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INSECTICIDAL EFFICACY OF ENDOPHYTIC FUNGAL EXTRACT OF *ANNONA SQUAMOSA* AGAINST *CALLOSOBRUCHUS CHINENSIS* (COLEOPTERA: BRUCHIDAE)

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ABSTRACT

Endophytic fungi are microorganisms that live inside the plant without causing any overt negative effect on plant tissues; rather they protect the host plant from pests and diseases. The insecticidal activity of endophytic fungal extract of leaf and seed of *Annona squamosa* against pulse beetle, *Callosobruchus chinensis* were studied. Endophytic fungi were isolated from the leaf and seed of *Annona squamosa*. The various concentrations of methanol and ethyl acetate crude endophytic fungal extracts were tested against *Callosobruchus chinensis*. The percent mortality was recorded after 96h.The insecticidal activity of the endophytic fungi isolated from leaf of *Annona squamosa* were (LD_{10}= 35.99mg/kg, LD_{50}= 70.03mg/kg) in methanol and (LD_{10}= 70.57mg/kg, LD_{50}= 112.4mg/kg) in ethyl acetate respectively. The fungi isolated from seed of *Annona squamosa* were (LD_{10}= 12.46mg/kg, LD_{50}= 37.33mg/kg) in methanol and (LD_{10}= 33.72mg/kg, LD_{50}= 65.11mg/kg) in ethyl acetate respectively. The mortality increases with increase in concentration of endophytic fungi. The methanol solvent extract showed more insecticidal property against *C. chinensis* due to endophytic secondary metabolites. Statistical variance, 95% confidence limits and regression equations are presented.

Figures : 02 References : 47 Tables : 02
KEY WORDS : *Annona squamosa, Callosobruchus chinensis,* Endophytic fungi, Mortality.

Introduction

The crop and store grain pest problems are nearly as old as the beginning of crop cultivation. With a greater awareness of hazards associated with the use of synthetic organic insecticide there has been an increasing need to explore suitable alternative methods of pest control. Farmers use different plant materials to protect store grain pest from pest infestation. Natural products in their crude form or plant extract provide unlimited opportunities as biopesticide. Heavy qualitative and quantitative losses occur due to heavy infestation of pulse beetle, *Callosobruchus chinensis* in the store grains[3, 18, 32, 34, 37, 44]. Various plants and their derivatives are used for controlling the storage pest [33].

Some species of Annonaceae have been used traditionally as insecticides. The powdered seeds and leaf juices of *Annona sp.* are used to kill head lice and body lice[28,40]. The annonaceous acetogenins extracted from tree leaves, bark and seeds have pesticidal or insect antifeedant properties[2, 27, 35,36].

Endophytes are microorganisms that grow within plants without causing any obvious symptoms of infection or disease[16,22]. Some of the endophyte microorganisms are thought to protect

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their host from attack by fungi, insect and mammals by producing secondary metabolites. Therefore plant associated microbes have the potential in pest control. The endophytic fungal metabolites showed pesticidal activity against major groundnut defoliator *S. litura*[30]. In several rye grasses that high fungi infection is correlated with a decrease in the attack frequency of the Argentine stem weevil, *Listronotus bonariensis*[15]. Several authors studied that the role of endophytic fungi in the control of insects[5,8,10-12,39].

The present study was directed to assess the endophytic fungi isolated from leaf and seed of *Annona squamosa* for the control of *Callosobruchus chinensis*.

Materials and Methods

Insect culture

Infected seeds were obtained from the local market and culture the insect, *Callosobruchus chinensis* in laboratory. The glass jars were cleaned and dried in oven at 60 to 70 ^{0}C. Fresh non-infected grains of *Vigna unguiculata* were purchased from the market and were manually screened to remove the infected or hollow grains. The disinfected grains were then washed and dried in the oven at 60 ^{0}C to kill the stages of life cycle of pests if any. Ten males and ten females were released in 500 g of these grains and allowed to maintain as stock culture.

