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Research Article

BIOCIDAL ACTIVITYOFDIFFERENT EXTRACT OF *GLYCOSMIS PENTAPHYLLA AGAINST* THE RICE WEEVIL *SITOPHILUS ORYZAE* (L.) (COLEOPTERA: CURCULIONIDAE)

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ABSTRACT

The rice we evil, *Sitophilus oryzae* is one of the major pests of stored grains. This study investigates the bio efficacy of the *Glycosmis pentaphylla* in controlling infestations of the rice weevil. Different doses of aqueous, ethanolic and acetone extracts of the leaves of the plant *Glycosmis pentaphylla* were checked and the results showed that high doses of the extracts were significantly more toxic to *Sitophilus oryzae*(L.) compared to lower doses. The efficacy of the extracts on the insects was dose-dependent. LD50 value was assessed. In the present investigation, acetone extracts of *Glycosmis pentaphylla* have shown very high toxicity to *Sitophilus oryzae* Linn. After the extract exposure of the plant, enzyme activity-digestive enzyme-amylase and protease, lactic dehydrogenase, Lipid, Nuclease and catalase of the insects were checked. Enzyme activity was found to be depleted compared to control. Acetone extracts of *Glycosmis pentaphylla* have exerted toxic effects on enzymatic parameters of *Sitophilus oryzae*, it proved to have strong insecticidal activity, So it could be integrated into pest management system.

INTRODUCTION

Insects have been causing tremendous losses not only to the crops growing in fields but also to post-harvest commodities during storage. In different ways stored products are attacked by insects. In storage, rice is damaged by a number of insect pests, particularly by rice we evil, *Sitophillus oryzae*(L.) (Coleoptera: Curculionidae). The rice weevil adult gathers and reproduces in stored grains and cause a great damage and loss according storage period (Asawalam *et al*., 2012) Synthetic chemicals were proved highly toxic to non-target organisms, entered in the food chain and put adverse impact on the environment. Insect pests have developed resistance to many commercially available synthetic pesticides. Resistance and toxicity problems of the synthetic insecticides have resulted in the necessity of finding more effective and healthier alternatives. Plants may provide potential alternative to chemical pesticides because they constitute a rich source of bioactive chemicals. Research reveals that extracts prepared from plants have a variety of properties including insecticidal activity, repellency to pests, anti feedant effects, insect growth regulation, toxicity to nematodes, mites and other agricultural pests, also antifungal, antiviral and antibacterial properties against pathogens (Prakash and Rao, 1997). *Glycosmis* ***pentaphylla*** is an evergreen medicinal herb widely present in India with aromatic leaves used in the *treatment* of cough, rheumatism, anaemia, jaundice and ascariasis (Gangarao & Jayaraju *et al.*2009) fever and liver complaints, eczema and skin affections (Mandal *et al*.2011).

The test plant would yield environmentally sound chemicals having no harmful effects on the non target organisms. Keeping this in view, the present study was carried out to test the efficacy of the different leaf extracts of the plant *Glyosmis pentaphylla* against the stored product pest *Sitophilus oryzae*.

MATERIALS AND METHODS

Culturing of test insects

The pest, *Sitophilus oryzae,* was collected from stored rice from a local shop. Fresh rice was washed and dried in sunlight. This rice was taken in containers and the insects were transferred to it. Thus stock cultures were prepared. Holes were drilled on the container lid for permitting the passage of air. The culture was maintained at room temperature. For getting newly emerged adults, 100 insects, including both male and female, from the stock were transferred to fresh rice. They were allowed to lay eggs on fresh rice. Then after 2 weeks, they were

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removed, and the rice containers were kept undisturbed. On the sixth week, new insects began to emerge in the containers. These insects were used for further studies. The stock culture was cleaned by sieving once in five days. This helps to remove the food waste and faecal matter of the insects to avoid fungal attack.

Preparation of aqueous extract of plants

The plant leaves were collected and washed well with distilled water. The leaves were ground without adding water. The ground mass was then transferred into a beaker containing 100 ml of distilled water. Then it was mixed well and kept for three days. After three days the mixture was filtered. Then this mixture was kept in a water bath at 60-70^{0}C. After drying, this residue is dissolved in water and made up to different concentrations.

