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Evaluation of anti-insect properties of *Acorus calamus* linn. essential oil emulsion formulation against pulse beetle, *Callosobruchus maculatus* (Fabricius)

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Abstract
Storage practices which help us to overcome food insecurity are greatly undetermined by varieties of factors. Post-harvest loss caused by unscientific storage, insects, rodents, microorganisms etc., are the main reasons for major food grain loss. Among them, insects play a major role. About 500 species of insects have been associated with stored grain products and nearly 100 species among them cause economic loss. Management of these insects relies heavily on the use of synthetic insecticides and fumigants, which resulted in many environmental and efficacy deterioration. Phyto insecticides are mooted as alternatives for effective and safe management of stored insects. Acorus calamus Linn. is one of the important phyto-insecticide which is known to possess wide variety of anti-insect properties. Still their commercial use is highly restricted due non availability of formulations. Hence, Essential oil emulsion formulation of A. calamus Linn. essential oil has been attempted. The results clearly indicated the supremacy of the formulations in effectively controlling the pulse beetle. When the essential oil emulsion formulation was tested, the highest mortality and FDI were observed in 10 and 7% concentrations at 200µL dose (98.32%; 93.32% and 75.45;70.04 respectively). It is also significantly reduced oviposition and progeny production of C. maculatus. Weight loss was also found to be significantly reduced.

Keywords: *A. calamus*, *C. maculatus*, essential oil, emulsion formulation, fumigant toxicity

Introduction
Post-harvest storage loss caused by insects, rodents, and micro-organisms undermine food security. Among them, stored grain insect pests cause huge economic loss globally (Pimentel 1991) [29]. In India alone, IGMRI (2015) estimated the annual loss due to stored grain insect pests at INR 1,300 million. Such avoidable colossal loss mandated scientific management of these pests using synthetic fumigants. However, their overuse resulted in efficacy and environmental deterioration (Jembere *et al*. 1995; Okonkwo and Okoye 1996) [17, 27]. Further, restrictions imposed on methyl bromide and phosphine (Fields and White 2002; Benhalim *et al.* 2004; EPA 2021) [10, 3] renewed research interests on alternatives. Traditional wisdom in the use of insecticidal plants bought through centuries offered ample scope for their exploitation as Phyto-insecticides (Isman 2006) [15].
Plant essential oils with their excellent volatile properties are suitable replacements for synthetic fumigants. *Acorus calamus* L. (Fam.: Acoraceae), an essential oil yielding plant, used traditionally in the management of stored product pests (Koul *et al.* 2008) has α, β and γ asarone (Streloke *et al*. 1989; Juan *et al*. 2009a&b; Liu *et al*. 2013) [40, 18, 19, 23]. It has variety of anti-insect action and is suitable for commercial exploitation (El –Nahal *et al*. 1989; Risha *et al*. 1990; Kim *et al*. 2003; Nandi *et al*. 2008) [8, 33, 20, 26].
Since the availability of formulation decides the commercial success of a Phyto-insecticide, the present study aims to evaluate the anti-insect properties of *A. calamus* essential oil emulsion formulation against stored legume pest *Callosobruchus maculatus* (Fabricius).

Materials and Methods
Rearing of test insect *C. maculatus*
The test insect *C. maculatus* was reared throughout the study period as described by Rahman and Talukder (2006) [31] at 28±2ºC temperature, 75±5% RH and 12h:12h light: dark photoperiod.

Extraction of Essential Oil From *A. calamus* Rhizome
A. calamus rhizomes purchased from the locally available country medicine shop, were air-dried, ground, and sieved (Akinkurolere *et al.* 2006; Singh 2011) [1, 28, 36, 38]. Essential oil was extracted using Clevenger apparatus (2000 ml capacity) and stored at 4°C in a refrigerator (Moretti *et al*. 2002; Kumar *et al.* 2007; Loni and Panahi 2015; Ejemen 2017 a & b) [25, 22, 37, 24, 6, 7].

