Allelochemical synergism and insect behavioural diversity

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Synergism in insect–plant interactions plays a pivotal role in increasing the bioactive efficacy of allelochemicals and also influences the behavioural diversity of insects. As herbivore-induced plant volatile blends act as communication signals to natural enemies, the role of synergism in tritrophic interactions is very significant. Pheromonal synergism being widespread in insects, the precise composition of the pheromone stimulus affects their orientation behaviour, different isomers tending to evoke diverse behavioural patterns.

Recognition of plant allelochemicals as agents influencing the behaviour of insect herbivores and their natural enemies is comparatively recent. The green leafy odours of plants, comprising mixtures of fatty acid derivatives such as C₆ aldehydes, alcohols and esters provided by the lipoxygenase pathway, form ‘aerial bouquets’, enabling phytophagous insects to reliably detect host plants. For instance, volatiles influencing searching behaviour include (Z)-3 hexanol, (Z)-3 hexenal, (E)-2 hexanol, (E)-hexanol and (Z)-3-hexanyl acetate. This reliability and detectability of volatile chemical stimuli from the first to the second trophic levels are of considerable significance. Subsequent feeding by phytophagous insects cause qualitative or quantitative changes, resulting in specific or nonspecific, direct or indirect modifications through alteration of their carbon/nutrition balance. Being of short-term duration, detectability of these compounds often poses a problem unlike those of the more durable blends of terpene volatiles emitted from insect-damaged plants. While different host species may cause differential release of plant compounds, synergism has come to play a pivotal role in increasing the bio-active efficiency of the allelochemicals. In many cases, systemic responses induced by insect feeding are mediated by elicitors that are transported from the infested to the uninfested sites. Because plant volatiles are known for their diversity of chemical components as well as their changeability in time, natural enemies become exposed to the mixture of compounds, enabling synergistic interactions. Besides, blends of pheromones cause sequential behavioural patterns and a highly adaptive, typically synergistic behaviour is evident in ants and other social insects.

Emphasis has been laid on the need for increased realization of the role that synergism plays in insect–plant interactions as well as tritrophic interactions. This will go a long way in pinpointing the compound(s) responsible for the behavioural diversity of insects. Of equal significance is the recognition of synergistic interactions between viruses and chemical pesticides and between viruses and other pathogens which have reduced the rate of application of chemical insecticides. Plant natural products influencing the efficacy of entomopathogens are known, as evidenced by the action of chlorogenic acid and polyphenol oxidase. These alter pathogenicity by acting directly on Btk².

Synergistic reaction of allelochemicals

Synergism is usually coaltive wherein a system of two components of which each alone causes no remarkable impact, but together provide a catapulting effect. A notable instance is gossypol, a phenolic sesquiterpene toxic to a variety of insects. It causes significant decrease in the survival, growth and development of several cotton pests. Pigment glands of cotton plant produce another sesquiterpene, caryophyllene oxide³, serving to synergize the growth-inhibiting effect of gossypol. Increased vigour of the boll weevil is evident through ingestion of gossypol, which suppresses the growth of gut bacteria. An enhanced phagostimulatory effect is seen when sinigrin and glucose interact as evident in Plutella xylostella. Similarly piperonyl butoxide is known to be an effective synergist in conjunction with diflubenzuron in the case of Spodoptera exigua increasing the toxicity of diflubenzuron⁴.

Synergistic effects of gallic, vanillic and salicylic acids are evident not only in their interaction with other plant allelochemicals, but also in their interactions with the endotoxins of Bacillus thuringiensis. Gallic acid occupies an important position in the overall phenolic metabolism of higher plants exhibiting synergistic interactions. Interestingly enough, majority of hydrobenzoic acids (p-hydroxybenzoic, salicylic, vanillic, syringic, protocatechuic) are relatively rarely encountered in plants, particularly in the herbaceous dicots. Various cinnamic acids (p-coumaric, caffeic and synapic acids) are ubiquitous in the tissues of higher plants formed normally as monoesters and rarely as bi-esters⁵ (Figure 1).
Effect of the addition of allelochemicals and/or entomocidal toxins in the diet, on the food consumption patterns of *Helioverpa armigera* larvae showed a reduction from 331 mg/day to 92 mg, 79 mg and 56 mg/day by supplementation with gallic acid, β endotoxins and resorcinol respectively. At higher concentrations of endotoxins with gallic acid, the feeding rate was reduced to a greater degree. Similarly the impact of vanillic and salicylic acids on the development, survival and adult emergence of *H. armigera* also varied greatly with the concentrations and combinations tested, as compared to individual phenols.

