Bio-Insecticide and Residual Effect of *Eucalyptus camaldulensis* Dehnh Essential Oil on *Callosobruchus maculatus* Fabricius in Stored Cowpea Grains

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ABSTRACT

Cowpea is one of the food legumes grown in Africa and the world due to its high protein. However, it has been faced with insect attack either in storage or in the field, which has caused huge economic losses. The major storage pest of cowpea grains is *Callosobruchus maculatus,* which causes severe losses of up to 100% in unprotected stored cowpea grains. Synthetic insecticides are used in controlling the beetles but are discouraged due to undesirable effects on humans and the environment. This study assessed *Eucalyptus camaldulensis* plant essential oil as an alternative to control stored cowpea grain weevils. Objectives of the study include identifying the compounds present in *E. camaldulensis* essential oil as a bio-insecticide, determining the residue of the essential oil in treated cowpea grains, and determining the proximate analysis of treated cowpea grains.

A susceptible cowpea variety Ife Brown cowpea grains were used with six treatments: 0.15, 0.25, 0.35 and 0.45mL/g of Eucalyptus essential oil (EO), Phostoxin (0.01g) and control (without treatment), all replicated four times and laid out in a completely randomized design. The essential oil was extracted with GC-MS to identify the compounds and residue of the essential oil in cowpea grains, also, proximate analysis of treated cowpea grains was determined using standard procedures. Data were collected on mortality of insects using Abbot's formula and Analysis of Variance (ANOVA) at $p<0.05$.

Results revealed 19 compounds (prominent monoterpenes and terpenes) from *E. camaldulensis* essential oil with highest value 27.74% obtained from Eucalyptol, followed by 12.68% from gamma Terpinene and Camphene with lowest value of 0.28% Toxicity of the essential oil on *C. maculatus* at 24 hours duration application gave 100% mortality at 3.50, 3.75 and 4.0 mL/g, respectively and compared favorably with phostoxin (100%), while the control gave highly significant reduction (12.5%). Essential oil residues in treated cowpea showed seven compounds with Caren having the highest concentrations (45.74 -2565.19 mg/L), while Bicyclogermacrene recorded the lowest (5.54 - 7.40 mg/L) throughout the applied treatments. Significant variations were observed in proximate analysis; the essential oil at 0.15 mL/g had highest values of 29.05% in crude protein and 5.60% in Ash over other treatments. Highest value of 11.81% was recorded from 0.45 mL/g for moisture content and 51.84% maximum value from 0.35 mL/g for carbohydrate.

This study revealed that *Eucalyptus camaldulensis* essential oil contained important insecticidal components, high toxicity on *Callosobranchus maculatus* on stored cowpea grains, with no toxic residue of the essential oil in the cowpea grains. Also, the essential oil showed no harmful effects on the nutritive components of the proximate analysis. Therefore, *Eucalyptus camaldulensis* essential oil could give food quality and safety when used as bio insecticide to protect cowpea grains in storage.

(Keywords: bio-insecticide, *Eucalyptus camaldulensis*, essential oil, contact toxicity, cowpea, food quality, food safety)

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp.) is a major staple food crop in tropical countries, cheap source of dietary protein especially in West Africa. Also, where it is a cheap source of dietary protein. it is also an essential protein source of protein in sub-Saharan Africa, particularly especially in the dry savannah regions where animal protein is rarely affordable (Adedire, *et al*., 2011; Isienyi, *et al*., 2020 and Sarr, *et al.,* 2022). The seeds are a major source of plant proteins and vitamins for man, the fodder is used as feed for animals, and the crop is also a source of cash of income.

The growing economic importance of cowpeas is due to its food value, which makes them a good supplement/complimentary source of protein for alternative animals sources of protein (Isienyi, *et al*., 2020). Cowpea contains 24.53 -28.70% protein, fat (1.51 and 3.02%), crude fiber (2.25 - 4.68%), and ash 3.06 and 4.30% (Fayinminnu and Adesiyan, 2013) and 64% carbohydrate. It is also a good source of calcium, iron, vitamins, and carotene (Adedire, *et al*., 2011). The crop has tremendous potential in contributing to the alleviation of malnutrition, specifically amongst the poor. Besides being low in fat and high in fiber, the protein in cowpea has been shown to reduce low-density lipoproteins that are implicated in heart disease (Frota, *et al*., 2015).

Cowpea is attacked by a complex of insect pests from seedling to harvest and storage. The chrysomelid *Callosobruchus maculatus* (Coleoptera: Chrysomelidae: Bruchinae) causes the major losses in stored grains. *Callosobruchus maculatus* is a store agricultural insect pest in Africa and Asia that ranges throughout the tropical and subtropical world (Deme, *et al*., 2019).

However, *C. maculatus* constitutes losses due to infestation between 87 to 100% within 3-5 months of storage have been reported (Ojiako and Adeyinka, 2013). Damage is done to the seeds by the exit holes created during the emergence of adult cowpea beetle and includes a reduction in kernel weight caused by the burrowing larvae as they feed; this also diminishes market value due to the presence of insects inside the kernels. Cowpea beetle (*C. maculatus*) infestation also decreases the germination potential of the seed (Munthali and Sondashi, 2004; Maina, *et al*., 2006).

The common control method against storage pests is treatment of produce with synthetic insecticides such as pirimiphos methyl, fenitrothion, methyl bromide, phosphine fumigant and sniper (Dichlorvos) (Daily Trust Newspaper Nigeria, 2020; Fayinminnu, *et al*., 2022). Mordue and Nisbet (2010); Longe (2016), reported that these chemicals appear effective. However, their use is being discouraged due to the problems of environmental pollution and contamination, the development of insecticide resistance by pests and injury to human health.

One of the explosive and argumentative issues affecting agricultural production is the perception that pesticide residues in food supplies are hazardous and pose serious health risks (FAO, 2005; Daily Trust Newspaper, Nigeria, 2020). This concern about the deleterious effects of inorganic insecticides has been expressed in most countries, irrespective of location and development ranking. Thus, a need to research alternatives to these synthetic pesticides that are easily accessible, affordable, environmentally friendly and safe for both producers and consumers (Fayinminnu, *et al.,* 2013). Also, the bioactive compounds obtained from seeds, leaves, flowers and roots of plants have been reported to be better than synthetic insecticides, due to being easily biodegradable, ecologically safe and broad-spectrum in action (Fayinminnu, *et al*., 2013; Isienyi, *et al*., 2020).

