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Efficacy of Essential Oils Extracted From Cinnamon and Rosemary against the Rat Fleas (*Xenopsyllacheopis*) and Evaluate Their Effects on Some Vital Processes in Rat Fleas and Albino Rat

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Abstract

Control of fleas was an important public health topic, so the purpose of this research was to calculate the insecticidal effect of cinnamon and rosemary oils for controlling adult fleas. The examination of phenolic compounds by GC-MSof the cinnamon and rosemary oil revealed isolation of 10 and 14 compounds for cinnamon and rosemary oil respectively. The lethal concentration (LC50) of cinnamon was 0.290 mg /L compared with 3.680 mg /L for rosemary oils. This result revealed that the cinnamon oils had a greater insecticidal effect than the rosemary oil. Levels of total proteins and acetyl cholinesterase in fleas (AchE) were decreased in group treated with cinnamon oil more than group treated with rosemary oil compared with control group, but level of GST in fleas increased in group treated with cinnamon oil more than group treated with rosemary oil compared with control group. Also, the level of Alpha- and beta- esterase in fleas increased in the group treated with rosemary oil more than group treated with cinnamon oil in contrast to the control group. No notable changes in serum biochemical, hematological parameters and pro-inflammatory markers in male and female rats. Therefore, cinnamon and rosemary oil are considered an eco-friendly and safe insecticide to keep adult fleas under control (*Xenopsyllacheopis)* but cinnamon is more powerful than rosemary oil.

Keywords: fleas; cinnamon oil; rosemary oils; insecticidal activity; acetyl cholinesterase.

1. Introduction

Around the world, adult fleas are ectoparasites of birds and animals. There are about 2525 species known to exist, of which 96% infest mammals and 4% in birds [1]. The illnesses that many species carry make them significant, particularly those from the genera *Ctenocephalides, Xenopyslla, and Pulex* [1, 2, 3, 4].The deadly infectious disease plague, or Plague, is spread by the rat flea, *Xenopsylla cheopis*, and is brought on by the Gram-negative bacterium *Yersinia pestis* [5].

Using pesticides is a standard approach to managing fleas (*Xenopsylla cheopis*). The extensive and widespread use of artificial insecticides often increases the risk of residual insecticidal buildup in the environment and the growth of resistance to insects [6]. Consequently, much research has focused on the potential use of plant extracts rather than synthetic insecticides to control arthropod vectors of tropical diseases that affect humans [7].

Numerous research studies have indicated that plant-based essential oils can serve as effective means of controlling insects. This is due to their ability to decompose into non-toxic products and their ability to be selective, all without harming living things or the environment [8, 9, 10].

Using Cinnamon *(Cinnamomum verum) and* rosemary *(Rosmarinus officinalis*) oils for fleas is a very effective remedy, since these oils have an anti-inflammatory, antiseptic, anti-parasitic and analgesic nature. Additionally, they are completely safe to use. Cinnamon can be used as an insect repellant. Many insect species have been targeted by cinnamon oils and their constituents, including cinnamaldehyde, which is an insecticidal chemical [11].Different studies showed that Cinnamon essential oil has antimicrobial, insecticidal, and acaricidal properties

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[12,13,14]. Many commercial pesticides contain rosemary (*Rosmarinus officinalis L., Lamiaceae*) oil as their active ingredient because of its insecticidal qualities [15],Because essential oils of rosemary contain important components including 1,8-cineole, α-pinene , camphor, and camphene, they are employed as an alternate method of pest control [16, 17,18,19,20].

Due to their ability to spread disease to both humans and animals, adult fleas are a major source of health issues. A new trend in the management of insects causing diseases is the use of natural pesticides rather than synthetic ones. Thus, the goal of the present investigation was to examine the impact of rosemary and cinnamon oils on the important processes of adult fleas, additionally assess their acute toxicity on rats.

2. Materials and methods

2.1. Reagents and Chemicals

Acetylcholine bromide, alkalinehydroxylamine,conc HCL, potassium salt of phosphate buffer,1-chloro 2,4-dinitrobenzene (CDNB), α-or β-naphthylacetate, acetone, diazoblue B , sodium laurylsulphate, Coomassie Brilliant blue G and ethanol were purchased from Elnasr for achemical company (Aborwash, Giza)

The biochemical kits, including those for AST (aspartate aminotransferases), albumin, ALT (alanine aminotransferases), urea, uric acid, creatinine, and TP (total protein) were obtained from Spinreact (Spain) Company. The Hb (hemoglobin concentration), HCT (hematocrit), MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), MCHC (mean corpuscular hemoglobin concentration), and count of platelets were measured using the Mindray BC-30S (China). Smears of blood were fixed, and the Wright-Giemsa stain obtained from Sigma-Aldrich Company (St. Louis, Missouri, US) was used to differentiate the white blood cells. TNF-alpha and IL-6 obtained from the Bioassay Technology laboratory (Shanghai, China).

2.2. Plant oils

The unit responsible for pressing and extracting natural oils at the PDIRI, NRC, Giza, provided the oils of rosemary and cinnamon.

2.3. GC-M analysis of oils

At the Central Laboratories Network, NRC, Giza, Egypt, a MS-detector (5977A) and GC (7890B) were fitted with an Agilent Technologies GC-MS system. A DB-5MS column (30 m x 0.25 mm internal diameter and 0.25 µm film thickness) was fitted to the GC. Helium used as the carrier gas in the analyses, with a splitless injection volume of 0.2 µl and a rate flow of 3.0 ml/min. The Program of temperature was the following: 40°C for one minute; 10°C/min rise to 200°C and hold for one minute; 20°C/min rise to 220°C and hold for one minute; 30°C/min rise to 320°C and hold for three minutes. The injector and detector were adjusted at 320 °C and 250°C. After it dissolved in chloroform, the sample was introduced into the GC.Mass spectra produced by electron ionization (EI) at 70 eV were obtained by employing a spectral range of m/z 50-550. Quad 150°C and mass temperature were 230°C. Numerous constituents may be identified by comparing the spectrum fragmentation pattern with those contained in the data from the Wiley and NIST MS Libraries.

