IMPORTANCE AND IMPLEMENTATION OF ESSENTIAL OIL OF PAKISTANIAN ACORUS CALAMUS LINN., AS A BIOPESTICIDE

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Abstract

The Pakistanian Acorus calamus (Fam.: Araceae) is locally found (Nasir, 1978) in Chitral, Peshawar, Jhelum valley, Thalawan valley, Mandi and Kashmir. The common name of A. calamus is sweet flag. It is found in marshy places and along the river banks from 600-2000m. The rootstock (modified stem) commonly called as rhizome, is medicinal and yields an essential oil used in the manufacture of soap, cosmetics and in the liquor industry. It is also used as medicine for stomach complaints, snake bite, as an insect repellent and for remittent fevers. The essential oil of A. calamus is volatile (Aromatic) and contains acorenone, β-gyrjunene, Isoshyobunine, β-asarone, Calamendiol, α-selinene, α-calacorene, Calamusenone, Camphene and Shyobunone as main active ingredients. In the present paper the essential oil of A. calamus was tested against dengue fever virus vector mosquito Ae. aegypti larvae (late 3rd instar) The LC50 was found to be 1250ppm by WHO-method, (Anon., 1970). The IGR effects were observed on lower i.e. 150, 300, 450, 600 and 750ppm dose. The abnormalities were increased with the increase of dose. Phytotoxicity on cotton crop was recorded @ 1.5 litre/acre, whereas no phytotoxicity was recorded on mango tree and coconut plants upto the dose of 2 litres/acre. While 70-90% control of mango hoppers on mango trees in Mirpurkhas Sindh and rhinoceros beetles on coconut plants in Hub, Balochistan was recorded. Anti-fungal activity was recorded on cuts and wounds on sheep in Hub, Balochistan. The acorus oil prevents the cuts and wounds from fungal growth and the cuts & wounds heal up rapidly as compared to control. Systematic control of root knot nematode was also recorded in cotton and brinjal plants by using 0.25% solution of calamus oil, given to the infected plants. The scale insects were also controlled by the same systematic method by using 0.5% dilution to the infected cotton plants. The control of mealy bugs on cotton, brinjal and Abutilon indicum was achieved by spray method using 0.5% solution, repeated weekly for one month in Sindh and Balochistan. Beside this the different aspects of acorus oil activity and suggestions for implementation as biopesticide in agriculture and health sector with special reference to Pakistan have been discussed in detail.

Introduction

Scientists in different parts of the world are working for the development and establishment of plant based pesticide, usually called as phytopesticide, botanical pesticide, biopesticide or natural pesticides (Sexana *et al.*, 1974; Chauhan *et al.*, 1987; Muhammed *et al.*, 1995; Pathak *et al.*, 2000; Alesso *et al.*, 2003; Azmi, 2004; Tanveer *et al.*, 2005; Verma *et al.*, 2006; Yan-Zhang *et al.*, 2007; Siddiqui *et al.*, 2009). The synthetic or artificial pesticides have resulted as the destroyer of common human health and our environment. The commercial pesticides have not only polluted the environment

but also produced the problem of resistance in targeted pest and poisoning of the food chain by different routes.

Synthetic and commercial pesticides are reported hazardous not only for beneficial insects but also for environment and especially for human beings (Ray & Philip 2000, Wilks, 2000), that is why alternates are being searched for safety of environment and humanity. Neem has been already chalked out and is being used in field at different levels. But the neem only can not fulfill the requirement commercially so there is a need to search out new plant origin pesticides. For this purpose several researchers are working in this direction such as Ermel *et al.*, (1991), El-Nahal *et al.*, (1994), Ying-Juan *et al.*, (2005), Ying-Juan *et al.*, (2007). Similarly when any plant product is found effective against insects/pests, then it is also tested against birds, fishes and mammals for its effects on them for the commercialization purpose, without this the product may not be registered or commercialized (Isman, 1997).

Therefore the present work aims to discuss different aspects of essential oil from *Calamus* for its implementation as biopesticide in Pakistan on field level in agriculture and health sector.

