RESEARCH ARTICLE

The Effect of Extracts and Phenolic Compounds Isolation from *Rosmarinus officinalis* Plant Leaves on *Tribolium castaneum* Mortality

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ABSTRACT

The current laboratory study was conducted to evaluate the efficacy of different concentrations (2.5, 5, 10, and 20%) of the ethanolic extract, ethyl acetate, and hexane at a concentration (1, 2, and 4%) of the leaves of *Rosmarinus officinalis* against adults of the insect *Trabolium castaneum* for three periods (24, 48, and 72 hours). Results showed that the ethanolic extract, ethyl phenolic components (catechol, 4-hydroxybenzoic acid, calic acid, vanillic acid, coumarin, and cinnamic acid) of rosemary leaves were detected using HPLC technology over a 24 hours treatment period, and their effect on the adult stage of the insect was investigated. The results showed that catechol, calic acid, vanillic acid, and cinnamic acid had a killing rate of 93.33% at a concentration of 20%. In contrast, 4-hydroxy benzoic acid and coumarin had a killing rate of 90% at a concentration of 20%.

Keywords: Anti-insect, HPLC, Isolation, Phenolic compounds, *Rosmarinus officinalis*, *Tribolium castaneum*.

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INTRODUCTION

The safe storage of food and grains against crop-damaging insects is a critical issue. According to recent estimates, around 8% of the world's total grain production is affected by insect and pest infestations.[1] As a result of this situation, there is a pressing need to develop environmentally acceptable techniques that can replace very harmful substances. Many stored food products and grains are damaged, which is a severe concern all across the world, especially in underdeveloped countries.[2] Insects attack many stored grain products, causing harm not just in stores but also during shipment and transportation of stored grains. According to estimates, these pests harm roughly 10 to 40% of stored grains in granaries and homes, as well as products. Furthermore, these insects have a negative impact on grain quality.[3] Many pests attack the embryos of stored grain, lowering the protein content of the grain as well as the percentage of seeds that germinate.[4]

Tribolium castaneum (*Coleoptera: Tenebrionidae*), a stored product pest, can cause losses in various durable stored products such as barley, corn, flour, millet, wheat, potatoes, sweet potatoes, dried fruit, nuts, and sorghum. This insect pest is a major and widespread pest of grain processing and storage.[5] *T. castaneum* is a serious pest in flour mills, the baked-goods industry, and retail establishments.[6] This pest is more damaging to processed cereals in the form of flour than to whole-grain cereals.[7] The benzoquinone released from this insect's abdomen gland causes an unpleasant odor when it is infested.[8] *T. castaneum*, popularly known as red flour, is an important stored grain pest that belongs to the Tenebrionidae family. It's a pest of stored goods all over the world.[9] *T. castaneum* is an Indo-Australian species that thrive in temperate climates. However, they can only survive the winter in well-protected settings, such as those with central heating.[10] Agricultural pest management has relied heavily on the use of synthetic chemical pesticides for field and post-harvest crop protection for the past half-century. However, these synthetic pesticides are poisonous and have negative environmental consequences by contaminating soil, water, and air, and their widespread usage has resulted in the development of resistance in some species as well as the widespread eradication of beneficial creatures. In addition, there is mounting evidence of synthetic insecticides' detrimental environmental and health effects, as well as increasingly strict pesticide environmental control.[11]

This has piqued interest in research into the use of plant extracts as insecticide alternatives to synthetic pesticides. Botanical derivatives may be safer for the environment than synthetic poisons, as certain secondary metabolites contained in plants could be exploited to generate novel insecticides.

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Insecticidal capabilities have been discovered in over 2000 plant species.[12] *Rosmarinus officinalis* L.(Rosemary) is an aromatic perennial herb native to the Mediterranean region with fragrant evergreen needle-like leaves.[13,14] It belongs to the Lamiaceae family as well. This plant contains phenolic acids, flavonoids, diterpenoids, triterpenes, and monoterpenes, which are included in the composition of essential oils and bioactive components. Many plant extracts are toxic to stored-product pests.[15-17] These botanical pesticides are more efficient against a number of pests, are easily biodegradable into certain innocuous compounds and may lead to the development of a variety of safe pest control agents.[18]

As a result, rosemary is a vibrant source of bioactive chemicals. As a result, the current study shows that various extracts of this plant could be employed as natural sources of a variety of bioactive components, particularly phenolic compounds, which could be valuable elements in complementary alternatives for warehouse insect management.

