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Chapter 7

**EFFECT OF ALLELOCHEMICAL ON DIGESTIVE AND BIOMOLECULES OF RICE
WEEVIL: *Sitophilus oryzae***

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INTRODUCTION

Rice (*Oryza sativa* Linn.) is an economically important crop and the most important staple foods for the world's population. More than 90% of the world's rice is produced and consumed in Asia. The rice plant is vulnerable to various kinds of pests, From the seeds to the stored grains. The rice losses occur when the milled grains are attacked by stored product insects, the important one is rice weevil, *Sitophilus oryzae* L. (Coleoptera: curculionidae). Damaging rice by the weevils is seriously affected the availability of food for a large number of people worldwide. Without protection, the weevils rapidly grow, develop, and damage the stored rice grains. The quality of rice grains is so poor that they do not meet the requirement for normal consumption, exportation, and industrial purposes. Chemical control of rice weevils has been uses, Such as rice fumigation with insecticides . Using synthetic chemicals as insect pest control has given rise to a number of problems, including adverse effects on the environment and human health. Recently, there has been considerable pressure from consumers opposed the use of synthetic insecticides in foods.

Rice weevil (*sitophylus oryzae*)

S. oryzae belongs to the order Coleoptera and the family Curculionidae. It grows and distributes in warm and tropical parts of the world. *S. oryzae* was first described by Linnaeus in 1763. The species name of *oryzae* was given because it was found in rice. The adult rice weevil is attracted by lights. The rice weevil is a small snout beetle which varies in size, about 3-4 mm long. Its morphology varies from a dull reddish brown to black with round or irregular pits on the thorax. There are four light reddish or yellow spots on the elytra (hardened forewings). The head of many adult weevils has a characteristic snout. Male rice weevils have shorter snout, wider and more distinct punctures than females. The Larvae of *S. oryzae* are legless, white to creamy with a small tan head. Jadhav (2006) described *S. oryzae* differs from *S. granarius*. The adult *S. granarius* is shiny reddish brown with elongated pit on thorax, whereas the adult *S. oryzae* is dull reddish brown with irregular shaped pit on the thorax and four light spots on the wing covers.

ECONOMICAL LOSS

The most common insect pest of stored corn in north carolina is the rice weevil, *Sitophilus oryza* (L.). General estimates of the amount of damage caused by the rice weevil in the state are not thought to be very accurate. The study reported here is an attempt to provide more accurate information than has heretofore been available on the actual weight loss and damage caused by weevils in different quantities of shelled corn stored at 60°, 70°, and 80° F and on the nutritive value of weevil- infested corn as determined by chemical analysis and by feeding tests with mice.

ALLELOCHEMICALS

Allelochemicals are a subset of secondary metabolites, produced by an organism (certain plants, algae, bacteria, coral, and fungi) which are not required for metabolism (i.e. Growth, development and reproduction) of that allelopathic organism but influence the growth, survival, and reproduction of other organisms or of that organism itself. Allelochemicals can have beneficial (positive allelopathy) or detrimental (negative allelopathy) effects on the target Organisms. Accordingly, it is extremely difficult to unambiguously demonstrate allelopathy in soil because of the complexity of allelochemical interference and its relationship to soil chemistry and the microbial process. However, with an increased understanding of soil degradation dynamics occurring in a few typical allelochemicals, such as wheat benzoxazinoids, and the ability to identify allelochemicals and microbial community structure in soil, an effort should now be directed toward understanding

the fate and impact on microorganisms of allelochemicals in the soil environment.

Flavonoids are widespread throughout the plant kingdom, and several reviews have examined their food sources and the bioavailability, metabolism, and biological activity of these compounds in humans. Flavones differ from other flavonoids in that they have a double bond between C2 and C3 in the flavonoid skeleton, there is no substitution at the C3 position, and they are oxidized at the C4 position. These compounds play a variety of roles in plants. Along with flavonols, they are the primary pigments in white- and cream-colored flowers and act as copigments with anthocyanins in blue flowers.