Eucalyptus camaldulensis, commonly known as the river red gum, is a tree native to Australia. It has smooth white or cream-colored bark, lance-shaped or curved adult leaves, flower buds in groups of seven or nine, white flowers and hemispherical fruit with valves extending beyond the rim (Isienyi, *et al*., 2020). Eucalyptus oil is a complex mixture of various compounds, including monoterpenes, sesquiterpenes and phenols, which vary in composition and proportion depending on the species (Brooker and Kleinig, 2006; Edris, *et al*., 2007).

The pesticidal activity of this oil is attributed to its components, including 1,8-cineole (Eucalyptol), citronellal, citronellol, citronellyl acetate, p-cymene, eucamalol, limonene, linalool, a-pinene, g-terpinene, a-terpineol, alloocimene, and aromadendrene (Batish, *et al*., 2008; Maciel, *et al*., 2010). Bioactivity of Eucalyptus oil depends on the type and concentration of its constituents, which can be influenced by factors like species,

season, location and extraction method (Brooker and Kleinig, 2006). The components of this oil work synergistically to produce its pesticidal effects, with 1,8-cineole being the most important compound responsible for its pesticidal properties (Duke, 2004).

The natural insecticidal properties and activities of Eucalyptus oil have been demonstrated to repel mosquitoes and other arthropods and serve as an anti-feedant against herbivores in various studies. Yang *et al.* (2004) found that *Eucalyptus globulus* oil and its major component, 1,8-cineole, showed pediculicidal activity against human head lice, outperforming commercial pediculides -delta-phenothrin or pyrethrum. Ceferino, *et al.* (2006) also showed the fumigant toxicity and repellent activity of Eucalyptus oil against permethrin-resistant human head lice. However, the bio-insecticide and residual effect of *Eucalyptus camaldulensis* essential oil on *Callosobruchus maculatus* (cowpea weevils) in stored cowpea grains has limited information.

This study, therefore, was carried out to offer a potential natural solution for sustainable agriculture and food security. Also providing an alternative cowpea preservative technique that is low-cost, accessible and safe. Therefore, objectives of the study were to (i) identify the chemical compounds present in E*ucalyptus camaldulensis* essential oil (ii) assess the efficacy of Eucalyptus *camaldulensis* essential oil as a bio-insecticide on stored cowpea grains, (iii) determine the residue of the essential oil in treated stored cowpea grains and (iv) determine the proximate analysis of treated cowpea grains with E*ucalyptus camaldulensis* essential oil.

MATERIALS AND METHODS

Experimental Site

Experiments were carried out in the Entomology Research Laboratory, Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan, under the ambient temperature of 29±6 °C and a 12:12 h period. The Department is located within latitude 7° 43"N and longitude 3° 54"E at an altitude of 200 m with annual rainfall between 1,250-1,500 mm spanning eight months (March October) with dry spell in August**.**

Source of Cowpea Seeds and Culture of *Callosobruchus maculatus*

Cowpea seeds, Ife Brown variety, were sourced from the Institute of Agricultural Research and Training (IAR&T), Moor Plantation, Ibadan, Nigeria. Twenty-four Kilner jars were filled with sorted, clean cowpea seeds of 250 g. Insect rearing was done using unsexed adult *C. maculatus* (cowpea weevils) obtained from an existing culture at the Entomology Research Laboratory, Department of Crop Protection and Environmental Biology, University of Ibadan, introduced into the jars to infest the seeds. Kilner jars were covered with 1- 2 mm mesh for aeration and to prevent the escape of the confined insects. After seven days, the beetles were removed from the culture, and the setup was maintained until the F1 generation (newly emerged adult beetles) was used for the experiment.

Eucalyptus Plant Collection

Fresh and matured *Eucalyptus camaldulensisis* leaves were collected in a 50 kg empty sack from the Department of Forestry and Forest Products, University of Ibadan, and identified at the Forestry Research Institute of Nigeria (FRIN) Herbarium, Ibadan, with a Voucher Number 111807

Extraction of Essential oil from Eucalyptus leaves

Eucalyptus camaldulensis leaf samples were extracted using hydro-distillation with a modified Clevenger's apparatus at the Research Laboratory, Department of Chemistry, University of Ibadan using the method of British Pharmacopoeia (1980). The leaves (100 g) were placed in a 5 L flask and water was added until the sample was fully immersed. The essential oil was trapped in 2.0 mL of hexane and carefully collected using a syringe and placed in a pre-weighed sample vial. The oil was collected, weighed and recorded; the yield was also stored in an amber vial for further analysis. The extraction process was repeated for the weighed leaf sample for three hours according to the British Pharmacopoeia specification.

Preparation of Concentrations of Eucalyptus Essential Oil

From a portion of the essential oil obtained above, a stock solution was prepared for each concentration in 100 mL of standard flax with methanol using the equation $C_1V_1=C_2V_2$. Four concentrations: 0.15, 0.25, 0.35, and 0.45 mL/g were prepared using the serial dilution method in methanol as the solvent.

Screening and Identification Method of Essential Oil from Eucalyptus leaves

The analysis was performed using a 8860A Gas Chromatograph Mass Spectrometry (GC-MS) coupled to a 5977C Inert Mass Spectrometer with an electron impact source (Agilent Technologies). The Gas Chromatography Mass Spectrometry (GC-MS) was carried out at CTX-ION Analytics Limited Ikeja, Lagos, Nigeria, using all instrumental conditions as reported by Adegoke and Alo (2013).

The mass spectrometer was operated in electron-impact ionization mode at 70 eV with ion source temperature of 230°C, quadrupole temperature of 150°C and transfer line temperature of 280°C. Scanning of possible compounds was from m/z 50 to 550 amu at 2.62s/scan rate and were identified by comparing measured mass spectral data with those in the NIST 14 Mass Spectral Library.

Experimental Design

Six treatments: 0.15, 0.25, 0.35, and 0.45 mL/g of *Eucalyptus camaldulensis* essential oil, Phostoxin (0.10g) and without treatment (control) were all replicated four times and laid out in a completely randomized design. The *Eucalyptus camaldulensis* essential oil was represented as follows: EUC A = 0.15mL/g of *Eucalyptus camaldulensis* essential oil, EUC B = 0.25mL/g of *Eucalyptus camaldulensis* essential oil, EUC C = 0.35mL/g of *Eucalyptus camaldulensis* essential oil, EUC D = 0.45mL/g of *Eucalyptus camaldulensis* essential oil.

