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EFFECT OF PHYTOECDYSTEROIDS ON GROWTH AND DEVELOPMENT OF INSECT

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ABSTRACT

There is a finite supply of physiologically active chemicals in plants. Numerous plants produce a variety of secondary metabolic chemicals, including alkaloids, terpenoids, phenolics, steroids, *etc*. These substances are typically believed to play a role in interactions between plants and insects. An analog of the hormones generated by insects during molting, phytoecdysteroids are a class of substances that plants make. In this review, an insect was used to test the 20-hydroxyecdysone's effects. This molecule is a part of the phytoecdysteroids group. Phytoecdysteroids replicate the effects of insect ecdysone hormone by binding to ecdysone receptors and eliciting similar reactions. In non-adapted (sensitive) insects, phytoecdysteroids cause responses at the wrong time and stage, leading to aberrant development, decreased fecundity and fertility, decreased energy stores and body weight, increased cannibalism, death rate and feeding detterency.

***Key words*: Biopesticide, Ecdysone-hormone, Insect, Phytoecdysteroids**

Introduction

In part to its fascinating potential for the creation of novel biopesticides with plant origins, the study of insect-plant interactions is currently one of the most actively researched fields in chemical ecology. Numerous secondary plant metabolites are involved in these interactions, which may affect how insects behave, grow or develop. Insect growth is limited by the cuticle as it hard and non-elastic. The cuticle must be replaced to increase in size of the body. In insects, two major endocrine hormones viz., ecdysone and juvenile, are present which coordinately regulate a variety of developmental processes, including moulting, metamorphosis, embryogenesis, larval growth and reproduction. Ecdysone is a major insect molting hormone, which is secreted from the prothoracic glands**.** Insect molting hormones ecdysone and its homologues are generally called ecdysteroids**.** At the time, large amounts of insect material were required to isolate milligrams of purified ecdysone, the first ecdysteroid identified**.** Since then, over 300 different ecdysteroid analogues have been identified from animal and plant sources. Ecdysteroid analogues derived from plants are called phytoecdysteroids (PE). When insects feed the plants with PE, they will prematurely moult, loose weight, or suffer other metabolic damage and die. Nakanishi *et al*., In 1966 were the first to isolate phytoecdysteroids (ponasterones a, b and c) from the plume pine, *Podocarpus nakaii* Hayata (Table 1). 20-hydroxyecdysone found in the wood of *Podocarpus elatus* R. and the rhizomes of *Polypodium vulgare* L. Spinach (*Spinacia oleracea* L., Chenopodiaceae) possess higher concentration (50 µg/g fresh weight) of phytoecdysteroid (Grebenok *et al*., 1994). Ecdysteroids have been detected in 27 families of the Pteridophyta, 10 families of Gymnosperm and 74 families of Angiosperm. Approximately 6 per cent of all plant species synthesize phytoecdysteroids (Dinan *et al*., 2001).

Distribution of Phytoecdysteroids

Phytoecdysteroid levels in plants are usually found

Table 1: Plant families and species containing phytoecdysteroids.

Sr. No.	Family	Common name	Species	Ecdysteroid isolated	Plant parts	Concentration (mg/kg)
1.	Lamiaceae	Bugleweed	*Ajuga iva* L.	Ajugasterone	Whole plant	100
2.	Verbenaceae	Chaste tree	*Vitex acunae* L.	20-hydroxyecdysone	leaves	880
3.	Convolvulaceae	morning glory	*Ipomea calonyction*	Ecdysone & Makisterone A	Seeds	130
4.	Asteraceae	Rhaponticum	*Rhaponticum integrifolium*	20-hydroxyecdysone & Integristerone A	Dry inflorescence	150
5.	Amaranthaceae	Ox knee	*Achyranthes bidentata*	20- hydroxyecdysterone & inokosterone	Seeds	250
6.	Podocarpaceae	Yellow silver pine	*Dacrydium intermedium*	20-hydrooxyecdysone	Dry bark	10,000
7.	Polypodiaceae	Golden polypody	*Polypodium aureum*	20-hydrooxyecdysone	Rhizomes	7
		Common polypody	*P. Vulgare*	ecdysone	Rhizomes	18

Modified from Kerkut, & Gilbert, (1985) *Comprehensive Insect Physiology, Biochemistry and Pharmacology*

to be 0.1% or less of their dry weight and have been isolated from all parts of plants in much higher amounts than those present in arthropods (Dinan *et al.,* 2001). PE are distributed in over 100 terrestrial plant families representing ferns, gymnosperms and angiosperms. 27 families: Pteridophyta (fern); 10 families: Gymnosperm; 74 families: Angiosperm. 300 different phytoecdysteroids have been identified (Table 2). Approximately 6 per cent of all plant species synthesize phytoecdysteroids (Lafont *et al*., 2002).