2.4. Biological activity studies

2.4.1. Fleas

The adult fleas employed in this research are from the *Xenopsylla Cheopis* species. They came from the Medical Entomology Research Institute in Giza, Egypt. The WHO-standardized sensitivity tests served as the foundation for the toxicity test technique [21]. Every flea that was chosen for its insecticidal properties belonged to the same generation and reproduced at the same locations.

2.4.2. Insecticidal activity

On adult fleas, the insecticidal effects of cinnamon and rosemary oils were fully realized. Insecticide susceptibility level measurement methods graciously provided 5 cm by 1.5 cm tapered paper that was impregnated with each concentration for each oil utilized [22]. Each test tube contained a test paper and a control paper that was only saturated with maize oil. Ten fleas were inserted into each tube by using the aspirator equipment. The exposure phase started when each tube sealed with fine-mesh gauze. Vertically arranged tubes in the rack under one of the halves of the kit box so that the fleas kept in darkness during the exposure period. Each concentration was replicated 3 times with concurrent control. After the exposure time (after 24 hours), the fleas examined and mortality counts calculated for 24 hours. Abbott's method used to adjust the mortality rate [23].The method described by Finney [24] and the logarithmic-probability used to evaluate the relationship between concentrations and mortality. The slope (b) function ratio was also calculated.

2.4.3. Preparation of insects for analysis

The insects were prepared following Amin's instructions [25]. 50 milligrams per milliliter of distilled water used to homogenize them. In a chilled centrifuge, homogenates are spun for 15 minute, at 8000 r.p.m., and 2 ºC. Supernatants were stored in a freezer at -20ºC until needed for biochemical experiments, and the deposits were disposed of. The

absorbance was measured using a spectrophotometer (Spectrophotonic 1201, Milton Roy Co., USA).

2.4.4. Total protein in fleas

Bradford's method [26] was employed to determine the total amount of proteins. Dissolved 100 mg of Coomassie Brilliant blue G-250 in 50 milliliters of 95% ethanol to create the protein reagent. Inserted 100 ml of 85% W/V of Phosphoric acid into the previous solution. One liter was the ultimate volume of the diluted solution. Test tubes were pipetted with 50µl of the test solution or, in the case of the standard curve preparation, 50µl of serial concentrations (from 10 to 100µg) of bovine serum albumin. Phosphate buffer (0.1M, pH 6.6) was added to the test tube to bring 1 ml. After adding five millimeters of reagent of protein, the contents are combined by vortexing or inversion. The absorbance measured at 595 nm after 2 minutes and before 1 hour.

2.4.5. Acetylcholinesterase (AChE) activity in fleas

Acetylcholinesterase (AchE) activity was estimated using themethodology outlined by Simpson et al. [27] using substrate (acetylcholine bromide). The mixture of reaction consisted of 200 µl of solution of enzyme, 0.5 ml phosphate buffer (0.067 M, pH7), and 0.5 ml acetylcholine bromide (3mM).incubated it for precisely thirty minutes, at 37 ºc. To the test tubes, 1 milliliter of alkaline hydroxylamine was added, then added 0.5 milliliters of Hcl. shaken mixture and after 2 minutes, added 0.5 ml of ferric chloride solution. At 515 nm, the drop in acetylcholine bromide brought on by AchE's hydrolysis was measured.

2.4.6. Glutathione S-transferase (GST) activity in fleas

Reduced glutathione (GSH) and 1-chloro 2,4-dinitrobenzene (CDNB) are conjugated via the glutathione -SH group, which is catalyzed by glutathione S-transferase (GST). S-(2,4-dinitro-phenyl)-L-glutathione, the conjugate, may be found using Habig et al. [28].200µl of flea homogenate, 100µl of GSH, and one milliliter of phosphate buffer (pH6.5) made up the mixture of reactions .25µl of the CDNB solution was added to initiate the reaction. CDNB and GSH concentrations increased to 5 mM and 1 mM, respectively. Incubated enzymes for five minutes, at 30˚C. Absorbance measured at 340 against a blank that included all the components except the enzyme to calculate the nano-mole substrate conjugated/min/flea.

2.4.7. Non -specific esterases activity in fleas

Applying naphthyl acetate (α- or β-) as substrates, respectively, the alpha or beta esterases (α- or β-esterases) were determined using Van Asperen's methodology [29]. 20µl of homogenate of fleas was added to 5 ml of α-or β-naphthylacetate, 0.1M phosphate buffer (pH7) and 1% acetone. After significant at P <0.05, when comparing means using Duncan's multiple range test.

precisely 15 minutes at 27˚C of incubation, 1 milliliter of color reagent (diazoblue), which was made by combining 1% diazoblue B with 5% sodium lauryl sulfate, was included in the mixture. The generated color measured at 600 or 555 nm for α- and β-naphthol resulted from reaction, respectively.

Curve of standard made by dissolving 20 mg of either naphthol in 100 milliliters of pH 7 phosphate buffer (stock solution). Aliquots of diluted solution (corresponding to 2,4,8,16, and 32 µg naphthol) in volumes of 0.1, 0.2, 0.4, 0.8, and 1.6 ml were pipetted into test tubes and topped off with 5 ml of phosphate buffer. After adding one milliliter of diazoblue reagent, the generated colour was measured as previously indicated.

2.5. Toxicological studies

A total of fifty albino rats, both male and female, weighing 107 ± 5 g, were obtained from the Animal House of the Medical Entomology Research Institute, Giza, Egypt. The rats were split into five groups (five male and five female per group). Under the ethical approval number IME00074 (22/3/2023), all rats are given humane treatment depending on the criteria of the National Institutes of Health (NIH publication 86-23 amended 1985) and the Animal treatment and Use Committee of the Research Institute of Medical Entomology. Rats were divided into:-**group 1**: rats obtained 1 ml of distilled water daily for 3 weeks by the oral route (control), **group 2**: rat received280mg/kg of cinnamon essential oil [30]. **Group 3**: rats received 140 mg/kg of cinnamon essential oil. **Group 4**: rats received 550mg/kg of rosemary essential oil [31].**Group 5**: rats received275mg/kg of rosemary essential oil. Anesthetized rats with isoflurane at the conclusion of the experiment. For biochemical, pro-inflammatory cytokine, and hematological investigations, samples of blood were extracted from the retro-orbital venous plexus using a vacutainer tube without any anticoagulant and a vacutainer tube with anti-coagulant like EDTA (ethylenediaminetetraacetic acid). The serum was extracted by centrifugation for 10 min, at 3000 rpm (600×g) and 4 °C using a Heraeus Labofuge 400R (Kendro Laboratory Products GmbH, Germany).