Toxicity of *Eucalyptus camaldulensis* Essential Oil against *Callosobruchus maculatus* in Stored Cowpea Grains

Twenty grams (20 g) of Ife brown cowpea grains were weighed into each of six 1 L Kilner jars. Two (2) mL of each 0.15, 0.25, 0.35, 0.45 mL/g concentrations of *E. camaldulensis* essential oil were introduced on the grains using a micro syringe, Phostoxin (0.01 g) was added to the grains and control (without treatment), afterwards each jar received four pairs of unsexed adults of C*. maculatus*. Data on mortality were collected at 24h after infestation, converted to a percentage and corrected using Abbott's formula (Abbott, 1925):

$$P_T = \frac{P_{o-} P_c}{100 - P_c}$$

Where,

P_T= corrected mortality (%),
P_o= observed mortality (%),
P_c= control mortality (%)

Extraction and Determination of *Eucalyptus* Essential Oil Residue in Stored Cowpea Grains

The *Eucalyptus camaldulensis* essential oil residue in stored cowpea grains was carried out using the UNEP/FAO/IAEA (1986). The procedures were stated below: Instrument used: Ultrasonic Bath: CLEAN 120-HD (China), Rotary Evaporator: BUCHI Rotavapor R-215 (Switzerland) and Analytical Balance: ADAM AAA250LE Weighing Balance (UK).

Reagents used: Acetone: GC Ultratrace Scharlau (Spain), n-Hexane: 96% GC Ultratrace Scharlau (Spain), Silica Gel: Loba Chemie (India) and Anhydrous Sodium Sulphate: Merck (US).

Five grams of properly homogenized cowpea grain samples were weighed into beakers and mixed with 10 ml n-hexane: acetone (1:1). The beakers were then placed into an ultrasonic bath and sonicated for 20 minutes. The mixture was allowed to settle, and the solvent layer was decanted and concentrated down to 2 mL using a rotary evaporator (UNEP/FAO/IAEA (1986)).

Cleanup Procedure of *Eucalyptus camaldulensis* Essential Oil Residue in Stored Cowpea Grains

Granular silica gel (Mesh Size 60- 200A) was activated by heating at 130°C for 16 h and stored in a desiccator. A glass column was packed with 5 g of silica gel and 1 g of Anhydrous Na_2SO_4 was added. n-Hexane (20 mL) was added to the column and eluted into a beaker. The 2 mL sample extract was quantitatively added to the top of the column. Another 10 mL of n-Hexane was added to the column and eluted to waste. Before the column head dried out, 10 mL (1+1) Dichloromethane + Hexane was added, and the eluent was collected. The eluent was then concentrated at 2 mL using a rotary evaporator and analyzed.

Proximate Analyses of the Cowpea Samples

Treated stored cowpea samples were analyzed at the Department of Animal Science, University of Ibadan, Ibadan, Nigeria. Cowpea seeds (25g) treated with various concentrations of *Eucalyptus camaldulensis* essential oil, Phostoxin, and no treatment (control) were ground into fine powder. The proximate analyses for % crude protein, ash, ether fat, crude fiber, dry matter, moisture content and carbohydrate were determined using AOAC (1998).

DATA ANALYSIS

Statistical Analysis

Data collected from the study were analyzed using the Analysis of Variance (ANOVA) with the SAS statistical package (SAS Institute, 2003). Means were separated using Duncan Multiple Range Test (DMRT) at 5 % probability level.

RESULTS

Identification of Compounds in Essential Oil from *Eucalyptus camaldulensis* leaves using GC-MS

Essential oil from the leaves of *Eucalyptus camaldulensis* was identified as containing nineteen (19) compounds as presented in Table 1 and Figure 1. The compounds were divided into various classes, such as Monoterpenoids, monoterpenes, terpenes, sesquiterpenoids, sesquiterpene, natural monocyclic phenolics, allyl chain-substituted guaiacol, and organoheterocyclic. Six compounds of Monoterpenoid were identified: camphene, eucalyptol, fenchol, endo-borneol, terpinen-4-ol, and alpha-terpineol. Eucalyptol compound recorded the highest percentage composition, accounting for 27.74% of the total volume of the essential oil. Three monoterpenes' compounds were identified: beta-myrcene, gamma-terpinene, and (+)-4-carene.

Gamma-terpinene had the highest percentage composition, accounting for 12.68% of the total volume of the essential oil. Two terpenes, alpha-pinene and beta-pinene, were identified. Alpha-pinene had the highest percentage composition, accounting for 7.99% of the total volume of the essential oil. Four sesquiterpenoids were identified: bicyclogermacrene, aromandendrene, epiglobulol, and (-)-spathulenol.

Bicyclogermacrene had the highest percentage composition (2.98%), while (-)-spathulenol had the lowest percentage composition (0.94%). Caryophyllene was the only identified compound in the sesquiterpene compound class, accounting for 0.48% of the total volume of the essential oil. Additionally, thymol (0.74%), eugenol (5.01%), and beta-longipinene (0.63%) were found in the essential oil, belonging to the natural monocyclic phenolics, allyl chain-substituted guaiacol, and organoheterocyclic compound classes, respectively.

Toxicity of Eucalyptus Essential oil on *Callosobruchus maculatus* treated with Different Concentrations at 12 and 24 Hours

The toxicity of *Eucalyptus camaldulensis* essential oil on insect mortality was significant ($p<0.05$) at both 12 and 24 hours after application, as presented in Table 2. At 12 hours, the highest mortality rate (100%) was observed with Phostoxin, followed by 0.45 and 0.35 mL/g of Eucalyptus essential oil (93.75 and 81.75%, respectively). The control treatment had the lowest mortality rate (12.50%). Observations at 24 hours revealed that Phostoxin and 0.45 and 0.35 mL/g of Eucalyptus essential oil showed similar highest mortality rates (100%), while the control treatment remained significantly lowest (12.50%).

Table 1: Identification of Compounds in Essential Oil from *Eucalyptus camaldulensis* leaves using GC-MS.