Mode of Action of Phytoecdysteroids

In insects, 20-Hydroxyecdysone (20HE) acts through the ecdysone receptor (nuclear receptor). The ecdysone receptor is a non-covalent heterodimer of two proteins

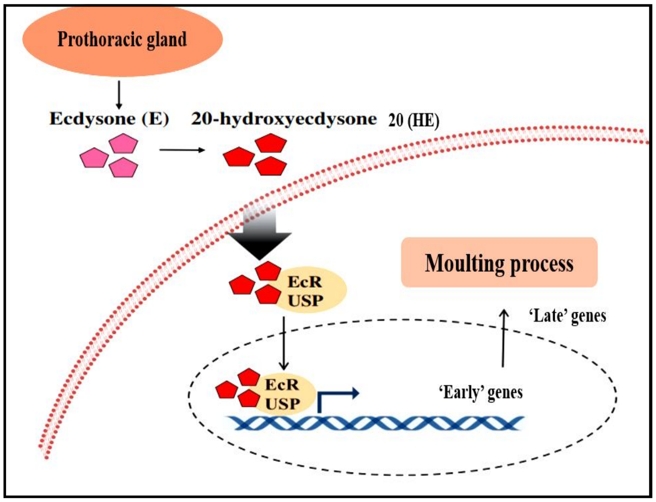

Fig. 1: Mode of action of phytoecdysteroids.

viz., the Ecdysone Receptor protein (EcR) and Ultra Spiracle Protein (USP). EcR must be dimerised with a USP for high-affinity ligand binding (Fig. 1). The binding of ecdysone to receptor leads to the activation of ecdysone responsive genes and many other genes. Which ultimately causes physiological changes that result in ecdysis. The ecdysone receptor also binds and activated by phytoecdysteroids. Thus, phytoecdysteroids can mimic 20-Hydroxyecdysterone of insects, bind insect ecdysone receptors and can elicit the same responses. This ultimately causes physiological changes that result in premature ecdysis (Chaubey, 2017).

Structure of Phytoecdysteroids

The various analogues differ in the number and site of hydroxylations, as well as the length and structure of the carbon side chain (Fig. 2). Glycosylated and acetylated ecdysteroids have been described both in nature and in the laboratory. Many more plants have the ability to 'turn on' the production of phytoecdysteroids when under stress, animal attack or other conditions. The term phytoecdysteroid can also apply to ecdysteroids found in fungi, even though fungi are not plants. Fungi that produce phytoecdysteroids include *Achyranthes bidentata, Tinospora cordifolia, Pfaffia paniculata, Leuzea carthamoides, Rhaponticum uniflorum, Serratula coronate, Cordyceps* and *Asparagus.*

Estimation of Phytoecdysteroids

Phytoecdysteroids can be extracted from the dried plant materials then estimated by several techniques *viz*.,

Table 2: List of different Phytoecdysteroids.

Sr. No.	Phytoecdysteroids
1	Ponasterone A
2	1-epi-integristerone
3	Paristerone
4	5α-polypodine B
5	Viperidinone
6	Turkesterone
7	22-dehydro-12 hydroxycyasterone
8	Sogdisterone
9	Silenosterone
10	Poststerone
11	4α-hydroxypinnasterol
12	Kaladasterone
13	Podecdysone B
14	Carpesterol
15	Ajugasterone B
16	Dacryhainansterone
17	Tenuifoliosides A and B
18	22-deoxy-20,21-dihydroxyecdysone
19	Inokosterone
20	Makisterone D
21	Amarasterone B
22	Gerardiasterone
23	Rapisterone
24	Venustone
25	Canescensterone
26	Rubrosterone
27	Carthamosterone
28	Taxisterone (22-deoxyecdysterone)
29	29-norcyasterone
30	Rapisterone C
31	22-oxo-cyasterone
32	Pra emixisterone
33	Stachysterone A
34	Stachysterone C
35	Decumbesterone A
36	Cheilanthones A and B
37	Makisterone A
38	Makisterone C
39	Amarasterone B
40	Sidisterone
41	Poststerone
42	29-norsengosterone

reversed phase thin layer chromatography, high-pressure liquid chromatography, column chromatography, supercritical fluid chromatography followed by mass spectrometry. High-pressure liquid chromatography is the most commonly used method for separation of ecdysteroids given by CSIRO, Australia, as described by Kerkut and Gilbert (1985).

Extraction of phytoecdysteroids from plant material

This flowchart shows extraction method (Fig. 3) of phytoecdysteroids from plant materials.