MATERIALS AND METHODS

Insects Used in the Study

The red flour beetle *T. castaneum* was employed in this study, which was obtained from stores infested with this pest and bred in the Insect Research Laboratory, College of Education for Pure Sciences, University of Mosul, Iraq.

Insect Breeding Environments

Sifted flour (zero flour) was mixed with dry bread yeast powder at a rate of 5% to produce the red flour beetle, which was done in an industrial setting.[19] Insects were placed in glass bottles with a capacity of 600 mL and a third of the size of the insect's industrial environment. A cloth with perforations was used to cover the mouth, which was then tightly sealed with rubber belts. They were then placed in an incubator at a temperature of 35 + 1°C and a humidity of 70 + 5%. The farm was renewed on a regular basis. New plantations were started with the use of young insects.

Plant Material

The RO was chosen as a plant that grows in Mosul's local environment. The plant parts were cleaned and then dried in the shade at laboratory temperature on big filter papers, stirring from time to time to prevent rotting, before being stored in appropriate paper envelopes until use. The plant variety under study at the College of Agriculture, University of Mosul's and Forestry was verified by plant taxonomy experts, using principles approved by researchers and with the assistance of Dr. Talal Taha Ali Suleiman, a member of the Ministry of Agriculture's Genetic Resources Committee.

Extraction by Continuous Extraction (Soxhlet)

The extraction process was carried out using a continuous Soxhlet Apparatus with a sequential solvent system (hexane, ethyl acetate, and ethanol) at a concentration of 1000 mL per 100 g of material (plant powder), i.e., 100 mL/10 g. The extraction process was repeated for each type of solvent used for a duration ranging from 72 to 48 hours or until the color of the solvent used in Soxhlet vanished, yielding the extract, which is a combination of the plant extract and the solvent employed. The extracts are concentrated using the Rotary Vaccum Evaporator (RVE) after each extraction procedure to obtain the Crude Extract, which is preserved in a dark-colored bottle with a tight-fitting top and kept in the refrigerator until usage.[20]

Column Chromatography (CC)

It is a glass column of various sizes filled with silica gel after thermal activation as an adsorbent used in the separation process, obtaining many sections or the so-called fractions by employing the consecutive solvent sequence system.[21] This approach implies the presence of a fixed medium (silica gel) and a mobile medium (the solvent), both of which have qualities that are exploited in the separation process, such as polarity and adsorption capacity, on the separated components or "fractions," which are put in sealed dark-colored vials and maintained in the refrigerator until needed.[21]

High-Performance Liquid Chromatography (HPLC)

The measurements were performed at the Samarra Pharmaceutical Company/Intravenous Solutions Factory in Nineveh, and the HPLC analysis was carried out according to the references. The HPLC from Agilent Technologies, an American company, was used to identify and purify the compounds using a C18 separation column (25 mm x 4.6 mm x 5mm) at a room temperature of 25°C. After being filtered and gases were ejected from it using an ultrasonic device (sonicator) at a flow rate of 1 = flow rate ml/min at a wavelength of 280 nm, the H3PO4 was utilized as a mobile phase.

RESULTS AND DISCUSSION

Extracts' Effect on *Tribolium castaneum*

Table 1 shows the influence of different concentrations (2.5, 5, 10, and 20%) of the ethanolic extract, ethyl acetate, and concentrations (1, 2, and 4%) of hexane extract of rosemary leaves on the percentage of killing throughout three time periods. The results show that the alcoholic extract has a significant and clear effect on the percentage of killing in the adult phase of *T. C.*, with the highest percentage of killing being 83.3 and 73.3, respectively, using ethanolic alcohol extract and ethyl acetate at a concentration of 20%, and within 24 hours of treatment.