Materials and Methods

Toxicity tests against dengue vector mosquito larvae were carried out by WHOmethod (Anon., 1970) and phytotoxicity test were done on cotton crop, mango trees and coconut plants. Furthermore, the systematic control of root knot was also done on brinjal and cotton plants. Moreover, the literature was studied; the results were extracted and were described, in the support of implementation of the essential oil of *Calamus* as biopesticide, specially in Pakistan and generally in the world.

Results and Discussions

A lot of work on *A. calamus* is in progress in different parts of the world, especially in Pakistan to emphasize the use of essential oil from *Calamus* in the agriculture sector particularly and in other health sector generally. The following results of present paper and the reports from different scientist and researchers will help in positive understanding for the use of essential oil as a biopesticide from *A. calamus*.

Natural and biodegradable: As the essential oil of Acorus is plant origin and it is natural, due to which it is easily biodegradable in sunlight, in soil, in air, in water and in the body of livings.

Residual effect: Being a natural product its residual effects have less persistency as compared to other commercial and synthetic pesticides. In the experiments, the residual effects of calamus oil has been recorded from one week to two weeks.

Locally available in Pakistan: The raw material may be collected locally on large scale level. As the source is local, so it will be cheep as compared to imported pesticides.

Lower mammalian toxicity: The experiments done by Naqvi & Tariq (2007) suggests the lower mammalian toxicity of the acorus oil. They reported the LD_{50} against small mammals, the Wistar strain (Albino rats/white rats) as 8.7 ml per kg by body weight which is much lower as compared to other synthetic pesticides. Perveen *et al.*, (2008)

also reported lower mammalian toxicity of *Cedrus deodara* root oil against the same wistar strain of Albino rats as 8.9 ml per kg by body weight. This was reported safe as compared to neem oil having LD_{50} as 5.0 gm/kg. In this sense the calamus oil is also safer as compared to neem oil and is equivalent to *C. deodara* root oil in toxicity against small mammal the white rats.

Lower bird toxicity: The experiments conducted by Tariq & Naqvi (2006) suggests the lower toxicity of acorus oil against poultry. They reported 0.8 ml per kg by body weight as LD_{50} . This quantity is impossible for any bird to take from field in local practice which is a positive point to our environment.

Insect repellent: It is aromatic in nature, due to which it acts as insect repellent, as reported by Tariq & Qadri (2001). They reported two hours repellency of acorus oil against dengue fever vector mosquitoes and 50% biting after two hours. Whereas 2.5 hours repellency and 34% biting in 2.5 hours was recorded by marketed repellents such as King & Mospel (Harish *et al.*, 1999; Ravikant *et al.*, 2007; Streloke *et al.*, 1989).

Fumigant activity: The acorus due to its aromaticity may be used as fumigant in store houses, warehouse and godowns for the protection of cereals, pulses and other store able food items. Several experiment have been conducted in this direction (Jilani *et al.*, 1988; Ali *et al.*, 1983; Akhtar *et al.*, 2004; Kouninki *et al.*, 2005; Akhtar, *et al.*, 2007).

Toxicity against stored grain pests: Acorus oil was tested against stored grain pest the *Sitophilus oryzae*, by filter impregnation method. The LC_{50} was found to be $3500\mu g/cm^2$ (Tanveer *et al.*, 2005), whereas by Cypermethrin it was $19\mu g/cm^2$ (Ahmed *et al.*, 1998, 2000).

Lower fish toxicity: According to the experimental data recorded by Tariq *et al.*, (2004), the toxicity tests were done in connection to compare with those of commercial pesticides. They reported acorus oil toxicity against two edible fishes *Labeo rohita* and *Cyprinus carpio* as 33 and 55ppm, respectively as LC_{50} by WHO-method (Anon., 1970) whereas the LC_{50} by permethrin (2.5EC) was 0.25 and 0.27ppm, respectively. This lower toxicity also supports the implementation of the acorus oil. Other reports are Kazmi *et al.*, (1991); Sultana *et al.*, (1996); Azmi *et al.*, (1997); (1999).

