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The Potential of Using Insecticidal Properties of Medicinal Plant *Gymnema sylvestre* (R.br) Against *Sitophilus oryzae* (L.)

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Abstract: Preservation of herbs in storage is essential for safer consumption. Insect pest damage leads to losses in quantity and quality of herbal products in storage. Hence to manage storage pests, botanicals extracted from the medicinal herb, *Gymnema sylvestre* were tested for their insecticidal activities. Seven different formulations of *G. sylvestre* leaves were studied for their effect on mortality and progeny production against stored product pest, *Sitophilus oryzae*. Adults of *S. oryzae* were exposed to the treated rice and the mortality was assessed after 24h, 72h, 7days, 14days and 21 days of exposure. Then all adults were removed and the treated substrate remained at the same conditions for an additional 30 days, after this interval the commodity was checked for progeny production. Exposed pest showed mortality in all formulations and the average mortality percentages indicated that the extracts used caused significant mortality on the target insect. Observed mortality percentage increased with increase in time intervals after application but the extract concentration had no significant effect. Cumulative mortality (71.1%) and progeny suppression (60.9%) were higher in leaf extract. Separately ethanolic extract was assessed for mortality by residual film assay and the mortality was 100% at 24h at 100mg dosage. LD_{50} value was found as 25mg and 17.5 mg for 24h and 48h, respectively .Therefore, these results indicate that *G. sylvestre* can be used for protection of stored products from infestations of stored product insect pest *S. oryzae*.

Key words: *Gymnema sylvestre* % Progeny production % Mortality % *Sitophilus oryzae* % Leaf extracts

INTRODUCTION

Gymnema sylvestre R.Br belonging to the family Asclepiadaceae is naturally found in tropical forests of India and Sri Lanka. This herb is best known for its ability treat of diabetes mellitus. In Sri Lanka, there are two varieties of *G. sylvestre* (Colombo and Jaffna) grown and are geographically differentiated. The leaves are used traditionally by the public and the Ayurvedic medical practitioners in indigenous medicine to treat diabetes mellitus. It has been identified as a neutraceutical compound with a complete functional food [1].

Nowadays considerable efforts have been focused on plant derived materials, potentially useful as commercial insecticides. Toxic effects of plant products on some pests have been studied by many researchers. Different types of plant preparations such as powders, solvent extracts, essential oils and whole plants are being investigated for their insecticidal activity including their action as fumigants, repellents, antifeedants, anti ovipositions and insect growth regulators [2, 3].

Sitophilus oryzae (Coleoptera: Curculionidae) is considered as a major pest of stored grain. Control of this insect relies heavily on the use of synthetic insecticides and fumigants. But their widespread use had led to some serious problems. Thus, arising need for residual free food encourages the development of alternative reduced risk methods for stored grain protection. On this way information on the insecticidal properties of *G. sylvestre* plant is not available. But larvicidal effect was studied against *Culex quinquefasciatus* larvae at the concentrations of 1, 2, 3, 4 and 5% up to three days. Larval mortality was 100% with the use of 5% concentration after 2 days [4]. Hence, this study was focused to investigate various *G. sylvestre* leaf extracts for insecticidal activity against pest of stored products *S. oryzae*.

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MATERIALS AND METHODS

Plant extracts were prepared following eight different procedures. They are,

Leaf Extract: Fresh leaves of *G. sylvestre* were crushed in grinder without any solvent and extract was used in the experiment.

Leaf Powder: Leaves were dried in open air and the dried leaves were pounded into fine powder using a blender. The plant powder was exposed to 60°C for 1 hour and then stored in air sealed polythene bags at room temperature before using.

Leaf Powder Extract: Leaf powder was extracted in water for one hour and strained. Extract was used in the experiment.

Leaf Powder Paste: Leaf powder was mixed with water and made into slurry and was used directly. Leaf powder extract contains water solubles only and Leaf powder paste contains all compounds.