Statistical analysis

The Statistical Package for Social Sciences is used to examine the data of biochemical, bioassay, pro-inflammatory cytokine, and hematological investigations (SPSS 26.0 for Windows). The SPSS program completed the probit analysis and mortality % using Finney's [24] and Abbott's formula [23] approaches.

The one-way analysis of variance (ANOVA) was used to investigate the results. Results are considered

3. Results

Analysis of phenolic substance of the cinnamon oil by GC-MS revealed isolation of 10compounds (Table

1) including 64.62%2-Propenal, 3-phenyl-,8.73% Phenol, 2-methoxy-4-(2-propenyl)-, 5.28%Acetic acid, cinnamyl ester,3.17%Benzene, 1,2-dimethoxy-4-(2-propenyl)-,3.02%Caryophyllene, 2.87% Linalool, 2.69% Benzyl benzoate, 2.15% Estragole, 2.07 %Benzaldehyde,1.4%1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-, while analysis of phenolic substance of the rosemary oil revealed isolation of 14 compounds (Table 2), including 26.91% Eucalyptol,20.48% D-Limonene,14.91%Bis(2-ethylhexyl) phthalate,14.01%Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S),8.18% Cyclohexane, 1-methylene-4-(1-methylethenyl)-,4.27%3-Carene, 3.49% Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-,(1S-endo), 2.79% Camphene, 2.16%.alpha-Terpineol, 1.9% Isobornyl acetate, 1.45% Isoborneol, 0.68 % Caryophyllene, 0.42 % D-Carvone,0.36%.beta.-Myrcene.

Both rosemary and cinnamon oils had activity as insecticides against adult fleas. The lethal concentration (LC_{50}) of cinnamon oil was 0.290 mg /L compared with3.680 mg /L for rosemary oils (Table 3). This result revealed that the cinnamon oils had greater insecticidal activity than the rosemary oil.

Table 1: Analysis of cinnamon oil using GC/MS

	RT	Name	Formula	Area	Area Sum %
1	**4.944**	**Benzaldehyde**	**C7H6O**	**22008241**	**2.07**
2	**7.062**	**Linalool**	**C10H18O**	**32925594**	**2.87**
3	**8.515**	**Estragole**	**C10H12O**	**24089704**	**2.15**
4	**10.003**	**2-Propenal, 3-phenyl-**	**C9H8O**	**736399604**	**64.62**
5	**10.769**	**Phenol, 2-methoxy-4-(2-propenyl)-**	**C10H12O2**	**97857193**	**8.73**
6	**11.324**	**Benzene, 1,2-dimethoxy-4-(2-propenyl)-**	**C11H14O2**	**35579712**	**3.17**
7	**11.53**	**Caryophyllene**	**C15H24**	**32605289**	**3.02**
8	**11.902**	**Acetic acid, cinnamyl ester**	**C11H12O2**	**40587603**	**5.28**
9	**11.982**	**1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-**	**C15H24**	**15728162**	**1.4**
10	**15.627**	**Benzyl benzoate**	**C14H12O2**	**30127025**	**2.69**

Table 2: Analysis of rosemary oil using GC/MS

	RT	Name	Formula	Area	Area Sum %
1	4.435	3-Carene	C10H16	39166544	4.27
2	4.681	Camphene	C10H16	2525409.3	2.79
3	5.145	Cyclohexane, 1-methylene-4-(1-methylethenyl)-	C10H16	75036034	8.18
4	5.305	.beta.-Myrcene	C10H16	3279418.3	0.36
5	5.986	D-Limonene	C10H16	187940202	20.48
6	6.06	Eucalyptol	C10H18O	246885545	26.91
7	7.845	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-	C10H16O	128502214	14.01
8	8.029	Isoborneol	C10H18O	13265409	1.45
9	8.183	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-	C10H18O	32016867	3.49
10	8.498	.alpha.-Terpineol	C10H18O	19859581	2.16
11	9.184	D-Carvone	C10H14O	3847096.1	0.42
12	9.762	Isobornyl acetate	C12H20O2	17455817	1.9
13	11.502	Caryophyllene	C15H24	6244964.3	0.68
14	22.053	Bis(2-ethylhexyl) phthalate	C24H38O4	136795044	14.91

Table 3: The insecticidal properties of cinnamon and rosemary oils against adult fleas

Oil	LC_{50} (mg/l)	Confidence limits		LC_{90} (mg/l)	Confidence limits		LC_{90}/LC_{50}	Chi-Square	Slope ± SE	Toxicity index (TI)	Toxicity Increase%
		Lower limit	Upper limit		Lower limit	Upper limit					
cinnamon	**0.290**	**0.246**	**0.333**	**0.627**	**0.553**	**0.742**	**2.162**	**1.712**	**3.798 ± 0.466**	**0.0787**	**92.13**
Rosemary	**3.680**	**3.130**	**4.142**	**6.656**	**6.005**	**7.666**	**1.808**	**1.932**	**0.431 ± 0.058**	**1**	**0.00**

lC_{50}: lethal concentration. Toxicity index (TI) = {(LC50 of cinnamon oil)/ (LC_{50} ofrosemary)}. TI lower than 1 mean the compound have high toxicity. Toxicity increase (%) = (TI ofrosemary – TI of cinnamon oil) x 100.

The Effect of rosemary and cinnamon oils on total proteins of adult fleas (Fig. 1) was investigated at LC50 dosages. Level of total proteins was decreased in group treated with cinnamon oil more than group treated with rosemary oil compared with control group. The effect of rosemary and cinnamon oils on acetyl cholinesterase (AchE) of adult fleas (Fig. 2) was investigated at LC_{50} dosages. Level of AChE was decreased in the group treated with cinnamon oil more than the group treated with rosemary oil compared with control group, but effect of rosemary and cinnamon oils on glutathione S-transferase (GST) of adult fleas (Fig. 3) showed increasing in level of GST in the group treated with cinnamon oil more than the group treated with rosemary oil compared with control group. in addition, effect of rosemary and cinnamon oils on alpha- and beta-esterase of adult fleas (Figs. 4 and 5) showed a significant increase in the level of alpha- and beta-esterase in the group treated with rosemary oil more than the group treated with cinnamon oil compared with control group.