Preparation of acetone and ethanol extracts of plants

For the extraction, soxhlet apparatus was used. About 25g powder of each plant leaves were extracted with 250ml ethanol and acetone. The extraction of each plant sample was done in about 12 hrs. After soxhlet extraction; the material was run on rotary evaporator. The extracts were concentrated on rotary evaporator by removing the excess solvent under vacuum. After evaporation of solvent with rotary evaporator the remaining extracted material was kept in a water bath for removing remaining solvent from the extracts. The extracts were stored at 4°C prior to application.

Treatments

The extracts were applied at different doses on Whatmann No. 1 filter paper and air-dried for an hour. The controls were treated with acetone or ethanol or distilled water only. The treated and control filter paper discs were placed singly at the bottom of plastic jars and 20gm of rice were placed on the papers. Ten insects *were* released in each plastic container. There were five replicates for each *treatment* and control. Observations were recorded on the seventh day of treatment.

Enzyme assay

Bioassay of enzyme was conducted on both control and insects, treated with sub lethal doses of *Glycosmis pentaphylla*.

Assay of digestive enzyme –a) Amylase

Activity of digestive enzyme-amylase was measured according to the method of Neolting and Bernfield, (1948)**.**The reaction mixture comprised 0.4 ml 1 % starch solution as substrate,0.2 ml Tris-HCl buffer of Ph 8.2. The reaction mixture was incubated at 37^0 c for 20 minutes and the enzyme activity was terminated by adding 1.2 ml 3,5 dinitro salicylic acid reagent and heating the mixture at 100^{o}c for 5 minutes. It was cooled and diluted to 10 ml by adding distilled water. A reaction mixture containing denatured enzyme prepared by heating the extract at 100^{0}c for 10 minutes was run side by side as blank while estimating the enzyme activities. The difference between the OD units of the experimental and blank samples was taken as actual OD units of the experimental sample.

The absorbency of the sample was read at 550 nm with a spectrophotometer. From the OD units' µg of maltose equivalents liberated was calculated using 0.01 % maltose solution as standard.

Protease

To estimate the Protease activity of adult *Sitophilus oryzae*by using the method of Sreekumar and Prabhu (1988) The incubation mixture comprised of 0.4 ml 1% casein (vitamin free) solution, 0.2 ml glycine NaOH buffer of P^H 9 and 0 .2 ml enzyme extract. It was incubated at 37^{0}c for 30 minutes and the reaction was terminated by adding 1.2 ml of 5% tri chloro acetic acid. The mixture was heated at 100^{0}c in water bath for 5 minutes to ensure complete coagulation of proteins. The mixture was then filtered through Whatman No.1 filter paper to obtain a clear solution and made up to a convenient volume of 10 ml for reading absorbency at 280 nm with spectrophotometer. 0.01 % tyrosin solution was used as standard. A reaction mixture containing denatured enzyme prepared by heating the extract at 100^{0}c for 10 minutes was run side by side as blank while estimating the enzyme activities. The difference between the OD units of the experimental and blank samples was taken as actual OD units of the experimental sample.

Assay of Lactic Dehydrogenase(LDH)

Activity of lactic dehydrogenase was measured according to the method of Annon(1984). For this purpose, 100 mg of insects were homogenized in 1.0 ml of 0.1 M phosphate buffer (pH 7.5) in ice bath and centrifuged at 10000 X g for 30 minutes in cold centrifuge at 4 °C. Supernatant was used as enzyme source. For determination of enzyme activity 0.05 ml of enzyme source was added to 0.50 ml of pyruvate substrate. Then, the contents were incubated at 37 °C for 45 minutes. Now 0.50 ml of 2,4- dinitrophenyl hydrazine solution was added and the contents were mixture and kept at the room temperature. After 20 minutes, 5.0 ml of 0.4 N NaOH was mixed and left for 30 minutes at room temperature. The optical density was measured at 540 nm and it was converted to LDH unit by drawing a standard curve. Standard was done by pippeting 0.2ml, 0.4ml, 0.6 ml, 0.8 ml and 1 ml of pyruvic acid. Standards were treated in the same way as the sample and the absorbance was measured at 540 nm.

Determination of lipids

Activity of lipid was measured according to the method of Zollner and Krich,(1962)20 mg of insects were homogenized in 10ml of chloroform-methanol mixture using a glass homogenizer, filtered through Whatman No.1 filter paper and to this was added 2ml of 0.9% NaCl solution. This mixture was shaken well and transferred to a separating funnel and was allowed to stand over night at 4°C.

