

Full Length Research Article

Potential of Phytocompounds as Antifeedants against the Adults of the Red Pumpkin Beetle *Aulacophora foveicollis* Lucas 1849 (Coleoptera: Chrysomelidae)

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Abstract

Red pumpkin beetle has been a limiting factor as a destructive polyphagous pest of cucurbit vegetables, and the extent of damage caused by this pest is severe. Phytochemical antifeedants are alternates to chemical pesticides to control agricultural pests, and has emphasized their potential as ecologically safe pesticides. In the present study, phytocompounds, viz., momordicine, nicotine, piperine, plumbagin and Rhein along with azadirachtin (standard reference) were tested for their antifeedant potential against the adults of *Aulacophora foveicollis* by leaf disc no choice bioassay for 24 hours at varied concentrations (10, 20, 30, 40 and 50ppm). Leaf discs treated with the phytocompounds showed high antifeedancy. Total feeding inhibition was recorded at the maximum concentration by azadirachtin, and a feeding deterrence of 74.1% to 93.6% was observed between 10 and 40ppm. Momordicine exhibited more than 50% feeding deterrence from 30ppm, and displayed a maximum of 92.1% feeding deterrence at 50ppm. The same trend was displayed by nicotine, piperine, plumbagin and Rhein with a maximum feeding deterrence of 93%, 86.4%, 89.2% and 94.3%, respectively at the highest concentration. The rate of feeding by the adult beetles was highly reduced by the phytocompounds as they exhibited more than 85% feeding inhibition. This study indicated that the tested phytocompounds inhibited the feeding of *Aulacophora foveicollis* by making the food unpalatable resulting in feeding deterrence.

Key words: *Aulacophora foveicollis*, Phytocompounds, Antifeedant mechanism behaviour

Red pumpkin beetle, the most destructive pest of cucurbit vegetables occurs throughout the year and causes severe percentage of damage to musk melon (80.63%), long melon (71.69%), ash gourd (13.88%), and snake gourd (7.63%) [1-6]. This pest is widely distributed over South-East Asia, Africa, Europe, Mediterranean region, Australia, Greece, Sri Lanka, Nepal, Burma, Iraq and India [7-8]. Beetles attack right after the germination and slow down the growth of plants due to serious attack. The grubs feed on root tissue and cause direct damage to the newly developed seedlings besides stem and fruits [9]. Adults prefer young seedlings, flowers and young tender leaves, and cause irregular holes on leaves leading to severe attack on the young seedlings of cucurbitaceous crops resulting in the death of plants [10-12]. The indiscriminate use of broad spectrum, persistent synthetic insecticides, unmindful of the resultant complexities inflicted upon agricultural environment has brought in problems due to pesticide residues, resistance by pests, secondary pest outbreak, and hazards to non-target organisms [13]. To overcome such problems relating

to insect pest mis-management, there is an urgent need to employ economically viable and environmentally safe pest control strategies [14]. Owing to these implications, alternates have been devised and most of the attention and focus has been paid on the use of natural products.

The concept of green pesticides which involves the development of green pesticide technology is gaining a lot of attention, and involves nature-oriented and beneficial pest control materials, which can contribute to reduce the pest population and increase food production, as they are safe, ecofriendly, and more compatible with the environmental components than synthetic pesticides. The concept of using insect antifeedants as crop protectants against destructive agricultural pests is intuitively attractive [15]. Antifeedants, also called 'feeding inhibitors' [16], or 'feeding deterrents' [17], is a substance that stops insects from feeding on plants, without killing them [18]. Antifeedants are naturally occurring compounds regarded as allelochemical belonging to allomones, and they offer first line of crop protection against notorious

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insects. According to Isman [19-20], any behaviour modifying substance that deters feeding or reduces food consumption by an insect through direct action on the taste organs (peripheral sensilla) in insects that taste bad to insects is an antifeedant or feeding deterrent. Discovery of antifeedants from plants has emphasized a potential method for the development of ecologically safe pesticides. Insect antifeedants are chemical substances that prevent or inhibit feeding of insects and such substances are naturally present in a plant. Phytocompounds with insect antifeedant potential can be used as a safer alternative to harmful chemical pesticides to control agricultural pests. Botanical antifeedants inhibit feeding or disrupt insect feeding and eventually insects starve to death owing to phytochemical constituents which possesses obvious antifeedant property against insects [21-22]. The concept of antifeedants attracted considerable attention ever since the

