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Experimental Induction of Insect Growth Regulators in Controls of Insect Vectors as well as Crops and Stored Products Pests

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Abstract: *Information on insect hormones has delivered a number of opportunities to enlist them or molecules related to them in the fight against insect pests. This article is a contribution to the knowledge on insect growth regulator (IGR), which is a substance or chemical that inhibits the life cycle of an insect. Insect growth regulators (IGRs) are typically used as insecticides to control populations of harmful insect including fleas, cockroaches, mosquitos and stored products pests. IGRs of the juvenile hormone type alter physiological processes essential to insect development and appear to act specifically on insects. IGRs affect certain hormones in insects or inhibit specific biochemical pathways or processes essential for insect growth and development. Three natural juvenile hormones have been found in insects, but not in other organisms. These are chitin synthesis inhibitors, juvenile hormone analogs and mimics, and anti-juvenile hormone agents. These do not kill insects immediately, but they can stop a pest population from reproducing until all of the pests have been died. IGRs may come from a blend of synthetic chemicals or from other natural sources, such as plants. Certain insects exposed to insecticides with growth regulating properties may die due to abnormal regulation of hormone-mediated cell or organ development. Future use of antagonists or inhibitors of hormone synthesis may be technically possible as an advantageous extension of pest control by IGRs in integrated pet management strategy.*

Keywords: *Insect hormones, Insect behavior, Insect reproduction and development, Neurophysiological activity*

INTRODUCTION

Insects because of their rigid exoskeleton can only grow by periodically shedding of their exoskeleton called molting that occurs repeatedly during larval development. Different hormones or hormone types are integral to the growth and reproductive functions in insects. Classically, hormones are chemical substances produced by specialized tissues (glands) and secreted into hemolymph, in which they are carried to target organs. Modernly, hormones are chemical substances that carry information between two or more cells at micromolar concentration or less. Hormones play an important role in insect behavior, and these hormones are mainly the neurohormones of the brain and of the corpus cardiacum, the juvenile hormone of the corpus allatum and the ecdysone of the prothoracic glands (Prabhu, 1985).

Molting and pupation require the hormone, Prothoracicotropic Hormone (PTTH) also called **ecdysiotropic hormone** or **prothoracotropin** neurohormone is secreted by two pairs of cells in the brain of the larva in insects. There are two prothoracic glands located in the thorax and after being released by neurosecretory cells of the brain, the thoracotropic hormone is carried by the hemolymph to the prothoracic glands, where it stimulates the release of steroid hormone ecdysone in insects that initiates molting. Juvenile hormone (JH) also called **neotenin** is secreted by two tiny glands behind the brain, the corpora allata that controls the retention of juvenile characters in larval stages. The hormone affects the process of molting, the periodic shedding of the outer skeleton during development and in adults it is necessary for normal egg production in females. As long as there is enough JH, ecdysone promotes larva-to-larva molts, with lower amounts of JH, ecdysone promotes pupation and complete absence of JH results in formation of the adult. So, if the corpora allata are removed from an immature silkworm, it immediately spins a cocoon and becomes a small pupa, and a miniature adult eventually emerges (Syed et al., 2017).

Juvenile hormone regulates development, metamorphosis and reproductive maturation in insects, therefore, interruption of JH biosynthesis has been considered as a strategy for the development of target-specific insecticides (Cusson et al., 2013).

Fundamentally, all chemicals used to control insects fall into one of three categories like neurotoxins, growth regulators and behavior modifiers.

1. Neurotoxins

Neurotoxins are toxins that are destructive to nerve tissue causing neurotoxicity in insects. Most chemicals used to control insects are neurotoxins which interfere with normal nerve function. Approximately, the only thing that kills quickly is a neurotoxin so chemicals that act on neurotransmissions are sought and developed as insecticides. In the early discovery and development of insecticides, efforts have been focused on chemistry rather than biology. Because all animals share basically the same neurochemical systems, neurotoxins are toxic to all animals. By inhibiting the ability for neurons to perform their expected intracellular functions or pass a signal to a neighboring cell, neurotoxins can induce systemic nervous system arrest or even nervous tissue death. Common examples of neurotoxins include lead, ethanol (drinking alcohol), glutamate, nitric oxide, botulinum toxin (Botox), tetanus toxin and tetrodotoxin (Adams and Olivera, 1994).