Isolation of endophytic fungi

The plant parts of *Annona squamosa* were collected from local area of Aurangabad and brought to the laboratory. Collected plant parts (leaf, seed)were gently rinsed in running tap water to remove adhered dust and debris. The plant parts were surface sterilized with 0.1% HgCl, for two minutes followed by washing in 70% ethanol, after that plant parts were washed with distilled water. Small pieces upto 1 cm were cut and transferred into petridishes containing Potato Dextrose Agar medium (PDA) supplemented with Chloramphenicol antibiotic and incubated at 25 ± 2p C for 3-5 days. The fungus grown out from the plates were subcultured in PDA slants. The fungal mycelia growing out of the sample plates were continuously subcultured and maintained in PDA plates[42].

Preparation of fungal extract

The 100 mg of mycelium and spores of obtained endophytic fungi were collected separately from leaf and seed and extracted in 100 ml of methanol and ethyl acetate solvent as stock solution.

Insecticidal bioassay

The methanol and ethyl acetate extracts of endophytic fungi were screened for insecticidal activity against *Callosobruchus chinensis*. For screening 30g seeds were shaken thoroughly with various concentrations of methanol and ethyl acetate endophytic fungal extracts of leaf and seed in each jar. The dose was prepared by mixing the isolated endophytic fungi with respective solvent and was applied to grains. One jar of control containing seeds treated with only respective solvent was maintained. The treated seeds were allowed to evaporate the solvent for 48 hours. Five male and five females emerged in a batch were released in each experimental and control jar containing 30g seeds and mortality was recorded after 24h upto 96h of treatment. The percent mortality was calculated after 96h and the observed data was subjected to probit analysis [9,14].

Results

The toxic effect of isolated endophytic fungal extract from leaf and seed of *Annona squamosa* were evaluated against pulse beetle, *Callosobruchus chinensis*. The numbers of dead *Callosobruchus chinensis* were counted after 24, 48, 72 and 96h at different doses of methanol and ethyl acetate crude extract of endophytic fungi. The total percent mortality was observed after 96h. Then the corrected mortality was calculated and the results are presented. The results showed that the mortality increased with increase in concentration at all doses (Tables 1,2 & Figs. 1,2).

The results of the probit analysis for the estimation of LD_{10}, LD_{50}, variance, 95% confidence limits and regression equation at 96h for the mortality of pulse beetle, *Callosobruchus chinensis* are presented (Table-2). In bioassay of methanol endophytic fungal extract of leaf and seed were LD_{10}= 35.99mg/kg, LD_{50}=70.03mg/kg and LD_{10}= 12.46mg/kg, LD_{50}=37.33mg/kg respectively, and in ethyl acetate leaf and seed endophytic fungal extract were LD_{10}= 70.57mg/kg, LD_{50}= 112.4mg/kg and LD_{10}= 33.72mg/kg, LD_{50}= 65.11mg/kg respectively. Among the various estimate of regression based probit analysis, the $\div^2$ values for the regression coefficients showed homogeneity to the data.

Discussion

Annona squamosa has insecticidal activity due to the presence of essential oil, terpenes,

TABLE-1 : Mortality percentage of *Callosobruchus chinensis* treated with endophytic fungal extracts isolated from leaf and seed of *Annona squamosa*

Plant parts	Solvent	Dose (mg/30g)	No of insect used	Mortality after 96h.	Percent mortality	Corrected mortality
	Control	-	10	-	-	-
Leaf	Methanol	1.5	10	3	30	30
		2.0	10	4	40	40
		2.5	10	6	60	60
		3.0	10	8	80	80
		3.5	10	10	100	100
	Ethyl acetate	2.5	10	2	20	20
		3.0	10	4	40	40
		3.5	10	5	50	50
		4.0	10	7	70	70
		4.5	10	10	100	100
Seed	Control	-	10	-	-	-
	Methanol	0.5	10	2	20	20
		1.0	10	4	40	40
		1.5	10	6	60	60
		2.0	10	8	80	80
		2.5	10	10	100	100
	Ethyl acetate	1.0	10	1	10	10
		1.5	10	3	30	30
		2.0	10	5	50	50
		2.5	10	7	70	70
		3.0	10	10	100	100

TABLE-2 : LD_{10}, LD_{50}values with variance, 95% confidence limits and probit analysis parameters for adult of *Callosobruchus chinensis* after 96h of treatment.