Resistance avoidance: The commercial pesticides used contains a single particular active ingredient in them. The genetic resistance develops against that single active ingredient usually a pure compound by detoxification of acetylcholinesterase enzyme gene (Ming-Jing *et al.*, 2007). While the essential oil of the acorus or any other plant usually contains 30-50 pure compounds, such as 41 compounds are reported by Siddiqui *et al.*, (2009) only from neem flower. Therefore, the gene get confused in developing resistant against multiple compounds in an essential oil of any plant. In acorus oil itself as well contains about 40 compounds (Vollinger, 1987; Naqvi & Tabassum, 1992; Tabashnik *et al.*, 2005).

Against agricultural pests: The essential oil of acorus may be successfully used in the agriculture sector because, due to aromaticity, it repells the pest of the crops. On the other hand it has been found toxic to insects of both types, the sucking and biting, chewing and

cutting pests. It has been found less toxic to pest as compared to commercial pesticides but with minimum side effects (Johri *et al.*, 2004; Naqvi *et al.*, 2006).

Safe and sound for fruits, vegetables and other live-stocks: Proliferation of synthetic pure compounds in our food chain by different routes is poisoning our food, due to which many side effects and hazards are being developed. The farmers do spray on vegetable crop for killing the pests, the vegetable absorbs the sprayed chemical on their surface and within few (2-4) days the vegetable is plucked and brought into the market which is directly eaten by the people such as cucumber, long cucumber, cabbage, brinjal pumpkin, lady finger (okra) etc., and thus sprayed chemical get way into the human body which results in the shape of different disease and complications. Similar is the case for fruit farms, cereal crops and pulses crops (Stepan *et al.*, 2005). But acorus oil may prove sound and safe in these spheres and will provide bio-security to human beings, non-targeted animals, fishes, birds, mammals, pollinators, predators and hunters (Basappa, 2007).

As insect growth regulator: Essential oil of calamus was found toxic against late 3^{rd} instar larvae of Dengue fever virus vector mosquito the *Aedes aegypti*. The LC₅₀ was found to be 1250ppm by WHO-method (Anon., 1970). When late 3^{rd} instar larvae were exposed to lower dose 150, 300, 450, 600 and 750ppm, the abnormalities were produced. The abnormalities were increased with the increase of dose. These produced abnormalities are due to the effect of IGR by acorus oil, because in control no abnormalities were recorded (Risha *et al.*, 1990; Verma *et al.*, 2006; Tariq *et al.*, 2009).

As grain protectant: Su (1991) reported that when calamus oil was applied at a dosage of 1000ppm to wheat or black-eyed peas, the oil completely protected the grains from infestation by *Sitophilus oryzae* and *Callosobruchus maculatus*, respectively. The oil, at the dosage of 500 and 250ppm, still gave high protection to wheat and peas from infestation by these insects, respectively. This reflects that acorus oil may be used as good grain protectant.

Acorus oil as anti-feedant: Tariq *et al.*, (2007) reported anti-feedant activity of Acorus oil against American bollworms (*Heliothis armigera*), spotted bollworm (*Earias fabia*) and pink bollworm (*Pectinophora gossypiella*). The results were comparable with standards. The bollworms and cutworms feed on the flower and vegetative parts of the plants, the acorus oil gave good results against cutworms and bollworms as anti-feedant in this connection.

Phyto-toxicity of acorus oil: During the present work the field application of acorus oil against agricultural pests in Sindh, Punjab and Balochistan was carried out and it was recorded that acorus oil gave phyto-toxicity on cotton crop at the dose of 1.5 litre/acre, because the cotton crop is a sensitive and thin leaves crop. But on the crops having hard leaves such as on mango tree (against mango hoppers) and coconut tree (against *Rhinoceros beetle*), it gave no phyto-toxicity @ 2 litre per acre, and gave good 70-90% control of mango hoppers in Mirpurkhas, Sindh and rhinoceros beetles on coconut plants in Balochistan.

