Plant metabolites: an alternative and sustainable approach towards post harvest pest management in pulses

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ABSTRACT

Grain legumes are an important source of proteins in vegetarian diet besides their role in biological nitrogen fixation. They are prone to heavy pest infestation both on and off the field. Pest associated losses are an important contributing factor towards declining per capita availability of grain legumes. Synthetic chemical pesticides have played an important role in crop preservation, however their incessant use has posed several environmental and human health concerns. Methyl bromide and phosphine are commonly used for the post harvest preservation of grain legumes. However, the former has to be phased out by 2015 as per the Montreal protocol whereas the latter is showing development of resistance to it by the insects. In the light of this, alternative, safer and sustainable strategies are needed for crop protection. Plants can serve as a rich source of bioactive chemicals for this purpose. Both primary as well as secondary metabolites can be evaluated against the target pests. The paper reviews the status of research in the area of use of plant metabolites in post harvest pest management of grain legumes. [Physiol. Mol. Biol. Plants 2009; 15(3) : 185-197] E-mail : vlmaheshwari@rediffmail.com

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INTRODUCTION

Nutrients are indispensable sources for survival of life forms and maintenance of ecosystem on earth. Agriculture has to play the most dominating role to fulfill the nutritional demands of an ever increasing global human population which is now about 6 billion and is likely to reach 8 billion by 2025 (Khush, 1999). Agricultural sector has been influenced by many factors, such as weather, manner of application of various inputs like seeds and fertilizers, precipitation/irrigation, climatic changes, disease/pest control in crops produced during pre- and post-harvest periods, government's pricing policies, marketing, credit supply etc. (Anonymous, 1996). In the last 50 years, many countries, including India, have enjoyed the fruits of modern agricultural technology for increasing the productivity of vegetables, crops, fruits, fodder and forest produce. Their remunerative cultivation necessitated the use of high crop yielding short duration varieties of seeds, fertilizers, improved irrigation system, plant

growth regulators (PGRs) and effective pest management. While all the above inputs increased plant growth rate and vigor, pesticides helped in preserving the vigor by controlling the pest(s) (Ramamurthy *et al.*, 1998). No doubt, these inputs have restored self reliance in food, but at the same time, their application introduced an emergence of certain pests, diseases and weeds, posing a greater threat to sustainability in crop production.

The pulses scenario

South Asian region, with about 1.25 billion people, is the largest pulse consuming region in the world. Pulse crops are important sources of dietary protein and calories in the food and feed products across the world, particularly South Asia, the Middle East and North Africa (Table 1). In some of these regions, animal protein is in short supply whereas in others, like India, religious preferences discourage meat production and consumption and therefore pulse crops have greater importance. Pulses contain almost 20-25% of the proteins and all the essential amino acids, except cysteine and methionine, which are available in smaller amount (Gopalan *et al.*, 1999). Pulses contribute close to 60 MMT in the global food grainary. Of this, India's contribution is

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approximately 12 to 14 MMT (Chaturvedi and Ali, 2002) making it the largest producer accounting for 27-28% of global production. The growth in production of pulses does not meet the demand of the growing population, resulting in an overall decline in per capita availability and higher prices for pulses (Mendki, 2002). India's per capita net availability of pulses has come down to 36 g/day as compared to 70 g/day in 1960 (Chaturvedi and Ali, 2002). Similarly, percent contribution of pulses *vis-a-vis* other food grains in total food grain production has also decreased (Chandra and Pental, 2003).

In India, pulses are cultivated on about 22 million hectares of land amounting to approximately 32 per cent of total area under their cultivation globally. Indian yield of pulses has been pretty much static for the last 40 years averaging between 561-672 kg/hectare. While the green revolution produced a three fold increase in wheat yields in India, pulses have not received the same level of attention in research. Pulses are grown on dry land during the winter season under less than ideal conditions. In India, pulses are cultivated in two seasons *viz.* *kharif* (summer crop; harvested in September) and *rabi* (winter crop; harvested in March/April). The major *kharif* pulses are green gram (moong bean), black gram (urad), pigeon pea (arhar) and cowpea while *rabi* pulses are chickpea, green pea and lentil. Average production of pulses in *kharif* was found to be less (450-500 kg/ha) than *rabi* (625-750 kg/ha).

