

1 **EFFECTIVENESS OF CERTAIN ESSENTIAL OILS ON**
2 ***SPODOPTERA LITTORALIS* (Boisd.)**

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4
5 ***Abstract***

6
7 The results revealed that the lowest concentrations (1%) of eucalyptus and clove
8 oils caused 36.66 & 13.3 % mortality, respectively while the highest conc. 5% of the
9 same oils caused 83.83 & 73.33% mortality, respectively against 4th instar larvae.
10 Moreover, mortality was a concentration-dependent response. While LC₅₀ values of
11 eucalyptus and clove oils were 1.9859 and 3.378 %, respectively indicating that
12 eucalyptus was more toxic than clove oil against 4th larval instar. The antifeeding
13 percentage values were significant different between eucalyptus and clove oil-treatments
14 either at low or high concentrations of both oils. Whereas, the maximum AF% was in the
15 eucalyptus and clove oils (at 5% conc.) were 73.52 and 65.73%, respectively. Therefore,
16 the tested EOs effectiveness was shown from acting either as biocides or antifeeding
17 agents against *S. littoralis* larvae.

18
19 **Keywords:** *S. littoralis*, eucalyptus oil, clove oil, LC_{50s}, antifeedant effects.

20
21 **Introduction:-**

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23 The cotton leafworm *S. littoralis* is considered as a destructive phytophagous pest
24 causing great losses in yield (Lanzoni et al., 2012). Essential oils (EOs) extracted from
25 plants are a good alternative source to synthetic pesticides (Pathak et al., 2022; Ngegba
26 et al., 2022). Essential oils have been demonstrated to have repellent, insecticidal and
27 growth inhibitory effects on insects as evaluated by fumigation, contact and ingestion
28 (Isman, 2020; Chaudhari et al., 2021). EOs proved to have efficient against an
29 enormous number of pests (Fabres et al., 2014; Liang et al., 2017; Benelli & Pavela,
30 2018; Isman, 2005, 2006; Kanda et al., 2017). The present work aims to determine the
31 toxicity and antifeedant effects of both eucalyptus EO and clove EO on *S. littoralis* 4th
32 larval instar.

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35 **Materials and Methods:-**

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37 **1-Insects**

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39 From the cotton leafworm division, Plant Protection Research Institute, Dokki, Egypt,
40 *S. littoralis* larvae were obtained. At 27±2 °C and 65±5 % R.H., larvae were reared on
41 castor bean leaves as described by (El-Dafrawi et al., 1964).

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44 **2- Chemicals**

46 From El-Captain Company, Cairo, Egypt, the commercial EOs of eucalyptus, *Eucalyptus*
47 *camaldulensis* and clove, *Syzygium aromaticum* were obtained.

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50 **2-Experiments**

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52 **2.1. Toxicity bioassay**

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54 The preparation of conc. (1, 2, 3, 4 and 5%) from stock solutions of the tested EOs was
55 done and leaf dip method was adopted in this study. Fresh castor leaves dipped in
56 different concentrations for treating 4th *S. littoralis* larval instar with EOs. The treated
57 leaves were applied to the 4th instar larvae as a mere feeding source for 48 hours. After
58 that the larvae were fed on the non-treated castor leaf discs. After 24 hours of treatment,
59 larval mortality was recorded. 3 replicates/ treatment were done with 10 larvae/ replicate.

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62 **2.2. Antifeedant assay (non-choice test):**

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64 Ten larvae/replicate of 4th instar were placed in each petri dish and introduced for them
65 the different conc. EOs - treated castor leaf. The leaves of all treatments were dipped in
66 each proposed concentrations and the control leaf discs were dipped in distilled water.
67 After 24 hrs. amount of leaf discs eaten by the larvae treated with EOs and control were
68 recorded. From the formula of **Singh and Pant (1980)**, the % antifeedant activity was
69 calculated.

70

71 **3. Statistical analysis:**

72

73 Probit analysis was adopted according to **Finney (1971)** using a software computer
74 program (2002). By using one-way analysis of variance (ANOVA), the resulted data
75 were statistically analyzed supported by Duncan's multiple range test (**1955**) running on
76 Co Stat statistical software (**1990**).