EFFECT OF ALLELOCHEMICAL ON INSECT

Allelochemicals constitute a major defensive weapon of plants to defend themselves against a broad range of phytophagous herbivores, so it is necessary to investigate the biosynthesis and the application of these metabolites to deploy them as an eco-friendly and sustainable pest management option. Recent analytical tools and techniques have enabled the elucidation of the role of secondary metabolites in plant defense. These metabolites are categorized into four different groups: terpenoids, phenolics, and nitrogen and sulfur-containing.

Terpenes are the largest group of plant secondary metabolites. Although most terpenes are important in plant defense, some terpenes (e.g., gibberellins) are involved in primary functions, such as plant growth and development. Terpenes comprise around 25,000 compounds with diverse functions including feeding deterrence, direct toxicity, or oviposition deterrence. Specialist herbivores can tolerate terpenoids and utilize them as an attractant to locate their host plants and as feeding stimulants.

Every day, the demand for natural products obtained from plants increases to be used as pest control agents. New pesticides are being developed using flavonoids, as they are an alternative to synthetic pesticides. They can inhibit enzymatic activity and prevent the growth of larvae insect species. Some flavonoids interfere in the process of moulting and reproduction of several insects, that is, they inhibit the formation of juvenile hormone (ecdysone). Flavonoids inhibit transcription oecdysone receptor-dependent genes. It has been reported that some types of flavonoids have had an effect on agricultural pests effect, oviposition, fecundity, mortality, weight reduction, and emergence of adults. Some flavonoids can influence agricultural pests depending on the concentration applied; if they are low, they do not affect them. Therefore, it is necessary to test the minimum concentrations

for the flavonoid to have an insecticidal effect.

The study of plant flavonoids in biology and medicine requires a multidisciplinary approach. Like other secondary metabolites, flavonoids have been shown to affect the feeding behaviour of insects although these compounds have generally received less attention in plant- insect studies than some others. Ermanin is an example. Despite recent studies such as that of Larsson et al. (1992) of the effect of taxifolinglucoside on larval pine sawflies, we know little of the ecological importance of these ubiquitous plant compounds. As a result, there is a lack of detailed data regarding the effect of flavonoids on insect growth and survival. However, there has been considerable progress in elucidating the biosynthetic pathways and genes responsible for synthesis of flavonoids. Therefore, flavonoids may constitute accessible secondary metabolites for genetic engineering aimed at host plant resistance.

Many flavonoids are known to inhibit the growth of insect larvae (Elliger et al., 1980a). The flavonols quercetin, rhamnetin and rutin have recently been evaluated for their effect on growth parameters and food processing efficiency of the southern armyworm, *Spodoptera eridania* Cramer (Lindroth and Peterson, 1988). Rutin may have third trophic level effects on an invertebrate predator of rutin-fed *manduca sexta* larvae. A test of a series of naturally occurring and synthetic flavones on growth of the navel transitella showed that an unsubstituted flavone was the most inhibitory the products of the oxidative reactions include quinones reduce the nutritive value palatability of the plant tissue to insect pests by cross-linking with the nucleophilic sidechains of proteins and free amino acids. Quinones also act as directly toxic to insect pests.

The primary metabolites of plants don't directly help encountering the insect attacks; rather serve as precursors for the synthesis of secondary metabolites. These secondary metabolites are involved in the plant defense against a variety of stresses and don't take part in normal growth and development of the plant. PSMs can be directly toxic to insect pests or can mediate signaling pathways of the production of plant toxins. Among the plant defensive traits, both the inducible and constitutive chemical barriers and nutritional content in a plant are considered to have significant effect on reducing the insect growth and development. The plant produced compounds Phytoecdysteroids are analogues of the insect hormone ecdysteroid, which controls developmental phases on insects from larva to adult. These compounds have been identified in asparagus (*Asparagus* spp.).