Reasons for declining pulses production

Analysis and retrospect suggest the following for the static or declining production of pulses (i) lack of modern agricultural practices, (ii) slow seed replacement (SSR), (iii) dependence on monsoon, (iv) lack of research inputs and (v) heavy damages due to both pre as well as post harvest infestation by microbes and insect pests (Chaturvedi and Ali, 2002). Insects, moulds and mites cause most proportion of damage and deteriorate grains by producing entomotoxins and mycotoxins (Morgan and Aldred, 2007). If the infestation level is high at the pre-harvest stage, post-harvest quality is also proportionately reduced and shelf-life is also lowered. Thus, on the one hand, a direct reduction in yield occurs, and, on the other hand, the harvested quality suffers (Wagacha and Muthomi, 2008).

Insects belonging to 150 different species are pests of 10 different crops of pulses (Pradhan, 1992). Among these, cutworms, aphids, stem borers and pod borers are responsible for damage at different pre-harvest stages of crops (Atwal and Dhaliwal, 2008). Microbes

and insect pests, in general, infest food grains during storage to what is termed as post-harvest infestation. According to one estimate, post harvest losses during storage may be as high as 25-30 per cent (Champ, 1985). This means that one-quarter of what is produced never reaches the consumer for whom it was grown and the efforts and money required to produce it are lost for ever. The problem of post harvest infestation is more severe in developing countries due to (i) lack of infrastructural knowledge and (ii) no or less use of grain protecting chemicals. Two major physical parameters influence grains during storage. They are temperature and humidity. The temperature remains in the zone of 30 ± 5 °C for most part of the year and is favorable for the infestation and propagation of majority of storage pests. Humidity, on the other hand, varies with season, being maximum during rainy and minimum in winter season. In a tropical country like India, duration of dry period is an extremely important factor for the long term storage of grains. Like temperature, higher humidity is also conducive for infestation and spoilage of stored grains (Mendki *et al.*, 2004).

Post harvest losses: The causative agents

(i) Microorganisms

The classes of fungi, most important in crop storage, are the moulds or microscopic fungi with optimum temperature for development above 20 °C. Unless precautions are taken, stored crop products form an ideal substrate for fungal growth and results in the production of mycotoxins (Bryden, 2007). Consumption of such commodities has resulted in human disease outbreaks. Naturally occurring toxins, inherently present in foods and either consumed as such or mixed up with grains, had been responsible for disease outbreaks (Bhat, 2008). From an ecological point of view, fungi may be divided into field and storage fungi. *Alternaria*, *Fusarium*, *Cladosporium* and *Helminthosporium* sp., invade the seeds before harvest (Multon, 1989; Kosiak *et al.*, 2004). These fungi develop only on seeds with high moisture content (22-25 %) and would perish under correct storage conditions. Storage fungi, mainly *Aspergillus* and *Penicillium* sp., develop on seeds with 12 to 18 % moisture content. One of the most important storage fungal species is *A. flavus*, which grows on, and causes deterioration of proteins, starch and oils; in particular, the quality of oil. Some strains produce the poisonous toxin-aflatoxin particularly in inadequately dried oil-seeds and cereals. *A. niger* is similar to *A. flavus* but the toxin produced is not so dangerous. *A. glaucus* is a very common group of molds able to grow on substrates

Photoplate 1. Representative post harvest pests (A) primary and (B) secondary insect pests

with very low moisture and high sugar content and are usually the primary invaders of stored crop products. *Penicillium* sp. is commonly associated with rots. The mycelium is bluish green and may be aerial or embedded in the substrate and widely dispersed. *Fusarium* sp. is a widely occurring species, as a fungus associated with storage rots and as a pathogen causing blights and blasts of cereals. It may survive in the seed and may continue growing during storage. Some species produce toxins on stored maize that has not been dried to safe moisture content. Two species belonging to the *Phycomycetes*, *Rhizopus otrhijus* and *Mucor pusillus* are also common on stored products (Multon, 1989).

Various bacteria which contaminate the grains during storage are *Pseudomonas maidis*, *P. fluorescens*, *Acetobacter oxydans*, *Escherichia coli*, *Enterobacter aerogenes*, *E. cloacae*, *Proteus vulgaris*, *Serratia marcescens*, *Streptococcus lactis*, *Bacillus cereus*, *Clostridium perfringens*, *Streptomyces albus* etc. (Multon, 1989).