2. Behavior modifiers

Behavior modification is a psychotherapy that seeks to extinguish or inhibit abnormal or maladaptive behavior by reinforcing desired behavior and extinguishing undesired behavior. Behavior-affecting chemicals, such as pheromones, have been discovered in the same way as IGRs, but tend to be even more specific. *Pheromones* are chemicals capable of acting like hormones outside the body of the secreting individual, to impact the behavior of the receiving individuals. *Pheromones* produced and released into the environment by an insect, affect the behavior or physiology of others of its species. Pheromones aid the sexes of a single species to find each other so that effort is not wasted in chasing mates of a different species.

3. Insect growth regulators

The origin of IGRs is entirely different, and their discovery is based on knowledge of how insects grow, develop, function and behave. They have been discovered in two ways. One way is to expose an insect to IGRs and observe abnormalities in how it develops, functions or behaves, and chemicals that produce desired effects are developed. Another mode is to find out what processes in the insects' development involve hormones, and to use those hormones as models to synthesize chemical analogs that will interfere with normal insect growth and development. Because IGRs act on systems unique to insects or shared with close relatives, they are less likely to affect other organisms.

Insect growth regulators can be used to prevent nsects from reaching maturity. Two commonly used regulators are juvenile hormone analogs and chitin synthesis inhibitors. **Juvenile hormone analogs** regulate morphological maturation and reproductive processes. They are highly specific to arthropods, have very low mammalian toxicity, and are effective at exceptionally low rates of application. Such compounds mainly include hydroprene and pyriproxifen. **Chitin synthesis inhibitors** prevent normal formation of chitin during molting. These compounds cause many of the affected nymphs to die during the molting process. Males that survive to the adult stage often have reduced life expectancies, whereas females tend to abort their oothecae (Brenner and Kramer, 2019).

The growth regulator effect may be seen in several ways. First are those molecules inhibiting metamorphosis, in other words, these are compounds preventing metamorphosis from taking place at the right time. Other compounds force the insect to go through an early metamorphosis, so that development takes place at a time not favorable for the insect. Lastly, it has been observed that certain molecules may alter hormones related to this function so that insects suffer malformations, are sterile or die. In fact, one of the most common entomological anecdotes tells that an experiment is being carried out simultaneously in the United States and Hungary. In the latter country the insects go through an additional immature stage before molting to adults. All methods have been reviewed but no difference is found until the paper towels used to provide water for the insects analyzed and then it is found that in the two countries the paper towels are made from different trees. In Europe they used *balsam* fir *Abies balsamea* L., (*family* Pinaceae), a coniferous tree, very common in Hungary, which contains a plant hormone which induced the additional molt. A more practical example is the case of basil plant *Ocimum basilicum* L., (family Lamiaceae) from which the compound juvocineme-II is extracted and which later on served as the model for synthesis of piriproxifen and fenoxicarb (Sarwar, 2016 a).

A professional fogging insecticide with two killing agents such as two synergists and an IGR are effective for devastating insect control. The product lineup, complete with insect growth regulators, insecticides and synergists, can help to control insects in stored commodities from start to finish (Sarwar, 2016 b).

Since IGR's address only the problem of reproduction, but do not actually kill an adult insect, it is always a good idea to use a knockdown-and-kill insecticide along with the IGR for ultimate population control. Insect growth regulators typically are effective for about 30 days when used for indoor treatments. On the other hand, in case of doing outdoor treatments it needs to make sure to purchase an IGR product that is photostable. If there is working to be spraying the product in areas of direct sunlight then photostable IGRs last longer in direct sunlight (Sarwar, 2016 c; 2016 d).

IGR's can come in a concentrated, spray, bait and aerosol forms for multipurpose application by producing environmentally compatible insect control. IGR concentrates like Precor and Gentrol are meant to be mixed with water and can then be used as crack and crevice, spot or fogging applications.