Systematic control of root knot nematode: Experiments were conducted during present work on infected plants of brinjal and cotton by root knot by using 0.25% solution of acorus oil given to plant roots. The infected plants become healthy and grow very well after the application of three to five times both in cotton and brinjal. The scale insects were also controlled by the same method by giving 0.5% dilution to the infected plants. The same was true for mealy bugs on cotton, brinjal and *Abutulon indicum*.

Calamus is store able: It may be available through the whole year, because the rhizomes of the calamus may be stored in dry condition and so may easily be transported anywhere, it is required.

Culture of calamus may be increased: It may also be grown in Pakistan to meet the requirements, commercially on large scale.

Anti-fungal activity: As the acorus extract is oil, the oils usually are anti-fungal in nature. Therefore, the acorus oil was also tested for cuts and wounds on sheep during present study, it prevents the cuts and wounds from fungal growth and the cuts and wounds heal rapidly as compared to control.

Other biopesticide of Pakistan: Nimbokil, a product of PCSIR, Pakistan (Tariq *et al.*, 2005), Biosal, a product of International Centre for Chemical & Biological Sciences (ICC&BS), Pakistan (Sial *et al.*, 2009).

Conclusion

Keeping in mind the work of different scientists and researchers as discussed in this manuscript about *Acorus calamus*, it may be suggested that the use of acorus oil is safe and sound as compared to synthetic and commercial pesticides. So it may be given preference on commercial pesticides for the use in agriculture and health sector.

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References

Ahmed, I., S.N.H. Naqvi, T. Alam, M.A. Azmi and R. Tabassum. 1998. Toxicity of cypermethrin and *Acorus calamus* extract on *Sitophilus oryzae*. *Proc. Pakistan Congr. Zool.*, 18: 31-36.

Ahmed, I., T. Ahsan, R. Tabassum, A. Azmi and S.N.H. Naqvi. 2000. Effects of Acorus calamus extract and cypermethrin on enzymatic activities in Sitophilus oryzae. J. Exp. Zool. India, 3(2): 169-173.

