Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 5, No. 5, p. 128-135, 2014
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Botanical pesticide and insects repellent prepared from *Tanacetum baltistanicum* endemic to Gilgit-Baltistan

Muhammad Ismail*, Farhad Hussain, Sajjad Ali

Department of Chemistry, Karakoram International University, University Road, Gilgit-15100, Gilgit-Baltistan, Pakistan

Article published on November 11, 2014

Key words: Botanical pesticides, insects, vegetables, fruits.

Abstract

Conventional pesticides and antiseptics are no more attractive for consumers where biotechnology and hyphenated state of art techniques are available to bring innovation and discoveries. The shift from the current, conventional market to new greenest paradigm can be possible by rational identification and amplifications of botanicals. These botanicals, in turns can be applied on field or processed further for better action and efficacy. The mountainous region of Gilgit-Baltistan (G-B) is one of the most appropriate places with huge and unique floral diversity having great potential for such discoveries. During the current study the four different solvent extracts from *Tanacetum baltistanicum*, an endemic species to G-B was thoroughly investigated to determine its antiseptic potential. The toxicity of only 1% w/v aqueous solution of ethyl acetate, methanol and water extractives against woolly or apple aphids: *Eriosoma lanigerum* (Hemiptera: Aphididae), mealy cabbage aphids: *Brevicoryne brassicae* (Hemiptera: Aphididae), cabbage caterpillar: *Pieris brassicae* (Lepidoptera: Pieridae) after 24 hours was found in the range of 85-95% population mortality, while hexane extractive from *T.baltistanicum* was interestingly found 100% repellent against Sri Lankan weevil: *Myllocerus undecimpustulatus undatus* (Coleoptera: Curculionidae) on pear plants. This is worth to mention here that no damage to any pods was observed during and after the application of the formulations.

***Corresponding Author:** Muhammad Ismail ✉ dr.ismail@kiu.edu.pk

Introduction

Current dissatisfaction with chemicals used to control pests in agriculture has pushed the development of safer and more sustainable materials and methods for farmers to use. Scientist are looking for a solution that provides acceptable control for growers, but at the same time allows the production of fruits and vegetables that are safe for consumers.

Biological control measures have been implemented to manage many serious pests, including arthropods and nematodes, but chemicals persist as the first choice of many growers. This is due to the ease of their use, their reliable efficacy and their relatively inexpensive cost which make it difficult to compete with chemicals over biological means. With the passage of time the botanical insecticide preparations became in place to offer a potential solution to this problem. These new pesticides can be formulated similarly to synthetic chemicals, but are safer for both the agricultural workers who are in direct contact with them, and the consumers who may be exposed to pesticide residuals when food is purchased in the marketplace (Farnsworth *et al.,* 1985).

The importance of botanical pesticides can be measured by the rapidly increasing growth of this discipline roughly by 10-15% per year. Approximately 3000 research publications have been published on bio/botanical pesticides between 2004 and 2012. Despite this effort, there remains much to be learned about the secondary chemicals of plants. It is estimated that only 1.5% of total plant species have been identified and recorded while 98.5% are yet to be explored. Out of this small proportion of know plants most of the research on natural products has been done on just a few plant species and 85% of known plant species have not been investigated yet (Butler 2004).

There are many plant secondary metabolites playing an active role as botanical pesticides against many microorganisms, insects and flies. In this connection, abamectin and emmamectin are the key examples of broad spectrum pesticides which are biologically very effective as a miticide to replace Dicofol, a traditional chemical miticide. Some other botanical pesticides include Nicotine, Nimbin, Pyrethrin, Rotenone, Matrine, etc (Duke *et al.,* 2010). In order to support the idea of botanical pesticides some well documented plants genera which are known to have a potential as insecticides from centuries with increasing interest in recent years are highlighted in Table 1.

Table 1. Plant genera with proven antiseptic properties.