S/N	IDENTIFIED COMPOUND	% COMPOSITION	COMPOUND CLASS
1	Camphene	0.28	Monoterpenoids
2	Alpha.-Pinene (α-Pinene)	7.99	Terpene
3	Beta.-Pinene (β-Pinene)	0.69	Terpene
4	Beta.-Myrcene (β- Myrcene)	0.75	Monoterpene
5	Eucalyptol	27.74	Monoterpenoid
6	Gamma-Terpinene (γ-Terpinene)	12.68	Monoterpene
7	(+)-4-Carene	1.12	Bicyclic monoterpene
8	Fenchol	0.38	Monoterpenoids
9	Endo-Borneol	0.92	Bicyclic monoterpenoids
10	Terpinen-4-ol	3.44	Menthane monoterpenoids
11	Alpha -Terpineol (α-Terpineol)	4.83	Menthane monoterpenoids
12	Thymol	0.74	Natural monocyclic phenolic compound
13	Eugenol	5.01	Allyl chain-substituted guaiacol
14	Caryophyllene	0.48	Bicyclic sesquiterpene
15	Aromandendrene	1.18	Aromadendrane sesquiterpenoids
16	Beta -Longipinene	0.63	Organoheterocyclic compounds
17	Bicyclogermacrene	2.98	Sesquiterpenoids
18	Epiglobulol	0.47	Aromadendrane sesquiterpenoids
19	(-)-Spathulenol	0.94	Aromadendrane sesquiterpenoids

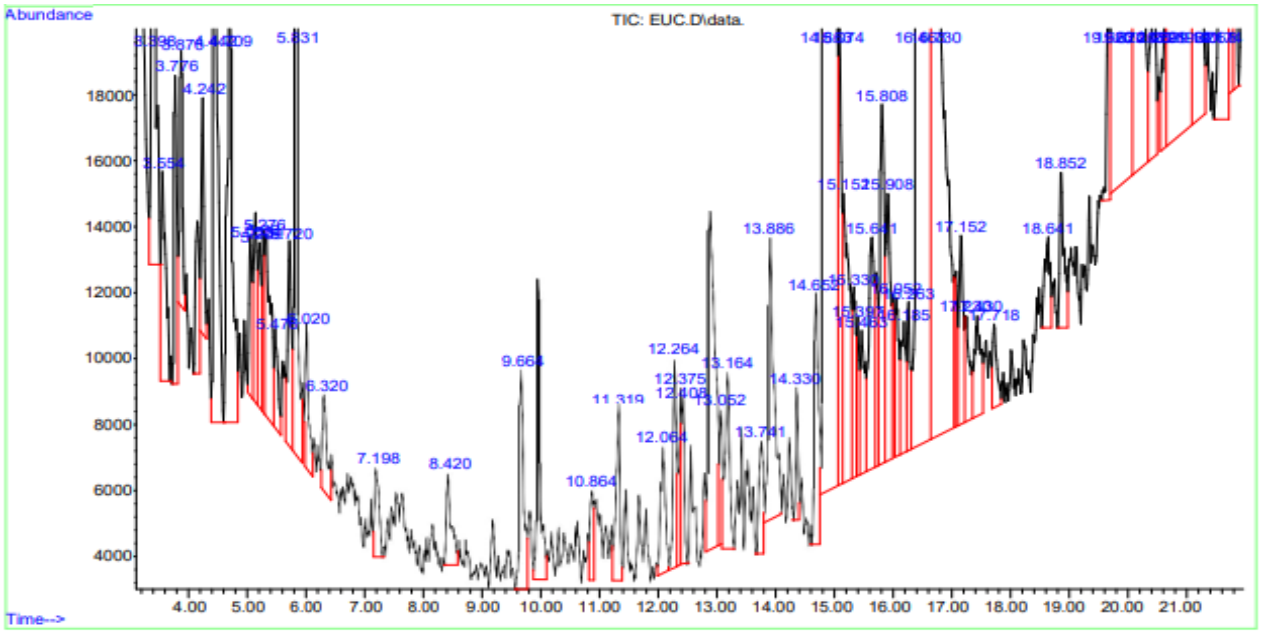

Figure 1: Identification of Compounds in Essential Oil from *Eucalyptus camaldulensis* Leaves Using GC-MS.

Table 2: Toxicity of Eucalyptus Essential Oil on Stored Cowpea Grains Beetle (*Callosobruchus maculatus*) Treated with Different Concentrations at 12 and 24 Hours.

Concentration (mL/g)	Mortality counts (%)	
	12 hours	24 hours
0.15	68.75b	75.00b
0.25	68.75b	87.5ab
0.35	81.75ab	100.00a
0.45	93.75ab	100.00a
Phostoxin (0.01 g/20 g)	100.00a	100.00a
Control(untreated)	12.50c	12.50c

Means followed by the same letter (s) within a column are not significantly different at ($p<0.05$) from each other using the Duncan Multiple Range Test.

Results indicated that *Eucalyptus camaldulensis* essential oil is toxic to the insects, with increasing doses resulting in higher mortality rates.

Determination of *Eucalyptus camaldulensis* Essential Oil Residue in Stored Cowpea Grains at 0.25 mL/g Concentration

Seven different compounds were observed in the treated cowpea grains` residue analysis, as shown in Table 3 and Figure 2. The highest concentration value (110.43 mL/g) was from (+)-4-Carene, followed by gamma. -Terpinene (103 mL/g), and the lowest value (7.41mL/g) was recorded from Bicyclogermacrene. The highest retention time (18.199 min) was observed in Ylangenal, while the lowest retention time (6.766 min) was recorded from alpha-Pinene. However, Eugenol was not detected in the residue analysis.

Determination of *Eucalyptus camaldulensis* Essential Oil Residue in Stored Cowpea Grains at 0.35 mL/g Concentration

The treated cowpea residue at 0.35 mL/g revealed seven compounds (Table 4 and Figure 3). The highest concentration was Ylangenal (66.15 mg/L), followed by gamma-Terpinene (47.55 mg/L). The lowest concentration was Eucalyptol (11.39 mg/L). The retention times ranged from 6.738 min (alpha-Pinene) to 18.170 min (Ylangenal). However, Eugenol and Bicyclogermacrene were not detected in the residue analysis.