- Akhtar, K., M.A. Azmi, S.N.H. Naqvi and R.M. Tariq. 2007. Effect of Acorus calamus extract and RB-b (Neem product) against Tribolium castaneum. Pak. J. entomol., 22(1&2): 5-9.
- Akhtar, K., M.A. Azmi, N. Gul and R.M. Tariq. 2004. Toxic effects of various plants extracts on Callosobruchus analis (F.). Pak. J. entomol., 19(1&2): 27-30.
- Alesso, E., R. Torviso, B. Lantano, M. Elrich, M., Liliana, G. Finkielsztein, Moltrasio, J.M. Aguirre and E. Brunet. 2003. Synthesis of 1-ethyl-2-methyl-3-arylindanes. Stereochemistry of five-membered ring formation (online) ARKIVOC 2003 (X): 283-297.
- Ali, S.I., O.P. Singh and U.S. Misra. 1983. Effectiveness of plant oils against pulse beetle Callosobruchus chinensis. Indian J. Entomol., 45(1): 6-9.
- Anonymous. 1970. World Health Organization. Insecticide resistance and vector control: 17th Report of WHO Expert Committee on insecticides: *WHO Tech. Report*, Ser. No. 443.
- Azmi, M.A. 2004. Toxicity of *Clerodendrum inerme* extract and cyhalothrim against *Rhizopertha dominica* PARC strain and their effects on acid phosphatase and cholinesterase activity. *Pak. J. Sci. Ind. Res.*, 47(5): 394-397.
- Azmi, M.A., S. Jahan, S.N.H. Naqvi, R. Tabassum, M. Jahan and M.F. Khan. 1997. Toxic effect of Tetranotriterpenoids (Neem products) and Deltamethrin (Pyrethroid) against Cyprinus carpio (common carp). *Proc. Pakistan Congr. Zool.*, 17: 171-177.
- Azmi, M.A., S. Jahan, S.N.H. Naqvi, R. Tabassum, M.F. Khan and K. Akhtar. 1999. Effect of tetranortriterpenoids (Neem products SDS) and deltamethrin (pyrethroid) on phosphomonoesterase activity in *Cyprinus carpio* (common carp). *Nat. Acad. Sci. Letters*, 22(7&8): 130-134.
- Basappa, H. 2007. Toxicity of biopesticides and synthetic insecticides to egg parasitoid, *Trichogramma chilonis* Ishii and coccinellid predator, *Cheilomenes sexmaculata* (Fabricius). *Journal of Biological Control*, 21(1): 31-36.
- Chauhan, S.P.S., A. Kumar, C.L. Singh and U.K. Pandey. 1987. Toxicity of some plant extracts against rice moth *Corcyra cephalonica* (Stainton) (Lepidoptera). *Indian J. Entomol.*, 49(4): 532-534.
- El-Nahal, A.K.M., G.H. Schmidt and E.M. Risha. 1994. Influence of vapours of *Acorus calamus* L. oil on the reproductivity of some stored product Coleoptera. *Pak. J. entomol. Karachi*, 9(1): 21-27.
- Ermel, K., H.O. Kalinowski and H. Schmutterer. 1991. Isolation and characterization of marrangin, a new insect growth regulating (IGR) substance from the seed kernels of the marrango tree, *Azadirachta excelsa* (Jack). J. Appl. Entomol., 112(5): 512-519.
- Harish, C., A. Nagender, D.K. Ahuja and S.K. Berry. 1999. Laboratory evaluation of plant-extracts as repellents to the rust red flour beetle, *Tribolium castaneum* (Herbst), on jute fabric. *International Pest Control*, 4(1): 18-20.
- Isman, M.B. 1997. Neem and other botanical insecticides: Barriers to commercialization. *Phytoparasitica*, 25(4): 339-344.
- Jilani, G., M.I. Khan and Ghiasuddin. 1988. Studies on insecticidal activity of some indigenous plant materials against the pulse weevil, *Callosobruchus analis. Pak. J. Entomol. Kar.*, 3(1): 21-32.
- Johri, P.K., R. Maurya, D. Singh, D. Tiwari and R. Johri. 2004. Comparative toxicity of seven indigenous botanical extracts against the infestative stage of three insect pests of agricultural importance. *Journal of Applied Zoological Researches*, 15(2): 202-204.
- Kazmi, M.A., S.N.H. Naqvi, W. Qureshi, A. Bukhsh and Z. Qureshi. 1991. Toxicity of Diflubenzuron and Malathion on freshwater carp *Cyprinus carpio*, Linnaeus. *Kar. Univ. J. Sc.*, 19(1&2): 181-188.
- Kouninki, H., E. Haubruge, F.E. Noudjou, G. Lognay, F. Malaisse, M.B. Ngassoum, A. Goudoum, P.M. Mapongmetsem, L.S.T. Ngamo and T. Hance. 2005. Potantial use of essential oils from Cameroon applied as fumigant or contact insecticides against *Sitophilus zeamais* Motsch (Coleoptera: Curculionidae). *Communications in Agricultural and Applied Biological Sciences*, 70(4): 787-792.