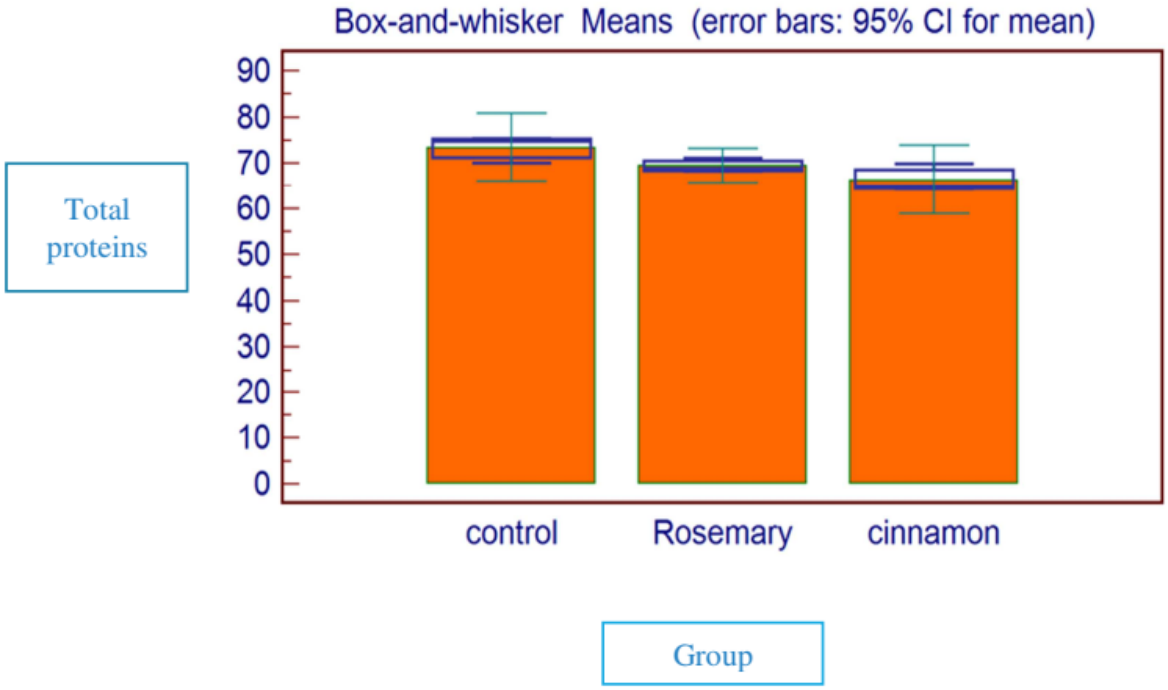

Figure 1: Influence of cinnamon and rosemary on total proteins in adult fleas

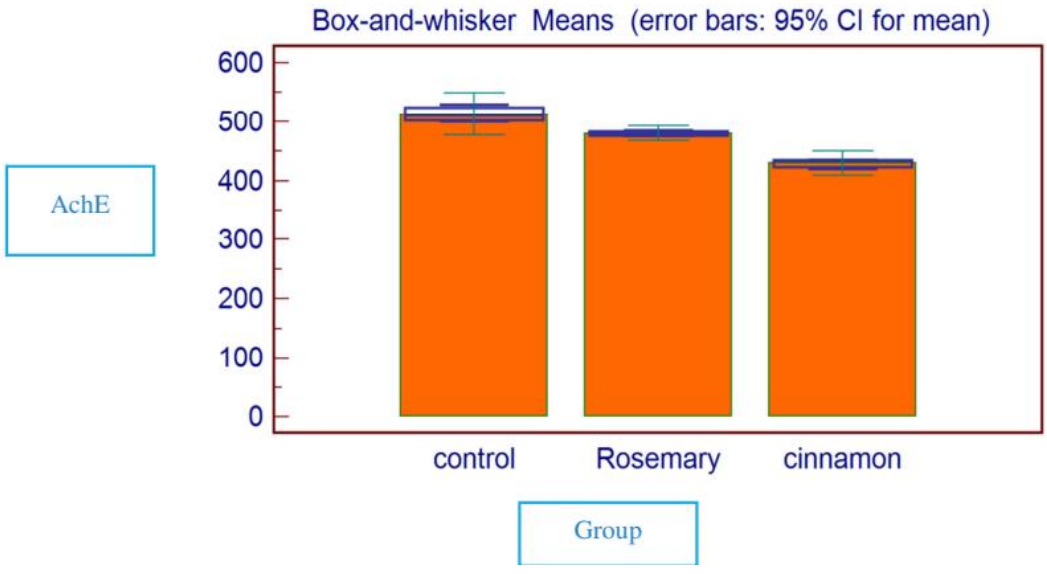

Figure 2: Influence of cinnamon and rosemary on acetyl cholinesterase (AchE) in adult fleas

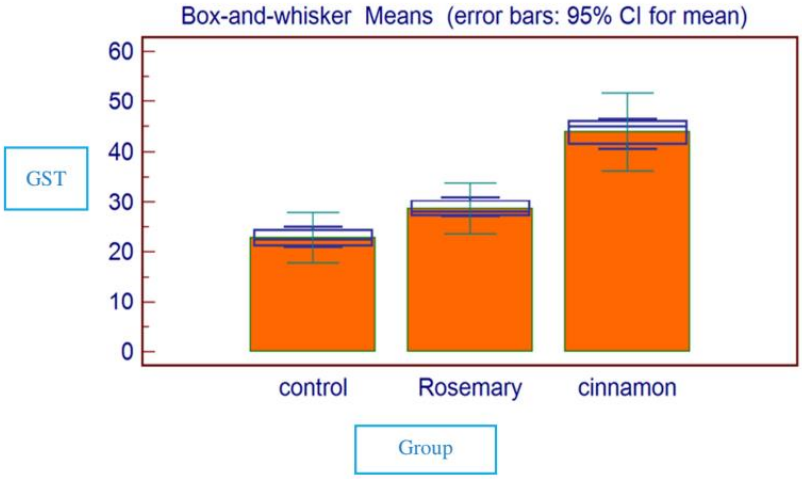

Figure 3: Influence of cinnamon and rosemary on Glutathione S-transferase(GST) in adult fleas.