(ii) Insect pests

Nearly one thousand species of insects have been associated with stored products throughout the world, majority of which belong to the Coleoptera (60 %) and Lepidoptera (8-9 %) (Atwal and Dhaliwal, 2008; FAO, 2009). Stored grain insects can be grouped as primary pests which are: (i) capable of successfully attacking, feeding and multiplying on previously undamaged grains, (ii) adapted to feed on a narrow range of commodities, (iii) able to cause distinctive damage, (iv) able to complete their development within a single grain, (v) selective in their egg laying behavior and (vi) unable to develop on the same food if the grains are ground/milled. Secondary pests, on the other hand, (i) can attack and breed in previously damaged grains on wide range of commodities, (ii) may cause non distinctive damage, (iii) may complete their life cycle within grains but never complete their development within a single grain, (iv) do not have a selective egg laying behavior and (v) can develop on the same food after it is ground.

Grains once infested get contaminated with insect excreta, secretions, cuticle casts, dead insects and frass generated by boring of the grains. During storage, the quality and quantity of the produce is also affected by birds, rodents and microorganisms (Rajendran, 1999). Insect and microbial attack proceeds increase in moisture content (Multon, 1989) followed by some biochemical changes in the grain legumes (Mendki *et al.*, 2001). In case of severe infestation, the flour turns grayish,

Table 1. Trends in share of pulses in nutrition

Per capita/day	World	Asia	India
Energy (Kcal)	56.00	51.90	117.4
Protein (g)	3.50	3.10	6.90
Fat (g)	0.40	0.40	1.00

(Source: Survey of Indian Agriculture, The Hindu, 2002)

mouldy, emits disagreeable pungent odour and becomes unfit for human consumption. Some of the important primary and secondary stored grain insect pests are listed in Table 2 and shown in Photo plate 1.

Pulse beetle, *Callosobruchus chinensis* (CC), (Coleoptera: Bruchidae)

The order Coleoptera is the largest order of insects and contains the most common and important stored product pests. Family Bruchidae contains several important field and stored crop pests (FAO, 2009). The seeds of nearly all legume species are vulnerable to attack by seed beetles, both in the field and in storage. Common insect pests of stored pulses are *Acanthoscelids obtectus* (Say), *Callosobruchus analis* (CA) (Fabricius), *C. chinensis* (Linnaeus), *C. maculatus* (Fabricius), *Zabrotes subfasciatus* (Boh) and *Sitophilus oryzae* (Atwal and Dhaliwal, 2008). Pulse beetle, *C. chinensis* causes substantial economic loss during the storage/preservation of pulses (Shu *et al.*, 1996). It is a notorious pest of green gram (*V. radiata*), Bengal gram (*C. arietinum*), red gram (*V. unguiculata*) and math (*Phaseolus aconitifolius*). Severe infestation reduces quality and germinability to under 20 % after 4 months only (Seck *et al.*, 1996) and as much as 90 % loss within 6 months of storage (Mendki *et al.*, 2001). In early stages, beetle larvae are whitish, with a light brown tinge on head, to become creamy white later. The mature larva is 6-7 mm long and causes damage to pulses by feeding on their endosperm (Mendki *et al.*, 2001). However, the adult beetle is 3-4 mm in length, oval in shape, chocolate/reddish brown in colour and has long erected antennae.

(iii) Life cycle

The pulse beetles breed freely from March to November and hibernate in the larval stage in winter. During January to April, adults appear and copulate immediately after emergence. The female lays small, oval shaped and scale like eggs just after a day. These are glued on the surface of the grains. While CC can lay 1-8 eggs per grain and metamorphose into adults in separate chambers, CA prefers to lay only one egg per grain.