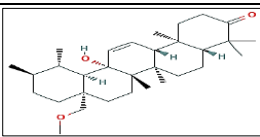
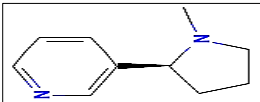
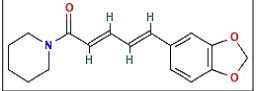
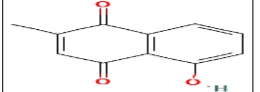
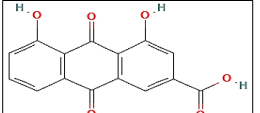
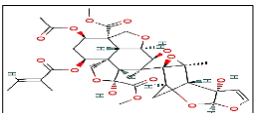
striking feeding deterrence effects of azadirachtin first documented in the desert locust *Schistocerca gregaria* by spraying it on its natural diet [23-24], followed by total feeding inhibition in lepidopteran larvae when its food was coated with azadirachtin [25]. The present investigation aims to study the antifeedant potential of a few selected phytocompounds on the red pumpkin beetle, *Aulacophora foveicollis*.

MATERIALS AND METHODS

Phytocompounds

Phytocompounds, viz., momordicine, nicotine, piperine, plumbagin, rhein and azadirachtin (standard antifeedant) were tested for their antifeedant potential on the adults of the red pumpkin beetle. The details of these phytocompounds are presented in (Table 1).

Table 1 Details of phytocompounds utilized for the present study

Phytocompound	Plant species	Plant family	Common name (English)	Vernacular name (Tamil)	Plant part	Plant habit	Structure	Molecular formulae (Molecular weight g/mol)
Momordicine	<i>Momordica charantia</i> L.	Cucurbitaceae	Bitter melon	Paagarkaai	Leaf	Climber / Herb		C ₃₁ H ₅₀ O ₃ (470.70)
Nicotine	<i>Nicotiana tabacum</i> L.	Solanaceae	Tobacco	Pukaiyilai	Leaf	Herb		C ₁₀ H ₁₄ N ₂ (162.23)
Piperine	<i>Piper nigrum</i> L.	Piperaceae	Black pepper	Kurumilagu	Seed	Climber		C ₁₇ H ₁₉ NO ₃ (285.34)
Plumbagin	<i>Plumbago zeylanica</i> L.	Plumbaginaceae	Chitrak	Chittiramoola m	Root	Herb		C ₁₁ H ₈ O ₃ (188.18)
Rhein	<i>Cassia fistula</i> L.	Fabaceae	Golden shower	Konrai	Flower	Shrub		C ₁₅ H ₈ O ₆ (284.22)
Azadirachtin	<i>Azadirachta indica</i> A. Juss	Meliaceae	Neem	Vaepam	Seed	Tree		C ₃₅ H ₄₄ O ₁₆ (720.70)

Aulacophora foveicollis

Aulacophora foveicollis adults collected from cucurbits plants near agricultural fields at Padappai, Kancheepuram, district, Tamil Nadu, India was brought to the laboratory at the Department of Zoology, Thiruvalluvar University, Vellore, Tamil Nadu, India and taxonomical identity was confirmed prior to rearing and mass culturing of the beetles. The collected beetles were released into the glass jars containing fresh pumpkin leaves which were provided as food, and maintained at room temperature of 27±2°C and relative humidity of 70-80%. The mouth of the glass jars was closed with muslin cloth held by rubber bands. Fresh pumpkin leaves were replenished on a daily basis. Cyclic generation of beetles on emergence were transferred to new rearing glass jars provided with fresh tender pumpkin leaves. This process of culture method was repeated and the culture was maintained throughout the study period.