S. No	Name of plant	Active principles	Uses/applications	Ref.
1	*Chrysanthemum* species	parthrins, both pure compounds and extracts are being used	insecticides, mosquito control agents, antifeedant, bite inhibition	Dessalgne *et al.,* 2011
2	*Tanacetum* species	parthrins, both pure compounds and extracts are being used	Insecticidal	Barnes *et al.,* 2007, Eizumi *et al.,* 2008, Casida 1980
3	*Piper* species	unsaturated amides, pipernonaline,pellitorine, guineensine, etc	Insecticidal	Lee 2009
4	*Azadirachta* species	Azadirachtin, azadirachtin A, and essential oil from the plant	insecticidal and toxic towards soft-bodied pests like aphids, mites, etc.	Rasheed 2002
5	*Syzygium* species	2-methoxy-4-(2-propenyl)- phenol and *trans*-caryophyllene	Insecticidal	Zeng *et al.,* 2010

The genus *Tanacetum* belongs to the family Asteraceae. These species have received considerable attention in recent years due to their known biological properties. The genus *Tanacetum* (syn. *Chrysanthemum*), containing *Ca*.200 species, is distributed throughout Europe and western Asia.

Preparations from the plants in the genus *Tanacetum* have been used to treat arthritis, asthma, constipation, dermatitis, earache, fever, headache, inflammatory conditions, insect bites, labor, menstrual disorders, potential miscarriage, psoriasis, spasms, stomach ache, swelling, tinnitus, toothache, vertigo, and worms. These plants also have been used as an insecticide (Pareek *et al.,* 2011, Maia *et al.,* 2011, Heptinstall *et al.,* 1992, Ayvaz *et al.,* 2010, Jbilou *et al.,* 2006, Polatoglu *et al.,* 2011). Chemicals extracted from *Tanacetum parthenium* have been used since ancient times for a variety of medicinal proposes, and recently has gained considerable prominence due to its ability to alleviate the symptoms of migraine headaches (Tian *et al.,* 2013), arthritis and psoriasis (Venkateswari *et al.,* 2008), and to inhibit blood platelet aggregation (Desneux *et al.,* 2007)). Parthenolide and a number of related sesquiterpene lactones are considered to be responsible for these activities. Due to its emerging need and importance the current research is paying great attention to this field of natural relevance (Isman 2008, Oparaeke *et al.,* 2005, Antonio 2009, Isman 2006).

In Pakistan there is no encouraging research on botanical pesticides. There are very few articles related to the hazardous effects of conventional pesticides and their applications (Khooharo, *et al.,* 2008, Zia *et al.,* 2009, Ahmad *et al.,* 2007, Akbar *et al.,* 2010), along with some research on microbial bio-pesticides (Ahmed, *et al.,* 2002, Akbar *et al.,* 2012, Sagheer *et al.,* 2008).

Gilgit-Baltistan (G-B) is basically rich in floral diversity with a wide spectrum of medicinal values, though no effort to isolate, identify and develop botanical pesticides has been established yet in this region. G-B enormous biodiversity imparts sufficient opportunities to establish botanical pesticides formulation units. Unfortunately to date no such unit is in existence in this region. However, plant products and materials are being used traditionally in most parts of the G-B. Use of sawdust, cow urine, tobacco leaves and stalks, sweet flag, hot pepper etc are not new methods of plant protection in most villages of G-B. Application of different pheromones, attractants, poison baits are also in practice. Recently, agro spray: a mineral oil is also getting popularity against sucking pests.

Based on above incentives and in order to make and identify an efficient replacements for currently used synthetic materials, we started working to strengthen the capabilities in the production and application of newer generation of botanical pesticides formulations with a view to reducing the risks in the use of toxic crop protection chemicals and their old formulations, ensuring safety to the environment, farmers, workers, and minimize the residues of pesticides on food crops. A valid mixture of botanical pesticides extractives from *Tanacetum baltistanicum* Podlech is thoroughly investigated and formulations were applied on field to determine the toxicity of each formulation (1% w/v) against target pests on apple trees: *Malus domestica* Borkh., cabbage, *Brassica oleracea var. capitata*, and pear trees: *Pyrus communis* L. The results were amazing and very unique. The toxicity of ethyl acetate, methanol and water extractives against woolly or apple aphids: *Eriosoma lanigerum* (Hemiptera: Aphididae), mealy cabbage aphids: *Brevicoryne brassicae* (Hemiptera: Aphididae), cabbage caterpillar: *Pieris brassicae* (Lepidoptera: Pieridae) after 24 hours was found in the range of 85-95% population mortality, while hexane extractive from *T.baltistanicum* was interestingly found 100% repellent against Sri Lankan weevil: *Myllocerus undecimpustulatus undatus* (Coleoptera: Curculionidae) on pear trees. In addition to this, no damage to any pods was observed during and after the application of formulations.