Table 2. A representative list of important stored grain pests

Scientific Name	Common Name	Host	Order	Family
A. Primary Pests				
<i>Cadra cautella</i> Walker.	Almond or Fig moth	Grains	Lepidoptera	Phycitidae
<i>Callosobruchus chinensis</i> L.	Pulse beetle	Pulses	Coleoptera	Bruchidae
<i>C. maculatus</i> L.	Pulse beetle	Pulses	Coleoptera	Bruchidae
<i>Corcyra cephalonica</i> Stainton	Rice moth	Rice	Lepidoptera	Pyalidae
<i>Plodia interpunctella</i> H.	Indian meal moth	Maize	Lepidoptera	Pyalidae
<i>Rhizopertha dominica</i> F.	Lesser grain borer	Rice, wheat	Coleoptera	Bostrychidae
<i>Sitophilus granarius</i> L.	Granary weevil	Wheat	Coleoptera	Curculionidae
<i>S. zeamais</i> Mots	Maize weevil	Maize	Coleoptera	Curculionidae
<i>S. oryzae</i> L.	Rice weevil	Rice	Coleoptera	Curculionidae
<i>Sitotroga cerealella</i> Olivier	Grain moth	Grains	Lepidoptera	Gelichidae
<i>Trogoderma granarium</i> E.	Khapra	Wheat	Coleoptera	Dermestidae
B. Secondary Pests				
<i>Cryptolestes ferrugineus</i> O.	Red rust grain beetle	Grains	Coleoptera	Cucujidae
<i>Cryptolestes pusillus</i> S.	Flat grain beetle	Grains	Coleoptera	Cucujidae
<i>Latheticus oryzae</i> W.	Long headed flour beetle	Flour	Coleoptera	Tenebrionidae
<i>Oryzaephilus surinamensis</i> L.	Saw toothed grain beetle	Rice	Coleoptera	Silvanidae
<i>Tribolium castaneum</i> H.	Red rust flour beetle	Wheat flour	Coleoptera	Tenebrionidae
<i>T. confusum</i> J.	Confused flour beetle	Wheat flour	Coleoptera	Tenebrionidae

(Source: Anonymous. 1990. Storage and Research Division, Indian Grain Storage Institute, Ministry of Food, Government of India)

Atwal and Dhaliwal (2008) reported that CC may lay 34-113 eggs @ 1-37 per day and CA lays 11-150 eggs in 2-22 days. The pest causes maximum damage during February-August when all its developmental stages simultaneously exist. The larvae feed and breed inside the grain, consuming its entire endosperm; partially infested grains develop fungus infestation and as a result emit foul odour. When the infestation is severe, the devastation is complete (Mendki *et al.*, 2001).

The Approach

Various strategies have been used to control pre- and post-harvest insect pests since times immemorial. Therefore, at this juncture, a brief review of (i) existing

insect pest management strategies, (ii) their merits and demerits and (iii) alternative strategies is presented in ensuing pages.

As per the latest reports, chemicals worth US \$ 26 billion are applied to crops all over the world, both on and off the field (Thakore, 2006). France, USA, Germany, Great Britain and Switzerland together produce almost 3/4th of this amount. Though, India is one of the largest producer of synthetic pesticides, usage of them is approximately 0.5 Kg/ha compared to about 3 Kg/ha in USA (Wahab, 2009). Pesticides worth Rs. 27 billion approx. were consumed in India in the year 2005 with cotton alone accounting for the major share (Wahab, 2009).

For post-harvest preservation, sun drying at the farmers' field is an important step. Sieving removes foreign materials as well as insect larvae, eggs and adults. The traditional storage bins can be replaced by air tight barns/silos so that nothing could enter and none mistakenly entered can survive in the absence of air. Nitrogen flushing and UV exposure can also be done to protect the barns. Contact pesticides break down quickly in tropical than temperate climate particularly when the humidity is high. Under these circumstances, insects are less susceptible to death. On the contrary, increased humidity promotes multiplication (Ducom, 1990). This necessitates use of pesticides and fumigants are higher than their recommended dose for controlling the pests (Mendki *et al.*, 2004). Aluminum phosphide and methyl bromide are the two most commonly used chemicals for fumigation of stored grains.

Insects were found to be more susceptible to death at higher CO₂ and low O₂ level during storage. Similarly, exposure to temperature 70–80 °C for a brief period is also found to be useful in controlling pest infestation during storage. Forced ventilation in commercial storage bins prevents moisture accumulation and development of heat spots which also helps in arresting infestation (Singh, 2003). Traditionally, a number of folk remedies have been tried for the preservation of grains during storage. The mode and extent of application of these remedies differed from state to state and offered varying degree of preservation (Mendki *et al.*, 2004).

Merits and demerits of synthetic/chemical pesticides

The synthetic/chemical pesticides are characterized by (i) rapid knock down effect, (ii) target specificity and (iii) relatively high residual toxicity. On the other hand, their repeated use has resulted in (i) disruption of biological control by natural enemies, (ii) caused development of resistance in pests (Verma and Dubey, 1999; Ramoutar *et al.*, 2009), (iii) led to resurgence of stored product insect pests, (iv) left undesirable effects on non-target organisms and (v) fostered environmental and human health concerns. According to the April 2000 decision of the Montreal protocol, however, methyl bromide, a proven ozone depleter in the atmosphere, is to be phased out by 2005 in advanced countries and by 2015 in developing countries (TEAP, 2000). Similarly, phosphine resistance is becoming more common and is a matter of considerable concern (Chaudhari, 1995).