Plant parts	Solvent	LD_{10} mg/kg	LD_{50} mg/kg	Variance	95% CL		Regression equations	x^2 (Degree of freedom)
					Lower	Upper		
Leaf	Methanol	35.99	70.03	0.002177	1.7539	1.9368	Y= -3.1833 + 4.4348x	0.3892 (2)
	Ethyl acetate	70.57	112.4	0.001086	1.9861	2.1153	Y= -8.0043 + 6.3414x	0.1455 (2)
Seed	Methanol	12.46	37.33	0.006109	1.4188	1.7252	Y= 0.7725 + 2.6893x	0.3002 (2)
	Ethyl acetate	33.72	65.11	0.002427	1.7172	1.9103	Y= -3.1353 + 4.4856x	0.0029 (2)

alkaloids- anonaine, roemerine, corydine, norcorydine, isocorydine and norisocorydine. The seed oil of *Annona squamosa* has been resulted to reduce the survival of leaf hopper, *Nephotettix virescens* (Hemiptera: Cicadellidae) and transmission of rice tungro virus[25,26]. The seed extract of *Annona squamosa* at 1.5 % concentration the highest mortality in *H. armigera* (43.33%) and 36.66 % mortality at 1% concentration in *S. litura*[41].

The isolated flavonoids from the aqueous extracts of *Annona squamosa* showed antimicrobial activity against all the common microbial contaminants of pulses and 80% insecticidal activity against *Callosobruchus chinensis*[20] at a concentration of 0.07 mg/ml-1. The adults *Tribolium castaneum* were repelled by contact with food medium treated with 2 and 5g *Annona squamosa* leaf powder/10 g flour[17]and the

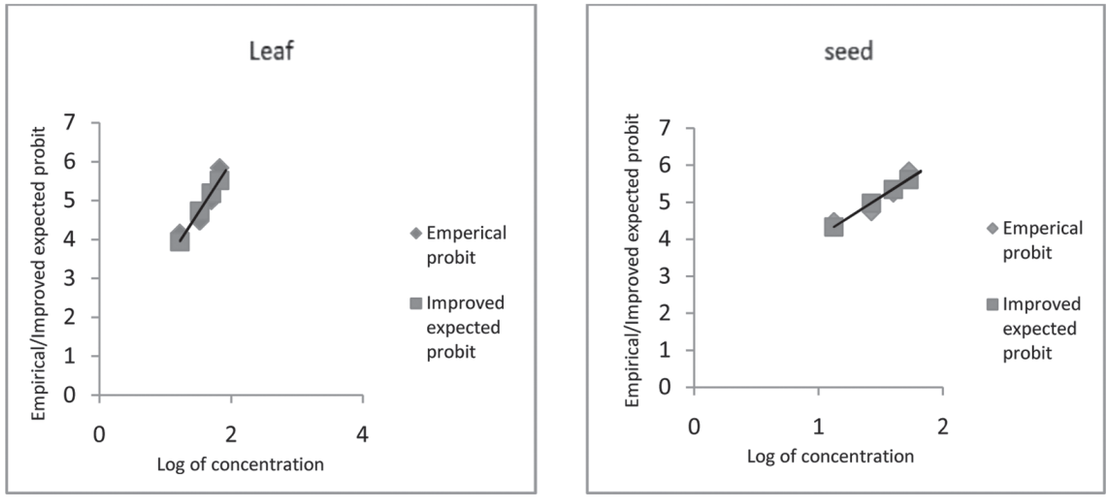

Fig. 1 : Regression and provisional lines for *Callosobruchus chinensis* exposed to methanol extract of endophytic fungi isolated from leaf and seed of *Annona squamosa* after 96h