In particular circumstances, the simultaneous application of two different types of chemicals may be necessary. For instance, IGRs only control the immature stages of insects, but if the adult stage must also be controlled, it will be necessary to apply another insecticide such as an adulticide. Nevertheless, choose a formulation that requires a different type of application. For example, if the IGR is applied as a spray, it would be preferable for the adulticide to be applied as an aerosol or smoke with rapid kill of the adults and little residual that might select for resistance buildup in surviving immatures (Sarwar, 2015 a; Sarwar and Salman, 2015 a).

Insect pests suppression in organic agriculture with approved oils and IGR are periodically utilized to reduce damaging pest populations (Sarwar and Salman, 2015 b; 2015 c).

For managing development of resistance to insecticides, there is always the potential for resistance in insects to IGRs and further studies are needed for improvement of effective protocols for their use in commodity protection (Sarwar, 2015 b; Sarwar and Salman, 2015 d).
Worldwide, mosquito larvicides derived from microbial organisms and insect growth regulators have been increasingly used to control mosquito larvae (Sarwar, 2015 c; 2015 d; 2015 e; 2015 f).

Methods by which Insect Growth Regulators work

Insect Growth Regulators (IGR's) are a type of chemicals that break the life cycle of an insect. Common active ingredients in IGR'S are hydroprene, methoprene and pyridine. Their common names are Precor, Gentrol, Nygard, Surge and Archer. As an insect grows, it molts by growing a new exoskeleton under its old one and then shedding the old one to allow the new one to swell to a new size and harden. IGRs prevent an insect from reaching maturity by interfering with the molting process. This in turn limits infestations because immature insects cannot reproduce. Because these IGRs work by interfering with an insect's molting process, they kill insects more slowly than traditional insecticides. Death typically occurs within 3 to 10 days, depending on the type of IGR product, the insect's life stage at the time the product is applied and how quickly the insect develops. Some IGRs cause insects to discontinue feeding long before they die.
IGRs affect certain hormones in insects and these do not kill insects immediately, but they can stop a pest population from reproducing until all of the pests have been died. Insect growth regulators are insecticides that mimic hormones in young insects. They disrupt when insects grow and reproduce and can control many types of insects including fleas, cockroaches and mosquitos. Although there are rarely fatal for adult insects, they can prevent reproduction, egg-hatch and molting from one stage to the next. Many IGR products are mixed with other insecticides that kill adult insects and these have generally low in toxicity to humans.
Eggs treated with IGRs may never hatch, but If the eggs do hatch, the young insect may not survive. The larva or worm-like stage may not be able to develop correctly into an adult after exposure to IGRs. Some larvae may stay in this juvenile stage until they die. A case like a moth's cocoon which usually protects the pupa, treatment with an IGR may prevent the pupa from becoming an adult and reproducing. Adults usually survive IGR treatments and continue to be a nuisance until they die naturally. However, the eggs they produce may not survive. Sometimes adults reproductive organs are affected and the adults become sterile.

1. Hormonal Insect Growth Regulators

Hormonal IGRs typically work by mimicking or inhibiting the juvenile hormone, which is one of the two major hormones involved in insect's molting synthesized by retrocerebral endocrine organs the corpora allata. IGRs can also inhibit the other hormone ecdysone, large peaks of which trigger the insect to molt. If JH is present at the time of molting, the insect molts into a larger larval form, but if absent, it molts into a pupa or adult. IGRs that mimic JH can produce premature molting of young immature stages thereby disrupting larval development. They can also act on eggs, causing sterility and disrupting behavior or disrupting diapause, which are the processes that cause an insect to become dormant before winter. IGRs that inhibit JH production can cause insects to prematurely molt into a nonfunctional adult. IGRs that inhibit ecdysone can cause pupal mortality by interrupting the transformation of larval tissues into adult tissues during the pupal stage.