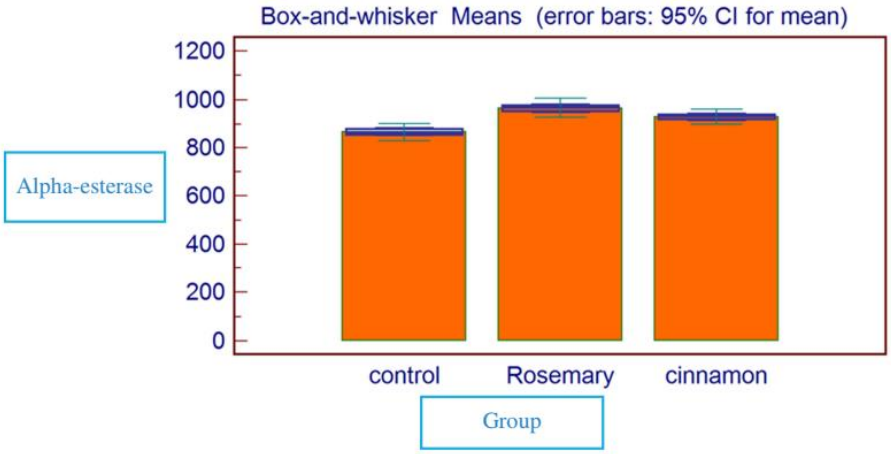

Figure 4: Influence of cinnamon and rosemary on Alpha-esterase in adult fleas

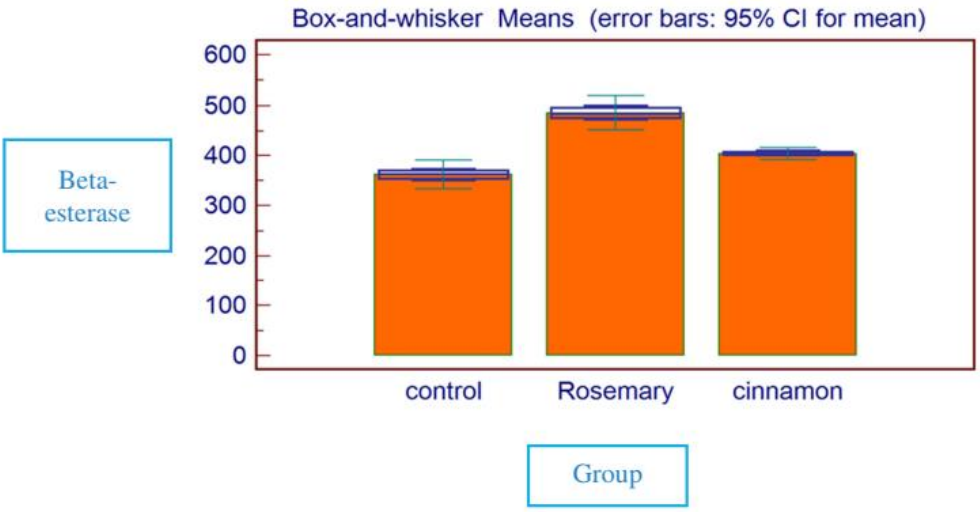

Figure 5: Influence of cinnamon and rosemary on Beta-esterasein adult fleas.

Hepatic (AST, ALT, albumin, TP) and renal (urea, creatinine, uric acid) biomarkers in female and male rats treated with rosemary and cinnamon oils for three weeks did not significantly change according to (tables 4 and 5) which displays the biomarkers of the kidney and liver in the rats, but this study shown that level of liver and kidney functions in males more than in females. Furthermore, neither the male nor the female treated rats showed any change in hematological characteristics like WBC, HB, RBC, MCV, MCHC, HCT, MCH, platelets, neutrophils ,monocytes, eosinophils , and lymphocytes (tables 6 and 7), but this study showed that hematological parameters increased in males than females .In addition, pro- inflammatory markers of males and females rats exposed to rosemary and cinnamon oils (tables 8 and 9) showed no significant alterations in serum TNF and IL6 levels, but this study showed that pro-inflammatory markers increased in males than females.

Table 4: Liver and renal indicators in female rats treated with oils of rosemary and cinnamon for three weeks

Biomarker	Treatment				
	Control	Cinnamon oil (140 mg/kg)	Cinnamon Oil (280mg/kg)	Rosemary oil (275 mg/kg)	Rosemary oil (550 mg/kg)
AST (u/l)	106.20 ± 5.08	108.22 ± 4.99	109.40 ± 6.08	104.01 ± 3.51	110.01 ± 4.25
ALT (u/l)	38.80 ± 5.40	36.20 ±3.66	39.0 ± 3.77	37.40 ± 3.55	40.83 ± 2.63
Albumin (g/dl)	3.22 ± 0.205	3.23 ± 0.279	3.25 ± 0.238	3.20±0.162	3.27 ± 0.249
Total protein (g/dl)	5.56 ± 0.463	5.53 ± 0.344	5.58 ± 0.479	5.54 ± 0.349	5.59 ± 0.336
Urea (mg/dl)	42.60 ± 1.88	39.40 ± 1.99	43.62 ± 1.74	38.0 ± 1.43	40.20 ± 2.40
Uric acid (mg/dl)	3.70 ±0.111	3.67 ± 0.196	3.73 ± 0.136	3.68 ± 0.257	3.71 ± 0.217
Creatinine (mg/dl)	0.76 ± 0.040	0.75 ± 0.036	0.77 ± 0.016	0.74 ± 0.025	0.79 ± 0.029

Values are mean±SE, n=5. At P<0.05, there is a negligible difference in the means of the same row.

