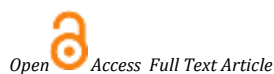


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Research Article

Formulation and Evaluation of Herbal Liquid Insecticide

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Abstract



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Extensive usage of commercially available synthetic pesticides against phytophagous insects has resulted in their bio-accumulation in the environment, leading to a rise in resistance and a decrease in soil biodiversity over time. In addition, 90% of the sprayed pesticides penetrate the different natural resources via runoff, exposing farmers and consumers of agricultural products to serious health risks. As a result, increasing emphasis has been placed on the creation of environmentally benign pesticides/insecticides that would enable an effective pest management system and minimise chronic exposures that contribute to illnesses. The utilization of the herbal active compounds with insecticidal activities is one such technique. Hence the aim of this study was to develop herbal insecticide formulations from extracts of leaves of *Azadirachta Indica*, *Datura Stramonium*, *Cascabela Thevetia* and seeds of *Annona Squamosa*. Insecticidal efficacy of developed formulations was tested in-vitro against *ballworm* larvae. The study unveiled its significance in developing herbal insecticidal formulations as an alternative to harmful synthetic chemical insecticides and a step forward towards development of a promising eco-friendly technology in crop protection.

Keywords: Insecticides, Biopesticide, *Azadirachta indica*, *Datura Stramonium*, *Annona Squamosa*, *Cascabela Thevetia*.

INTRODUCTION

Insecticides are chemicals used to kill insects, which have a wide application in medicine, agriculture, and industry. They have the potential to alter ecosystems and are toxic to animals as well as humans. Insects are an essential creation of nature and need food for their survival. They can be beneficial, harmful, or neutral, and can directly affect the health of humans or the environment. Insecticides can be natural or artificial that can stop the growth of such insects in the crop field, animal houses, or even inside our homes. There are various insecticides and pesticides available in the market under the name of various brands, and some insecticides are banned in different regions of the country due to their harmful effects on the environment ¹.

Pesticide effects on food production are a major issue, as more and more crops are needed to meet the growing demand. This has led to the use of pesticides to increase crop yield per acre. These chemicals affect wildlife, insects, water quality and air quality. Bees play a significant role in the pollination of the foods that we eat, and if the bee population was to have a significant decrease in their population, it could have a dramatic effect on the way our food is pollinated and crop yield. Pesticides can also be found in a lot of the foods you eat, such as fruits and vegetables, which are sprayed to lessen insect damage and improve yield.

When the insects repeatedly exposed to the insecticides build up resistance until finally, they contain either a little or no effect at all. The insect's reproduction is much quicker - they are capable of producing a new generation every 3 to 4 weeks. Thus, the resistance builds up rapidly. Here, the insecticides

will kill more than the intended organisms, which are risky to humans. Besides, when these insecticides get mixed with water sources through drift, leaching, or runoff, they result in harming the aquatic wildlife. When birds drink this contaminated water and eat the affected insects, they also die. A few examples of insecticides, such as DDT, were banned in the US because it affects the predatory birds' reproductive abilities ².

Chemical pesticides have been an accepted part of the global food industry for decades. However, recent news such as reports that traces of glyphosate to cancer and generate concern over how the chemical accumulates in the air, soil and water supply. Currently, there are limited natural options that food producers can use to remove pests, weeds, nematodes and diseases. But those that are on the market, compete with the well-established and soon to be banned chemicals, with some already existing natural products deemed better than their toxic competition. Using natural insecticides is the organic way to get control over the pests, they work for a longer period of time. The process of preparing natural insecticides is easier as compared to the synthetic ones. These are mainly extracted from plants or ground. Therefore, they don't need more effort or investment to be prepared. So, they are available at pocket friendly cost. The growth of natural insecticides is projected to outpace that of chemical pesticides, with compound annual growth rates between 10-20%. Added benefits include reduction or elimination of chemical residues, therefore easing export, enabling delay in the development of resistance by pests and pathogens to chemicals and shorter field re-entry, biodegradability and production using agricultural raw materials versus fossil fuels and low risk to non-target organisms, including pollinators ³.

There are four modes of action for natural insecticides: ingestion, contact, deterrence, and disruption of developmental processes. When moth larvae absorb the pesticide and get poisoned, this is known as ingestion. Contact poisoning occurs when the larvae are killed through their skin or other tissues. The pesticide acts as a deterrent when it inhibits the moth larvae from eating, causing them to starve. Lastly, some insecticides, specifically neem tree oil, disturb the hormones that regulate moulting and other activities⁴. In the present study we have developed herbal insecticide formulations from extracts of leaves of *Azadirachta Indica*, *Datura Stramonium*, *Annona Squamosa* and seeds of *Cascabela Thevetia* and insecticidal efficacy of developed formulations was tested in-vitro against ballworm larvae.