A clear biphasic layer was formed with the lower phase containing all the lipids. It was removed and the volume was made up to 10ml by the addition of chloroform. This was transferred to a 50ml beaker and the solvent was allowed to evaporate at 50-60°C for 5 hours. Then 5 ml of concentrated sulphuric acid was added to it, mixed well, placed in boiling water bath for ten minutes and was then cooled to room temperature. 0.2ml of this was taken in a test tube and 5ml of phosphovanillin reagent was added. Mixed well and was allowed to stand for half an hour. Standard was prepared by

mixing 0.2ml of standard cholesterol and 5ml of phosphovanillin reagent. Read test and standard against the blank at 520nm

Determination of Nucleic Acids

Level of nucleic acids in the whole body extracts of *Sitophilus oryzae* (Linn.) was estimated according to method of Scheidner (1957). For this purpose a total 500 mg of *Sitophilus oryzae* were fed with sublethal doses of different solvent extracts of *Glycosmis pentaphylla* separately. Insects were scarified and homogenized in 5% TCA with glass-glass homogenizer at 15,000Xg for 25 minutes.

DNA Estimation

For DNA estimation, 0.2 ml of supernatant was taken and it was diluted by adding 3.8 ml of distilled water. Then 4.0 ml of diphenylamine reagent (1 gm of diphenylamine, 100 glacial acetic acid and 2.5 ml of conc. H_2SO_4) were added to it. The mixtures were kept in boiling water bath for 10 minutes. A blue colour was developed in the solution which is measured at 595 nm (O.D.)

RNA Estimation

For RNA estimation 0.2 ml of supernatant was taken and it was diluted by adding 4.8 ml of distilled water. Now 2ml of orcinol reagent (1 gm orcinol, 100 ml conc. HCl and 0.5 gm ferric acid) was added to it. The solution was kept in boiling water bath for 10 minutes, a green colour was developed, which was measured at 660 nm.

Statistical analysis of data

The data obtained are recorded as mean± standard deviation. For testing the significance of the data obtained, statistical analysis were carried out using ANOVA ($p \leq 0.05$) using SPSS software. LD 50 was calculated using probit analysis.

RESULTS

Effect of plant extracts on mortality of insects

The total number of adult insects surviving after the *treatment* was recorded for seven days consecutively. The percent mortality was then calculated. Acetone and Ethanol extracts of the plant showed significant mortality compared to the aqueous extract. (Table 1) No mortality was seen in the case of control. LD 50 was calculated using probit analysis

Table 1 Effect of plant extracts on mortality of insects

Dose (%)	Mortality (%) *Glycomis pentaphylla*		
	Acetone extract	Ethanol extract	Aqueous extract
1%	41±0.44	41±0.44	31±0.40
5%	48±0.20	46±0.09	42±0.20
10%	75±0.45	74±0.14	51±0.45
15%	81±0.54	75±0.14	59±0.44
20%	84±0.45	78±0.45	70±0.40

Table 2 LD 50 of different extracts of *Glycosmis pentaphylla* on *Sitophilus oryzae*

Plant	Extracts	95 % Confidence limit		
		LD 50(%)	Lower limit	Upper limit
Glycomis pentaphylla	Aqueous	10.141	3.155	4.870
	Ethanol	4.385	2.849	5.640
	Acetone	4.067	9.278	11.003

Table 3 Sub lethal doses of adult insects

Plant	Sub lethal dose(%)		
	Aqueous	ethanol	acetone
Glycomis pentaphylla	10	4	3.8

Determination of Enzymes

Sub-lethal concentration of different extract of *Glycosmis pentaphylla* showed significant alterations in the biological activity of certain metabolic enzymes in *sitophilus oryzae* (L). Acetone extract has shown higher inhibitory activity against the enzymes and significantly reduced the body content of Amylase(8.16±0.04) Protease (8.04±0.01), LDH (5.98±0.02), Lipid (2.94±0.01), Nuclease-DNA(0.194±0.01) RNA (0.165±0.01) and catalase (1.36±0.01) (Table 4)