2. Chitin synthesis inhibitors

Chitin synthesis inhibitors work by preventing the formation of chitin, which is a carbohydrate needed to form the insect's exoskeleton. With these inhibitors, an insect grows normally until it molts. The inhibitors prevent the new exoskeleton from forming properly by causing the insect to die. Death may be quick or can take up to several days depending on the insect. Chitin synthesis inhibitors can also kill eggs by disrupting normal embryonic development. Chitin synthesis inhibitors affect insects for longer periods of time than hormonal IGRs. These are also quicker acting, but can affect predaceous insects, arthropods and even fish. Such types of compounds include benzoylurea pesticides. If chitin synthesis inhibitors are employed, and when an insect molts, it cannot form a new exoskeleton, causing the insect to die (Tunaz and Nedim, 2004).

Insecticides with growth regulating properties may adversely affect insects by regulating or inhibiting specific biochemical pathways or processes essential for insect's growth and development. Some insects exposed to such compounds may die due to abnormal regulation of hormone-mediated cell or organ development. Other insects may die either from a prolonged exposure at the developmental stage to other mortality factors (susceptibility to natural enemies, environmental conditions) or from an abnormal termination of a developmental stage itself. Insect growth regulators may come from a blend of synthetic chemicals or from other natural sources, such as plants. The chemical composition of hormones indigenous to insects is now being studied and used as a basis for developing analogs or mimics against insects (Ahmad and Sarwar, 2013; Sarwar, 2013 a; 2013 b).

Types of Insect Growth Regulators

There are three different types of IGRs, each of which has a different mode of action as mentioned below:-

1. Chitin synthesis inhibitors

These prevent the formation of chitin, which is a carbohydrate that is an important structural component of the insect's exoskeleton. When treated with one of these compounds, the insect grows normally until the time to molt. When the insect molts, the exoskeleton is not properly formed and it dies. Death may be quick, but in some insects it may take several days. As well as disrupting molting, chitin synthesis inhibitors can kill eggs by disrupting the normal development of the embryo.

2. Juvenile hormone analogs and mimics

When applied to an insect, these abnormal sources of juvenilizing agent can have striking consequences. For example, if the normal course of events calls for a molt to the pupal stage, an abnormally high level of juvenilizing agent will produce another larval stage or produce larval-pupal intermediates. Juvenoid IGRs can also act on eggs. They can cause sterilization, disrupt behavior and disrupt diapause, which is the process that triggers dormancy before the onset of winter. Theoretically, all insect systems influenced by juvenile hormone are potential targets for a juvenoid IGR.

The early juvenoid IGRs have true analogs of juvenile hormone and are unstable when exposed to ultraviolet light. This seriously limits their use in plant protection. Another group of juvenoid IGRs called juvenile mimics has been discovered. Entomologist found that extracts of many plant tissues have juvenilizing effects, but they have different chemical structures from juvenile hormones and are much more stable. They have been used as models to synthesize some highly effective and stable juvenile hormone mimics, which have potential to control tree fruit insect pests.

3. Anti-juvenile hormone agents

Anti-juvenile hormone agents cancel the effect of juvenile hormone by blocking of juvenile hormone production. For instance, an early instar treated with an anti-juvenile hormone agent molts prematurely into a nonfunctional adult. Two compounds which possess anti-juvenile hormone activity in insects have been isolated and identified from floss flower plant *Ageratum houstonianum Mill.*, (Asteraceae). These compounds, called precocenes, have been shown to inhibit the development of the insect corpus allatum and destroy it in sensitive species. A disadvantage of these chemicals is that these are so selective that they may not be economical for a manufacturer to develop (Bowers, 1981).

Insect growth regulators (IGRs) and pest control

Insect growth regulators (IGRs) are pesticides that do not usually kill insects outright, but instead affect the ability of insects to grow and mature normally. IGRs either block the insect's ability to turn into an adult or cause it to change into an adult before it is physically able to reproduce. Sometimes IGRs are called 'birth control; for insects and if immature insects are not able to molt into reproductive adults, the population will eventually die out. IGRs have been developed commercially and are being used to control insect pests in agriculture, forestry, public health and stored products. IGRs affect the biology of treated insects, for instance, both embryonic and post-embryonic development, reproduction, behavior and mortality. Abnormal morphogenesis is the observed effect of IGR action on the insects (Mondal and Parween, 2000).