Demonstration of synergism between two natural products, β-asarone and menthol has shown the involvement of enzyme induction. Bioactivation of β-asarone is synergized by menthol, an MFO inducer, synergism resulting from metabolic bioactivation by an enzyme. Similarly, toxicity of xanthotoxins to *Heliothis zea* caterpillars has been known to be increased by myristicin. Other synergists like safrole and apiol occur alongside with xanthotoxin in Umbelliferae as also fagaramide, asararin and sesamin in Rutaceae. The occurrence of these compounds at sublethal concentration suggests their having a primary role as phytosynergists.

**Synergism in tritrophic interactions**

Equally relevant is the fact that a number of plants damaged by insects emit chemical signals in large amounts that guide natural enemies to the concerned insects. To be effective, the emitted volatiles should be clearly recognized from the background odours, often coinciding when natural enemies forage. Besides, the green leaf odours mentioned earlier, after several hours of feeding result in the release of compounds such as linalool, (3-E)-4,8-dimethyl-1,3,7-nonatriene, α-trans bergamotene, β-farnesene, (E)nerolisol and (3E,7E)-4,8,12 trimethyl 1,3,7,11 tridecatetraene and indol. The insects are able to recognize differences in the ratio between these green leafy compounds. Programming or imprinting to specific plant environments is typical of some parasitoids. Some compounds are short range ones, not very volatile, but requiring high concentration for eliciting responses. Related strains or chemotypes of a given plant also tend to differ in attraction if their volatile chemical profiles are different. However, a combination of cues elicit better responses by female successful parasitoids. The mites *Tetranychus urticae* and *Panonychus ulmi* are known to affect several host plants, including apple leaves and release volatiles after feeding, eliciting different responses from predatory mites. While the relative percentages of chemical constituents differed between blends of volatiles, two compounds, viz. (E,E)-α-farnesene and (Z)-3-hexan-1-yl-acetate, with farnesene make up more than 50% of the blend. This confirms that plants are the major sources affecting variation in chemical information available to natural enemies. Thus the environmental effects of allelochemical variation become important.

In tritrophic interactions, herbivore-induced, carnivore attractants play an important role. Herbivore caterpillars regurgitate salivary β-galactosidase. This elicits plant response, usually in the form of release of volatiles which increase host detectability by natural enemies. Herbivore-induced plant volatiles provide natural enemies with information about plant species, plant cultivars and the herbivore insect species infesting the plant. Thus the herbivore-induced volatiles are essential information source for natural enemies and act as communication signals. In spite of these, our understanding of the role of plant odours in phytochemical-insect responses is very limited. It is clearly evident that the overall function of volatiles in plant-insect interactions is to act as chemical signalling systems with volatile blends promoting efficacy of interactions. Both quantitative and qualitative differences in the blends of these volatiles play important roles in such functioning.