77

78

79 **Results and discussion:-**

80

81 In table (1) and figure (1), the results revealed that the low concentrations (1, 2 & 3%)
82 of eucalyptus oil caused 36.66, 46.66 & 63.33% mortality, respectively while 4 & 5%
83 conc. of this oil caused 76.66 & 83.83% mortality. Also, it was observed that as the oil
84 concentrations increased the mortality percentages were increased. These results are in
85 agreement with those of **Barboucha et al. (2024)** who showed that concentration-
86 dependence of *E. camaldulensis* EO effects on *Tribolium castaneum* mortality. Also,
87 **Obembe et al. (2024)** showed that bioactive compounds, isopulegol, citronellol and
88 citronella within different species of *Eucalyptus* sp. have insecticidal activity on *A.*
89 *gambiae*. While **Nurdjannah & Bermawie, 2012; Tian et al., 2015; Rismayani &**
90 **Laba, 2015; Martínez et al., 2018; Armijos et al., 2019; Vargas-méndez et al. (2019)**

91 showed that the main active ingredient in clove leave oil is eugenol (80–88%), eugenol
 92 acetate and caryophyllene which was effective on different insect pests.

93

94 **Table (1):** Mortality % against *S. littoralis* larvae after treatment with clove and
 95 *eucalyptus* oils.

96

Concentrations %	Treatments	Mortality %
Control	-----	6.66 ^p ±0.069
1	Clove	13.3 ^o ±0.83
	<i>Eucalyptus</i>	36.66 ^l ±0.842
2	Clove	33.33 ^m ±1.09
	<i>Eucalyptus</i>	46.66 ^l ±0.956
3	Clove	43.33 ^j ±1.27
	<i>Eucalyptus</i>	63.33 ^g ±1.228
4	Clove	60.00 ^h ±1.63
	<i>Eucalyptus</i>	76.66 ^c ±1.238
5	Clove	73.33 ^d ±1.44
	<i>Eucalyptus</i>	83.83 ^a ±1.342
L.S.D_{0.05%}		1.402

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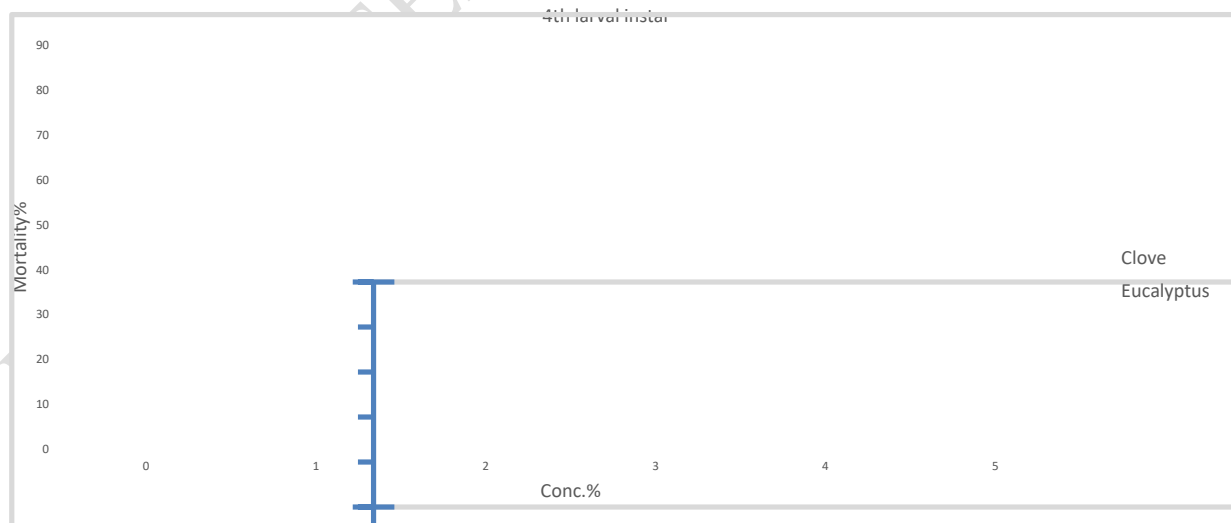
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105 **Figure 1:** Mortality % against *S. littoralis* larvae after treatment with clove and
 106 *eucalyptus* oils.