Antifeedant bioassay

The experiment was conducted using leaf disc no choice bioassay method [26] with minor modifications [27]. For the

bioassay, the adults of *Aulacophora foveicollis* from the F₁ generation culture was used. Fresh pumpkin leaf discs (1350 mm²) were prepared in varying concentrations (5, 10, 20, 30, 40 and 50ppm). Abaxial and adaxial surface of the host leaves were sprayed with these above-mentioned concentrations prepared for each phytocompound, and air dried. The treated leaf disc was then kept inside an individual transparent glass cage (45 × 45 × 45cm). A single pre starved unsexed adult was introduced into the cage, and thereafter the cage was closed with muslin cloth. The adult was allowed to feed on the treated leaf disc for a period of twenty-four hours. A similar condition was maintained for control wherein the leaf disc was sprayed with distilled water. A total of three trials with five replicates per trial were carried. At the end of the experiment, unconsumed area of leaf disc was measured with the aid of a leaf area meter and per cent antifeedant index was calculated based on the formula of Sadek [28]. Data was subjected to statistical analysis to determine differences in response between the treated bioassays and control, and the differences were considered as significant at P=0.05 level.

$$\text{Antifeedant index (\%)} = \frac{\text{Leaf disc area consumed in control} - \text{Leaf disc area consumed in treated}}{\text{Leaf disc area consumed in control} + \text{Leaf disc area consumed in treated}} \times 100$$

RESULTS AND DISCUSSION

Antifeedant bioassays

Antifeedant bioassays against insects have been used for decades as a means of elucidating the activity of many phytochemical components. The major goals achieved by employing bioassay techniques are to determine the roles of naturally occurring chemicals, identify the mechanism of resistance in crop plants and to find various insect control agents. The basic design to study antifeedants is to present to an insect a substrate with the candidate chemical and to measure the response of the insect. Therefore, substrate choice and presentation are important factors for a successful bioassay [29]. The same was followed in this study. Natural substrates

could be whole plant, leaves, leaf discs, or specialized substrates such as twigs, and for the present study it was leaf discs. Leaf discs are commonly used in preference or consumption bioassays with chewing insects. These assays are important in estimating the biological potential of the antifeedant effect of plant extracts in screening studies, and they correspond as much as possible to the conditions of the practical application. However, it must be emphasized that these assays are short term. For the purpose of practical use, further bioassays must be performed in phytochemical compounds which exhibits the best biological activity, and the same was investigated in this study. Koul [30] reported on the individual types of biological assays used for evaluating antifeedant efficiency. In general, bioassays can be divided into two groups according to the mode of the experiment: a choice or a no-choice bioassay [31]. The principle is that insects can choose either control or treated discs (choice) or insects may be exposed to the test substance only (no choice). The no-choice situation often is more representative but at the same time is very sensitive and this was used in the present study.