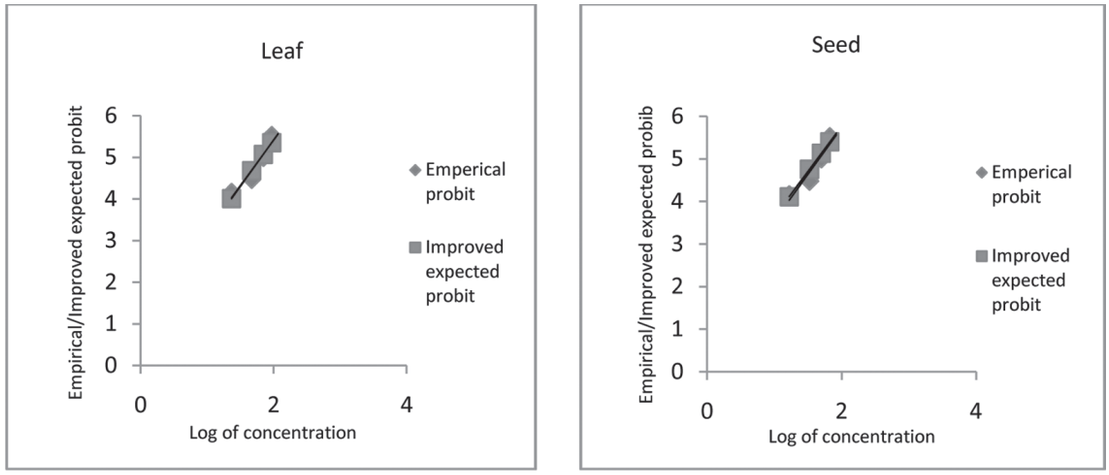

Fig. 2 :Regression and provisional lines for *Callosobruchus chinensis* exposed to ethyl acetate extract of endophytic fungi isolated from leaf and seed of *Annona squamosa* after 96 hours

seed oil is larvicidal against the rusty grain beetle *Tribolium castaneum*[23].

Several studies documented the growth and development inhibition properties of plant extracts on pulse beetle, *Callosobruchus chinensis.* The *Nerium indicum* bark extract was effective as insecticidal property against pulse beetle, *Callosobruchus chinensis*[31]. The biopesticidal effect of natural saponin isolated from *Acacia concinnna* against pulse beetle, *Callosobruchus chinensis*[7].

The endophytic microorganisms are those that inhabit the interior of plants, especially leaves and branches and stems, showing no apparently harm to the hosts[4].Endophytic fungi have received considerable attention in the last 20 years because of their capacity to protect hosts against insects pests and pathogens. Toxic metabolites produced by endophytic microorganisms in many plants can greatly reduce the population of associated insects. Toxic metabolites produced by endophytic microorganisms in many plants can greatly reduce the population of associated insects. The first reported example of plant protection to elm trees by an endophytic fungus, *Phomopsis oblonga* against the beetle, *Physocnemum brevilineum*[46].

The extracts of foliar fungal endophytes isolated from *Picea rubens*. (red spruce) needles were toxic to the forest pest *Choristoneura fumiferana*. (eastern spruce budworm) in dietary bioassays[29,43]. Toxic metabolites produced by endophytic fungi (*Epichloe* and *Neotyphodium* species) in fescue grasses greatly reduce the populations of associated herbivorous insects. These fungi produce various alkaloids that affect herbivore growth[13].The seven genera of endophytic fungi residing in *Vitex negundo* and mycelium extract of *Penicillium* sp. and *Fusarium* sp. revealed pesticidal activity[45].

Various agro active compounds such as herbicidal and pesticidal compounds have been isolated from endophytic fungi[21]. The metabolites of endophytic fungi showed pesticidal activity against *Spodoptera litura*[30].

The ethyl acetate extract of endophytic *Alternaria alternata* induced significant inhibitory effects on survival and reproductive potential of *Spodoptera litura*[19]. The antifeedant effects of *Trichilia connaroidae* fungal extracts were observed on *Peridroma saucia* and *S. litura* larvae [47]. Generally, the mortality depends on concentration[6,38]. Such a dose dependent mortality with spores of fungi was observed in *Sitophilus zeamais*[1] and *S. litura*[24].