The aim of this research was to improve the life of local inhabitants of the G-B region in particular and all over Pakistan in general through agricultural developments, fruits and vegetables free from chemical residues and production of greener environment friendly botanicals ensuring consumer protection by replacing the conventional pesticides. In the end, this research was designed to benefit the

local communities to improve social, economical and environmental aspects of their lives through scientific discoveries applicable in agriculture.

Material and methods

Plant material

The whole plant material of *T.baltistanicum* Podlech was collected from the Sumayar Valley of district Hunza-Nagar, Gilgit-Baltistan from an altitude of 5100-5300 m. above the sea level in August 2013. The plant was identified by Dr. Sujjad Haider, a Resident Botanist at Karakoram International University (KIU), and a specimen was kept for reference at the Herbarium of the department of biological sciences, KIU.

Extraction and fractionations

Dried and ground plant material of *T.baltistanicum* (2.9 Kg) was extracted three times with distilled methanol and the solvent was evaporated on a rotary evaporator. The crude methanolic extract (ca. 204 g) was then successively partitioned by solvent-solvent fractionation into three major fractions, *n*-hexane (50 g), ethyl acetate (60 g), and water (94 g) (Fig. 1). All the 4-major fractions, methanol, *n*-hexane, ethyl acetate, and water extractives were further processed to make the botanical formulation (1% w/v) to test its toxicity against wooly or apple aphids, mealy cabbage aphids, cabbage caterpillar and Sri Lankan weevil on pear trees.