Neem allelochemicals azadirachtin and nimbin were administered to different larval

instars of the tobacco armyworm, *Spodoptera litura* orally in artificial diet, topically or via injection. Nutritional analyses revealed strong antifeedant and growth regulatory effects of azadirachtin which were independent of each other. While salannin and nimbin induced concentration dependent feeding deterrence only; nimbin was inactive to the 1000 ppm level against this insect species.

RESULT AND DISCUSSION

Broadly speaking, plant resistance against insects can be grouped into two categories. The first one is constitutive resistance, which includes the inherited ability of the host plant to defend itself against the insect pests, regardless of biotic or abiotic factors. The second is induced resistance, which appears as a response to attack by insect herbivores, diseases or abiotic factors. Constitutive and induced resistance can be direct or indirect. In direct resistance, both morphological traits and secondary metabolites act as direct defense strategies to resist insect herbivores. In indirect resistance, plants rely on natural enemies of the herbivores to protect them. Herbivore-induced plant volatiles (HIPVs) emitted upon an insect damage are known to provoke indirect resistance. The HIPVs attract predators and parasitoids, which reduce the damaging caused by insect pests.

ESTIMATION OF PROTEIN

protein concentration of control and flavone treated insect group. From the result control group possess protein concentration is 0.82 mg/ml. 1% treated group possess 0.64 mg/ml. 2% treated group possess 0.47 mg/ml. And 3 % treated group possess 0.31 mg/ml. And result showed that during treatment with flavone protein concentration is decreased.

ESTIMATION OF TOTAL CARBOHYDRATE CONTENT

carbohydrate concentration of control and flavone treated insect group. From the result control group possess carbohydrate concentration is 2.59 mg/ml. 1% treated group possess 2.38 mg/ml. 2% treated group possess 2.26 mg/ml. And 3 % treated group possess 2.19 mg/ml. And result showed that during treatment with flavone carbohydrate concentration is decreased. Total carbohydrates content in insect on treated with flavone was found to be less than control. This comes in agreement with Fell *et al.* (1982), Rajendra (1990), and Shakoori and Saleem (1991) who suggested that carbohydrates converted to proteins in detoxification mechanism against toxicants that enter the animal body.

ESTIMATION OF LIPID

lipid concentration of control and flavone treated insect group. From the result control

group possess lipid concentration is 0.55 mg/ml. 1% treated group possess 0.45 mg/ml. 2% treated group possess 0.31 mg/ml. And 3 % treated group possess 0.19 mg/ml. And result showed that during treatment with flavone lipid concentration is decreased. The present results also showed that treatment with different concentration of flavone affected biochemical activities which lead to the disturbance in total lipid content. The reduction in lipid profile indicates a negative effect of the flavone on lipid metabolism and peroxidation. The decline in lipid quantity may be due to shift in energy metabolism towards lipid catabolism as the result of insecticidal stress induced by the compound.

ESTIMATION OF LIPASE

lipase enzyme activity of control and flavone treated insect group. From the result control group possess lipase activity is 343.58 $\mu\text{moles}/\text{min}/\text{mg}$ protein. Specific activity of lipase of 1% treated group is 533.86 $\mu\text{moles}/\text{min}/\text{mg}$ protein. Specific activity of lipase of 2% treated group is 602.38 $\mu\text{moles}/\text{min}/\text{mg}$ protein. Specific activity of lipase of 3% treated group is 142.06 $\mu\text{moles}/\text{min}/\text{mg}$ protein. And result showed that during treatment with 1 and 2 % concentration of flavone, specific activity of lipase is increased than control group and at 3 % treatment specific activity of lipase is decreased than control group. In the present study the lipase activity in 1 and 2 % treated rice weevil 55.38 and 75.32 % increase respectively but in 3 % treatment specific activity of lipase is decreased 58.65 %

Most of the inhibitors are proteins that inhibit the enzymes by forming a complex by blocking the active site of enzymes which finally leads to reduction in its catalytic activity. Different digestive enzymes inhibitors from cereals and beans (*Phaseolus vulgaris*) have different molecular structures, leading to different modes of inhibition and different specificity profiles against a diverse range of digestive enzymes. The inhibition occurs mainly via interactions within the enzyme substrate binding site, the aromatic residues lining the active site play an important role; the sub sites are usually occupied by structural elements originating from the inhibitor molecule; the structure-segments and loop regions strongly involved in the inhibition process are likely to correspond to flexible components of the free structures of the molecules. Both mammalian and insect specific digestive enzymes dual inhibitors act through a common mechanism using alternative ways (Gupta *et al.*, 2013).