Results and Discussion

Effects of Phytoecdysteroids on Insects

Inhibition of pupation and adult emergence: Kubo *et al.*, (1983) observed larvae ingested with methanol extract undergoing apolysis thrice without occurring ecdysis. Larvae had a 3 head capsule with unfunctional mouthpart and finally larvae died due to

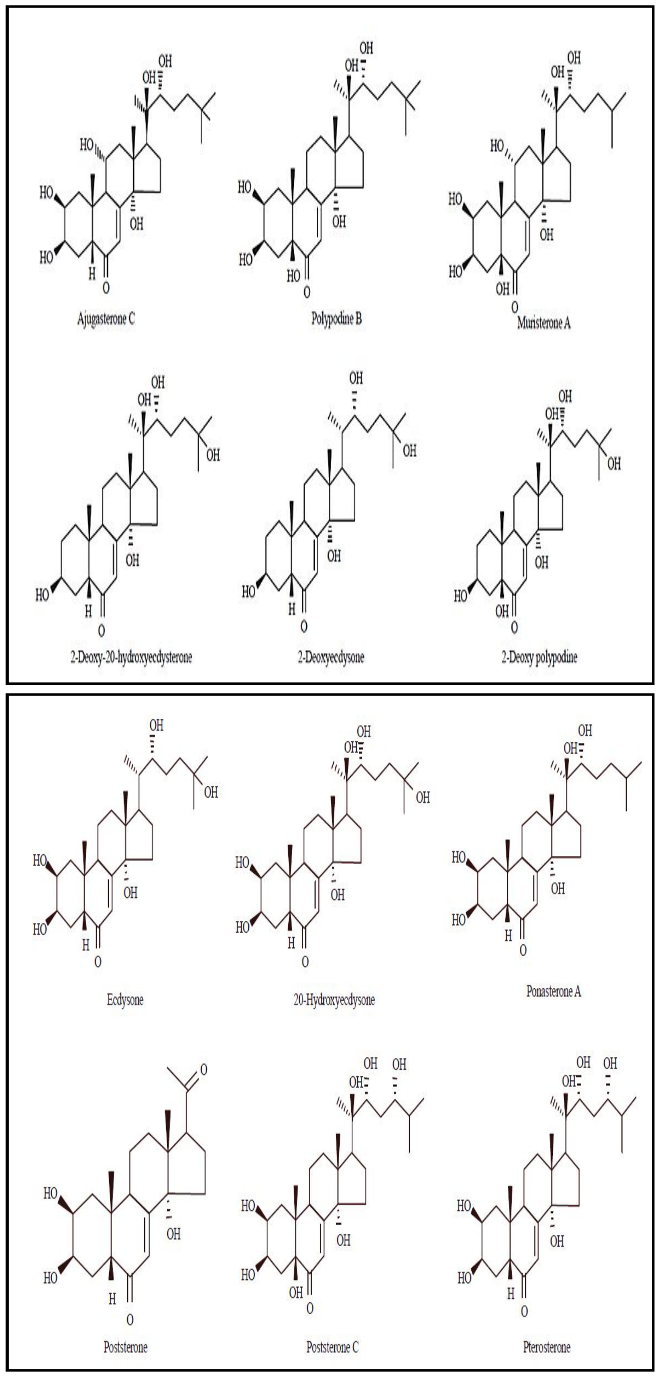

Fig. 2: General structure of ecdysteroids.

starvation. Rharrabe *et al.*, (2009) reported that larvae feed 5g of wheat flour mixed with different PE @ 200 ppm and after 24 days they observed less cumulative pupation (%) observed in Makisterone A followed by Ponasterone A. Sun *et al.*, (2015) observed the morphological changes in diamondback moth, *Plutella xylostella* L. larva and pupa caused by ingestion of exogenous dietary 20-hydroxyecdysone. Tatun *et al.*, (2018) concluded that the red rust flour beetle, *tribolium castaneum* H. Larva injected with 20he @ 300 ng/insect during last larval instar showed morphological abnormalities in pupal and adult stage. Rharrabe *et al.*, (2019) reported that the larva of T. *Castaneum* feeding on wheat flour mixed with 1200-ppm 20-hydroxyecdysone recorded adult emergence up to 30 per cent, whereas it was cent per cent in control. Taha-Salaime *et al.*, (2020) concluded that cotton leafworm, *Spodoptera littoralis* B. larva fed castor leaves sprayed with phytoecdysteroid fraction @ 250 µg/µL for 4 days were unable to complete their pupation.