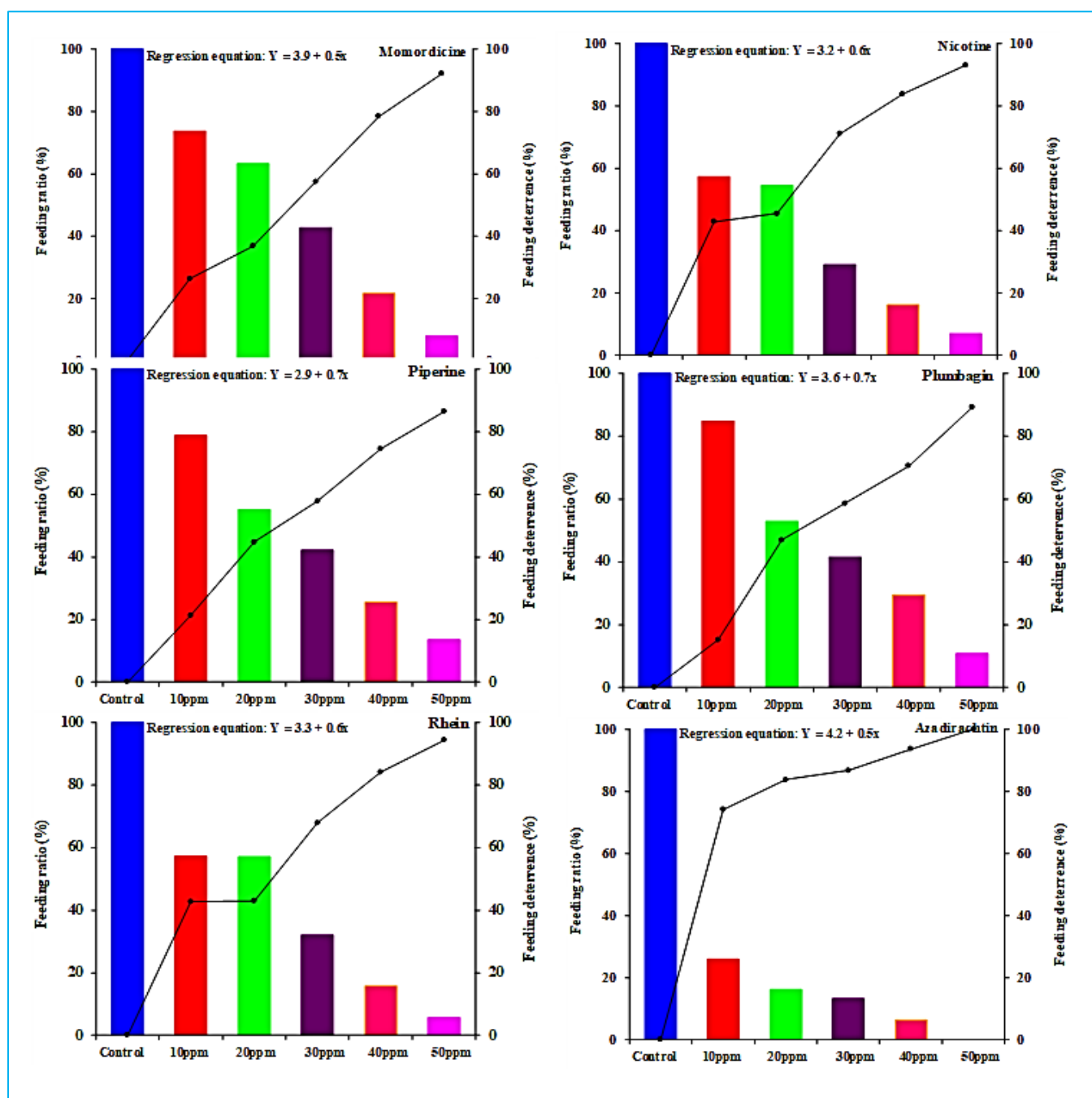


Fig 1 Antifeedant potential of phytocompounds on *Aulacophora foveicollis* adults