Table 5: Liver and renal indicators in male rats treated with oils of rosemary and cinnamon for three weeks

Biomarker	Treatment				
	Control	Cinnamon oil (140 mg/kg)	Cinnamon Oil (280mg/kg)	Rosemary oil (275 mg/kg)	Rosemary oil (550 mg/kg)
AST (u/l)	110.20 ± 4.50	112.0 ± 4.90	115.40 ± 6.37	107.5 ± 3.55	112.2 ± 4.19
ALT (u/l)	39.88 ± 5.57	37.20 ±3.75	42.55 ± 3.79	38.40 ± 3.57	41.99 ± 3.62
Albumin (g/dl)	3.40 ± 0.303	3.38 ± 0.389	3.42 ± 0.328	3.41 ±0.259	3.49 ± 0.239
Total protein (g/dl)	5.75 ± 0.562	5.76 ± 0.432	5.80 ± 0.577	5.73 ± 0.448	5.84 ± 0.546
Urea (mg/dl)	47.66 ± 2.28	45.40 ± 2.90	50.62 ± 2.75	48.55 ± 2.45	52.25 ± 3.41
Uric acid (mg/dl)	4.30 ±0.221	4.28 ± 0.269	4.35 ± 0.226	4.32 ± 0.355	4.37± 0. 317
Creatinine (mg/dl)	0.80 ± 0.065	0.78 ± 0.046	0.85 ± 0.053	0.82 ± 0.055	0.87 ± 0.097

Values are mean±SE, n=5. At P<0.05, there is a negligible difference in the means of the same row.

Table 6: Complete blood picture of female rats received rosemary and cinnamon oils

Hematological Parameters	Treatment				
	Control	Cinnamon oil (140 mg/kg)	Cinnamon oil (280mg/kg)	Rosemary oil (275 mg/kg)	Rosemary oil (550 mg/kg)
WBC (10^3/cmm)	11.08 ± 0.294	11.09 ± 0.529	11.10 ± 0.439	11.07 ± 0.182	11.10 ± 0.212
RBC (10^6/cmm)	4.93 ± 0.282	4.95 ± 0.272	4.98 ± 0.210	4.97 ± 0.331	4.98 ± 0.289
HB (g/dl)	11.18 ± 0.419	11.19 ± 0.213	11.20 ± 0.321	11.20 ± 0.324	11.21 ± 0.439
HCT (%)	36.75 ± 1.68	36.77 ±1.35	36.79 ± 1.51	36.76 ± 1.54	36.78 ±1.63
MCV(FL)	62.25 ± 2.35	62.26 ± 2.83	62.29 ± 2.25	62.27 ± 2.39	62.30 ± 3.14
MCH(PG)	20.59 ± 0.612	20.60 ± 0.823	20.63 ± 0.825	20.61 ± 0.597	20.64 ± 0.528
MCHC (%)	31.95 ± 0.812	31.97 ± 0.713	31.99 ± 1.15	31.96 ± 0.835	32.00 ± 0.813
Platelet count (10^3/cmm)	471 ± 28.71	472 ± 25.53	477 ± 22.73	473 ± 18.37	475 ± 16.92
Neutrophils (%)	30.40 ± 3.21	30.42± 2.18	30.45 ± 2.18	30.43 ± 2.53	30.46 ± 2.23
Lymphocytes (%)	65.60 ± 3.38	65.61 ± 2.80	65.63 ± 2.42	65.63 ± 3.41	65.65 ± 2.15
Monocytes (%)	2.90 ± 0.537	2.93 ± 0.517	2.95 ± 0.616	2.94 ± 0.314	2.96 ± 0.724
Eosinophils (%)	1.70 ± 0.214	1.71 ± 0.244	1.72 ± 0.245	1.70 ± 0.214	1.73 ± 0.234
Basophils (%)	0	0	0	0	0

Values are mean±SE, n=5. At P<0.05, there is a negligible difference in the means of the same row.

Table 7: Complete blood picture of male rats received rosemary and cinnamon oils

Hematological Parameters	Treatment				
	Control	Cinnamon oil (140 mg/kg)	Cinnamon oil (280mg/kg)	Rosemary oil (275 mg/kg)	Rosemary oil (550 mg/kg)
WBC (10^3/cmm)	12.11 ± 0.284	12.15 ± 0.420	12.17 ± 0.329	12.12 ± 0.163	12.18 ± 0.222
RBC (10^6/cmm)	5.10 ± 0.242	5.11 ± 0.371	5.14 ± 0.215	5.12 ± 0.321	5.15 ± 0.269
HB (g/dl)	12.20 ± 0.429	12.21 ± 0.233	12.25 ± 0.321	12.22 ± 0.324	12.26 ± 0.419
HCT (%)	37.55 ± 1.28	37.56 ±1.35	37.58 ± 1.60	37.56 ± 1.44	37.58 ±1.63
MCV(FL)	63.35 ± 2.25	63.35 ± 2.43	63.37 ± 2.15	63.36 ± 2.19	63.37 ± 2.04
MCH(PG)	21.50 ± 0.622	21.51 ± 0.425	21.54 ± 0.715	21.50 ± 0.487	21.54 ± 0.548
MCHC (%)	32.78 ± 0.414	32.78 ± 0.513	32.70 ± 1.15	32.77 ± 0.525	32.80 ± 0.813
Platelet count (10^3/cmm)	480 ± 24.21	482 ± 23.12	485 ± 20.71	484 ± 14.62	485 ± 12.82
Neutrophils (%)	31.20 ± 2.21	31.21± 2.18	31.24 ± 2.12	31.22 ± 1.63	31.22 ± 2.08
Lymphocytes (%)	66.10 ± 2.38	66.12 ± 1.81	66.15 ± 2.32	66.11 ± 3.21	66.12 ± 1.15
Monocytes (%)	3.05 ± 0.437	3.06 ± 0.417	3.09 ± 0.218	3.06 ± 0.234	3.08 ± 0.614
Eosinophils (%)	1.75 ± 0.210	1.77 ± 0.224	1.77 ± 0.245	1.76 ± 0.234	1.77 ± 0.214
Basophils (%)	0	0	0	0	0

Values are mean±SE, n=5. At P<0.05, there is a negligible difference in the means of the same row.