Gymnemic Acid Powder: Leaf powder (100g) was extracted in boiling water (1 L) followed by through shaking for ten minutes. Then the mixture was allowed to cool to room temperature and strained. This operation was repeated three times with the same powder. This hot water-extraction solution was adjusted into pH 2 with a 2N hydrochloric acid solution to give a precipitate containing crude gymnemic acid [1]. The resulting suspension was cooled in a refrigerator at 5°C for 5 hours. The supernatant solution was decanted and the separated precipitate (gymnemic acid) was transferred into a tray and dried in an oven at 60°C for 12 hours.

Gymnemic Acid Paste: The separated precipitate (gymnemic acid) was used in the experiment

Gymnemagenin Extract: The separated precipitate was adjusted into pH 7 with a 1N solution of KOH to give the gymnemic acid in the form of K salt called gymnemagenin. The resulting mixture (gymnemagenin) was transferred into a tray and dried in an oven at 60°C for 24 hours [5] and then this was dissolved in water.

Ethanol Extract: 100g leaf powder was successively extracted by means of cold extraction with the ethanol. In the first step, the powder was soaked with intermittent shaking with ethanol for three days at room temperature and thereafter the supernatant was filtered through Whatman No 1 filter paper to remove the fine particles. This extraction procedure was carried out eight times with the same solvent. Then the extracts obtained in eight times were combined together and the solvent in the extract was removed by using rotary evaporator under reduced pressure. Then the crude extract was transferred in to a glass vial and stored until used. The yield of the extract was also determined by measuring the weight.

Test Insects and Commodity: The adults of *S. oryzae* were taken from the culture that was kept on rice at room conditions. All individuals used in the test were 7 days old. Untreated clean rice was used as diet.

Bio Assay: All tests were conducted at room temperature and RH (about 27°C and 50%). Test solutions were prepared of seven different extracts other than ethanol extract. Leaf and leaf powder extracts were prepared using 2g, 4g and 6g leaves and powder. For other extracts 0.2g, 0.4g and 0.6g doses were used. Extracts were mixed with the diet at the mentioned concentrations. Each sample (20g) was placed in a small rearing chamber. In the meantime, control experiments was carried out without any treatments. Three replicates were set up for the treated and control insects. To assess the effects of different extracts on mortality and progeny production twenty *S. oryzae* adults were introduced in the rearing chamber and then covered with muslin cloth. The chambers were kept undisturbed until counting. Dead adults were counted after 24 hours, 72hours, 7days, 14days, and 21 days. After 21 days of mortality count, all adults (dead and alive) were removed and the rearing chambers were left in the same condition for an additional period of one month. Then the chambers were opened and the emerged individuals were counted. All the emerged individuals were adults, because the larvae of this species developed inside the grain kernels [6].

Dose-Mortality Test Through Residual Film Assay: Extracts of 25, 50, 75, 100, 200 and 300mg were separately mixed with 1ml of ethanol. The 100mg extract was diluted by serial dilution method for obtaining 10, 1, 0.1, and 0.01mg/ml. The prepared doses were used in the experiment of residual film assay. For each dose one ml was dropped on a Petri dish (90 mm diameter) in such a way that it made a uniform film over the Petri dish. Then the Petri dishes were air-dried leaving the extract on it. After drying even aged 7 days old ten *S. oryzae*