Wing and Aller (1990) grouped IGRs into three categories: (i) juvenile hormones (JHs) and their analogues (JHAs) also called as juvenoids; (ii) ecdysone agonists and (iii) chitin synthesis inhibitors (CSIs) or moult inhibitors (MIs). The physiological and behavioral processes in insects are controlled by the juvenile hormones, ecdysones and moulting hormones. Juvenile hormones and ecdysones regulate the morphogenetic changes during metamorphosis. Ecdysones are secreted for varying periods in the larval stadium. Withdrawal of JHs and ecdysones induces the secretion of molting hormones, and the insects ecdyse. Recent advances in structural analysis and synthesis have resulted in the ready availability of synthetic analogues and mimics of these hormones.

Some IGRs are juvenoids, man-made chemical mimics of the juvenile growth hormones that occur in an insect's body. For instance, cockroach nymphs that have been exposed to juvenoid IGRs either never molt into adults or they develop into sterile adults that cannot reproduce. Cockroach adults that are exposed to IGRs as nymphs develop short, twisted wings and a darker body color. IGRs also can affect insects that are exposed later, in the adult stage, by blocking the development of viable eggs. Eggs that are exposed to IGRs may not hatch. Other IGRs are chitin synthesis inhibitors. They block the development of chitin (the insect's 'skin'), so that the insect cannot form a new exoskeleton or shed the old one. These affected insects die during the molting process. Some termite baits contain chitin synthesis inhibitors. The baits are carried back to the termite colony, affecting other worker termites as well.

Common insect growth regulators are methoprene used against fleas, ants, stored product pests, mosquitoes and many others; hydroprene is used against cockroaches, fruit flies, bed bugs and stored product pests; pyriproxyfen employed against fleas and cockroaches; and hexaflumuron, diflubenzuron and noviflumuron engaged against termites. IGRs are packaged as total release aerosols (foggers), crack and crevice aerosols and emulsifiable concentrates.

The IGRs may be considered viable grain protectants and therefore as potential components in stored-product integrated pest management. Insect growth regulators (two juvenile hormone analogues [fenoxycarb and pyriproxifen], four chitin synthesis inhibitors [diflubenzuron, flufenoxuron, lufenuron, and triflumuron], one ecdysteroid agonist [methoxyfenozide], and one combination of chitin synthesis inhibitors and juvenile hormone analogues [lufenuron plus fenoxycarb]) have been tested in the laboratory against adults of larger grain bore *Prostephanus truncates* (Horn) in maize and against adults of *lesser grain borer Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae) in wheat. The tested IGRs have been applied in maize at three doses (1, 5 and 10 ppm). All IGRs have been very effective (88.5% suppression of progeny) against the tested species at doses of 5 ppm, while diflubenzuron in the case of *P. truncatus* or lufenuron and pyriproxyfen in the case of *R. dominica* completely suppressed (100%) progeny production when they applied at 1 ppm. At all tested doses, the highest values of *R. dominica* parental mortality have been observed in wheat treated with lufenuron plus fenoxycarb (Kavallieratos et al., 2012).

Chemicals that interfere with the normal maturation of insects by disturbing the hormonal control of metamorphosis are the **juvenile hormone mimics,** such as **juvenoids** (fenoxycarb, hydroprene, methoprene, pyriproxyfen). These halt development so that the insect either fails to reach the adult stage or the resulting adult is sterile and malformed. As juvenoids deleteriously affect adults rather than immature insects, their use is most appropriate to species in which the adult rather than the larva is the pest, such as fleas, mosquitoes and ants. The **chitin synthesis inhibitors** (diflubenzuron, triflumuron) prevent the formation of chitin, which is a vital component of insect cuticle. Many conventional insecticides cause a weak inhibition of chitin synthesis, but the benzoylureas (also known as benzoylphenylureas or acylureas, of which diflubenzuron and triflumuron are examples) strongly inhibit formation of cuticle.