Antifeedant phytochemicals

In the present study, leaf discs treated with the phytochemicals showed high antifeedancy. Total feeding inhibition was recorded at the maximum concentration by azadirachtin, and a feeding deterrence of 74.1% to 93.6% was observed between 10 and 40ppm. Momordicine exhibited more than 50% feeding deterrence from 30ppm, and displayed a maximum of 92.1% feeding deterrence at 50ppm. The same trend was displayed by nicotine, piperine, plumbagin and rhein with a maximum feeding deterrence of 93%, 86.4%, 89.2% and 94.3%, respectively at the highest concentration (Figure 1). Feeding deterrence by botanicals have been reported earlier by the present authors against *Spodoptera litura* [32-34], and *Raphidopalpa foveicollis* [27]. In the present investigation, the rate of feeding by the adult beetles was highly reduced by the phytochemicals as they exhibited more than 85% feeding inhibition. Neem based insecticides for the management of this beetle as feeding inhibitor are reported [35]. Leaf extracts of *Momordica charantia* [36-37], *Ageratum conyzoides* [38], *Cassia fistula*, *Leucas aspera*, *Plumbago zeylanica* and *Streblus asper* [27], whole plant extracts of *Mentha longifolia*, *Mentha piperita*, *Mentha spicata*, *Ocimum basilicum*, *Ocimum canum*, *Pogostemon heyneanus* and *Salvia officinalis* [39], seed extracts of *Pachyrhizus erosus* and *Strychnos nuxvomica* [40], and seed kernel extract of *Azadirachta indica* [41] were considered effective feeding deterrents against the red pumpkin beetle. The standard antifeedant phytochemical azadirachtin, a complex tetranortriterpenoid limonoid has established itself as a pivotal antifeedant ingredient [42-43], as it inhibits the feeding behaviour making the food unpalatable resulting in feeding deterrence [44]. Lakshmi *et al.* [45] revealed nimbecidine from neem was effective in reducing the leaf damage caused by red pumpkin beetle. The present study results corroborated with the reports of these phytochemicals as antifeedants. Chandravadana and Pai [36], Chandravadana [46] and Abe and Matsuda [37] reported momordicin, a triterpenoid compound exhibited significant antifeedant property against *Aulacophora foveicollis*, and Ling *et al.* [47] reported it against *Plutella xylostella*. Nicotine, an alkaloid functions as an antifeedant against *Spodoptera littoralis* [48]. Several allelochemicals isolated from *Piper* species are reported as antifeedants [49]. Piperine, an alkaloid functions as an antifeedant against *Spodoptera exigua* [50]. Plumbagin, a naphthaquinone acts as an antifeedant against *Spodoptera litura* and *Achaea janata* [51], and against acridids, *Sphenarium purpurascens*, *Dactyloctenium aegyptium* and *Phoetaliotes nebrascensis* [52]. Duraipandiyan *et al.* [53] stated rhein, a quinone compound exhibited antifeedant property against *Helicoverpa armigera* and *Spodoptera litura*. Antifeedant property of phytochemicals against adult coleopteran pests have been reported. Frazier [54] signified antifeedants in alkaloids, chromenes, coumarins, cucurbitacins, flavonoids, phenolics, phenols, polyacetylenes, quassinoids, quinines, quinones (anthra and naphtho), saponins, tannins, terpenes (tri and sesqui) and terpenoids, and all these secondary metabolites are reported as potent insect antifeedants [29], [31], [55-63]. Amongst them, the potent antifeedants belong to the terpenoid group, which has the greatest number and diversity of known antifeedants compounds [62].

Antifeedancy mechanism

Antifeedants deter phytophagous insects against food consumption. Food selection among insect herbivores is a highly specialized phenomenon as the choice of food is based primarily upon contact chemoreception of various allelochemicals [54], [64]. The acceptance or rejection by a phytophagous insect of a plant species as a suitable host

depends on the capability of the insect's chemosensory system to detect plant tissues with levels of feeding stimulants and feeding deterrents [29], [65-67]. Mechanism of action through which feeding inhibition can be established in insects by antifeedants are divided into two categories: (i) inhibit feeding through sensory perception, wherein phytochemicals have unpalatable taste to insects [16], [68]. (ii) inhibit feeding by postingestive, toxic effects resulting in sick insects without appetite [69-72]. Antifeedants act through one, or both of these types of mechanisms of action, and the mechanism of action of antifeedants varies between insect species. Behavioral mechanisms provide a system of avoidance of non-host chemicals by which insects select their food, though the molecular basis for action of chemical deterrents on both gustatory and olfactory sensory systems in insects is poorly understood. Among plant antiherbivore chemistry, a strong link does not exist between feeding deterrence and internal toxicity in insects, suggesting that behavioral rejection is not an adaptation to ingested effects but more an outcome of deterrent receptors with wide chemical sensitivity [73].

Acceptance of host plants by herbivores requires chemoreception of favorable levels of phagostimulants relative to plant antifeedants [74]. Antifeedant effects are highly correlated with the sensory response of chemoreceptors on the insect mouthparts [75]. Antifeedants work by producing feeding inhibition stimulant such that it disturbs the stimulation perception for eating. Mouth parts such as maxillae and its receptor cells are highly sensitive to antifeedant phytochemicals, and ultimately lead to feeding inhibition [76]. Isman [19-20] stated that antifeedants possess a peripherally mediated behaviour modifying substance that act directly on the chemosensilla of the insect, through a direct action on peripheral sensilla (taste organs) in insects resulting in feeding deterrence. Antifeedants mode of action is directed at the taste cells. A typical gustatory sensillum in an insect contains receptors selective deterrents. Majority of antifeedants perform by stimulating a deterrent receptor that directs a signal 'do not feed' to the feeding center in the insect's central nervous system. At the same time some are thought to block or otherwise impede with the perception of feeding stimulants, whereas others may cause erratic bursts of electrical impulses in the nervous system, stopping the insect from acquiring appropriate taste information on which it may choose a proper feeding behavior [20]. Antifeedants also produce an anorexic effect, a consequence of action on either the feeding center in the suboesophageal ganglion of the central nervous system or on the alimentary canal [19]. Murdock *et al.* [77] suggested that plants can also psychomanipulate insects by interfering with information processing in the central nervous system. Yet, the mechanism of antifeedant action is still unfamiliar, and it can be only supposed that antifeedants depress or terminate insect feeding through mechanisms that involve chemosensory-based food rejection.