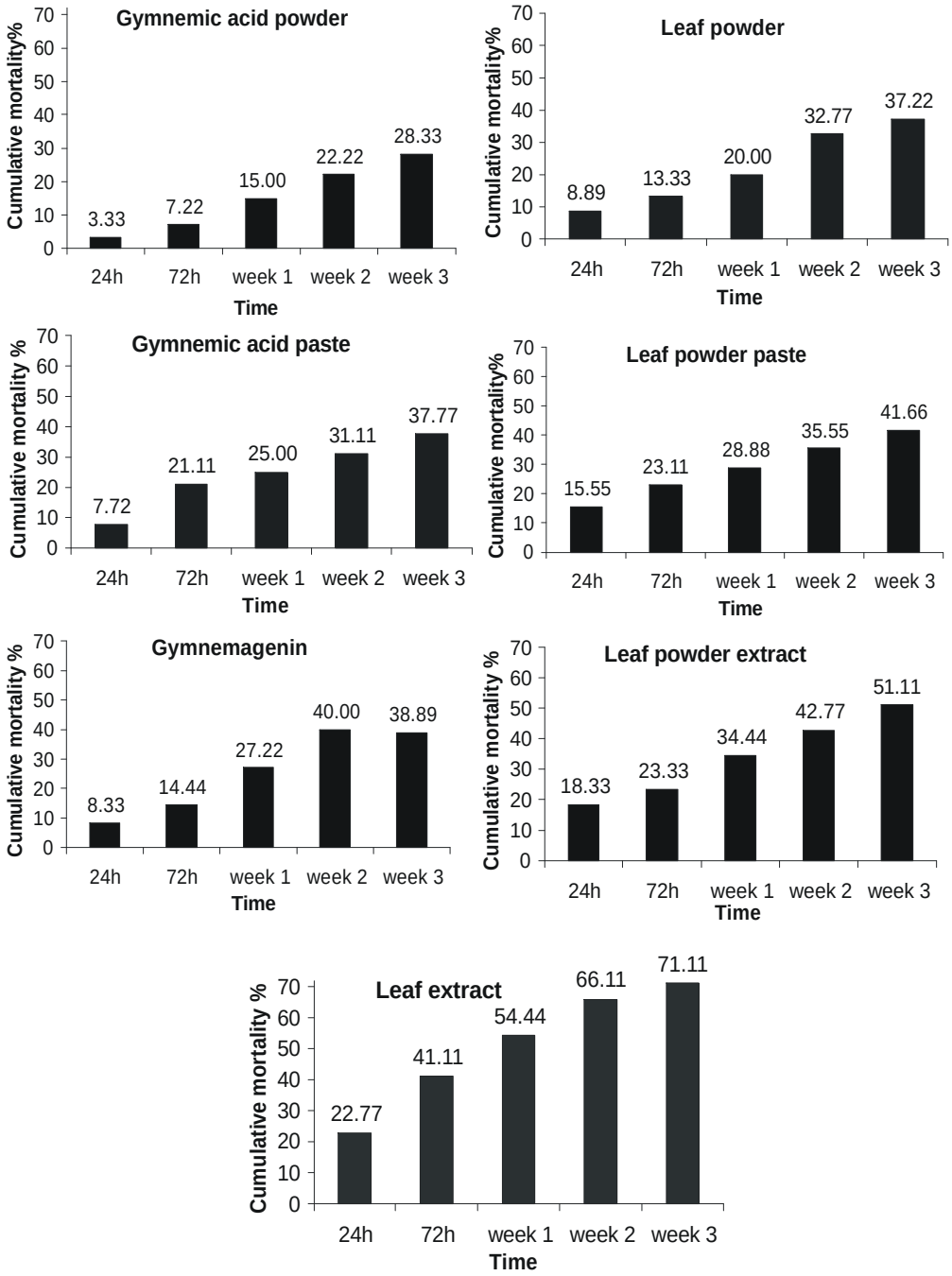

Fig. 1: The percentage mortality of S. oryzae adults exposed for different periods on rice treated with seven different formulations of G. sylvestre leaves.

adults were released into each Petri dish separately. The treatments were replicated thrice. A control batch was also maintained with the same number of weevils and evaporating the solvent only. The treated weevils were placed undisturbed. The mortality of the weevils was counted after 24 and 48 hours of treatment [7].

$$\text{Progeny reduction (\%)} = \frac{\text{Number of progeny in control-Number of progeny in treatment}}{\text{Number of progeny in control}} \text{ X } 100$$

Data Analysis: Mortality data was analyzed by using statistical programme SAS version 8 .The percentage of reduction in progeny production was determined by the following formula described by Khoshnoud [6].

Table 1: The percentage of reduction in progeny production of *S. oryzae* on rice treated with different formulations of *G. sylvestre* leaves.