Determination of *Eucalyptus camaldulensis* Essential Oil Residue in Treated Cowpea Grains at 0.45 mL/g Concentration

The result in Table 5 also revealed seven compounds in the treated cowpea residue analysis, as shown in Table 5. The highest concentration value of 2562.19 mg/L was recorded from (+)-4-Carene, followed by alpha.-Pinene (1735.08 mg/L), while the lowest value (11.39 mg/L) was from Bicyclogermacrene. The highest retention time (18.165 min) was observed in ylangenal, while alpha.-Pinene had the lowest retention time (6.675 min). However, Eugenol was not detected in the residue analysis, as shown in Table 5 and Figure 4.

Proximate Analyses of Cowpea Grains Treated with *Eucalyptus camaldulensis* Essential Oil

Results presented in Table 6 showed the proximate composition of cowpea grain samples treated with *Eucalyptus camaldulensis* essential oil, phostoxin, and control. Significant differences ($p < 0.05$) were found in Crude Protein (% CP): EUC A (0.15 mL/g essential oil) had the highest value (29.05%).

Table 3: Determination of *Eucalyptus camaldulensis* Essential Oil Residue in Stored Cowpea Grains at 0.25 mL/g Concentration.

S/N	Compounds	Retention time (RT)	Concentration (mL/g)
1	alpha.-Pinene	6.766	28.12
2	Eucalyptol	8.575	86.02
3	gamma.-Terpinene	9.181	103.88
4	(+)-4-Carene	9.576	110.43
5	Eugenol	0.000	N.D.
6	Bicyclogermacrene	15.338	7.41
7	Ylangenal	18.199	51.24

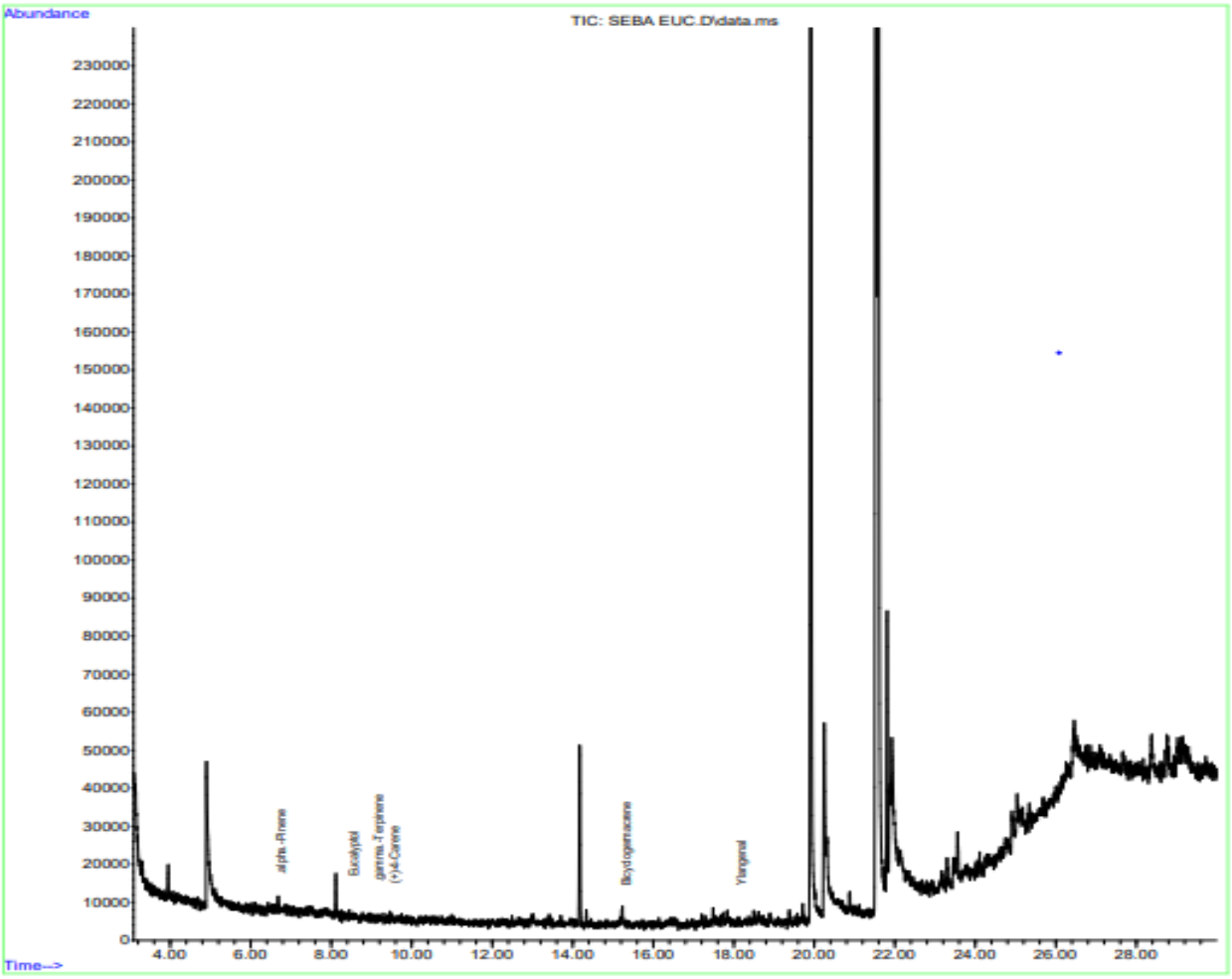

Figure 2: Determination of *Eucalyptus camaldulensis* Essential Oil Residue in Stored Cowpea Grains at 0.25 mL/g Concentration using GC-MS.

Table 4: Determination of *Eucalyptus camaldulensis* Essential Oil Residue in Treated Cowpea Grains at 0.35 mL/g Concentration.