Need of alternative strategies

The increased concern of public about resistant strains/insects, toxic residues on food and feed, human safety, erratic supply and prohibitive costs associated with the synthetic/chemical insecticides has given impetus to search for alternative methods to combat the attack of various insect pests at different stages of crop production and storage. Moreover, stringent Integrated Pest Management (IPM) strategies and high cost involved in research and development has also hindered the process of technology development in the field

Table 3. A profile of alternative strategies for post-harvest grain preservation

Strategy	Target pests	References
CO ₂ as fumigant	<i>L. sericorne</i>	Newton <i>et al.</i> , 1993
	<i>O. surinamensis</i>	Krishnamurthy <i>et al.</i> , 1993
	<i>T. castaneum</i>	Hodges and Surendro, 1996
	<i>S. granarius</i>	ICIPE, 2006
Biogas as fumigant	<i>C. chinensis</i>	Subramanya <i>et al.</i> , 1994
	<i>S. oryzae</i>	ICIPE, 2006
	<i>T. granarium</i>	
	<i>T. castaneum</i>	
	<i>L. sericorne</i>	
High temperature treatment	<i>S. cerealella</i>	Sharma <i>et al.</i> , 1997
	<i>S. oryzae</i>	ICIPE, 2006
	<i>R. dominica</i>	
Low temperature treatment	<i>P. interpunctella</i>	Mason <i>et al.</i> , 1997
Ultrasonic waves	<i>T. confusum</i>	Pradzynska, 1998
	<i>T. castaneum</i>	Shayesteh and Barthakur, 1997
	<i>P. traneatus</i>	
	<i>O. surinamensis</i>	
Use of natural enemies	<i>P. interpunctella</i>	ICIPE, 2006
Irradiation/Micro-wave treatment	<i>T. confusum</i>	Langlinais, 1998
	<i>C. pusillus</i>	ICIPE, 2006
Pheromones	<i>P. operalella</i>	Trematerra, 1997
	<i>L. sericorne</i>	Howlader and Ambadkar, 1995
	<i>C. chinensis</i>	Ismam, 1994

of synthetic pesticides (Arthur, 1996). Some of the alternative strategies for preservation being tried by different workers on various post harvest pests are summarized in Table 3.

Integrated Pest Management (IPM)

Of the different alternative methods for controlling various pests, Integrated Pest Management (IPM) has so far received most attention because of its approach and inbuilt advantages. IPM has been defined in many ways and implemented under an array of connotations. Previously, the term 'integrated control' was used which is now broadened to explore use of all biological, cultural and artificial practices under the term IPM. The most common hazardous effect of chemical/synthetic pesticides is on natural enemies. In the light of IPM strategies, modification of pesticide use practices is the most suitable way of diminishing the negative impact on natural enemies. In other words, IPM programmes have been operated with 'pesticide management' rather than 'pest management' objectives (Lewis *et al.*, 1997). Another important aspect in selection of pesticide use under IPM is the selectivity of the pesticide. Similarly, it has been recognized that plant derived secondary metabolites can be developed into products suitable for IPM based on their (i) selectivity and (ii) no or little side effect on non-target organisms or environment (Park *et al.*, 2003; Lehman *et al.*, 2007). Various aspects of IPM and biological control have been reviewed for storage systems in the tropics previously (Haines, 1984). Pheromones are exocrine secretions used by insects for finding mates, aggregation, alarming, tracking or trailing (Varma and Dubey, 1999). Pheromones, being behavior controlling chemicals or semio-chemicals, represent another promising option to chemical/synthetic pesticides and find important role in insect control within IPM. Use of insect growth regulators which can act as mimics of juvenile hormone, inhibitors of chitin synthesis or agonists of ecdysone thereby disrupting the normal development of insects is also proved to be promising insect control strategy. Similarly, some entamo-pathogenic nematodes have been found to be useful in pest management in storage grains (Wahab, 2009). In the scenario of development of pesticide resistance by key pests, legal restrictions on essential pesticides, consumer attributes and the development of biological control methods other than chemical pesticides, IPM has gained importance.