Extracts	Progeny reduction percentage of *S. oryzae*
Gymnemic acid powder	13.6
Leaf powder	20.6
Gymnemic acid paste	29.3
Leaf powder paste	48.6
Leaf extract	60.9
Leaf powder extract	58.9
Gymnemagenin extract	54.3

Table 2: The mean percent mortality of *S. oryzae* on rice treated with ethanol extract of *G. sylvestre* leaves

Extract concentration		Mean percent mortality	
mg	mg/cm^2	24 h	48 h
300	1.178	100.0 (5.2983)a	100.0 (5.29834)a
200	0.785	100.0 (5.2983)a	100.0 (5.29834)a
100	0.392	100.0 (5.2983)a	100.0 (5.29834)a
75	0.294	83.33 (5.1145)ab	90.00 (5.19299)a
50	0.196	76.66 (5.0307)b	86.66 (5.15373)a
25	0.098	16.66 (3.4591)c	46.66 (4.53091)b
10	0.039	0 (0.0000)d	26.66 (3.95958)c
1	0.0039	0 (0.0000)d	0 (0.0000)d
0.1	0.00039	0 (0.0000)d	0 (0.0000)d
0.01	0.000039	0 (0.0000)d	0 (0.0000)d
Ethanol		0(0.0000)d	0 (0.0000)d

For biofilm assay, the percent mortality was subjected to statistical analysis [8, 9]. The dose mortality relationship was expressed as a median lethal dose (LD_{50}).

RESULTS AND DISCUSSION

Extracts of medicinal plants have potential to repel the insects and often kill them due to the availability of toxic material found in their plant parts. Similarly experiments on the different plant extracts of *G. sylvestre* was conducted against stored product insect, *S. oryzae* to understand the insecticidal properties and the results are found promising.

Effect of Various Extracts on Mortality: Mortality percentages of *S. oryzae* in all extracts used were significant with control mortality. Mortality of *S. oryzae* adults increased with increase of exposure interval for all tested extracts (Figure 1) but the concentration had no effect. The mortality effects of the different extracts can be arranged in a descending order as follows : leaf extract> leaf powder extract > leaf powder paste> gymnemagenin> gymnemic acid paste> leaf powder> gymnemic acid powder. The results of this study showed that these extracts are effective against *S. oryzae* but the effectiveness is determined by the characteristics of the extract and exposure interval. One of the most interesting findings of this study is the dissimilar efficacy of extracts of the same plant species and therefore leaf extract was superior in causing mortality and progeny reduction against *S. oryzae* than others. Longer intervals are needed to the satisfactory level of mortality. The findings of this study agree with the earlier reports that most of the plant extracts have insecticidal properties and control pest through affecting the biological activities [10-13].

Effect of Various Extracts on Progeny Production: One of the basic characteristic of an effective grain protectant is the ability to reduce progeny production in the treated commodity. This is shown in the results with using seven extracts (Table 1). Progeny production of *S. oryzae* was highly reduced when rice was treated with leaf extract. Females of *S. oryzae* laid their eggs on the kernel and newly hatched larvae were exposed to extract before entering the kernel. The extracts suppressed oviposition or killed the larvae. These results suggested that variations existed in using different extracts.

Effect of Ethanol Extract on Mortality: The dose mortality of the ethanol extract against *S. oryzae* has been found strongly effective and the findings are given in Table 2. The extract showed 100% mortality against *S. oryzae* at 100mg and the range of activity was short. LD_{50} value was found as 25mg and 17.5 mg for 24h and 48h, respectively. The mortality was found to be dose dependant.

CONCLUSION

Storage and preservation of medicinal herbs has been given more concern to use the herbal parts for the preparation of medicine. Identifying the insecticidal or repellant properties of medicinal herbs would help to preserve the herbs ecofriendly from insect pest damage. Insecticidal properties of medicinal herb, *G. sylvestre* have been tested against common stored product coleopteran insect pest *S. oryzae*. The results obtained from the assay revealed that all extracts of *G. sylvestre* leaves have shown varying levels of insecticidal property against *S. oryzae*. This is the first record of reporting insecticidal properties of *G. sylvestre* against storage pest. The importance of *G. sylvestre* for the control of storage pest has been proved. This can be used as an alternative for the chemical methods and with this the safer storage of medicinal herbs will be ensured.

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