S/N	Compounds	Retention time (RT)	Concentration (mg/L)
1	alpha.-Pinene	6.738	13.54
2	Eucalyptol	8.580	11.39
3	gamma.-Terpinene	9.169	47.55
4	(+)-4-Carene	9.450	45.74
5	Eugenol	0.000	N.D.
6	Bicyclogermacrene	0.000	N.D.
7	Ylangenal	18.170	66.15

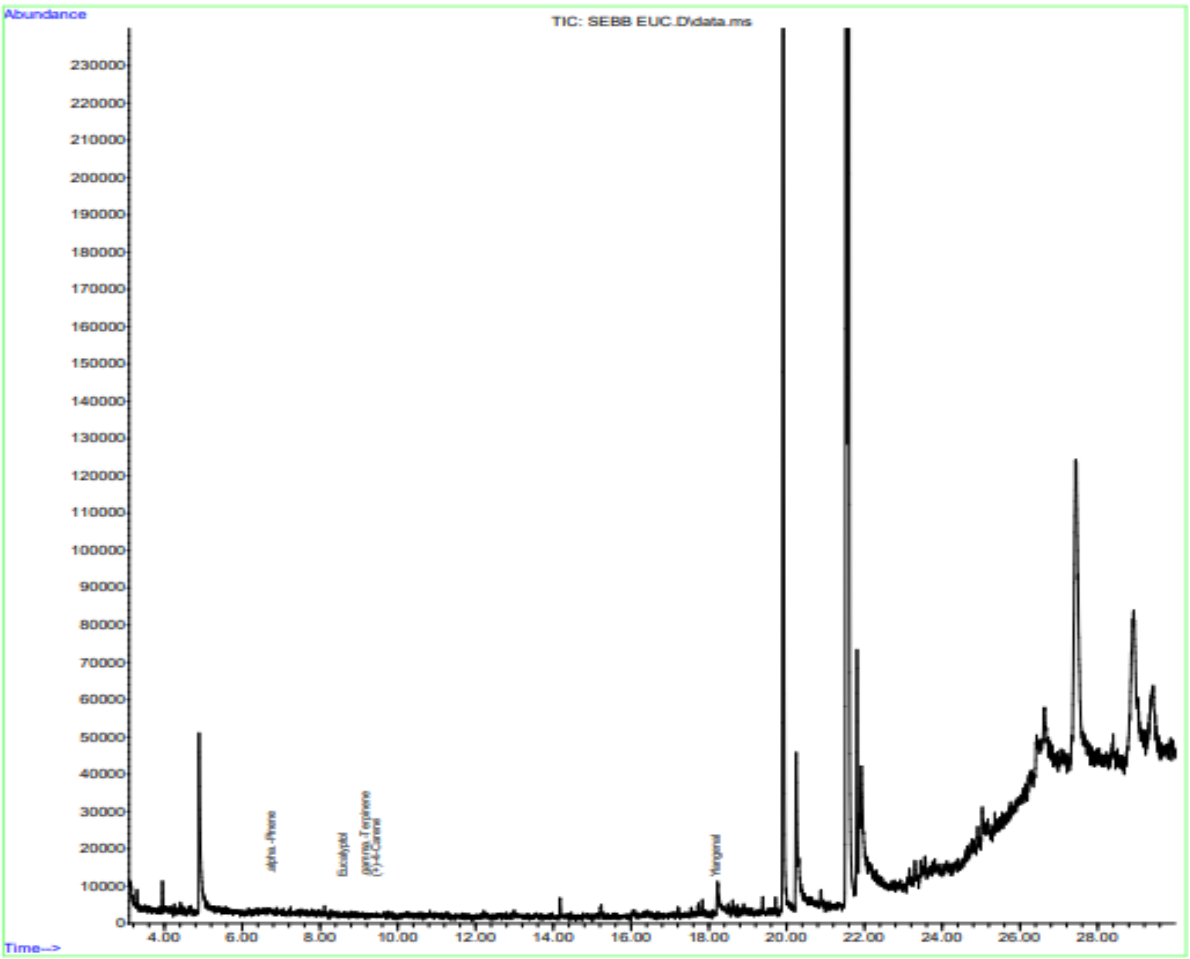

Figure 3: Determination of *Eucalyptus camaldulensis* Essential Oil Residue in Treated Cowpea Grains at 0.35 mL/g Concentration using GC-MS.

Table 5: Determination of *Eucalyptus camaldulensis* Essential Oil Residue in Stored Cowpea Grains at 0.45 mL/g Concentration.

S/N	Compounds	Retention time (RT)	Concentration(mg/L)
1	alpha.-Pinene	6.675	1735.08
2	Eucalyptol	8.546	41.24
3	gamma.-Terpinene	9.061	97.35
4	(+)-4-Carene	9.473	2563.19
5	Eugenol	0.000	N.D.
6	Bicyclogermacrene	15.452	5.54
7	Ylangenal	18.165	120.56