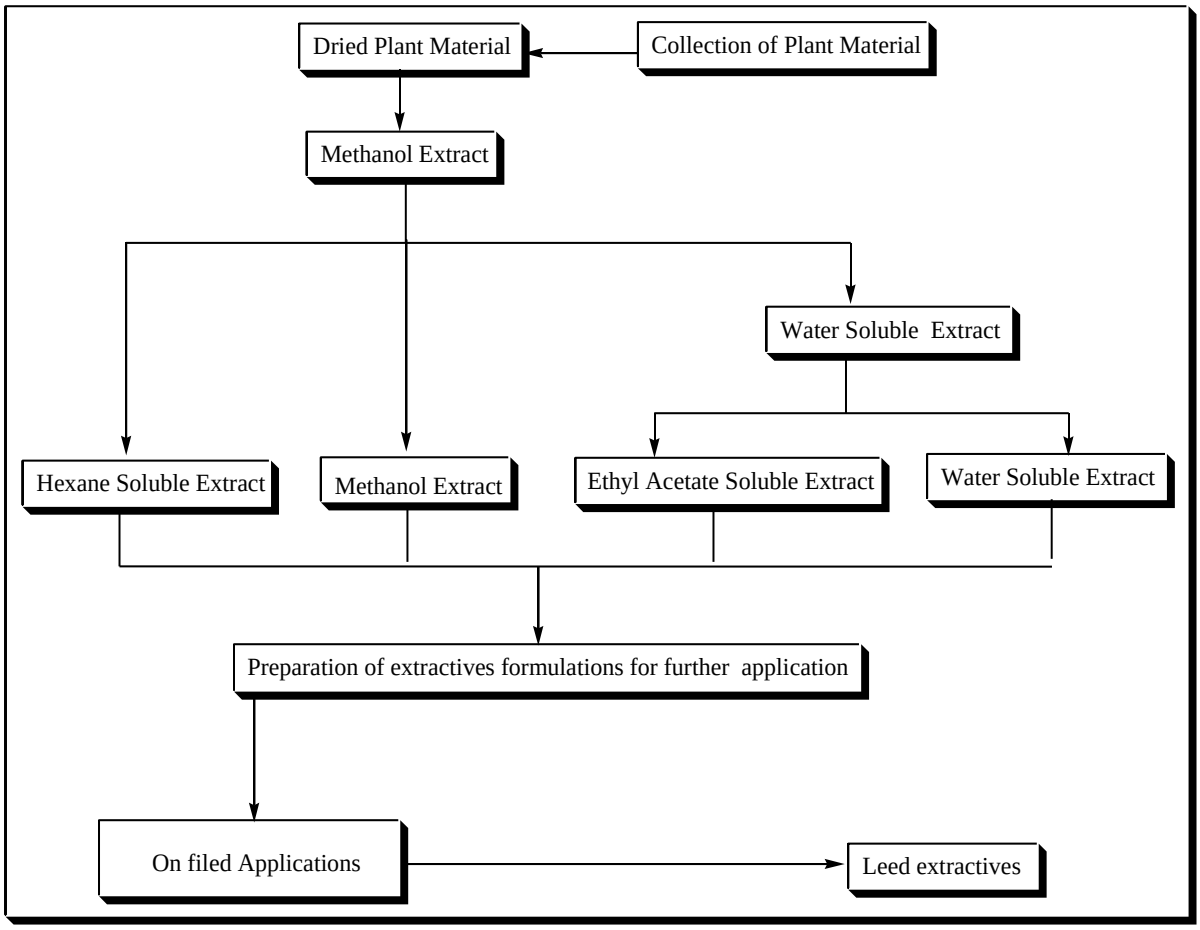

Fig. 1. Work Plan for the preparation and application of botanical extractives from *T.baltistanicum*.

Preparation of botanical formulations

The spray formation was made by weighing 5 g each of methanol, *n*-hexane, ethyl acetate, and water extracts in 500 ml flasks separately, and dissolved with 100 ml distilled water. To each flask further added 5 g of bar soap and 5 g of starch to make the solution viscous and sticky. The final volume of each flask was made to 500 ml by addition of distilled

water to make 1% (w/v) solutions of methanol, *n*-hexane, ethyl acetate, and water formulations. These formulations ware then labeled and shacked well before tacking to on-field application. These formulation were applied on field and observations were taken immediately after spay at the intervals of 1 h as well as 24 h after the treatment.

On-field applications

The trials were conducted on the research forms of the Agriculture Department, Kachura, Skardu, situated in northern areas of Pakistan. The field used for each trial had an area of about 25 m^2. The treatment consists of four different formulations of *T.baltistanicum* extracts (methanolic, ethyl acetate, *n*-hexane and water) against woolly or apple aphids: *Eriosoma lanigerum* on apple trees, mealy cabbage aphids: *Brevicoryne brassicae* and cabbage caterpillar: *Pieris brassicae* on cabbage, and Sri Lankan weevil: *Myllocerus undecimpustulatus undatus* on pear trees. For each treatment the sample area were divided in to five equal quadrants while taking $1/5^{th}$ area as a control. The wooly aphids on apple and aphids on cabbage were sampled from the control area before the spraying between 7:00 am and 8:00 am by using magnifying lens and collection net. The insects were assessed by randomly picking 10 plants per plot placing them in a glass bottle containing 30% ethanol and next day the insects found were identified and counted, while the butterfly caterpillar on cabbage and flea beetles and black bugs on pear was visibly counted on 5 plants in the same quadrants.

Results and discussion

From the last one decade, the interest in botanical insecticides has been increased due to environmental concerns and growing insect resistance to conventional insecticides. Botanical insecticides are derived from plants which are greener in nature.

The only 1% aqueous solution (w/v) of four different extractives from *T.baltistanicum* showed various degrees of pest species reduction and insect repellent activities. These very diluted aqueous formulations from *T.baltistanicum* also offered highest level of protection to both flowers and pods. The most active insecticidal fraction from *T.baltistanicum* was methanolic extractive with 95% mortality rate on 24 hours of treatment while hexane extractive was found to be most potent with 100% repellent activity against *M.undecimpustulatus undatus* on leaves of pear trees.

The hexane formulation was applied on pear trees (*P.communis* L.) infected by Sri Lankan weevil: *M.undecimpustulatus undatus*. It was interesting that 5% of *M.undecimpustulatus undatus* were found dead after half an hour of the spray and the application of extractive, while the plants was found free from all insect pests in 24 h of the treatment. These are amazing results and this fraction of *T.baltistanicum* can be now used a potent botanical insect repellant against *M.undecimpustulatus undatus* (Table 2). In addition to this, the application of this extractive can be expended to determine its toxicity against other insect pests in future.

Table 2. Summary of results of *T.baltistanicum* as insecticide and insect repellent.

