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Larvicidal efficiency and stability studies of various extracts of *Ocimum basilcum* and *Ricinus communis*

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Abstract

In the present work, aqueous, hexane, ethyl acetate and ethanollic extracts of leaves and seeds of *Ocimum basilcum* (Basil) and *Ricinus communis* (Castor) were evaluated for their larvicidal efficiency. An attempt has also been done to study their stability by storing the extracts at 4^{0} C for thirty days and they were used in a same tests of the fresh extracts so as to evaluate their degradation rate in their tested properties. The results indicate that the tested extracts exhibited potent larvicide property. Stability studies suggest that, except the aqueous extracts, all the tested extracts showed a good stability and retained larvicidal potency.

Keywords: Larvicidal potency, herbs ,stability testing

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1.0 Introduction

Because mosquitoes are the most important single group of insects in terms of public health importance, which transmit a number of diseases, repeated use of synthetic insecticides for their control has disrupted natural biological control systems and lead to resurgences in mosquito populations. It has also resulted in the development of resistance, undesirable effects on non-target organisms and fostered environmental and human health concern, which initiated a search for alternative control measures. Plants are considered as a rich source of bioactive chemicals and they may be an alternative source of mosquito control agents [1], [2] and [3].Synthetic chemical larvicides continue to be applied for controlling mosquitoes as a solution of vector problems [4]. Many of these chemicals are toxic to human, plant and animal life, and resistance can be problematic in maintaining control, especially with organophosphate and pyrethroid larvicides. As a result, researchers are currently investigating natural subtances to use as insecticides for controlling larval mosquitoes. Phytochemical insecticides have received much attention, as they are considered to be more environmentally biodegradable and considered safer than synthetic insecticides [5].

Insecticides should reduce vector populations, be target-specific, break down quickly, and have low toxicity to humans and other animals. Although, synthetic insecticides have been an important part of vector management for many years, the disadvantages and risks of using them have become apparent. Some synthetic insecticides leave unwanted residues in food, water, and the environment. Some are suspected carcinogens, and low doses of many insecticides are toxic to mammals let alone other animal groups. As a result, many people are looking for less hazardous alternatives to conventional synthetic insecticides [6]. The search for alternatives, whether means, practices or chemicals, synthetics or naturals for vector control has received increased attention. [7], [3]. There are several reports on the phytochemical constituents and on some of the pharmacological activities of these plants. However, limited, or no, data are available on their side effects or toxic effects in the literature. Secondary metabolites include phenolic components of the cell wall, lignification of cells and the presence of polyphenols such as condensed tannins. Soluble secondary compounds such as cyanogenic glycosides, isoflavonoids and alkaloids can also be toxic to animals. Not all plant defense reactions adversely affect plant nutritional quality and not all anti-nutritional metabolites aid plant survival [8]

2.0 Materials and methods

All biological materials viz plant materials, third larvae instars' of *An. Arabien*sis used in this study were collected from Khartoum state, Sudan; The Plant materials have been collected, dried under shade at room temperature; and then ground to a fine powder using an electric grinder. The leaves powder was kept in a plastic bag. The same procedure was followed to obtain and keep the seeds powder. A conventional Soxhlet apparatus was used to prepare the different chemical extracts. 500 gms of the prepared powder of each plant part was first enrolled inside a filter paper; considerable volume of Hexane was added to a 2-litres Soxhlet round bottom flask. Approximately 50-60 siphonings were excuted during the 12 hours period. The flask was placed on a rotatory evaporator and the solvent was removed under vacuum at this stage, all non-polar substances like fats, lipids, waxes were considered to be extracted. The residue was then extracted using Ethyl acetate and furthermore was re-extracted in the Soxhlet using Ethanol as a polar solvent for 12 hours so as to extract all polar substances. One set of extracts was use freshly, while the other set of crude extract was kept in dark glass bottles covered tightly with aluminum foil, labeled then stored in a refrigerator at four degrees Celsius for one month after preparation till needed for experiments.

Concentrations of 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/l were then obtained by adding distilled water to the initial volumes to be completed to 250 ml then transferred to suitable dishes for experimentation.