Table 8: Pro-inflammatory indicators in female rats treated with oils of cinnamon and rosemary

marker	Treatment				
	Control	Cinnamon oil (140 mg/kg)	Cinnamon oil (280mg/kg)	Rosemary oil (275 mg/kg)	Rosemary oil (550 mg/kg)
TNF (pg/ml)	46.74 ± 4.18	47.62± 2.62	49.40 ± 3.23	45.27 ± 2.28	50.02 ± 2.55
Il6 (ng/ml)	1.08 ± 0.149	1.13 ± 0.260	1.15 ±0.182	1.07 ± 0.327	1.11 ± 0.371

At P<0.05, there is a negligible difference in the means of the same row.

Table 9: Pro-inflammatory indicators in male rats treated with oils of cinnamon and rosemary

marker	Treatment				
	Control	Cinnamon oil (140 mg/kg)	Cinnamon oil (280mg/kg)	Rosemary oil (275 mg/kg)	Rosemary oil (550 mg/kg)
TNF (pg/ml)	50.44 ± 3.18	51.60 ± 2.62	55.40 ± 2.33	52.20 ± 2.48	55.02 ± 2.55
Il6 (ng/ml)	1.50 ± 0.129	1.51 ± 0.340	1.57 ±0.172	1.52 ± 0.427	1.57 ± 0.241

At P<0.05, there is a negligible difference in the means of the same row.

4. Discussion

The rat flea, or *Xenopsylla cheopis*, is an insect that lives as an ectoparasite on birds and mammals [32, 33]. There are 2,500 species of fleas in the world that are known to exist. It is most famous for being one of the transporters of the plague bacterium *Yersinia pestis*, which kills many people [34].Apart from its function as a carrier of bacterium of plague, this species is also capable of transmitting other human infections, including *Rickettsia typhi*, the bacteria responsible for flea-borne typhus, and two distinct tapeworm species [32, 35].Rat flea prevention methods relied on the application of chemical pesticides. Due to insect resistance to many insecticides and the negative health impacts on humans and ecological systems, conventional flea control methods are currently ineffective [36, 37].

This study used natural insecticides such as cinnamon and rosemary against rat flea. The examination of phenolic compounds by GC-MS of the cinnamon oil identified the isolation of ten substances (Table 1).These results concurred with the information provided by Zhang *et al*.[38] who found that cinnamon oil contains some compounds including Aldehydes (such as Benzaldehyde, and Cinnamaldehyde), Alcohols (such as Linalool, and Benzyl alcohol), Ethers (such as Estragole).Knauth *et al*.[39] and Kumar *et al*. [40] found that cinnamon oil contained oxygenated compounds and Hydrocarbons (i.e. β-caryophyllene, cinnamyl acetate, benzyl benzoate, eugenyl acetate and linalool), But analysis of phenolic substances of the rosemary oil by GC-MS revealed isolation of 14 compounds (Table 2). These results concurred with the information provided by Sienkiewicz *et al*.[41]and Mohammed *et al*.[42]Who noticed that rosemary oil contained Camphene,

Bornyl acetate, Limonene,Car-3-ene, α-Terpineol, The insecticidal activity of oils derived from cinnamon and rosemary was found to be effective against adult fleas (*Xenopsylla cheopis*). After 24 hours, the probit analysis yielded the lethal concentration (LC50) (Table 3).The lethal concentration (LC_{50}) of cinnamon was 0.290 mg /L compared with 3.680 mg /L for rosemary oils, while LC90for cinnamon oil was 0.553mg/l compared with 6.656 mg/l for rosemary oil. Cinnamon oil's toxicity index was 0.0787 with a 92.13% increase in toxicity compared to the rosemary oil. These findings suggested that cinnamon oil was more harmful than rosemary oil against adult fleas of the *Xenopsylla cheopis* species. Cinnamyl ester, 1,2-dimethoxy-4-(2-propenyl),acetic acid, benzene, caryophyllene, linalool, 1,5,9,9-tetramethyl-, Z,Z,Z, benzoate, estragole, benzoaldehyde, 1,4,7,-Cycloundecatriene, and other bioactive compounds may be the reason for cinnamon oil's high efficacy in killing fleas. These outcomes matched the information provided by Hassan *et al*.[43] who found that cinnamon oil has high efficacy in destroying fleas due to the presence of benzene methanol, dimethyl acetate, cinnamaldehyde,camphor,2-[2-(3-Hydroxybutyl) phenyl]ethanoic acid . Kim *et al*.[44] found that cinnamon oils could be useful as insecticides for the control of *M. pruinosa*, Also Abd-allah and Youssef.[45] found that cinnamon oil has high effect in controlling of cotton mealy bug *Phenacoccus solenopsis.* Estevam Ribeiro *et al*. [46] found that cinnamon oil can inhibit activity of acetylcholinesterase so it has high efficacy in killing fleas. In addition, Mahran *et al*. [14] showed that Cinnamon essential oil has significant larvicidal activity. Rosemary oil works rather well against several insects and mites. Numerous pests and insects are susceptible to the ovicidal and larvicidal actions of rosemary's fragrant vapor [47, 48]. Miresmailli & Isman[49] and Isman *et al*.[50] found that toxic effect of rosemary oil due to its chemical composition such as1,8-Cineol, Camphor, d-Limonene, β-Caryophyllen, α-Terpineol which have insecticidal effects. Also Selma *et al*. [51] demonstrated rosemary's insecticidal effectiveness against *E.ceratoniae*, a significant date pest.

Protein was important for the development of the adult from the pupa stage and the growth of some insects [52]. Wilkinson [53] reported that Proteins serve in the production of enzymes that detoxify microsomal systems, which aid in the removal of toxins ingested by insects. Proteins are the main components of an insect that are responsible for binding external chemicals. Usually, the difficulty of protein synthesis is closely linked to nucleic acid metabolism [54]. Our study found that the level of total proteins was decreased in the group treated with cinnamon oil more than the group treated with Myrcene, β-Caryophyllene, and Borneol.

rosemary oil compared with control group (Fig. 1) at doses equal to LC50. El-Barky *et al*. [55], and Assar *et al*. [54] revealed that using pesticides such as spinetoram, hexaflumuron, teflubenzuron, and emamectin, the total protein content of *S. littoralis's* fourth instars was reduced. Radwa*et al*. [56] found that cinnamon oil decreased protein content in 6th instar larvae of *Corcyra cephalonica*. Soltani *et al*. [57] found that rosemary oil decreased protein contents in adults of the *Oryzeaphilus surinamensis*, or saw-toothed grain beetle. Decreasing in level of proteins by cinnamon and rosemary oils due to their bioactive compounds exist in both of them [42, 43].