S. No.	Extractive/ formulation	Pests	Observation after treatment (% mortality)	
			After spray (30 min)	After 24 h
1	Hexane	Sri Lankan weevil	5	10 100% repellent
2	Ethyl acetate	mealy cabbage aphids and cabbage caterpillar	5	90
		woolly or apple aphids	10	85
3	Methanol	woolly or apple aphids	15	95
4	Water	woolly or apple aphids	10	90

The ethyl acetate formulation was applied on both apple trees (*M.domestica* Borkh.) affected by woolly or apple aphids: *E.lanigerum* and cabbage (*B.oleracea var. capitata*) attacked by mealy cabbage aphid: *B.brassicae* and cabbage caterpillar: *P.brassicae*. The toxicity and efficacy of ethyl acetate extractive against *B. brassicae* and *P.brassicae* on cabbage showed 5% population mortality immediately after the treatment while 90% of subject insect were found dead after 24 h of post treatment. This extractive was further tested against *E.lanigerum* on apple where 10% of these pests were killed after 1 h and 85% after 24 h of post treatment (Table 2).

The methanolic formulation from *T.baltistanicum* was applied against *E. lanigerum* on apple where it was found most potent with 15% dead population after 1 h and 95% after 24 h of post treatment. The formulation of water extractive was also tested against *E.lanigerum* on apple trees where it showed the toxicity rate of 10% and 90% mortalities after 1 h and 24 h of post treatment, respectively (Table 2).

These findings will really help to improve the life of local inhabitants of the GB region in particular and all over the world in general through agricultural developments, fruits and vegetables free from chemical residues and production of greener environment friendly botanicals ensuring consumer protection by replacing the conventional pesticides. In addition to this, it will also benefit the local communities to improve social, economic, and environmental aspects of their lives through scientific discoveries applicable in agriculture.

In conclusion these investigations should be intensified on the use of this material against some other common pests and also need to consider related plant species for its potential as botanical insecticides. Moreover, this research should be extended to isolate and find out the active ingredients of these formulations by using advanced state of art chromatographic and spectroscopic techniques. The results presented in this study have shown that the botanical formulation from *T.baltistanicum* is effective, safe, and cheap for the control of many insect pests without damaging the pods and degrading the environment.

Acknowledgements

The author (M. Ismail) is grateful to Social Economic and Environmental Development (SEED), a joint program of Italy and Pakistan for financial support to conduct this research. Special thanks also go to Dr. Sujjad Haider for identification of plant material, and Mr. Mehboob Ali for assistance during the bench work as well as field trips.

References

Ahmad S, Khan IA, Hussain Z, Shah SIA, Ahmad M. 2007. Comparison of biopesticide with some synthetic pesticides against aphids in rapeseed crop. Sarhad Journal of Agriculture **23,** 1117-1120.

Ahmed S, Saleem MA, Rauf I. 2002. Field efficacy of some biopesticides against maize and jowar stem borer, *Chilo partellus* (Pyralidae: Lepidoptera). International Journal of Agriculture & Biology **4,** 332-334.

Akbar MF, Haq MA, Parveen F, Yasmin N, Sayeed SA. 2010. Determination of synthetic and bio-pesticides residues during aphid (*Myzus persicae* (Sulzer) control on cabbage crop through high performance liquid chromatography. Pakistan Entomologist **32,** 155-162.

Akbar MF, Haq MA, Yasmin N, Naqvi SN, Khan MF. 2012. Management of potato leaf hopper (*Amrasea devastans* Dist.) with biopesticides in comparison with conventional pesticides on autumn potato crop. Pakistan Journal of Zoology **44,** 313-320.

Antonio BD. 2009. Botanical pesticides: A part of sustainable agriculture in Babati District Tanzania. Bachelor's Thesis, Sodertorn University College, Tanzania.

Ayvaz A, Sagdic O, Karaborklu S, Ozturk I. 2010. Insecticidal activity of essential oil from different plants against three stored-products insects. Journal of Insect Science **10,** 1-13.

Barnes J, Anderson LA, Phillipson JD. 2007. Herbal Medicines. Edn 3. London, UK: Pharmaceutical Press, 10-380.

Butler MS. 2004. The role of natural product chemistry in drug discovery. Journal of Natural Products **67**, 2141-2153.

Casida JE. 1980. Pyrethrum flowers and pyrethroid insecticides. Environmental Health Perspectives **34,** 189-202.

Desneux N, Decourtye A, Delpuech JM. 2007. The sublethal effects of pesticides on beneficial arthropods. Annual Review of Entomology **52,** 81-106.

Dessalgne FH, Mekonnen SA, Indris BA. 2011. Variability of pyrethrum (*Chrysanthemum cinerariaefolium*) clones for chemical traits grown at Bekoji and Meraro of south eastern Ethopia. International Journal of Medicinal and Aromatic Plants **1**, 166-176.