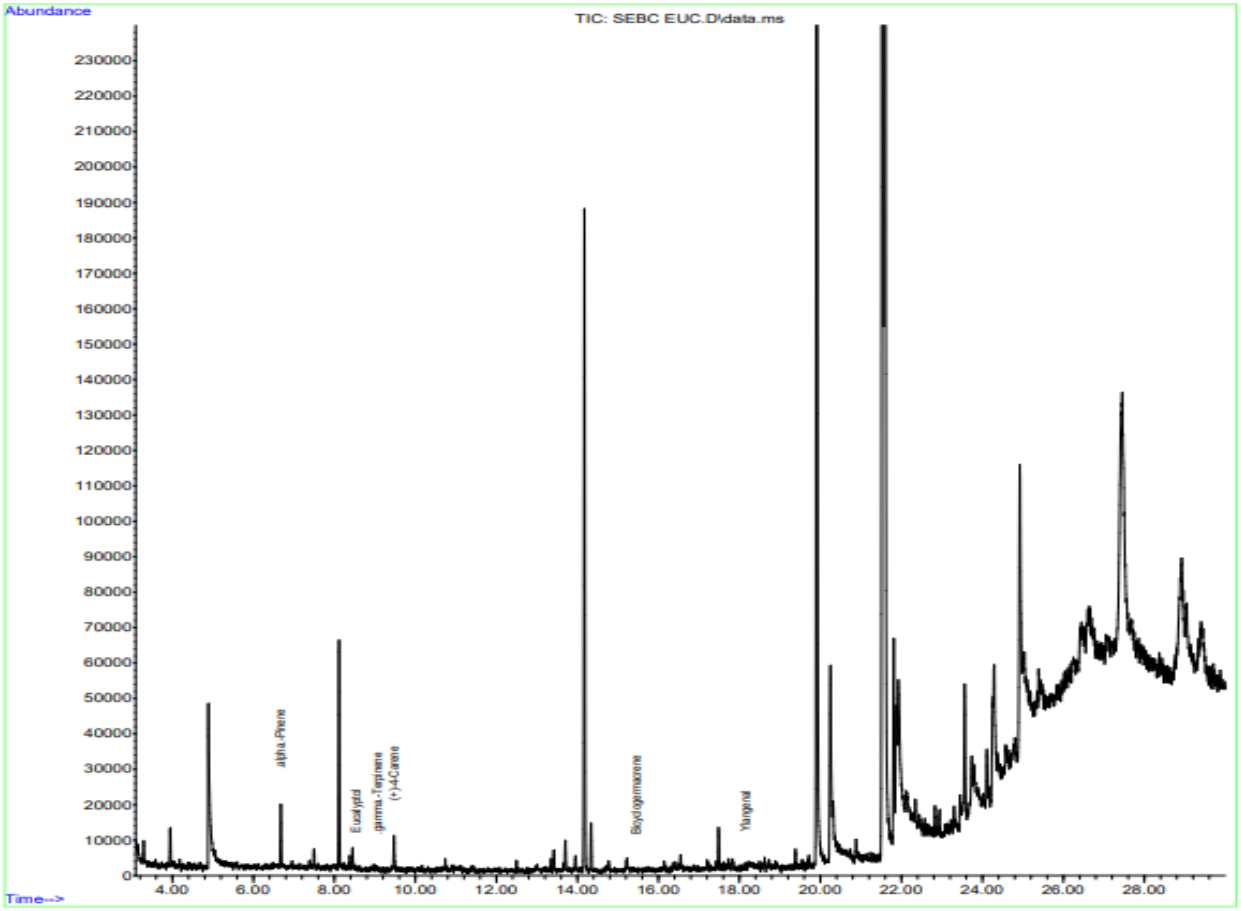

Figure 4: Determination of *Eucalyptus camaldulensis* Essential Oil Residue in Stored Cowpea Grains at 0.45 mL/g Concentration using GC-MS.

Table 6: Proximate Analysis of Cowpea Grains Treated with *Eucalyptus camaldulensis* Essential Oil.

Treatment	Crude Protein %	Ash %	Ether (Fat) %	Crude Fibre %	Dry Matter %	Moisture Content %	CHO %
EUC A	29.05a	5.60a	5.25a	4.50a	90.05a	9.95b	45.65e
EUC B	24.50c	5.20ab	4.80a	4.90a	89.99a	10.01b	50.59b
EUC C	23.63d	5.00ab	5.10a	4.80a	90.37a	9.63b	51.84a
EUC D	26.60b	2.59c	5.10a	4.70a	88.19a	11.81a	49.20c
Phostoxin	26.25b	5.00ab	4.60a	4.20a	89.77a	10.23b	49.72c
Control	23.10d	4.80b	4.90a	4.60a	88.89a	11.11a	46.59d

Means followed by the same letter (s) within a column are not significantly different at ($p<0.05$) from each other using the Duncan Multiple Range Test

EUC A = 0.15mL/g of *Eucalyptus camaldulensis* essential oil, EUC B = 0.25mL/g of *Eucalyptus camaldulensis* essential oil, EUC C = 0.35mL/g of *Eucalyptus camaldulensis* essential oil,EUC D = 0.45mL/g of *Eucalyptus camaldulensis* essential oil. Phostoxin = 0.01g/20g, Control = Untreated cowpea grains.

The Ash Content: EUC A had the highest value (5.60%) but was not significantly higher than phostoxin and control. Ether Extract (E.E): EUC A had the highest value (5.25%), followed by the control and phostoxin. The Crude Fiber Content: Control had the highest value (4.60%), followed by EUC A and phostoxin. The Dry Matter (DM) Content: EUC A and phostoxin had higher values (90.05% and 89.77%) than the control (88.89%). The Carbohydrate (CHO) Content: EUC C (0.35 mL/g essential oil) had the highest value (51.84%), followed by phostoxin and control. The Moisture Content (MC): EUC D (0.45 mL/g *Eucalyptus camaldulensis* oil) and control had higher values (11.8% and 11.11%) than phostoxin and other Eucalyptus oil treatments.

DISCUSSION

This study investigated the bio-insecticidal and residual effects of *Eucalyptus camaldulensis* essential oil on *Callosobruchus maculatus* (cowpea beetles) in stored cowpea. The essential oil was found to contain seven major compounds: Eucalyptol, gamma-Terpinene, alpha-Pinene, Eugenol, alpha-Terpineol, Bicyclogermacrene and Terpinen-4-ol. These findings are consistent with previous research by Dogan, *et al*. (2012), which identified similar compounds in *Eucalyptus camaldulensis* essential oil. The major component was Eucalyptol (1,8-cineole) and mostly of monoterpenes, these findings are similar to those from Tsiri, *et al*. (2003) and Cimanga. *et al*. (2002). Though, reports have shown that Monoterpenes possess strong insecticidal toxicity against numerous insect species, particularly those attacking stored products (Ajayi, *et al*., 2014; Bett, *et al*., 2016; Fayinminnu, *et al*., 2023).

However, a new compound, Ylangenal, was detected, potentially resulting from interactions between the storage container and the essential oil. This highlights the need for further research on the impact of container characteristics on the composition and effectiveness of essential oils, as Hall, *et al.* (1997), suggested. The presence of Ylangenal may have implications for the oil's bio-insecticidal properties and residual effects.

The toxicity of *Eucalyptus* essential oil was observed in the mortality rate of stored cowpea grain beetle (*Callosobruchus maculatus*), which recorded a highly significant value of about 100% at 24 hours after application. This could be due to the essential oil's insecticidal properties of the identified compounds, which have also been demonstrated by these researchers, Bignell, *et al.* (1996), Derwich, *et al.* (2009), Khemira, *et al. (*2012) using *E. camaldulensis* essential oil. The higher mortality rate of the treated cowpea grains signified the potency of the insecticidal oils. Several earlier studies have demonstrated the efficacy of different essential oils in protecting

grains against major stored product insect pests (Maina, *et al*., 2012; Dauda, *et al*., 2014), especially on *Callosobruchus* beetles (Tripathi, *et al*., 2003; Lee, *et al*., 2004, Fayinminnu, *et al.,* 2023)

The insecticidal properties of *Eucalyptus camaldulensis* essential oil were likely due to the presence of monoterpenes, particularly Eucalyptol, which is volatile and lipophilic. These compounds can quickly penetrate insects and disrupt their physiological functions, making them effective against stored product pests like *Callosobruchus maculatus*, which is in line with the report of Aref, *et al.* (2015). This study suggests that *Eucalyptus camaldulensis* essential oil is a viable alternative to synthetic insecticide such as phostoxin for controlling cowpea pests in storage. The oil's effectiveness increased with concentration and exposure time from 12 h to 24 h, leading to higher mortality rates in adult *Callosobruchus maculatus*. These findings support using *Eucalyptus camaldulensis* essential oil as a natural and sustainable pest control method for stored cowpea grains.