Stereotypically, juvenoids are used in urban pest control and inhibitors of chitin synthesis have greatest application in controlling beetle pests of stored grain. However, IGRs (pyriproxyfen) have been used also in field crops, for instance, in citrus, however, many growers are interested in using IGRs, such as pyriproxyfen and buprofezin, to control red scale *Aonidiella aurantii* (Maskell) (Hemiptera: *Diaspididae*). The experimental application of methoprene (often used as a mosquito larvicide) to wetlands resulted in both direct toxic and indirect food-web effects.

Neem *Azadirachta indica* A. Juss (family Meliaceae) derivatives are another group of growth regulatory compounds with significance in insect control. Their ingestion, injection or topical application disrupts molting and metamorphosis, with the effect depending on the insect and the concentration of chemical applied. Treated larvae or nymphs fail to molt, or the molt results in abnormal individuals in the subsequent instars. In particular, the main active principle of neem, azadirachtin, may act as an anti-ecdysteroid by blocking binding sites for ecdysteroid on the protein receptors. Neem-based products appear effective under field conditions against a broad spectrum of pests, including phytophagous insects of most orders (such as Hemiptera, Coleoptera, Diptera, Lepidoptera and Hymenoptera), stored-product pests, certain pests of livestock and even some mosquito vectors of human diseases. Opportunely, honey bees and many predators of insect pests, such as coccinellid and beetles spiders, are less susceptible to neem, thus making it very suitable for Integrated pest management (IPM) also known as integrated pest control (IPC) (Sarwar 2017; 2019).

The newest group of IGRs developed for commercial use comprises the molting hormone mimics (tebufenozide), which are ecdysone agonists that appear to disrupt molting by binding to the ecdysone receptor protein. They have been used against immature insect pests, especially lepidopterans successfully. There are a few other types of IGRs, such as the anti-juvenile hormone analogs (precocenes) that disrupt development by accelerating termination of the immature stages (Gullan and Cranston, 2014).

Application timing of Insect Growth Regulators

For the practical application of IGRs in pest management systems, three possible modes of exposure by surface contact, vapor action and ingestion of treated media have been investigated. IGR's exert their influence during limited periods of the insects life cycle, resulting in a variety of morphological and physiological deformities. For example, most reports on the effectiveness of IGRs on stored grain insects have emphasized that those possessing juvenile hormone activity must be available to target insects early in the last larval instar and must be maintained to be effective. Bugs, locusts and other hemimetabolous insects are usually only sensitive to juvenile hormone analogues or mimics shortly after the last larval ecdysis during the first third of the last larval instar, or after adult emergence, where JH then has an ovicidal effect. Larvae of most holometabolous insects such as Lepidoptera and Coleoptera are sensitive only at the end of the last larval instar, while the pupae are sensitive for several hours or at most a few days after the last larval ecdysis. Therefore to be effective in the field, juvenile hormone analogues must be applied at critical times to be effective and persist long enough to expose all members of the population during periods of sensitivity to juvenile hormone. They must be stable and effective for several weeks under field conditions if these control agents are to be of any practical use.

Certain strains of insects of medical importance including mosquitoes and houseflies, and field pests together with aphids that exhibit resistance to insecticides can also show some level of cross resistance to certain IGRs. Therefore, the candidate IGRs should be evaluated for their potential of cross-resistance with the appropriate target species before a full evaluation of these compounds can be ascertained.

Conclusion

This article highlights information on insect growth regulators (IGRs), which are compounds that affect insect growth via interference with their metabolism or development. Definitely, the only certain conclusion from this interpretation is that IGRs have devastating effects on their target insects. Several features of IGRs make them attractive as alternatives to the usage of broad-spectrum insecticides. Because they are more selective, these are less harmful to the environment and more compatible with pest management systems that include biological control. Finally, in spite of their slower speed of kill the insect pests than the faster acting neurotoxic insecticides, the three classes of insect growth and development disrupting insecticides are well suited for use in insect pest control. They are also suited for resistance management programs due to their novel and different modes of action. Although IGRs are in their infancy as a control measure in field and post-harvest storage, undoubtedly the potential of IGRs has a role to play in future strategies involving an integrated approach for control of insect pests.

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