The results also showed an interaction effect between the type of plant extract used and the concentration used, which had a significant and clear effect on the percentage of killing, which was 20, 33.3, 66.7, and 83.3% in the treatment of ethanolic extract and 16.7, 26.7, 56.7, and 73.3% in the treatment of ethyl acetate extract, respectively.

The interaction between time and concentration showed significant differences in the proportion of killings, with the maximum killing rate of 78.3 percent at 20% concentration and the lowest killing rate of 18.3 at 2.5% concentration. When treated with ethanol extract for 24 hours, the interaction impact between the extract and time was 40.7%.

Table 1: Shows the effect of different concentrations of(R.o) ethanolic extract, ethyl acetate, and hexane on the percentage of T.C. deaths in the adult phase following exposure to various periods.

Extract	*Time/hour* \ *Concentration*	*Mortality of adult %* 2.5	5	10	20	*Control*	*Interference between time and extract*	*Extracts effect*	*Time effect*
Ethanol	24	20 e	33.3 d	66.7 bc	83.3 a	0.0 f	40.7 a		
	48	20 e	33.3 d	66.7 bc	83.3 a	0.0 f	40.7 a		
	72	20 e	33.3 d	66.7 bc	83.3 a	0.0 f	40.7 a		
Ethyl acetate	24	16.7 e	26.7 de	56.7 c	73.3 ab	0.0 f	34.7 b		
	48	16.7 e	26.7 de	56.7 c	73.3 ab	0.0 f	34.7 b		
	72	16.7 e	26.7 de	56.7 c	73.3 ab	0.0 f	34.7 b		
Interference between con. and extract	Ethanol	20 g	33.3 e	66.7 c	83.3 a	0.0 h		40.7 a	
	Ethyl acetate	16.7 g	26.7 f	56.7 d	73.3 b	0.0 h		34.7 b	
Interference between con. and time	24	18.3 d	30 c	61.7 b	78.3 a	0.0 e			37.7 a
	48	18.3 d	30 c	61.7 b	78.3 a	0.0 e			37.7 a
	72	18.3 d	30 c	61.7 b	78.3 a	0.0 e			37.7 a
Concentration average		18.3 d	30 c	61.7 b	78.3 a	0.0 e			
Extract	*Time/hour* \ *Concentration*	*Mortality of adult %* 1	2	4	*Control*	*Average*			
Hexan	24	76.7 c	90 b	100 a	0.0 d	66.7 a			
	48	76.7 c	90 b	100 a	0.0 d	66.7 a			
	72	76.7 c	90 b	100 a	0.0 d	66.7 a			
	Average	76.7 c	90 b	100 a	0.0 d				

According to Duncan's Multiple Range Test, numbers with distinct letters differ statistically at the 5% probability level in each field.

Table 2: Shows the retention time (RT) (min) for a number of phenolic compounds isolated from R.O., as well as the retention time (RT) for the reference compounds using HPLC.

The separated fraction of the column	*Peak. No*	*Plant sample (R.t)*	*Standard sample (R.t)*	*Identification of compounds*
I	1	2.797	2.715	Catechol
	2	2.904	2.901	4- Hydroxy benzoic acid
II	1	2.072	2.019	Gallic acid
	2	3.017	3.031	Vanillic acid
	3	3.146	3.165	Coumarin
III	1	3.601	3.593	Cinnamic acid

