score was far less than hydroprene treatments alone suggesting that pretreatment with plumbagin does not lead to the enhancement but antagonism of activity of hydroprene in all doses tried in these studies. The highest level of antagonism was seen when pretreatment of nymph with 1.25 μg plumbagin led to the decrease in morphogenetic score from 1.21 with hydroprene alone to that of 0.625 with co-treatment with hydroprene.

The sequential treatment of last instar nymphs with hydroprene at 0.016 μg/insect followed by plumbagin at 5 μg/insect 4 days later led to the synergistic morphogenetic response over that of any of these insect growth regulators (figure 3).

Interaction of Plumbagin with Ecdysterone in ***D. koenigii***

Pretreatment of nymphs, 4 day old (96-120 hr), with plumbagin at a dose of 5 μg/insect was followed up by further treatment 0, 1, 2 and 3 days later with ecdysterone at an uniform dose of 1 μg/insect (figure 4). Treatment of nymphs with plumbagin alone caused morphogenetic effect to the extent of 2.04 while ecdysterone did not evoke any morphogenetic effects. Ecdysterone treatment 2 days after plumbagin treatment at the age of 6 days successfully rescued the plumbagin effects in *D. koenigii* to the extent of nearly 60% while others failed.

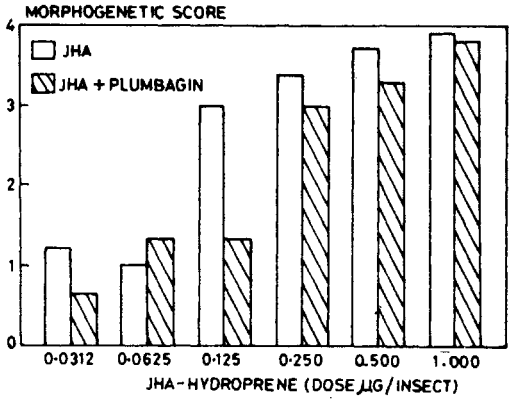

Figure 2 Interaction of juvenile hormone analogue, hydroprene with plumbagin treated last instar nymphs of *Dysdercus koenigii*. Insects, 0-24 hr old, were pretreated with plumbagin at 1.25 μg each

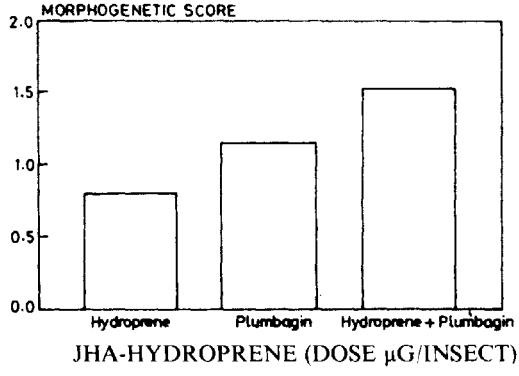

Figure 3 Synergistic interaction of hydroprene and plumbagin in last instar nymphs of *Dysdercus koenigii*. Insects, 0-24 hr old were pretreated with hydroprene at 0.016 μg/insect followed by treatment with plumbagin at 5 μg/insect 4 days later

Discussion

Effect of plumbagin as insect growth regulator was reported extensively in *Dysdercus* spp. (Joshi et al. 1988, Gujar & Mehrotra 1988). Age-dependent response of *D. koenigii* to plumbagin showed the efficacy of plumbagin even at late age in the last instar nymphs, about to metamorphose to adult stage after 144-168 hr. This unusual activity of plumbagin is its characteristic not found normally with chemicals acting like hormones on neuroendocrine system. Studies carried out by Joshi and Sehnal (1989) reported that intensity of staining of prothoracic glands increased with increasing age of nymphs of *D. cingulatus* treated with plumbagin. Plumbagin was shown to affect ecdysteroid synthesis *in vitro* and *in vivo* in *D. cingulatus*. Further studies by them also

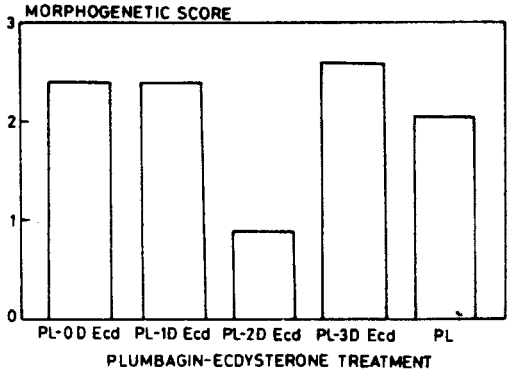

Figure 4 Interaction of plumbagin with ecdysterone in last instar nymphs of *Dysdercus koenigii.* Insects, 4-day old, were pretreated with plumbagin 5 μg/insect followed by further treatment with ecdysterone at an uniform dose of 1 μg/insect 0, 1, 2 and 3 days later

showed that ecdysteroid, makisterone A, was at its peak between 4th and 5th day of nymphs of *D. cingulatus* which correlated with high degree of morphogenetic effects in *D. koenigii* found in the present investigations. These studies indirectly proved that plumbagin was highly effective at the peak synthetic/secretory activity of prothoracic glands.

Studies on interaction of plumbagin with juvenile hormone effect showed:

(1) Pretreatment of nymphs with juvenile hormone analogue at a sublethal dose of 0.03125 μg/insect leads to the enhancement of activity in cotreatments with plumbagin. Obviously juvenile hormone analogue is acting even at lowest dose of 0.03125 μg at the transcriptional level by affecting programming of oncoming metamorphosis events while plumbagin action is at a time when prothoracic glands are not in active synthetic/secretory stage and hence less effective as shown in the studies on age dependent response of nymphs to plumbagin.

(2) Pretreatment of nymphs with plumbagin is antagonistic to the activity of juvenile hormone analogue in *D. koenigii.* It is difficult to conjecture reasons for these effects which may be possibly due to multiple action of plumbagin at different loci in neuroendocrine system, since plumbagin showed growth inhibitory and morphogenetic effects depending upon the time of treatment of last instar nymphs.

(3) Synergistic effects of these two kinds of insect growth regulators could be achieved first by treatment of nymphs with juvenile hormone analogue and then preferably at mid-instar stage with plumbagin as shown in figure 3. This has been possible due to optimization of morphogenetic effect, first by juvenile hormone in the early stage and then by antiecdysteroid effect by plumbagin in the later mid-instar stage.

Although ecdysterone is not a naturally available ecdysteroid in *Dysdercus,* it was able to rescue the effects of plumbagin. Joshi and Sehnal (1989) reported rescue of growth "inhibitory effects" of plumbagin treatment in last instar nymphs of *D. cingulatus,* 0-24 hr old, with makisterone A. The ability of makisterone A to rescue these effects of plumbagin suggested that makisterone A was persistent enough to counteract growth inhibitory effects of plumbagin in young last instar nymphs when prothoracic glands were not in secretory/responsive condition to prothoracicotropic hormone from the brain. In present studies, critical presence of ecdysterone after a mid-instar stage at 144-186 hr age was needed to rescue the "morphogenetic effects" of plumbagin and to signal oncoming events of metamorphosis to adult stage in *D. koenigii.* Plumbagin treatment of last instar nymphs of *D. koenigii* at early age has growth inhibitory effects in contrast to morphogenetic effects observed on nymphal treatment at

mid-instar stage. These effects could possibly be attributed to changes in morphogenetic hormonal levels under *in vivo* conditions, depending upon the time of application.

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