The majority of pesticides are toxic because they decrease the activity of AChE (acetylcholinesterase), which is accountable for degrading acetylcholine (ACh), a neurotransmitter that is crucial for the central nervous system (CNS) of insects and rodents [58,59]. Competitive inhibitors of AChE disrupt the function of autonomic ganglia and the Ach-controlled neuromuscular, parasympathetic, and sympathetic effect or junctions [60, 61].Our study found that level of AChE was decreased in the group treated with cinnamon oil more than the group treated with rosemary oil compared with control group (Fig2) at doses equal to LC50. Prerna *et al*. [62] and Hassan *et al*.[43] reported that cinnamon oils break down acetylcholinesterase enzyme, therefore it considered an anti-acetylcholinesterase and this effect is due to bioactive compounds that exist in it. Ilkay *et al*. [63] and Fatemeh *et al*. [64] found that *Rosmarinus officinalisL* decreased activity of AChE because of phenolic compound found in it.

GST (the glutathione transferases) is a type of enzyme that has an important role in intracellular transport, hormone production, and protection against oxidative damage in addition to aiding in the detoxification of various xenobiotics like pesticides [65]. It plays a part in pesticide resistance as well. Insecticides can be broken down by GSTs through the facilitation of conjugation or reductive dehydrochlorination reactions with reduced glutathione, which results in the production of more easily excreted water-soluble metabolites. Furthermore, they aid in the elimination of harmful oxygen free radical species that are generated as a result of pesticide use [66].The higher GST activity levels are indicative of diverse insect strains that are resistant to different kinds of pesticides. Our study showed the effect of Rosemary and cinnamon oils on Glutathione S-transferase (GST) of adult fleas (Fig3) at doses equal to LC50 since, It showed an increase in the level of GST in the group treated with cinnamon oil more than the group treated with rosemary oil compared with control group. Identical results provided by Mohammad *et al*. [67] who found that cinnamon oil induces the genes for glutathione

S-transferase and Krzyżowski *et al*. [19] discovered that exposure to rosemary oil increased the activity of GST, indicating the development of oxidative stress. On the contrary, Ita *et al*. [68] discovered that the 3th instar of C. *maculatus* and T. *castaneum* both exhibit decreased glutathione transferase activity when exposed to cinnamon oil.

The esterase type of enzymes breaks down ester bonds found in various insecticides; as a result, these enzymes may contribute to resistance to the primary chemicals used in control efforts [69]. According to Claire *et al*. [70], esterase activity rose as pests that were resistant to pyrethroid, carbamate, and organophosphate insecticides, including heliothine and spodopteran species. The present study revealed effect of rosemary and cinnamon oils on Alpha- and beta esterase of adult fleas (Fig4 and 5) at doses equal to LC50 since, It showed an increase in the level of Alpha- and beta esterase in the group treated with rosemary oil more than group treated with cinnamon oil compared with control group. Jun-Hyung *et al*. [71] found that rosemary (*Rosmarinus officinalis L.)* doesn't inhibit activity of general esterases enzymes and may increase them in *Trichoplusia ni*. On the contrary, Ita *et al.* [68] discovered that in both T. *castaneum* and C. *maculatus*, the third instar, cinnamon oil reduces esterase activity.

Results of this study on enzymes suggest that the toxicity of cinnamon and rosemary oils on adult fleas is due to the following mechanisms: 1-cinamon and rosemary oil effect on growth and development of the adult fleas by decreasing in level of total protein [56, 57].2-cinnamon and rosemary oil effect on neurotransmitter in the central nervous system by decreasing level of AChE [64, 72].

Female and male rats participated in a three-week sub-acute oral toxicity research of cinnamon and rosemary oils (Tables 4, 5, 6, 7, 8, 9). The rats treated with 275 mg/kg or 550 mg/kg of rosemary oil and 140 mg/kg or 280 mg/kg of cinnamon oil didn't exhibit any indicators of toxicity or mortality, according to the results. Moreover, there is an insignificant change of both oils on hepatic function (total protein, AST, ALT, and albumin), renal functions (creatinine, urea, and uric acid), hematological parameters, and pro- inflammatory markers (TNF and IL6). However, this study demonstrated that males had higher amounts of pro-inflammatory markers, hematological and biochemical parameters, and these differences were caused by sex hormones, which are involved in many aspects of reproduction, differentiation, growth, and homeostasis. The main effects of sex hormones are on the shape and function of the reproductive organs as well as the development of features unique to men and women. In addition, number of studies have revealed an additional crucial role in controlling the structure and/or function of almost every tissue and organ, including the kidneys, liver, brain, and bones, which results in sexual dimorphism (gender and sex differences) in a range of characteristics [73] Additionally, variations in muscle mass are linked to variations in liver and renal enzymes [74]. Therefore, cinnamon and rosemary oils are considered safe and eco-friendly insecticides for controlling adult fleas (*Xenopsylla cheopis*) but the toxic effect of cinnamon oil was higher than the toxic effect of rosemary in killing of fleas.

5. Conclusions

Following GC-MS analysis, the presence of 10 and 14 compounds, respectively, was found in the oils of cinnamon and rosemary. The current study's findings showed that, in comparison to rosemary oil, cinnamon has a higher insecticidal efficacy against fleas. When comparing the LC50 values for rosemary and cinnamon oils, they are 0.290 mg/L and 3.680 mg/L, respectively. Since cinnamon and rosemary did not cause any harm to rats, they are regarded as a safe and environmentally friendly insecticide for managing adult fleas (*Xenopsylla cheopis).*

6. Conflict of interest

No conflict of interest.

7. References

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