107

108 Table (2) showed that the sublethal concentration to kill 25% (LC₂₅) and lethal
 109 concentration to kill 50% (LC₅₀) of eucalyptus oil were 0.9214 and 1.9859 %,
 110 respectively against *S. littoralis* 4th larval instar. While LC₂₅ and LC₅₀ of clove oil were
 111 1.9407 and 3.378 %, respectively against 4th larval instar. Eucalyptus essential oil is rich
 112 in monoterpenes and sesquiterpenes. On 4th *S. littoralis* larval instar, Eucalyptus and
 113 clove EOs possess toxic effect against it (Yassin, 2013; Ibrahim & Abd El-Kareem,
 114 2018).

115
 116 **Table 2:** Toxicity indices (LC₂₅ and LC₅₀) of the essential oils (eucalyptus and clove oils)
 117 on *S. littoralis* 4th larval instar.

Treatments	LC%	Conc. %	95% Fiducial limits		Slope	Chi ²
			Lower	Upper		
<i>Eucalyptus</i> oil	25%	0.9214	0.6478	1.1595	2.0223±0.2454	4.8066
Clove oil		1.9407	1.6663	2.1799	2.8021±0.291	1.7893
<i>Eucalyptus</i> oil	50%	1.9859	1.6833	2.2731	2.0223±0.2454	4.8066
Clove oil		3.378	3.0599	3.7732	2.8021±0.291	1.7893

119
 120 * LC₂₅ and LC₅₀ values are significant (P< 0.05) whenever confidence intervals do not
 121 overlap.

122
 123 **Barboucha et al. (2024)** noticed that *Eucalyptus camaldulensis* oil contains spathulenol,
 124 cryptone and p-cymene as the major compounds (Barboucha et al., 2024). *Pectinophora*
 125 *gossypiella* adults were tested with *E. globulus* EO rich in 1,8-cineole (61.60%), p-
 126 cymene (12.40%), limonene (11.50%) for insecticidal activity using in contact assay
 127 (Kobenan et al., 2022). The phytochemical spectra of *E. pyrocarpa*, *E. andrewsii* and *E.*
 128 *siderophloia* reveal the presence of α -eudesmol, p-cymene, α -pinene, α -phellandrene and
 129 β -phellandrene (Filomeno et al., 2017). While Ribeiro et al., 2018 when applied
 130 eucalyptus EOs against *Ascia monuste* larvae found that after 72 h of the topical exposure
 131 at dose 30 μ g/ mg of insect with *E. sphaerocarpa* EO rich in α -phellandrene (15.1%), p-
 132 cymene (16.7%), 1,8-cineole (37.9%) and *E. tindaliae* EO rich in α -pinene (40.6%) and
 133 1,8-cineole (48.2%) caused ~50% mortality rate. In clove EO, the major compounds were
 134 humulene oxide (4.84%), γ -cadinene (5.01%), α -humulene (10.8%), 2-propenoic acid
 135 (12.2%), caryophyllene oxide (18.3%), caryophyllene (24.5%) caryophyllene (24.5%)
 136 and eugenol (27.1%) (Plata-Ruedaa et al., 2018). Vargas-Méndez et al. (2019) showed
 137 that (iso)eugenols and clove oil are a good choice as models for developing new
 138 insecticides against *Spodoptera frugiperda*.

139
 140 While the effect of different concentrations of eucalyptus and clove oils on the
 141 antifeeding percentage (AF%) of *S. littoralis* 4th instar was presented in table (3) and
 142 figure (2). The percentage antifeeding indices of *S. littoralis* 4th instar treated with lower
 143 conc. (1, 2 & 3%) of eucalyptus oil were 38.95, 49.61 and 59.27%, respectively whereas
 144 those of clove oil at the same conc. were 22.48, 40.15 and 50.77%, respectively. In
 145 connection with high conc. (4 and 5%), the feeding deterrence index values of eucalyptus