MATERIAL AND METHODS

The raw materials used in formulation were taken from their respective trees in Chhindwara, M.P. All raw materials were cleaned, dried properly in shade and evaluated for foreign matter. The drugs were then crushed and sieved through a sieve in order to obtain a powder. All the other chemicals were used of analytical grade.

Authentication of Crude Drugs

The crude drugs were identified morphologically and microscopically and compared with standard pharmacopoeial monograph. The voucher specimen were allotted as: *Azadirachta Indica* (DFW/AI/01/2022), *Datura Stramonium* (DFW/DS/02/2022), *Annona Squamosa* (DFW/AS/03/2022) and *Cascabela Thevetia* (DFW/CT/04/2022).

Pharmacognostic evaluation of crude drugs

The studies of sensory characters provide the simplest and quickest means by which identity, purity and quality can be measured. If a sample is found to be significantly different in terms of colour, consistency, odour, it not is considered as not fulfilling the requirements. The untreated samples were examined under diffused sunlight for color identification. A small portion was examined by slow and repeated inhalation of the air over the materials to determine the odor of drugs. The strength of odour like weak, distinct, strong is first determined and then the odour sensation like musty, moldy, rancid, fruity, aromatic, etc. was determined.

Determination of foreign matter

It determines the amount of impurities present in sample. 20gms of sample to be examined was weighed and was spread out in a thin layer. The foreign matter was detected by inspection with unaided eye. It was then separated and weighed. Thereafter the percent was calculated [5].

Determination of moisture content (loss on drying)

It determines the amount of moisture that is water drying off from the sample appearing to contain water as the only volatile constituent. 10gm of the sample after accurately weighing was placed in a tared evaporating dish, dish was dried at 105 degrees for 5 hours and was weighed. Then again the tared dish was dried in oven for one hour and cooled in desiccator and again the weigh was taken. This process was continued until the difference in weigh was not more than 0.01 gram⁶.

Preparation of *A. indica* leaves extract

The leaf samples were collected and washed carefully with water to remove dust and foreign materials. Then the washed leaves (200 gm) were dried under shade at temperature (25°C) for 7 days. After drying the leaf samples (150 gm) were ground into a powder form using a grinder for 30 seconds.

The dry leaf powder samples (150 gm) were extracted with methanol solvent (350 ml) for 3 days using Soxhlet extractor until complete extraction. After extraction, the sample was filtered with filter paper (Whatmann No. 1). The methanol solvent was evaporated using a rotary evaporator under pressure for 30 min resulting in a semi solid crude extract. The crude extracts was transferred into a separatory funnel and finally extracted by different solvents with increasing polarities followed the sequence of hexane, chloroform, methanol and water to give hexane, chloroform, methanol and water fractions, respectively. After extraction all crude extracts were put inside the fume hood for the solvents to evaporate. After the solvent was completely evaporated the hexane crude extracts, chloroform crude extracts, methanol crude extracts and water crude extracts of *A. indica* were obtained for preliminary phytochemical screening and estimate the effects of these extracts on larval survival⁷.

Preparation of *D. innoxia* leaves extract

Leaves of *D. innoxia* were collected, shortly prior to each experiment, washed and left for 7 days to dry under shade. The dry leaves were ground into fine powder using an electric blender. Water extract was prepared using 80 g of leaf powder was mixed in one liter of distilled water in a conical flask, thoroughly shaken manually and left to stand for 24 h, then strained through a fine mesh to obtain the stock solution (8% w/v). Regarding organic solvents extraction, each 75 g of *D. innoxia* leaf powder was extracted separately by hexane and methanol in a soxhlet apparatus for 14 h, and dried in a rotary evaporator⁸.

Preparation of *C. Thevetia* leaves extract

Fresh leaves (100g) of *Cascabela Thevetia* plant was crushed and soaked in 400ml ethanol for three days with occasional shaking. After three days the extract was centrifuged at 5000rpm for 10 minutes. Clear supernatants were allowed to dry at 45°C in incubator for few days.

Preparation of *A. Squamosa* seeds extract

The mature custard apple fruits were collected from local market and the seeds were separated from the fruits manually. The separated seeds were washed with water and shade dried. The dried seeds were crushed using hammer mill and the grinded material was screened through fine mesh sieve. For preparation of 2 percent aqueous seed extract, the 20 g of seed powder was mixed with 1 liter distilled water and this mixture was soaked overnight. It was then screen through muslin cloth and the volume of the passing liquid (extract) was maintained to 1 litre by adding distilled water⁹.