The study found that the essential oil of *Eucalyptus camaldulensis* is safe for use as a bio-insecticide, as none of the identified compounds would pose a toxic threat to humans and animals. Eugenol, a compound that causes liver damage when accumulated in large amounts in the body system of humans (Carvalho, *et al.,* 2022), was not detected during the process of cowpea residue analysis due to its high volatility in the atmosphere.

The highest values in proximate compositions of cowpea grain samples were from essential oil treatments. This revealed standard, which is a strong indication of essential oil capacity to enhance the nutritive compositions of the grains, to improve and meet the demands of the key nutrients (Sharma and Thakur, 2014) The higher nutritive compositions, such as crude protein, ash, ether (fats) and carbohydrate recorded from essential oil treated cowpea grains could be linked to anti-nutritional composition. Likewise, the harsh taste of the essential oil could affect the palatability of grains, hence interrupting the feeding behaviors (Daayf and Lattanzio, 2008) and thus strengthening their resistance to insect infestations (Lattanzio, *et al*., 2008).

The proximate composition of cowpea samples obtained, as shown in the crude protein level, varied between 29.05% for treated and 23.10% for untreated. The results are comparable to those reported in previous studies using neem and moringa essential oils (Gayan, *et al*. 2006; Ilesanmi and Gungula, 2016), with some variations likely due to differences in storage duration. However, the results on protein values were similar to the outcomes of the study by Fayinminnu, *et al*. (2023) using *Plecthranthus amboinicus* essential oil on *Callosobruchus maculatus.* Therefore, it could be conditioned that preserving cowpea with Essential Oil is a safe and effective method in protecting the grains against insect attack and hence improving their protein quality.

Ash content in this study ranged between 5.6 and 2.59%. The decrease in ash content with an increase in concentrations of the Eucalyptus essential oil implies that the higher its level, the lower the infestation of the cowpea, hence less endosperm is eaten up. The proportion of the seed coat could thereby be higher in samples treated with a high concentration of the essential oil and the component being enriched with ash as reported by Gayan, *et al*. (2006).

Treated cowpea samples with Eucalyptus essential oil showed high moisture content (9.63-11.81%). This agrees with the work of Ilesanmi and Gungula (2010; 2016) and is similar to the reports of Fayinminnu and Adesiyan (2013). Reasons for this could be an effect of differences in the relative humidity of the studied environment during the treated cowpea grains storage period. However, reports by Anderson and Alcock (2013) and Fayinminnu, *et al*. (2023) gave the most acceptable safe moisture level of 10-12% for good grain storage, which was attained in this study. Likewise, this level is less than the critical (14%) moisture value for cereals, which might be due to the elimination of water, through absorption by the essential oils into the cowpea grains. Moisture barrier for the cowpea grains in storage might have been formed by *Eucalyptus camaldulensis* oil even when the relative humidity was high.

The high levels of carbohydrate content obtained were within some of the treated cowpea samples in storage with neem-moringa seed oils from the work of Ilesanmi and Gungula (2016); Fayinminnu, *et al* (2023) through *Plecthranthus amboinicus*. This result might be due to the cowpea carbohydrate contents being bulky within the endosperm of the grains. Similarly, revealing

the levels of *Eucalyptus camaldulensis* essential oil that could preserve the grains against beetle infestation, whereby the carbohydrate contents were not affected (Ojimelukwe, *et al*.,1999; Ilesanmi and Gungula, 2016).

Above and beyond, *Eucalyptus camaldulensis* essential oil is easily biodegradable; hence, the use of its components to protect cowpea grains against *Callosobrunchus maculatus* in storage may not pose problems to man and the environment. Also, the main constituent of the essential oil is Eucalyptol (1,8 cineol), which was detected in the residue of cowpea grains, having human health benefits. Eucalyptol serves as an antioxidant, anti-inflammatory, antibacterial, antimicrobial and a decongestant (Healthline, 2024). The outcome of this study suggested that *Eucalyptus camaldulensis* essential oil is a safe and effective bio-insecticide for controlling cowpea pests, with potential application in stored product protection.

CONCLUSION AND RECOENDATIONS

This study revealed that *Eucalyptus camaldulensis* essential oil has potential as a natural insecticide for controlling *Callosobruchus maculatus* (cowpea beetle) in stored cowpea grains. All concentrations of *Eucalyptus camaldulensis* essential oil performed well, particularly 0.35 and 0.45 mg/L, which were as effective as the synthetic pesticide phostoxin in giving 100% mortality on cowpea grain beetle.

These findings demonstrate the great botanical potential of *Eucalyptus camaldulensis* essential oil in protecting stored cowpea grains against *Callosobruchus maculatus* infestation, provided timely applications are made. Moreover, the proximate composition of cowpea grains treated with 0.15, 0.25, 0.35, and 0.45 mL/g of *Eucalyptus camaldulensis* essential oil remained unchanged, indicating that the essential oil is a potent and safe bio-insecticide for use against *Callosobruchus maculatus* on stored cowpea grains.

Based on the study's findings, using *Eucalyptus camaldulensis* essential oil at concentrations of 0.35 and 0.45 mg/L are recommended to control *Callosobruchus maculatus* in stored cowpea grains. This natural insecticide has demonstrated similar toxicity to the synthetic insecticide phostoxin.

Use of *E. camaldulensis* essential oil offers a cost-effective and sustainable solution, as it is biodegradable and minimises environmental pollution risks. It is a safer alternative with no food residues and environmental contamination. The eco-friendly nature of *Eucalyptus camaldulensis* essential oil makes it an attractive solution for Integrated Pest Management (IPM) strategies in stored product protection. By adopting this recommendation, farmers can reduce their reliance on synthetic insecticides and use a natural, sustainable and effective solution to control cowpea pests, hence providing safe and quality grains.

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