146 oil were 68.29 and 73.52%, respectively whereas those of clove oil were 57.76 and
 147 65.73%, respectively at the same concentrations. The maximum AF% was in the
 148 eucalyptus oil (at 5% conc.) and the least AF% was at the 1% concentration and it was
 149 38.95 and 73.52%, respectively. The feeding deterrence percentage (AF%) was increased
 150 with increasing the concentrations, so at the lowest conc. (1%) of clove oil the AF% was
 151 22.48% and at the highest conc. (5%) the AF% was 65.73%. Meanwhile, data clearly
 152 indicate that both EOs have high feeding deterrence activity at high concentrations. Also,
 153 the same trend was noticed by **Selvam & Ramakrishnan (2014)** who mentioned that the
 154 antifeedant activity is directly proportional to the increase in the concentration of EOs.
 155 Clove oil has potential larvicidal and antifeedant effects on *S. litura* 3rd larval instar
 156 (**Fateha et al., 2021**). Also, **Karemu et al. 2013**, showed the high repellent effect of *E.*
 157 *camaldulensis* oil against *Sitophilus zeamais*. Certain major compound found within
 158 Eucalyptus oil had a high antifeedant index on *S. frugiperda* larvae at conc. 1000 mg/MI
 159 (**Vargas-Méndez et al., 2019**). **Ebadollahi & Setzer (2020)** showed that *E.*
 160 *camaldulensis* oil has repellent effect when applied on *T. confusum* adults. The
 161 insecticidal or repellent properties of EO from *E. camaldulensis* against *Sitophilus spp.*,
 162 are related to their chemical composition (**Ahouandjinou et al., 2021**).

163
 164 **Table (3):** Antifeeding index % against *S. littoralis* larvae after treatment with clove and
 165 *eucalyptus* oils.
 166

Concentrations %	Treatments	Antifeeding index %
Control	-----	0 ^r ±0.00
1	Clove	22.48 ^q ±0.81
	<i>Eucalyptus</i>	38.95 ^o ±0.825
2	Clove	40.15 ⁿ ±0.951
	<i>Eucalyptus</i>	49.61 ^k ±0.919
3	Clove	50.77 ^j ±0.985
	<i>Eucalyptus</i>	59.27 ⁱ ±0.970
4	Clove	57.76 ^s ±1.0326
	<i>Eucalyptus</i>	68.29 ^c ±1.024
5	Clove	65.73 ^d ±1.089
	<i>Eucalyptus</i>	73.52 ^a ±1.048
L.S.D _{0.05%}		1.052

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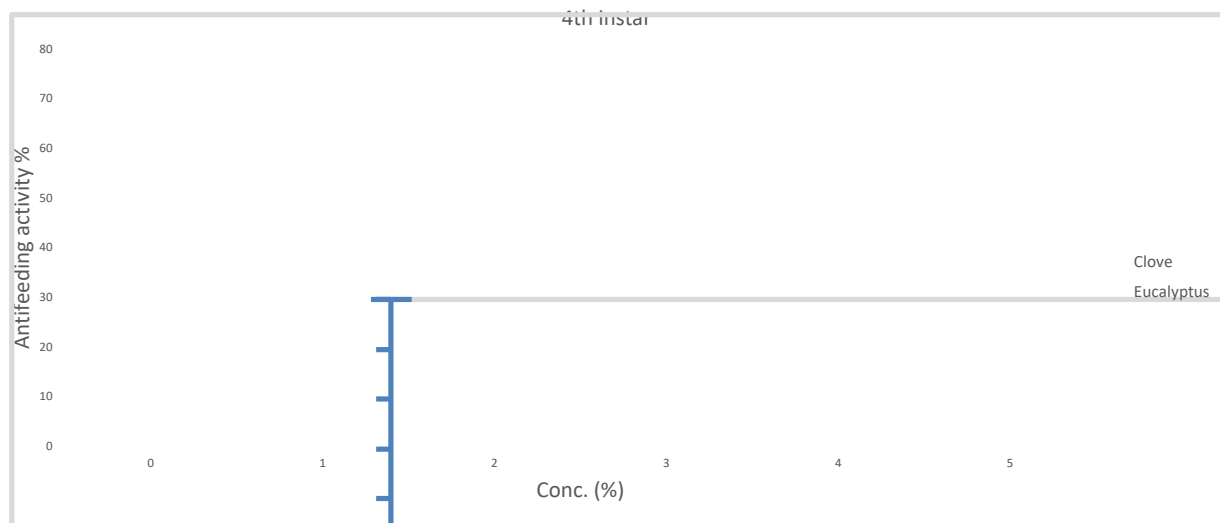


Figure 2: Antifeeding index % against *S. littoralis* larvae after treatment with clove and eucalyptus oils.

Conclusion:-

The EOs of eucalyptus as well as clove acted as larvicidal and antifeeding agents against *S. littoralis* larvae and have potential to be exploited as botanical insecticides used as a part of IPM of this pest and its related species.

Competing interests:

No competing of interests was obtained.

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