Table 4 Effect of sublethal doses acetone extract of *Glycosmis pentaphylla* on Amylase, Protease, LDH, Lipid, Nuclease and catalase of *sitophilus oryzae*(L)

Parameters	Control	aqueous	ethanol	acetone
Amylase (Units/mg protein)	14.32±0.01	11.92±0.02	9.26±0.02	8.16±0.04
Protease(Units/mg protein)	12.63±0.04	11.22±0.02	9.06±0.01	8.04±0.01
LDH(μ/min/mg)	8.32±0.01	7.92±0.04	7.08±0.03	5.98±0.02
Lipid(μg/gm)	6.36±0.01	4.65±0.04	3.95±0.03	2.94±0.01
DNA(Units/mg)	0.484±0.01	0.412±0.04	0.306±0.03	0.194±0.01
RNA(Units/mg)	0.434±0.01	0.312±0.04	0.296±0.03	0.165±0.01
Catalase(mM/min/g)	3.81±0.01	2.92±0.04	1.98±0.03	1.36±0.01

Values are mean ±SE of five replicates. All values are significant at $p \leq 0.05$ level of significance.

DISCUSSION

In the present study, it was found that both solvent and aqueous extracts of selected plants-*Glycosmis pentaphylla* had toxic effect against *Sitophilus oryzae* Linn. Significant mortality of the insect pest was observed in a dose dependent manner .Reports show that deterrent effects of plant compounds are concentration dependant and may lead to complete mortality at high concentration (Jermy,1990). The toxicity was possibly caused by the bioactive compounds present in the leaves of the selected plants. Many of these compounds are insect repellents or act to alter insect feeding behavior, growth and development, ecdysis and behaviour during mating and ovi position (Duke, 1990).

In this study Enzyme activity was found to be depleted compared to control. The activity of digestive enzyme – Amylase and Proteae were decreased in acetone treated sets of *Glycosmis pentaphylla* compared to other. The reduction of amylase and protease activity by plant extracts could be due to the plant defense compounds that act on insect gut enzymes - amylases, and proteases (Ryan 1990; Franco *et al*. 2002). Also, the reduction of this enzyme activity could be due to a cytotoxic effect of different extracts on epithelial cells of the midgut, which synthesize amylase (Jbilou *et al*. 2008).

Botanical insecticides affected the LDH activity ie, there was a decrease in LDH activity at different extract tested. The effect of the biopesticides resulted in a considerable decrease in enzyme activity, indicating strong enzyme inhibition. A decrease in LDH activity shows reduced metabolism in the insect and may be due to the toxic effects of plant derivatives

on membrane permeability, on the gut epithelium (Senthil Nathan *et al*., 2005, 2005a).

Lipases play a major role in storage and lipid mobilization. These enzymes are also the basic components in many of physiological process like, reproduction, growth, and defense against pathogens. In this study the amount of lipid is decreased in acetone treated sets of *Glycosmis pentaphylla* compared to other. Senthil-Nathan *et al*. (2006a) showed that treating C. medinalis, the rice leaf folder, with Btk (Bacillus thuringiensis Kurstaki), NSKE (neem seed kernal), and VNLE (Vitexnegundo leaf extract) (azadirachtin and neem components) sharply decreased the activity level of lipase in the midgut. Present study shows that highest reduction in nuclease activity-DNA and RNA were obtained in acetone extract treated diet of *Glycosmis pentaphylla* than other. Protein and Nucleic acid synthesis may be blocked at cellular level and catabolism get increased which results into low availability of protein and nucleic acid.

Catalase is an anti oxidant enzyme in insects. Reduction in catalase activity shows the inability of the insects in overcoming the stress occurred due to plant extract treatment. However, this imbalance in enzyme level indicates inhibition of important metabolic pathways (Ishaaya, I. and J.E. Casida, 1980) Enzyme activities have also been used as susceptible indicators of stress when the insects are treated with extract treated diet.

Hence, all significant changes in the level of enzyme and mortality indicates very high insecticidal activity of the *Glycosmis pentaphylla* extracts towards the *Sitophilus oryzae*This study arise scope for further investigations on the use of botanical products as alternatives of synthetic chemical pesticides in the effective control of the serious pest *Sitophilus oryzae*. Therefore, it is recommended that active ingredients from *Glycosmis pentaphylla* could be used for preparation of herbal insecticidal formulation to control stored grain insects.

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