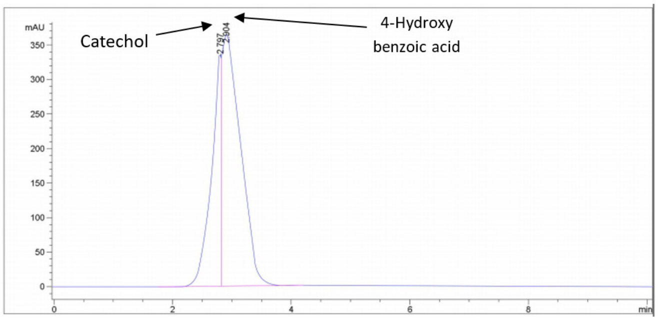
The adult phase's resistance is due to the presence of an advanced cuticle layer that covers the outer part of the body wall, giving it rigidity and impermeability to some extent, as protein and fatty compounds, among other things, enter into the cuticle's composition, making it impermeable to water, protecting the body from dehydration and entering into the composition of the cuticle. Chitin is a soft, water-permeable material that is not soluble in water, organic solvents, acids, or dilute bases.[22] Because these extracts contain phenolic compounds, alkaloids, and terpenes,[23,24] explained that the cause of death is the toxic effect during contact with the body's surface and the penetration of the chemical compounds of the ketocal extracts through the flexible areas or the respiratory openings, causing paralysis and rapid death.

Separation and Identification of Phenolic Compounds using HPLC

Because the principle of HPLC work relies on the physical separation of the active substance through two phases, the stationary phase, which is the column, and the mobile phase, which is a liquid and means solvent. It is one of the most important methods used to separate and identify compounds present in any sample.

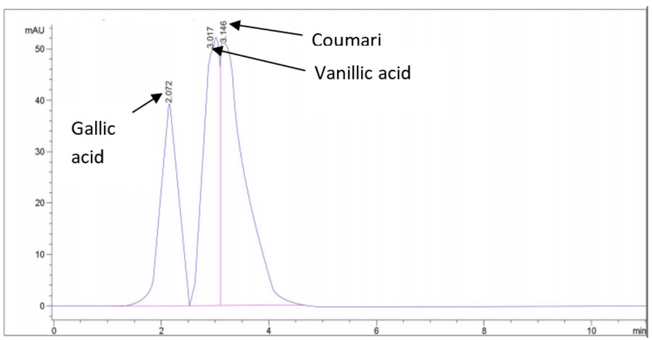
The retention time of isolated phenolic compounds (Rt) was calculated and compared to the retention time of standard phenolic compounds in this study using the HPLC technology (Rt). And by detecting chromatographic responses at a wavelength of 280 nm, this technique was able to create a curve for each standard component along with its retention duration. The number and type of phenolic chemicals are both very clear.

As shown in Figures 1 to 3 straight from Table 2, (R.o) plant includes phenolic chemicals (catechol, 4-hydroxybenzoic acid, calic acid, vanillic acid, coumarin, and cinnamic acid). When comparing the RT of the separated phenolic compounds that were proven to be present in the separated parts of the column to the RT of the standard phenolic compounds, the presence of compounds (catechol and 4-hydroxybenzoic acid) was diagnosed in the first part by comparing the detention time of these compounds (2.797 and 2.904) with the detention time for the standard phenolic compounds. Finally, in the third part, the diagnosis of the cinnamic acid compound with a retention time of 3.601 compared to the control sample (3.593), and therefore the third part is a single compound using HPLC detection.



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.797	VV	0.1528	3668.59497	332.29471	31.2878
2	2.904	VB	0.2987	8056.72119	363.35004	68.7122
Totals :				1.17253e4	695.64475	

Figure 1: The curve of separated and identifiable phenolic compounds from a fraction (I) of R.o) plant using HPLC.)



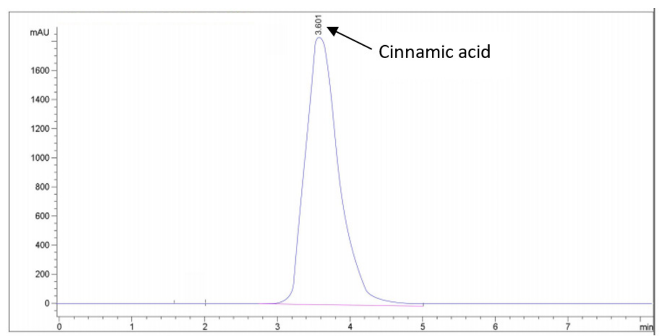
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.072	BV	0.2846	793.20123	39.38089	23.1830
2	3.017	VV	0.2425	972.75677	51.94072	28.4309
3	3.146	VB	0.4410	1655.52234	51.03307	48.3861
Totals :				3421.48034	142.35468	

Figure 2: The curve of separated and identifiable phenolic compounds from fraction (II) of R.o plant using HPLC.