**Pheromonal synergism**

Sex pheromones are single, two component or multi-component chemical blends, with chain lengths varying from C4 to C14. They occur in different proportions with several other chemicals and are present in a range of related and unrelated species. The precise composition of the pheromone stimulus affects orientation behaviour, and different isomers can evoke very different behavioural patterns. Such blends as Z(9) and Z(11) tetracosenyl...
Table 1. Examples of pheromone mixtures of well-known Indian insect pests showing synergism enabling increased sexual responses

<table>
<thead>
<tr>
<th>Pest Name</th>
<th>Pheromone Components</th>
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<tbody>
<tr>
<td>Agrotis ipsilon</td>
<td>(Z)-11-dodecanyl acetate, (Z)-9-tetradecanyl acetate</td>
</tr>
<tr>
<td>Chilo suppressalis</td>
<td>(Z)-11-hexadecenal, (Z)-13-octadecenal</td>
</tr>
<tr>
<td>Helicoverpa armigera</td>
<td>(Z)-11-hexadecanal, (Z)-9-hexadecanal</td>
</tr>
<tr>
<td>Pectinophora gossypiella</td>
<td>(Z,E)-7,11-hexadecadienyl acetate, (E,Z)-7,11-hexadecadienyl acetate</td>
</tr>
<tr>
<td>Phthorimaea operculella</td>
<td>(E,Z)-4,7-tridecadienyl acetate, (E,Z,Z)-4,7,10-tridecatrienyl acetate</td>
</tr>
<tr>
<td>Plutella xylostella</td>
<td>(Z)-11-hexadecanal</td>
</tr>
<tr>
<td>Spodoptera litura</td>
<td>(Z,E)-9,11-tetradecadienyl acetate, (Z,E)-9,12-tetradecadienyl acetate</td>
</tr>
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acetate in the ratio of 1 : 3 are common. Often the blend produces a greater response than any individual component, and this synergism is widespread in insects that produce pheromone ‘cocktails’. When the blend comprises of four components, males are attracted to more complex ratios such as 60 : 40 : 2 : 200, when the components are (Z) and (E)-11-tetradecenyl acetate, (Z)-9-tetradecenyl acetate and dodecenyl acetate. The proportion of the components varies according to the abundance of the species. Certain parasitic tachinids and braconids and other parasitoids locate their hosts by tuning to their (hosts) long distance sex-attractant pheromones.

Synergistic effects of terpenoid mixtures on bark beetles are well known. Frontalin of males synergizes with exobrevicomin of females and myrcene sequestered directly from oleoresin of trees, to produce a rich ‘pheromone bouquet’. The cumulative lure of all the three pheromones is synergistic, causing aggregation of beetles. Many lepidopteran pests have aldehydes and ketones as sex pheromones. Some pheromonal compounds of Helicoverpa zea such as hexadecanal, (Z)-7 hexadecanal, (Z)-9-hexadecanal and (Z)-11 hexadecanal influenced the host-seeking behaviour of Trichogramma pretiosum. The application of a blend of these chemicals increased parasitism rates in treated plants. Table 1 provides the composition of some sex pheromones of major lepidopteron pests in India. In Callosobruchus chinensis a mixture of methyl-branched saturated hydrocarbons and (E)-3,7-dimethyl-2-octone-1,8-dioic acid elicit copulatory effects of males, the copulation releasing hormone being erected. The combination of a highly stimulating excitant like formic acid and a direction stimulus, like undecane, results in excitement in ants which rapidly ‘home in’ very accurately. A combination of two hydrocarbon with formic acid releases a more intense response than one hydrocarbon. In Myrmica rubra the primary alarm pheromone 3-octanone is an attractant, while 3-octanol is an arrestant, together producing klinokinesis in excited workers. Phenomonal synergism which incites sequential behavioural responses, is much evident in several insects.

Briefly, there is no dearth of information on allelochemical synergism. Besides, augmenting the resistance mechanisms in plants against insects, the role of allelochemical synergism in tritrophic interactions is essentially to enhance the behavioural diversity of natural enemies, enabling more efficient biological control. Pheromonal efficiency through regulation of population build up of several agricultural pests by intercepting the communication signal between sexes is an added feature. With biopesticide industries cropping up rapidly, recognition of synergistic interactions should lead the way towards augmentation of the efficacy of the bio-active compounds, notably plant-based or βt-based products. BUGSAC, a herbal preparation made from 46 plant extracts such as neem, pepper, tulsi is a notable instance, acting as a non-toxic, ecofriendly pesticide effective on several pests, contributing to synergistic trends in the chemical amalgam.


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