The extracts were subjected to chemical according to the method described by [9], [10] and [11] to know the presence of various phytoconstituents..The larvicidial assays were conducted according to the standard procedure [12]. The results were subjected to normal descriptive statistical tests, size, variance and mean. The corresponding corrected mortalities were transferred to a Probit. Log-dose and the corresponding Probit were submitted to Probit analysis to calculate the regression equation, the correlation R^2 and the slope. From the regression equation, the LC_{50} was calculated. Computerized statistical analyses were done using Excel, Microsoft Office program 2003 and Statistical Package for Social Sciences, SPSS 13.0, 2004.

3.0 Results and discussion

3.1 General properties of the prepared extracts

From the foregoing experiments, some of the general properties both chemical and physical of Basil and Castor leaf and seed extracts were further analyzed and studied.

Table-1: The general properties of the prepared made test extracts

Plant	Part	Solvent used	Colour		pH		Density		Melting point, ˚C	
			fresh	kept	fresh	kept	fresh	kept	fresh	kept
Basil	leaf	Water	Light green	Slight green	6.64	6.05	1.03	0.98	295	323
		Hexane	Green	Slight green	6.60	6.50	0.72	0.71	285	295
		Ethyl acetate	Yellow	Yellow	7.61	7.48	0.98	0.98	226	236
		Ethanol	Green	Green	7.65	7.80	0.86	0.85	280	294
	seed	Water	White	white	9.20	8.90	1.52	1.65	266	345
		Hexane	Light	Slight yellow	8.05	8.00	0.85	0.83	256	269
		Ethyl acetate	Light brown	Slight brown	8.53	8.42	0.94	0.95	255	262
		Ethanol	Light	Slight yellow	8.89	9.40	0.85	0.82	257	268
Castor	leaf	Water	Light green	Slight green	8.14	7.96	1.01	1.48	268	354
		Hexane	Green	Slight green	8.03	7.85	0.70	0.73	278	281
		Ethyl acetate	Yellow	Yellow	7.79	7.72	0.90	0.90	257	263
		Ethanol	Green	Green	8.28	8.51	0.87	0.91	265	281
	seed	Water	White	white	8.80	9.23	1.68	1.75	267	308
		Hexane	Light	Colorless	7.12	7.38	0.85	0.84	278	279
		Ethyl acetate	Cream	Cream	6.30	6.25	0.96	0.95	232	237
		Ethanol	Light white	Colorless	7.76	7.90	0.84	0.83	294	298

The pH value were ranged between 6.30 for Ethyl acetate extract of Castor seeds which was the highest acidity obtained, while the Basil seed Water extract achieved 9.20 as the highest pH value. Although the Aberrant density of 1.685 and 1.523 representing the aqueous seed extract of Castor and Basil respectively. Density value is almost around 1.00 value. Density was slightly changed, but moderate regarding the water extracts. The behavior of the water extract was same considering the Melting point. Generally, comparing the result of general properties of the

freshly made extracts with the one month kept extracts (Table-1), showing that there was no noticeable change in colour, pH value, density and melting point regarding chemical extracts, but there is slight change regarding pH value, density and melting point in the aqueous extracts.)

3.2 Chemical properties of the extracts

Each plant part was introduced to a qualitative analysis which was carried out. The results of these primary tests were illustrated in the table-2.

Table-2: Results of qualitative phytochemical tests

Plant	Basil		Castor	
Part used / Test	Leaf	Seed	Leaf	Seed
Molisch's test for Carbohydrates	+	+	+	+
Barfoed's test for monosaccharides	+	+	+	+
Fehling's test for free reducing sugars	+	+	+	+
Fehling's test for Combined Reducing Sugars	+	+	+	+
Test for Tannins	+	+	+	+
Borntrager's test for anthraquinones	–	–	–	–
Liebermann-Burchard test for steroids	+	+	+	+
Test for terpenoids	+	+	+	+
Test for Saponins	+	–	+	+
Ferric chloride test for flavonoids	+	+	+	+
Test for alkaloids	+	+	+	+
Test for Soluble Starch	+	–	+	+