Anti-herbivory compounds are secondary metabolites of plants suppressing herbivore insects. They can be divided into several subgroups: nitrogen compounds including alkaloids, cyanogenic glycosides and glucosinolates, terpenoids and phenolics. The diversity of angiosperms during the Cretaceous period is associated with the sudden increase in speciation in insect. Parallel to their evolution, selective bio-chemical processes in plants

resulted in defensive adaptations against insect herbivores. First, insects' bit or chewed on plants. However, the coevolution of vascular plants and insect species caused new patterns of feeding to emerge, such as sap sucking, leaf mining, gall forming and nectar feeding.

CONCLUSION

The phenomena of allelopathy and phytotoxic interactions between plants are strongly expanding branches of biological science. Allelochemicals, as a group of substances also called bio communicators, seem to be a fruitful challenge for combining traditional agricultural practices and new approaches in pest management strategies. Allelochemicals have already been used to defend crops against pathogens, insects or nematodes, parallel to some attempts to use them for weed control. Crop rotation, cover crops, dead and living mulches are being employed in agriculture. Both in natural and agricultural ecosystems allelopathic interactions are involved in practically every aspect of plant growth, as they can play the role of stimulants and suppressants. Complex plant-plant and plant-microbe interactions in ecosystems and currently developing studies on molecular, cytological and physiological levels bring us to a better understanding of processes occurring around us. The ancient knowledge of well-known toxic properties of water extracts of a variety of allelopathic plants give us a basis that could be used in the creation of a novel approach in weed control. Some allelochemicals, mainly these that are mentioned in the text above, may act as a starting point for production of new bioherbicides with novel target sites, not previously exploited, as the understanding of their mode of action is still growing. Creation of bio-herbicides based on allelochemicals generates the opportunity to exploit natural compounds in plant protection and shows the possibility to cope with evolved weed resistance to herbicides. Despite the fact that we have extensive knowledge about the chemical nature of natural compounds, we can synthesize its analogues, and we have basically explored its phytotoxic potential, we still have insufficient data. Until recently, most studies on phytotoxicity have been conducted under laboratory conditions due to the ability to eliminate other environmental factors such as temperature, soil texture and its chemical and physical properties. Such approach allows the recognition of only direct effects of allelochemical action. There is still a great need to transfer laboratory data into field conditions. Such experiments are not willing to be taken on due to troublesome field experiments dependent on environmental conditions and a few year repetitions. New tools of molecular genetics, proteomics and metabolomics profiling as well as modern and sophisticated methods of chemistry and biochemistry will lead to the creation of substances, maybe based on the structure of particular compounds occurring in nature, which could be

used without any risks as selective and eco-friendly herbicides. The aim of the present study is the evaluation of change in digestive and biomolecules activities in flavone treated rice weevil, *Sitophilus oryzae* and the objectives are treatment with different concentration of flavone (1, 2 and 3 %) on rice weevil *Sitophilus oryzae* and estimation of protein, carbohydrate, lipid, digestive enzymes like α -amylase, protease and lipase. The result of the present study is at higher concentration of flavone activities of digestive enzymes and concentration of biomolecules inhibited. It is important to point out that inhibition of the digestive enzymes including amylase, lipase and protease indicates that these enzymes play no role in the digestion of tested compounds or the tested compound may act as an antifeedant and may decrease the susceptibility of insect pest against these compounds.

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