- Ming-Jing, Q., X. Xin-Jun, H. Zhao-Jun and J. Xiao-Jing. 2007. Advances in studies of acetylcholinesterase gene variation associated with insect resistance. *Chinese Bulletin of Entomology*, 44(2): 191-194.
- Muhammed, F.A., Z. Khan, S.N.H. Naqvi and J. Ahmad. 1995. Efficacy of *Nerium indicum* crude extract as compared with cypermethrin against adults of *Piezodorus hyberni* (Gmelin) (Hemiptera: Pentatomidae). *Proc. 1st Intl. Cong. Entol.*, pp. 189-194.
- Naqvi, S.N.H. and R. Tabassum. 1992. Probable development of resistance against neem extract (RB-a) and cyfluthrin (Solfac 10% EC), in *Musca domestica* L. (PCSIR strain). *Pak. J. entomol. Karachi*, 7(1-2): 9-16.
- Naqvi, S.N.H. and R.M. Tariq. 2007. Mammalian toxicity of *Acorus calamus* (AC) and *Annona squamosa* (AS) oil against Albino rats. *Pak. J. entomol. Karachi*, 22 (1&2): 37-38.
- Naqvi, S.N.H., R.M. Tariq, S.M.N. Zafar and M.R. Attique. 2006. Efficacy of Acorus calamus (AC) rhizome oil and Annona squamosa (AS) seed oil against sucking pests of cotton at CCRI-Multan as compared to Mospilan and Tamaron. Pak. J. entomol. Karachi, 21(1&2): 23-27.
- Nasir, Y.J. 1978. Araceae. Flora of Pakistan N o. 120. In: (Eds): E. Nasir and S.I. Ali. Department of Botany, University of Karachi and National Herbarium, Agricultural Research Council, Islamabad.
- Pathak, N., P.K. Mittal, O.P. Singh, D.V. Sagar and P. Vasudevan. 2000. Larvicidal action of essential oils from plants against the vector mosquitoes, *Anopheles stephensi* (Liston), *Culex quinquefasciatus* (Say) and *Aedes aegypti* (L.). *Int. Pest Control*, 42(2): 53-55.
- Perveen, R., S.N.H. Naqvi, M.A. Azmi, R.M. Tariq, M. Ahmed and S. Mehmood. 2008. Determination of mammalian toxicity of *Cedrus deodara* root oil, against Albino rats (Wistar strain). *Pak. J. entomol. Karachi*, 23(1&2): 1-4.
- Ravikant, U., G. Jaiswal and N. Yadav. 2007. Toxicity, repellency and oviposition, inhibitory activity of some essential oils against *Callosobruchus chinensis*. *Journal of Applied Bioscience*, 33(1): 23-28.
- Ray, D.E. and J.F. Philip. 2000. Pyrethroid insecticides: Poisoning syndromes, synergies and therapy. *Journal of Toxicology Clinical Toxicology*, 38(2): 95-101.
- Risha, E.M., A.K.M. El-Nahal and G.H. Schmidt. 1990. Toxicity of vapours of *Acorus calamus* L., oil to the immature stages of some stored product (Coleoptera). *J. stored Prod. Res.*, 26(3): 133-137.
- Sexena, B.P.E., B.D. Rohden and B. Veriag. 1974. Morphological changes in the *Thermobia domestica* under the influence of *Acorus calamus* oil vapours. *Separatum Experimentia*, 30: 1298.
- Sial, I.M., M.A. Kazmi, Q.B. Kazmi and S.N.H. Naqvi. 2009. Toxicity of Biosal (Phytopesticide) and permethrin (Pyrethroid) against common carp, *Cyprinus carpio. Pakistan J. Zool.*, 41(3): 235-238.
- Siddiqui, B.S., S.T. Ali, R.M. Tariq, T. Gulzar, M. Rasheed and R. Mehmood. 2009. GC-based analysis of insecticidal constituents of the flower of *Azadirachta indica* A. Juss. *Natural Product Research*, 23(3): 271-283.
- Stepan, Ticha, R.J., J. Hajslova, T. Kovalczuk and V. Kocourek. 2005. Baby food production chain: Pesticide residues in fresh apples and products. *Food Additives and Contaminants*, 22(12): 1231-1242.
- Streloke, M., K.R.S. Ascher, G.H. Schmidt and Neumann. 1989. Vapor pressure and volatility of β-asarone, the main ingredient of an indigenous stored-product insecticide, *Acorus calamus* oil. *Phytoparasitica*, 17(4): 299-314.
- Su, Helen, C.F. 1991. Laboratory evaluation of toxicity of calamus oil against four species of stored product insects. J. Enomol Sci., 26(1): 76-80.
- Sultana, S.H., M.A. Kazmi, S.N.H. Naqvi, M.S. Aslam and R. Tabassum. 1996. Toxicity and effect of neem products (RB-b and SDC) and OP (Dimethoate) on protein pattern of a fresh water fish. *Proceedings of the UNESCO Workshop on Coastal Aquaculture*, pp. 131-137.