Repeating of the same tests for the same prepared extracts, but one month after preparation gave the same results.Aromatic and amino acids play an important role in the life of insects. Aromatic acids may serve as egg-hatching factors for mosquitoes, and as insecticides or repellents. Although the toxicity of a few aromatic acids has been studied with mosquito larvae, no detailed studies on the structural relationship for aromatic acid toxicity have been reported [13] but recently numerous feeding experiments have been carried out which show that, secondary compounds accumulated in plants are toxic to insect herbivores, some of these compounds have been, and are still, used as insecticides [14].Alkaloids are insecticidal at low concentrations and frequently toxic to vertebrates. They are nitrogenous organic molecules with varying structures. Their mode of action varies but many affect acetylcholine receptors in the nervous system (e.g., nicotine), or membrane sodium channels of nerves (e.g., veratrin). Alkaloids are found in large quantities in many members of the Berberidaceae, Fabaceae, Solanaceae and Ranunculaceae families, all of which are used extensively as traditional insect repellents [14]. This valuable literature is completely agree with the current research results.The forgoing results is also compatible the literature of phenolic compounds. Phenols, sometimes called phenolics, are a class of chemical compounds consisting of a hydroxyl group (OH^-) attached to an aromatic

hydrocarbon group. The simplest of the class is phenol (C_6H_5OH). The functions of phenols are diverse, contributing to cell wall structure, flower color and defense against both vertebrate and invertebrate herbivores. Important phenolics in terms of insecticidal and repellent function are the flavonoids, which are characteristic compounds of higher plants. There are three important insect repellent flavonoid groups. Firstly, the flavones found in the Labiatiae, Umbelliferae and Compositae, and are quite new in evolutionary terms. The second important group is the isoflavonoids found mainly in the Leguminosae: an example of which is the highly insecticidal compound rotenone present in *Derris eliptica*. Rotenone is a potent mitochondrial poison. The other main phenolic groups important in deterring insects are the tannins; they are found throughout the plant kingdom and exhibit toxicity by binding to proteins [14].

Table-3: LC_{50} of the different extracts on the 3rd in star larvae of *An. arabiensis* after 24 hours of exposure

plant	Part used	Extraction medium	LC_{50} part per million^{-1}Litre	
			Fresh	Kept
Basil	Leaf	Water	1320	3240
		Hexane	1012	1156
		Ethyl acetate	0390	0402
		Ethanol	0705	0810
	Seed	Water	2258	4684
		Hexane	1154	1347
		Ethyl acetate	1159	1220
		Ethanol	0657	0673
Castor	Leaf	Water	1051	1869
		Hexane	0760	0834
		Ethyl acetate	0439	0481
		Ethanol	1108	1315
	Seed	Water	1102	1474
		Hexane	0851	1001
		Ethyl acetate	1216	1239
		Ethanol	0763	0800

3.3 Toxicity of Basil and Castor on 3rd instar larvae of *An. arabiensis*

Table-3 is shows the LC_{50} of both leaves and seeds of Basil and Castor extracts using water, hexane, ethyl acetate and ethanol in part per million^{-1}Litre. The lower the concentration of an extract, the best is the insecticide, because when the concentration is low, more quantities are needed and may be more time (Table-3) sum up LC_{50} of the different extracts on 3rd instar larvae of *An. arabiensis* after 24 hours, for both freshly made and one month kept extracts.In order of testing of extracts activity one month after preparation; Each extract used in the bioassay was prepared and kept at room temperature for one month and then used in bioassay against 3rd instar larvae of *An. arabiensis* that is to compare the larvicidal effect and to evaluate ability for storage before use (Table-3). Comparing the result of the freshly made extracts with the one month kept extracts (Table-3) showing there were no significant differences between the chemical extracts, but regarding the aqueous

extracts the differences were found significant.

4.0 Conclusion

The current study assured the Basil and Castor plants have valuable larvicidial effect against *An. arabiensis.* Except of the water extraction, the fear of degradation of the natural plants prosperities by keeping them for a long time is not right.

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