Reduction in fecundity and fertility: Radi *et al.*, (2011) found that ecdysterone extracted from bugleweed, *Ajuga iva* plant had significantly reduced fecundity (75%), while cyasterone from the same plant caused reduction in fecundity (50%) of *Bemisia tabaci*. They also found that ecdysterone significantly reduced egg fertility (56%) as compared to control. Sun *et al.*, (2015) found that adult feed 10 per cent honey solution mixed with different concentration of 20HE mg/mL after 10 days they observed lowest fecundity, hatching (%) and non-embryonated eggs in 0.50 mg/mL of 20HE 128.6, 86.97 (%) and 9.74 (%), respectively.

Reduction in energy reserves and body weight: Exposure of Indian meal moth, *Plodia interpunctella* H. larva to 20HE decreased protein, glycogen and lipid content in larva as compared to untreated larva (Rharrabe *et al.*, 2009). Ingestion of 20-hydroxyecdysone also caused significant decrease in protein content and inhibited alpha-amylase activity in *T. castaneum* larva (Rharrabe *et al.*, 2019).

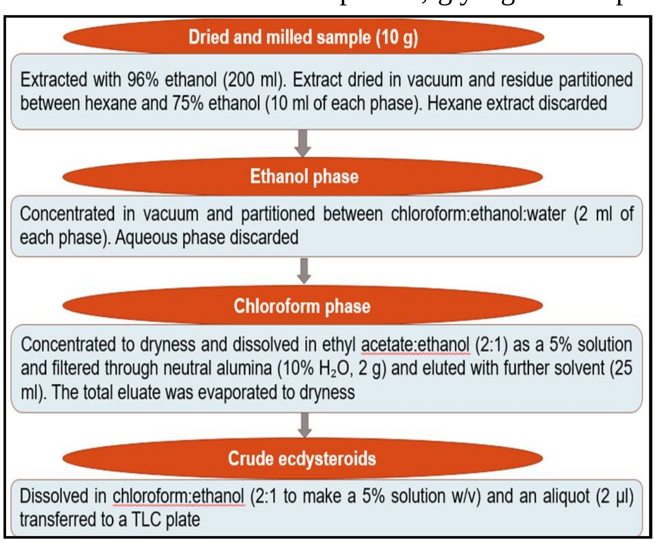

Fig. 3: Flowchart for extraction of phytoecdysteroids.

Cannibalism: Rharrabe *et al.*, (2009) observed that larva of *P. interpunctella* fed wheat flour mixed with 20 HE @ 50 ppm showed 26.7 per cent cannibalism, whereas the rate of cannibalism in control never exceeded 7 per cent.

Mortality: Colorado potato beetle, *Leptinotarsa decemlineata* S. larva feeding on potato leaves sprayed with 0.01% 20HE suffered 53.3 per cent mortality (Zolotar *et al.*, 2001). Rharrabe *et al.*, (2010) recorded the highest mortality (84%) of *P. interpunctella* larva @ 200 ppm concentration of makisterone A followed by ponasterone A (64%) after 22 days.

Feeding detterency: Spray application of 20HE @ 5 g/L inhibited the feeding of Japanese beetle, *Popillia japonica* N. adults in soybean leaves under choice and no-choice assays (Russell *et al.*, 2017).

Improvement of silk yield: Oral administration of phytoecdysteroids from chaff-flower, *Radyx achyranthes* L. to larva of *B. mori* at 48 hrs of 5^{th} instar significantly improved economic traits *viz.*, weight of mature larva, posterior gland, coccon and coccon shell (Nair *et al.*, 2005).

Advantages of Phytoecdysteroids

- Phytoecdysteroids are compounds with low mammalian toxicity.
- The LD_{50} values in mice are 6.4 and > 9 g/kg, using intraperitoneal and oral administration of 20-Hydroxyecdysone, respectively.
- Phytoecdysteroids like makisterone A, ponasterone A are very effective at low dosage.
- As compared to chemicals they are cheaper and available from plants.

Limitations of Phytoecdysteroids

- Phytoecdysteroids are effective against monophagous insect while polyphagous insect are tolerant.
- *Heliothis virescens* (Kubo *et al.*, 1983), *Heliothis armigera* and *Lacanobia oleracea* (Blackford and Dinan, 1997) have developed effective detoxification mechanisms against ingested phytoecdysteroids.
- Phytoecdysteroids are environmentally unstable.

Conclusion

Phytoecdysteroids are plant derived moulting hormones, which are analogues of insect moulting hormones. Phytoecdysteroids mimic insect ecdysone hormone, bind to ecdysone receptors and elicit same responses as insect ecdysone hormone. Phytoecdysteroids induce responses at inappropriate time and stage causing, abnormal development, reduction in fecundity and fertility, reduction in energy reserves and body weight increased cannibalism, death rate and feeding detterency in non-adapted (sensitive) insects. Its use also improves silk yield in sericulture.

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