The finding of the present investigation revealed that the endophytic fungal extract isolated from leaf and seed of *Annona squamosa* possess remarkable biopesticidal property against *Callosobruchus chinensis*. The study needs further investigation to find out active ingredients responsible for insecticidal properties against wide range of store grain pest and to reach any final recommendations.

References

1. ADANE, K., MOORE, D. AND ARCHER, S.A. (1996) Preliminary studies on the use of *Beauveria bassiana* to control *Sitophilus zeamais* (Coleoptera: Curculionidae) in the laboratory. *J. Stored Prod. Res.* **32** :105-113.
2. ALKOFAHI, A., RUPPRECHT, J.K., ANDERSON, J.E., MCLAUGHLIN, J.L., MIKOLAJCZAK, K.L. AND SCOTT, B.A. (1989) Search for new pesticides from higher plants. in: Arnason, J.T., Philog ene, B.J.R. and Morand, P. [Eds.] *Insecticides of Plant Origin. ACS Symp.* **387** 25-43.
3. ATWAL, A.S. AND DHALIWAL, G.S. (2005) Agricultural pests of South Asia and their management 5th edition. *Kalyani Publishers, New Delhi.* PP 390.
4. AZEVEDO, J.L. (1998) Microorganisms endofíticos. In: Ecologia Microbiana. Melo, I.S. and Azevedo, J.L. (edts.).*Editora EMBRAPA, Jaguariuna, Sao Paulo, Brazil.* 117-137.
5. AZEVEDO, J.L., MACCHERONI, JR. W., PEREIRA, J.O., LUIZ, DE ARAUJO W. (2000) Endophytic microorganisms: a review on insect control and recent advances on tropical plants. *Electronic J. Biotech.* **3** (1) : 40-65.
6. BAI, K.S. AND KANDASAMY, C. (1985) Laboratory induced mortality of *Spodoptera litura* fed on the leaf discs of castor treated with the extracts of *Vitex negundo* and *Stachytarpheta urticifolia. Indian J. Agri. Sci.* **55**: 760-761.
7. BAJAD, P.N. AND PARDESHI, A.B. (2016) Biopesticidal effect of natural saponin containing plant extract of *Acacia concinna* on pulse beetle, *Callosobruchus chinensis* (Linn.) (Coleoptera :Bruchidae). *Int. J. Curr. Res.* **8**(10): 39698-39701.
8. BREEN, J.P. (1994) Acremonium-endophyte interactions with enhanced plant resistance to insects. *Annu. Rev. Entomol.* **39**: 401-423.
9. BUSVINE, J.R. (1971) A Critical Review of the Techniques for Testing Insecticides. *Commonwealth Agricultural Bureau, London*. PP 345.
10. CARROLL, G. (1991) Fungal associates of woody plants as insect antagonists in leaves and stems. In: Microbial mediation of plant-herbivore interactions. Barbosa P, Krischik VA and Jones CG (edts.). *New York, John Wiley and Sons.* PP 253-271.
11. CARROLL, G. (1995) Forest endophytes: pattern and process. *Can. J. Bot.* **73**(1): S1316-S1324.
12. CLAY, K. (1989) Clavicipitaceous endophytes of grasses: their potential as biocontrol agents. *Mycol. Res.* **92**: 1-12.
13. CLAY, K. AND SCHARDL, C. (2002) Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. *Am. Nat.* **160**: 99-127.
14. FINNEY, D.J. (1947) Probit Analysis. Cambridge University Press, London, 333pp.
15. GAYNOR, D.L. AND HUNT, W.F. (1983) The relationship between nitrogen supply, endophytic fungus and Argentine stem weevil resistance in ryegrass. *Proceedings of the New Zealand Grassland Association.* **44**: 257-263.
16. GE, H.M. (2007) Penicidones A-C, three cytotoxic alkaloidal metabolites of an endophytic *Penicillium* sp. *Phytochemistry.* **69**: 571-576.
17. HUSSAIN, M. M., ALI, S.H., RAHIM, A. AND MONDAL, K.A.M.S.H. (1995) Studies on the repellent effect of two indigenous plants, biskantali (*Polygonum hydropiper*) and ata (*Annona squamosa*) leaf on *Tribolium castaneum* Herbst. *Bangladesh J. Sci. Indust. Res.* **30**: 81-85.
18. ISLAM, M.S., HAQUE, M.A., AHMED, K.S., MONDAL, M.F. AND DASH, C.K. (2013) Evaluation of Some Spices Powder as Grain Protectant against Pulse Beetle, *Callosobruchus chinensis* (L.). *Universal J. Plant Sci.* **1**: 132-136.
19. KAUR, H.P., SINGH, B., KAUR, A. AND KAUR, S. (2013) Antifeedent and toxic activity of endophytic *Alternaria alternate* against tobacco caterpillar, *Spodoptera litura. J. Pest. Sci.* **86**: 543-550.
20. KOTKAR, H. M., MENDKI, P.S., SADAN, S.V.G.S., JHA, S.R., UPASANI, S.M. AND MAHESHWARI, V.L.