- Tabashnik, B.E., T.J. Dennehy and Y. Carriere. 2005. Delayed resistance to transgenic cotton in pink bollworm. Proceedings of the National Academy of Sciences of the United States of America, 102(43): 15389-15393.
- Tanveer, A., J. Ahmed, N. Yasmeen, R. Tabassum, A. Azmi and M. Shoaib. 2005. Effectiveness of cypermethrin 10. EC and Acorus calamus extract in comparison with Danitol, methoprene and neem extract and their effect on total protein contents of Sitophilus oryzae L. Intl. J. Biol and Biotech., 2(4): 951-954.
- Tariq, R.M. and S.N.H. Naqvi. 2006. Avian toxicity of *Acorus calamus* (AC) and *Annona squamosa* (AS) oil against chicken (poultry). *Pak. J. entomol. Karachi*, 21(1&2): 29-30.
- Tariq, R.M. and S.S. Qadri. 2001. Repellent activity of some local plant's oil, two commercial repellents, di-methyl phthalate and non-alcoholic itter against dengue vector mosquitoes. *Pak. J. entomol. Karachi*, 16(1&2): 7-10.
- Tariq, R.M., S.N.H. Naqvi and S.M.N. Zafar. 2009. Two indigenous aquatic weeds *Lemna minor* and *Spirodella* spp., gave promising biological control of mosquito larvae with Rainbow fish on field level in Karachi, Sindh, Pakistan. *Pak. J. Bot.*, 41(1): 269-276.
- Tariq, R.M., S.N.H. Naqvi, N. Yasmeen, M.A. Azmi, M. Aslam, S.M.N. Zafar, K. Gabol and M. Masood. 2005. Toxicity of three neem pesticides against mosquito larvae. *Int. J. Biol. Biotech.*, 2(4): 963-966.
- Tariq, R.M., S.N.H. Naqvi, S.M.N. Zafar and A.S. Burrero. 2007. Toxic effects of botanical pesticide, from Acorus calamus (AC) and Annona squamosa (AS) against Bollworms at ARI-Tandojam, Sindh-Pakistan. Pak. J. entomol. Karachi, 22(1&2): 31-36.
- Tariq, R.M., S.N.H. Naqvi, S.M.N. Zafar and T.A. Saqib. 2004. Piscean toxicity of rhizome stem oil of Acorus calamus and seeds oil of Annona squamosa against Labeo rohita and Cyprinus carpio. Pak. J. entomol. Karachi, 19(1&2): 31-32.
- Verma, P.R., T. Subburaju and N. Balakrishnan. 2006. Larvicidal activity of Artemisia nilagirica (Clarke) Pamp. and Ocimum sanctum Linn. – A preliminary study. Journal of Natural Remedies, 6(2): 157-161.
- Vollinger, M. 1987. The possible development of resistance against neem seed kernel extract and deltamethrin in *Plutella xylostella*. *Proc.* 3rd *Int. Neem Conf.* (Nairobi 1986), pp. 543-554.
- Wilks, M.F. 2000. Pyrethroid induced paresthesia: A central or local toxic effect? Journal of Toxicology Clinical Toxicology, 38(2): 103-105.
- Yan-Zhang, H., Y. Chang-Ju, X. Dong, R.O. Akinkurolere and Y. Ying-Juan. 2007. Contact and repellency activities of ethanol extracts from twenty medicinal plants against *Rhizopertha dominica* (Fab.) (Coleoptera: Bostrichidae). *Acta Entomologica Sinica*, 50(2): 118-123.
- Ying-Juan, Y., X. Dong and Y. Chang-Ju. 2005. Bioactivities of extracts from 21 species of plants against maize weevil. *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). *Acta Entomologica Sinica*, 48(5): 692-698.
- Ying-Juan, Y., Y. Chang-Ju, X. Dong and H. Yan-Zhang. 2007. Bioactivities of extracts from *Acorus gramineus* on four stored grain pests. *Acta Entomologica Sinica*, 50(3): 309-312.

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