Botanical pesticides

Plants may provide potential alternatives to currently used insect-control agents because they constitute a rich source of bioactive chemicals. Since these are often (i) active against a limited number of species including target insects, (ii) biodegradable to non-toxic products, and (iii) potentially suitable for use in integrated pest management, they could lead to the development of new classes of safer insect-control agents. Much effort has, therefore, been focused on plant-derived materials for potentially useful products as commercial insect-control agents. Over 2000 plants belonging to some 60 plant families are known to exhibit insecticidal activities (Dev and Koul, 1997). Naturally occurring plant materials or products derived from such plant materials are termed as botanical insecticides. Botanical insecticides were extensively used (1800 to 1940 A.D.) until the discovery of synthetic insecticide, DDT. They may be crude preparations of plant parts such as powders or dusts (neem leaf dust, pyrethrum flower dusts etc.), aqueous or organic solvent extracts, used as liquid concentrates/dusts with carriers (Weinzierl, 1998; George *et al.*, 2008). A list of major plant derived pesticides is provided in Table 4. Traditionally used botanical pesticides and recent development in them have been reviewed by Walia and Koul (2008).

Secondary metabolites in plant defense

Plants produce an amazing variety of organic components and metabolites, which are called as secondary metabolites. The primary metabolites, carbohydrates, lipids, nucleotides and peptides are shared by all living organisms and are central to life processes. The secondary metabolites on the other hand are derived from primary metabolites and their functions include protecting the plant against diseases, pests, herbivores, environmental stress etc. Various plant metabolites have been found to be useful in controlling pests by acting as anti-feedants, repellents or behavior modifiers (Isman, 2008; George *et al.*, 2008). A number of plant families are known to produce alkaloids, phenolics and oils, which have been used for insect control since a long time. These secondary plant metabolites having insecticidal/pesticidal properties are of several types, *viz.*, repellents, anti-feedants, phagostimulants and toxins (Krishnayya and Rao, 1996; Nivsarkar *et al.*, 2001; Isman, 2006). The important groups of secondary metabolites are (i) alkaloids, (ii) terpenoids and (iii) flavonoids. Since last 8-9 years, we have been working actively working on devising strategies for minimizing losses in pulses during storage primarily using plant derived secondary metabolites. We have successfully demonstrated the use of papaya leaf dust (Mendki *et*

Table 4. A profile of major botanical insecticides

Botanical insecticides	Sources	Mode of action	Mammalian LD ₅₀	Target insect (s)
Pyrethrum	Flowers of <i>Chrysanthemum cinerariaefolium</i>	Interferes with nerve transmission	> 1000	On fleas, ticks and lice
Rotenone	Roots of <i>Derris</i> sp., <i>Lonchocarpus</i> and tropical legumes	Disrupts energy metabolism in mitochondria	Dermal >1000 Oral 25 to 3000	Beetles
Sabadilla	Seeds of <i>Schoenocaulon officinale</i> and <i>Veratrum album</i>	Interferes with Na and K ion movement in nerve axons	Oral near 4000	Bugs and thrips
Ryania	Stems of <i>Ryania speciosa</i>	Activates Ca ion release channels	Oral near 1000 Dermal near 4000	Caterpillars and thrips
Nicotine	Leaves of <i>Nicotiana</i> sp.	Mimics acetylcholine to overstimulate receptor cells	Oral near 3 to 188	Aphids, thrips and mites
Azadirachtin	Leaves, bark and seeds of <i>Azadirachta indica</i> and <i>Melia azedarach</i>	Growth hormone analogue	Oral > 5000	Soft-bodied insect pests
Limonene/linalool	Citrus oils	Stimulation of sensory nerves	Oral > 5000 Dermal > 3500	Fleas and ticks
Acetogenins	<i>Annona</i> and <i>Asimina</i> sp.	Inhibition of mitochondrial respiration	—	Soft-bodied insect pests

LD₅₀ estimates: mg of toxin/kg of body weight for test animals

LD₅₀ = dose required to kill 50% of test animal population; higher numbers indicate lower toxicity
(Sources: Weinzierl, 1998; Gopinathan and Sudhakaran, 2008)