(2002) Antimicrobial and pesticidal activity of partially purified flavonoids of *Annona squamosa*. *Pest Manag. Sci.* **58**: 33-37.

21. KUSARI, S., LAMSHOFT, M., ZUHLKE, S. AND SPITELLER, M. (2008) An endophytic fungus from *Hypericum perforatum* that produces hypericin. *J. Nat. Prod.***71**: 159–162.
22. LUGTENBERG, B.J., CHIN-A-WOENG, T.F. AND BLOEMBERG, G.V. (2002) Microbe– plant interactions: principles and mechanisms. *Antonie Leeuwenhoek*. **81**(1): 373–383.
23. MALEK. M.A. AND WILKINS, R.M. (1994) Effects of *Annona squamosa* L. seed oil on the pupae and adults of *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae). *Bangladesh. J. Entomol.* **4**: 25-31.
24. MANJULA, K., ARJUNARAO, P. AND NAGALINGAM, B. (2004) Record of *Nomuraea rileyi* (Farlow) Samson on *Helicoverpa armigera* Hubner in kharif Groundnut. *Indian J. Plant Prot.* **32**: 125.
25. MARIAPAN, V. AND SAXENA, R.C. (1983) Effect of custard apple oil and neem oil on survival of *Neophotettix virescens* (Homoptera:cicadellidae) on rice turgo virus transmission. *J. Econ. Entomol.* **76**: 573-576.
26. MARIAPAN, V., JAYARAJ, S. AND SAXENA, R.C. (1988) Effect of non-edible seed oil on survival of *Neophotettix virescens* (Homoptera:cicadellidae) on rice turgo virus transmission. *J. Econ. Entomol.* **81**: 1369-1373.
27. MCLAUGHLIN, J.L., ZENG, L., OBERLIES, N.H., ALFONSO, D., JOHNSON, H.A. AND CUMMINGS, B. (1997) Annonaceous acetogenins as new natural pesticides: recent progress. in: Hedin, P.A., Hollingworth, R.M., Masler, E.P., Miyamoto, J. and Thompson, D.G. [Eds.]. *Phytochemicals for Pest Control. ACS Symp.* Ser. No. 658. pp117-133.
28. MORTON, J.F. (1987) Fruits of Warm Climates. Media, Inc., Greensboro, NC, USA.
29. MILLER, J.D. (2008) Sumarah M.W. and Adams G.W., Effect of a rugulosin- producing endophyte in *Picea glauca* on *Choristoneura fumiferana*. *J. Chem. Ecol.* **34**: 362–368.
30. NAMASIVAYAM, S.K.R., SEKAR, S. AND BHARANI, R.S.A. (2014) Pesticidal activity of endophytic fungal metabolites against major groundnut defoliator *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae). *J. Biopest.* **7**(Supp.): 116-121.
31. PARDESHI, A. AND ZAMBARE, S.P. (2012) Biopesticidal effect of *Nerium indicum* bark extracts on pulse beetle, *Callosobruchus chinensis* (Linn.). *Int. J. Sci.* **1(**2) : 122-125.
32. PATEL, N.G. (2011) A Study on Control Measure of Pulse Beetle *Callosobruchus chinensis* (L.) Bruchidae: Coleoptera. *Int. J. Biotech. Biosci.* **1**: 25-35.
33. RAHMAN, A. AND TALUKDER, F.A. (2006) Bio efficacy of some plant derivatives that protect grain against the pulse beetle, *Callosobruchus maculatus*. *J. Insect Sci.* **6**(3): 19-25.
34. RAJA, M. AND WILLIAM, S.J. (2008)Impact of volatile oils of plants against the Cowpea Beetle *Callosobruchus maculatus* (Fab.) (Coleoptera: Bruchidae). *Int. J. Integr. Biol.* **2**: 62-69.
35. RATNAYAKE, S., RUPPRECHT, J.K., POTTER, W.M. AND MCLAUGHLIN, J.L. (1992) Evaluation of various parts of the paw paw tree, *Asimina triloba* (Annonaceae) as commercial sources of the pesticidal annonaceous acetogenins. *J. Econ. Entomol.* **85**: 2353-2356.
36. RUPPRECHT, J.K., HUI, Y.H. AND MCLAUGHLIN, J.L. (1990) Annonaceous acetogenins: review. *J. Nat. Prod.* **53**: 237-276.
37. SAGHEER, M., HASAN, M., ALI, Z., YASIR, M., ALI, Q., ALI, K. AND KHAN, F.Z.A. (2013) Evaluation of essential oils of different citrus species against *Trogoderma granarium* (Everts.) (Coleoptera: Dermestidae) collected from Vehari and Faisalabad districts of Punjab, Pakistan. *Pakistan Entomologist.* **35**: 37-41.
38. SAHAYARAJ, K. AND SEKAR, R. (1996) Efficacy of plant extracts against Tobacco caterpillar larvae. *Int. Arac. Newsletter.***16**: 38-39.