Duke SO, Cantrell CL, Meepagala KM, Wedge DE, Tabanca N, Schrader KK. 2010. Natural toxins for use in pest management. Toxins **2**, 1943-1962.

EIzumi E, Morello LG, Ueda-Nakamura T. 2008. *Trypanosoma cruzi*: Antiprotozoal activity of parthenolide obtained from *Tanacetum parthenium* (L.) Schultz Bip. (Asteraceae, Compositae) against epimastigote and amastigote forms. Experimental Parasitology **118**, 324-330.

Farnsworth NR, Akerele O, Bingel AS. 1985. Medicinal plants in therapy. Bulletin of World Health Organization **63**, 965-981.

Heptinstall S, Awang DW, Dawson BA, Kindack D, Knight DW. 1992. Parthenolide content and bioactivity of feverfew (*Tanacetum parthenium* (L.) Schultz Bip.). Estimation of chemical and authenticated feverfew products. Journal of Pharmacology and Pharmacotherapeutics **44,** 391-5.

Isman MB. 2006. Botanical pesticides, deterrents, and repellents in modern agriculture and increasingly regulated world. Annual Review of Entomology **51,** 45-66.

Isman MB. 2008. Perspective botanical pesticides: for richer, for poorer. Pest Management Science **64,** 8-11.

Jbilou R, Ennabili A, Sayah F. 2006. Insecticidal activity of four medicinal plant extracts against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). African Journal of Biotechnology **5,** 2936-940.

Khooharo AA, Memon RA, Mallah MU. 2008. An empirical analysis of pesticide marketing in Pakistan. Pakistan Economic and Social Review **46,** 57-74.

Lee HS. 2009. Pesticidal constituents derived from Piperaceae fruits. Agricultural Chemistry & Biotechnolog **48,** 65-74.

Maia MF, Moore S. 2011. Plants-based insect repellents: A review of their efficacy, development and testing. Malaria Journal **10,** 1-15.

Oparaeke AM, Dike MC, Amatobi CI. 2005. Botanical pesticide mixtures for insect pest management on cowpea, *Vigna unguiculata* (L.) walp plants–2. The pod borer, *Maruca vitrata* Fab. (Lepidoptera: *pyralidae*) and pod sucking bug, *Clavigralla tomentosicollis* stal (Heteroptera: *coreidae*). Agricultura Tropica Et Subtropica **38,** 33-38.

Pareek A, Suthar M, Rathore GS, Bansal V. 2011. Feverfew (*Tanacetum parthenium* L.): A systematic review. Pharmacognosy Review **5,** 103-110.

Polatoglu K, Karakoc OC, Gokce A, Goren A. 2011. Insecticidal activity of *Tanacetum chiliophyllum* (Fisch. & Mey) var. *monocephalem* grierson extracts and a new sesquiterpene lactone. Phytochemistry Letters **4,** 432-435.

Rasheed M. 2002. Studies on the chemical constituents of *Azadirachta indica* A. Juss (Neem), Ph. D. Thesis, University of Karachi, Pakistan.

Sagheer M, Ashfaq M, Hasan M, Rana SA. 2008. Integration of some biopesticides and *Trichogramma chilonis* for the sustainable management of rice leaf folder, *Cnaphalocrocis medinalis* (Guenee) (Lepidoptera: *pyralidae*). Pakistan Journal of Agriculture Science **45,** 69-74.

Tian YQ, Zhang ZX, Xu HH. 2013. Laboratory and field evaluation on insecticidal activity of *Cicuta virosa* L. var. *latisecta* Celak. Industrial Crops and Products **41,** 90-93.

Venkateswari G, Krishnayya PV, Rao PA, Murthy KVMK. 2008. Bioefficacy of abamectin and emamectin benzoate against *Spodoptera litura* (Fab.). Pesticide Research Journal **20,** 229-233.

Zeng L, Lao CZ, Cen YJ, Liang GW. 2010. Study on the insecticidal activity compounds from the essential oil from *Syzygium aromaticum* against stored grain insect pests. 10th International Working Conference on Stored Product Protection. Julius-Kühn-Archiv **425,** 766-771.

Zia MS, Khan MJ, Qasim M, Rahman A. 2009. Pesticide residue in the food chain and human body inside Pakistan. Journal of Chemical Society of Pakistan **31,** 284-291.