Antifeedancy behaviour

Insects are suppressed from biting once contact has been made with plant material leading to antifeedance (suppressants), which deter insects from feeding after they have bitten the plant material, i.e., inhibition by gustatory responses [78]. After having approached a potential food plant, herbivorous insects mostly start palpating the leaf surface, followed by taking some test bites and eventually feeding. In the case of a non-host plant, or when a plant is treated with antifeedants, initiation of feeding stops at some moment during this process because sensory information on the unpalatable food source is received by the brain, where a rejection response

is generated. Behavioural response of insects to antifeedants can change after the first moment of exposure. Instances of a declining sensitivity, and food aversion learning occurs in some insect species, wherein sensitivity to antifeedants increases over repeated exposures, separated by intervals of several hours to several days [79]. In insects, their sense of taste is located in hair like structures called taste hairs, or papilla like structures on the mouthparts. Axons from taste receptor cells directly project to the suboesophageal ganglion [80], that on its turn is connected to the brain. It is thought that major processing of taste information occurs in the suboesophageal ganglion [81]. The chemosensory taste hairs contain sensory taste receptor cells of which the dendrites, while feeding, come into contact with plant chemicals which enter the taste hairs through a small pore at the tip, leading to electrical signals (action potentials) produced by the sensory taste receptor cells. After action potentials are generated in the different sensory taste cells the sensory information is sent to the brain via a sensory nerve. In this way, the insects find the code for acceptable and unacceptable food [79]. Schoonhoven [29] neural coding of antifeedancy have shown to affect sensory responses in different ways: (i) stimulation of deterrent cells tuned to diverse plant compounds that deter feeding; (ii) stimulation of receptor cells with a broad sensitivity spectrum that includes secondary plant compounds; and (iii) inhibition of the response of receptor cells that are sensitive to feeding stimulants. Hence, feeding behaviour is directed by the central nervous system, wherein information from not only the chemical taste organs, but also from other body parts and from environmental factors is processed. Nevertheless, many factors play a role in the direction of insect feeding behaviour, such as developmental state, degree of satiety, host plant of insect, temperature and

light. This means that the behavioural response on antifeedants depends not only on its taste or post-ingestional effects, but also on additional factors, that should be standardized when comparing the response to an array of antifeedants. Further, dietary experience influences the ability of insects to taste plant chemicals that serve as signals of suitability or unsuitability, because certain dietary constituents suppress the development of taste sensitivity to deterrents in an insect [82]. Avoidance of allelochemicals, when looked at from a behavioural point of view, is the outcome of interactions with chemoreceptors characterized by broad sensitivity to a spectrum of deterrents [73]. Although research of chemoreceptors is important for a general understanding of efficiency of individual antifeedant substances, from the practical point of view, experiments based on simple biological tests are those mostly used in antifeedancy studies. Nevertheless, it should be emphasized that most of the antifeedant research is in the preliminary trial stage, although the activity of many phytochemicals and plant extracts are known [30]. Yet, it is expected that in the near future efficacious phytochemicals will be formulated as antifeedants, from the huge floral biodiversity provided by nature.

CONCLUSION

A possible way of using antifeedants in crop protection could be a combination of several antifeedants with different mechanisms of action, one that acts through sensory effects and one that has toxic properties, or one that stimulates a deterrent cell and another that inhibits cells sensitive to feeding stimulants. Such combinations may prevent the occurrence of habituation, or may synergistically increase the feeding inhibiting effect.

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