al., 2000), fly ash, a byproduct and waste of thermal power stations (Mendki *et al.*, 2001) *Calotropis procera*, (Mendki *et al.*, 2005 ; Salunke *et al.*, 2005), *Annona squamosa* (Kotkar *et al.*, 2002), *Ricinus communis* (Upasani *et al.*, 2003) in the preservation of pulses from pulse beetle, *C. chinensis* during storage. The bioactive secondary metabolites isolated and identified by various chromatographic and spectroscopic techniques were found to be flavonoides in the leaves of *Calotropis procera* and *Annona squamosa* (Kotkar *et al.*, 2002) and triterpenoides (long chain fatty alcohol) of *Annona squamosa* in seeds (Kotkar, 2004). These metabolites have shown promising activities against the pulse beetle, *C. chinensis* affecting its survival by interfering with its various biochemical systems (Salunke, 2006). Similarly, the essential oil of *Cymbopogon martinii* was effective in protecting stored wheat (*Triticum aestivum*) and gram (garbanzo bean, *Cicer arietinum*) from infestation of the beetles *Callosobruchus chinensis* and *Tribolium castaneum* (Kumar *et al.*, 2007). Though, essential oils are found to be effective in post harvest pest and diseases management, a comprehensive phytochemical and toxicity profile will have to be done before their large scale applications (Dubey *et al.*, 2008).

What are cyclotides?

Of late, several macro-cyclic polypeptides-considered

members of cyclotide family have been isolated particularly from members of Family Rubiaceae and Violaceae (Brussalles *et al.*, 2001 ; Craik *et al.*, 2002). These peptides show a range of biological activities like uterotonic (Gran, 1973), hemolytic (Schopke *et al.*, 1993), anti-microbial (Tam *et al.*, 1999) anti-HIV (Bokesch *et al.*, 2001), insecticidal (Jennings *et al.*, 2001) and cytotoxic (Lindholm *et al.*, 2002) activities (Table 5). It has been suggested that they may be involved in plant defense (Craik, 2001) and in fact, perhaps represent the perfect host defense proteins because of their peculiar structural characteristics (Svangard *et al.*, 2003). Presence of several isoforms within a single species further supports this assumption (Jennings *et al.*, 2001 ; Svangard *et al.*, 2003). These cyclic peptides are made of 29-31 amino acids with six conserved cysteine residues forming the so called 'cyclic cystine knot' (CCK motif) making them extremely stable and rigid so that they can co-exist with other proteases (Trabi and Craik, 2002). *H. armigera* larvae fed on diet containing Kalata B1- a cyclotide obtained from an African plant, *Oldenlandia affinis* showed remarkable effect on growth and development (Craik, 2001). However, the mechanism of action and structure-activity relationship (SAR) associated with its various activities have not been elucidated so far (Daly *et al.*, 2004).

Table 5. A brief profile of cyclotides with major activity obtained from different plants

Peptide	Source	Function	Reference
MCOTI	<i>M. cochinchinensis</i>	Trypsin inhibitor	Gustafson <i>et al.</i> , 2000
MCOTII	<i>M. cochinchinensis</i>	Trypsin inhibitor	Gustafson <i>et al.</i> , 2000
Varv peptide (A,E)	<i>V. tricolor</i>	Cytotoxic	Goransson <i>et al.</i> , 1999
Varv peptide (B-H)	<i>V. arvensis</i>	Cytotoxic	Hernandez <i>et al.</i> , 2000
Cycloviolasin O ₂	<i>V. odorata</i>	Cytotoxic	Svangard <i>et al.</i> , 2003
Vitri A	<i>V. tricolor</i>	Cytotoxic	Goransson <i>et al.</i> , 1999
Kalata B1 2006	<i>O. affinis</i>	Oxytixic agent, antimicrobial property	Cleason <i>et al.</i> , 1998, Craik,
Kalata B2	<i>O. affinis</i>	Insecticidal property	Craik, 2006
Circulin A	<i>C. parviflora</i>	Anti HIV and Antimicrobial property	Shankerav <i>et al.</i> , 2006
Circulin B	<i>C. parviflora</i>	Anti HIV and Antimicrobial property	Seample <i>et al.</i> , 2006
Circulin C, D, E, F	<i>C. parviflora</i>	Anti HIV activity	Shankerav <i>et al.</i> , 2006