Preparation of herbal insecticidal formulation

Herbal insecticidal formulation was prepared by taking 10gm of each extract and was stirred continuously in distilled water to form a homogeneous mixture.

Table 1: Composition of the herbal insecticidal formulation

Sr.No	Local Name	Biological Name	Part Used	Quantity
1.	Neem	<i>Azadirachta Indica</i>	Leaf	10gm
2.	Datura	<i>Datura Stramonium</i>	Leaf	10gm
3.	Sitafal	<i>Annona Squamosa</i>	Seed	10gm
4.	Kaner	<i>Cascabela Thevetia</i>	Leaf	10gm

Evaluation of herbal insecticidal formulation

The prepared formulation was visually inspected for organoleptic properties like appearance, color and odor. 10gm of the sample after accurately weighing was placed in a tared evaporating dish, dish was dried at 105 degrees for 5 hours and was weighed. Then again the tared dish was dried in oven for one hour and cooled in desiccator and again the weight was taken. This process was continued until the difference in weight was not more than 0.01 gram. The pH of the prepared formulation was determined using digital pH meter. Viscosity of the prepared formulation was determined using Brookfield's viscometer⁸.

Insecticidal activity

Acute toxicity of crude extracts and developed formulation was determined on the *ballworm* larvae of stored grain pest, following exposing them to its various concentrations for 24, 48 and 72 hours in different experiments¹⁰.

RESULTS AND DISCUSSION

The studies of sensory characters provide the simplest and quickest means by which identity, purity and quality can be measured. If a sample is found to be significantly different in terms of colour, consistency, odour, it is not considered as not fulfilling the requirements. Results of organoleptic evaluations are given in table 2 which confirms the authentication of plant parts.

Table 2: Macroscopic Evaluation Of Crude Drugs

Sr.No	Botanical Name	Colour	Odour	Size	Shape
1.	<i>Azadirachta Indica</i>	Green	sweet	2-3cm long	Elongated to oblong
2.	<i>Datura Stramonium</i>	Dark green	Acrid	1.5-2cm long	Trumpet shaped
3.	<i>Annona Squamosa</i>	Dark green- Brown	Sweet	3-6cm long	Broadly oblonged
4.	<i>Cascabela Thevetia</i>	Light green	Sweet	5-15cm long	Linear-lanceolate

Table 3: Moisture contents of plant material

Sr.No	Sample	% moisture content
1.	Neem	5.88 - 0.47
2.	Datura	2.91 - 0.33
3.	Sitaphal	3.49 - 0.56
4.	Kaner	4.19 - 0.17

Total extractive values of all the selected plant materials in different solvents was determined and reported in table 4.

Table 4: Extractable Matter

Sr.no	Crude drug	Petroleum ether	Chloroform	Ethanol	Water
1.	<i>Azadirachta Indica</i>	0.32 ± 0.07	0.49 ± 0.03	2.66±0.90	6.88±1.04
2.	<i>Datura Stramonium</i>	0.57 ± 0.01	0.76 ± 0.03	1.93±0.44	5.66±0.78
3.	<i>Annona Squamosa</i>	0.66 ± 0.15	0.78 ± 0.21	9.82±0.78	12.35±0.63
4.	<i>Cascabela Thevetia</i>	0.34 ± 0.01	0.59 ± 0.03	0.67±0.07	0.90±0.02

The herbal insecticide formulation was found to be off white in color and characteristic odor. The pH of the liquid herbal insecticide formulation was found to be 7.9. The viscosity of the prepared liquid herbal insecticide formulation was found to be 1925cps. Additional possible studies would involve testing different pesticide dosages and spraying frequencies, as well as spraying the larvae and their food separately to

determine if the insecticide is effective through ingestion or as a deterrent as opposed to dying through touch.

During this study, the contact action of crude extract was not found which indicated no significant mortality occurred in all tested concentrations up to 48 hours in larvae. However, after 72 hours exposure 10% mortality occurred in both larval

instars. On treating ball worms with the sample, a gradual decrease in their growth was observed. The treatment was

given in every three days and keeping them in individual containers (Figure 1).



Figure 1: Representing effect of herbal insecticide on ball worm larvae

CONCLUSION:

The finding of new insecticides is of great economic importance both from the agronomic and preventive medicine point of view. The reason for using new natural pesticides is that these are active at highly acceptable levels, biodegradable and do not leave toxic residues while the commonly used phosphorous and chlorinated insecticides contaminate the environment. The insecticidal activity appears to be due to the presence of its active principles. The use of plant derived toxicants as insecticides may allow a reduction in the health hazards of synthetic pesticides and the high degree of insecticidal activity of the new formulation. This present study establishes the insecticidal activity.

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