Linares *et al*.[25] discovered various phenolic compounds in the alcoholic extract of rosemary using HPLC, including (syringic acid, guanic acid, carnosol, rosmarinic acid, napetrine, hesperidin, and luteolin), while Kheiria *et al*.[26] discovered 11 phenolic compounds in the plant extract. Calic acid, caffeic acid, ferulic acid, rosmarinic acid, coumaric acid, carnosol, carnosic acid, hesperidin, luteolin, apigenin, and quinone were found utilising HPLC technology in Rosemary (rosmarinic acid, carnosol and carnosic acid as the main extracts).

Phenolic Compounds' Effect on *Tribolium castaneum*

The effect of phenolic compounds (catechol, 4-hydroxybenzoic acid, calic acid, vanillic acid, coumarin, and cinnamic acid) isolated and identified by HPLC from rosemary plants at concentrations (10 and 20%) in the percentage was shown in Table 3. The percentage of killing in the adult phase of *T. castaneum*, results show that there are significant differences in the mortality rate of adult phase members treated with different concentrations compared to the control treatment, as shown in the table, with the highest rate of killing (81.66%) when treated with 4-hydroxy benzoic acid, vanillic acid, and



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.601	VB	0.5849	8842.89218	1807.00378	100.0000
Totals :				8842.89218	1807.00378	

Figure 3: Curve of separated and identifiable phenolic compounds from fraction (III) of R.O. plant using HPLC.

Table 3: Shows the effect of phenolic compounds separated from(R.o) using HPLC on the percentage of kill in the adult phase of TC.

Insect type	*Identification of compounds*	*Of adult deaths%* 10	20	*The average*
Tribolium castanium	catechol	66.67 e	93.33ab	80.0000 c
	4- Hydroxy benzoic acid	73.33cde	90.00 b	81.6667bc
	Gallic acid	66.67 e	93.33ab	80.0000 c
	Vanillic acid	70.00 de	93.33ab	81.6667bc
	Coumarin	66.67 e	90.00 b	78.3333 c
	Cinnamic acid	70.00 de	93.33ab	81.6667bc
	control	0.00 f	0.00 f	.0000 d
The average		59 b	79 a	

According to Duncan's multiple range test (DMRT), numbers with distinct letters in each field differ statistically at the 5% probability level.

cinnamic acid. This was notably different from the lowest rate of killing, which was 78.33%, when the coumarin compound was used. The table also shows the effect of the interaction between concentration, compound, and phase on the percentage of killing, which had a significant and clear effect on the percentage of killing, as the killing rate was 93.33 percent when treated with catechol, calic acid, vanillic acid, and cinnamic acid at 20% concentration, which differed statistically from the control treatment, and 90% when treated with 4-fluorouracil. In general, the death rate of adult phase individuals increases as the concentration of the compound increases, with the highest rate of killing reaching 79% at a concentration of 20%, which differed significantly from the lowest rate of killing at 59% at a concentration of 10%.

The impact of rosemary extract against the southern cowpea beetle *Callasobruchus Maculatus* was validated by Krzyzewski *et al*.,[27] as was the effect of alcoholic extract of rosemary on the adult stages of the red flour beetle and Khabra beetle by Panezal *et al*.[28] Also, the effect of alcoholic extract of rosemary against the adult stages of *Tribolium confusum* and *Acanthoscelides obtectus* was studied by Gokturk *et al*.[29]

CONCLUSION

Because of the diversity of chemical components in rosemary leaves, extracts have been found to have anti-insect capabilities, according to the findings of this study. The intrinsic and phenolic components of the rosemary plant are the most effective and important of the many components discovered in the plant. Bioactive rosemary particles have the potential to be used in the creation of a variety of natural pesticides that are both environmentally benign and effective.

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