39. SAIKKONEN, K., FAETH, S.H., HELANDER, M. AND SULLIVAN, T.J. (1998) Fungal endophytes: A continuum of interactions with host plants. *Annu. Rev. Ecol. Syst.* **29**: 319-343.

40. SECOY, D.M. AND SMITH, A.E. (1983) Use of plants in control of agricultural and domestic pests. *Econ. Bot.* **37**: 28-57.

41. SONKAMBLE, M. M., DHANORKAR, B.K., MUNDE, A.T. AND SONKAMBLE, A.M. (2000) Efficacy of indigenous plant extracts against *Helicoverpa armigera* (Hubner) and *Spodoptera litura* (Fabricius) under laboratory conditions. *J. Soils Crops.* **10**: 236-239.

42. STROBEL, G. AND DAISY, B. (2003) Bioprospecting for microbial endophytes and their natural products. *Microbiol. Mol. Biol. Rev.* **67**: 491-502.

43. SUMARAH, M.W., PUNIANI, E., SORENSEN, D., BLACKWELL, B.A. AND MILLER, J.D. (2010) Secondary metabolites from anti-insect extracts of endophytic fungi isolated from *Picea rubens*. *Phytochemistry.***71**:760-761.

44. TESFU, F. AND EMANA, G. (2013) Evaluation of *Parthenium hysterophorus* L. powder against *Callosobruchus chinensis* L.(Coleoptera: Bruchidae) on chickpea under laboratory conditions. *African J. Agri. Res.* **44**: 5405-5410.

45. THYAGARAJAN, R. AND NAMASIVAYAM ,S.K.R. (2011)Searching of biological activities of endophytic fungi residing in leaves of *Vitex negundo* (L). *J. Pharm. Res.* **4**: 1654-1655.

46. WEBBER J. (1981) A natural control of Dutch elm disease. *Nature, London.* **292**: 449-451.

47. XIE, Y.S., ISMAN, M.B., GUNNING, P., MACKINNON, S., ARNASON, J.T., TAYLOR, D.R., SANCHEZ, P., HASBUN, C. AND TOWERS, G.H.N. (1994) Biological activity of crude extracts of *Trichilia* species and the limonoidhirtin against lepidopteran larvae. *Biochem. Syst. Ecol.* **22**: 129–136.