In our laboratory, we have screened few members of Family *Rubiaceae* and *Violaceae* for the presence of bioactive protein(s) with pesticidal activity. Since we have been working on storage pest management in grain legumes by alternative and sustainable methods, bioefficacy against pulse beetle, *C. chinensis* was taken as a marker tool. Of the many plants screened, three namely; *V. odorata* (*Violaceae*), *G. gummifera* and *A. cadamba* (*Rubiaceae*) have shown good adulticidal, ovicidal and ovipositional deterrent activities in our studies. The basis of range of bioactivities of circular protein is thought to be their potential harmful interactions with membranes (Craik *et al.*, 2003; Craik *et al.*, 2006). Hemolytic action is the most convenient measurable marker for the presence of circular proteins (Craik *et al.*, 2003; Barry *et al.*, 2003). Indeed, the cyclotides isolated earlier have been reported to be mildly hemolytic (Daly *et al.*, 1999; Daly and Fraik, 2000; Barry *et al.*, 2003). In our studies also, the partially purified proteins have shown mild hemolytic action consistent with earlier reports. A linear protein like BSA doesn't cause RBC hemolysis (Prakash *et al.*, 2008). The hemolytic action of our extracts could not be abolished even after keeping the proteins in boiling water bath for 15 minutes and treating them with 2 different exopeptidases (Collagenase and Trypsin) indicating the stability shown by circular proteins only (Prakash *et al.*, 2008). Our results apparently provide the first ever clue towards presence of circular proteins in indigenous members of Family *Rubiaceae* and *Violaceae* (Prakash *et al.*, 2008).

Plant proteins have been exploited commercially, e. g. enzymes papain and chymopapain from papaya, bromelain from pine apple juice, malt extract from barley etc. (Balandrin *et al.*, 1985). However, their use as biologically active compound has been rather limited, primarily because of the limitations imposed by their chemical structures. Cyclotides with their small and rigid structure have the potential to overcome these bottlenecks to be used as effective, economical and eco-friendly insecticide in future. The approach has inherent advantage of vertical integration and value addition as the gene(s) can be isolated and cloned in microbes for their production on commercial scale without sacrificing the environment.

After highlighting the use of various plants and their major secondary metabolites in insect pest management, it is necessary to throw some light on their advantages and disadvantages.

Merits and Demerits of Botanical pesticides

Generally, botanical pesticides are renewable, biodegradable and least persistent in the environment. They exhibit low mammalian cytotoxicity and as a result are safer to humans, animals, fish and other non target organisms (Dhaliwal and Koul, 2007; Isman, 2008). Similarly, chances of accumulation of residues in food and fodder and disturbance of soil micro flora are less as compared to synthetic pesticides. Biopesticides offer more targeted activity against a desired pest as opposed to conventional pesticides. Critical analysis suggests several bottlenecks in the success of biopesticides. Standardization has been the biggest constrain in their potential and marketability compared to synthetic pesticides. Economical production of the active agent remains another major limitation, Fundamental difference in the mode of action, bioefficacy, availability of raw materials, environmental fate, human toxicity etc. are some of the other potential grey areas. Identification of effective and stable chemical entity, development of simple and reliable bioassays, cost effective extraction methods and shelf stable formulations of plant derived products are need of the hour. Also, scale up and automation, quality assurance, consumer perception and economic viability of biopesticides will have to be improved (Gopinathan and Sudhakaran, 2008). Botanicals, with a few exemptions, tend to be rather slow acting making them less popular among the end users. Regulatory barriers and availability of cost effective competing products limit the use of biopesticides (Dubey *et al.*, 2008).

Market potential of biopesticides

Though, current share of biopesticides in global scene is small, it is growing at an average annual rate of over 10 percent. At this rate of growth, it will have a market share of close to 1 billion US \$ in the next 4-5 years which will be approximately 4 % of the total pesticide market (Thakore, 2006). Presently, more than 1,000 products formulated using over 200 active ingredients are registered for use as biopesticides globally (Gopinathan and Sudhakaran, 2008). The domestic market of biopesticide is still in its infancy, in spite of existence for last couple of decades, they are still considered as marginal products primarily due to lack of awareness of their advantages (Sarla, 2008). Estimates by experts indicate that biopesticides occupy a less than 3 % market share in India (Wahab, 2009). The regions that likely to see the substantial growth in biopesticide consumption are Europe followed by Asia and Latin America (Thakore, 2006).

CONCLUSION

In the light of differences in geo-climatic zones and biodiversity, the plant kingdom still remains an untapped vast reservoir of new molecules endowed with massive biopesticidal potential. However, deriving new biopesticidal principle(s) from plants remains a complex and time consuming task, because it needs interdisciplinary skills of isolation, purification, characterization, synthesis of standards (new/standard chemicals) and their screening for biological effect(s). While plant extracts may afford additive/synergistic action of several weak and strong biopesticidal activities, their purification and structure determination is essential for standardization, as also for bioefficacy improvement. Agriculture must become economically viable and ecologically sustainable without degrading the resource base so that future generations would get proper and sufficient food. In the grim scenario of mounting hazards and cost of synthetic chemical pesticides, natural chemistry of plants shows a ray of hope for environment and human friendly and sustainable pest management in future.

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