Development of Essential Oil Emulsion Formulation
Essential oil emulsion formulation was prepared by dispersing 1, 3, 5, 7 and 10 ml of essential oil in 99, 97, 95, 93 and 90 ml of aqueous mucilage of one per cent w/v Tween 80 to obtain 1, 3, 5, 7 and 10 per cent v/v

concentrations using a turbo emulsifier (Remi) at 10000 rpm for 5 minutes. The prepared formulation was stored separately in glass vials at 4°C under airtight conditions for further use (Moretti *et al*. 2002) [25].

Evaluation of Fumigant Toxicity under Laboratory Conditions

50µL, 100µL, 150µL, 200µL of each concentration was soaked in cotton and kept inside the aluminum foil fold and enclosed all the sides by using a stapler. The pouch was perforated by using pins (5 holes) (6cm×4cm) and kept separately. They were introduced into separate plastic containers (400g capacity) containing 250g of freshly harvested un-infested green gram seeds and twenty pairs of *C. maculatus* adults. The containers were closed airtight (modified after Pandey *et al.* 2011) [28]. Cotton swab soaked with pure essential oil and emulsified water served as positive and absolute controls. Each experiment was replicated three times.

Beetles not showing any response after exposure to the gentle heat of a 60 W lamp were regarded dead (Rahman and Schmidt 1999) [31]. Mortality was recorded every 24-hours till 72-hours.

After one week, number of eggs laid on treated seeds (Ts) and control seeds (Cs) were recorded, and the Per cent Oviposition Deterrence (POD) was calculated using the formula POD = [(Ts-Cs)/Cs] X 100.

These eggs were maintained until adult emergence. Data on number of adults emerged both in treated (Tn) and control (Cn) sets were recorded, and per cent egg hatch and per cent inhibition rate were calculated: Percent egg hatch = Total egg hatched / Total eggs laid x 100; Percent inhibition rate = [(Cn-Tn)/Cn] X 100 (Rahman and Talukder 2006; Jayakumar 2010; Singh 2011) [30, 16, 28, 36, 38].

After two months, final weight of seeds (W) was recorded in treated and control sets and from the initial weight (W_1), per cent weight loss was calculated: % Weight loss = [(W_1-W) / W_1] X100 (Jayakumar 2010; Shukla *et al*. 2011) [16, 36]. Similarly Feeding Deterrence Index (FDI) was calculated based on consumption in control (C) and treatment (T): FDI = (C − T) / (C + T) × 100 (Isman *et al*. 1990) [14].

Statistical Analysis

The data gathered are statistically analyzed using SPSS software

Result and Discussion

Influence of *A. calamus* Essential oil Emulsion Formulation on Fumigant Toxicity

The different concentrations of essential oil emulsion formulation and non-formulated essential oil were tested against *C. maculatus* at 50 - 200µl doses. In all the doses tested (50 to 200µl), and hours of observations (24,48 & 72 hours), non-formulated essential oil performed superior to other treatments. Further, non-formulated essential oil treatment imparted quick effect and recorded cent percent mortality at 24 hours after the treatment itself except at 50 µl dose, wherein it provided 96 percent kill (Fig. 1).

The essential oil emulsion formulation at 10 and 7 % concentrations were observed to impart poor mortality effect (24 and 17 percent respectively) 24 hours after treatment. However, it continued to impart mortality at 48 and 72 hours after the treatment (15 percent respectively) (Table 1). Similar results were noticed in 100, 150 and 200 µl doses. Among all doses and concentrations tested, maximum mortality of 35 percent alone was recorded 24 hours after treatment in 10 percent concentration of 200µl dose (Table 2 - 4). However, the cumulative mortality observed in 10 and 7 percent concentrations of essential oil emulsion formulation at 100, 150, and 200µl doses treatments clearly indicated the sustained release of *A. calamus* essential oil and imparted more than 70 percent mortality. Further, mortality effects were dose-dependent increasing with increasing dose (Fig 3).

In all the doses tested (50 -200 µl), 24 hours after treatment all the treatments provided significantly different effect than the control (p=0.000). When compared with 48 and 72 hours after treatments, the treatments were significantly different from control and the p value also supported the results (p= 0.000 respectively).

Similar results were recorded by Dhivya *et al*. (2019) [5] in *A. calamus* nanoemulsion at 1% concentration which recorded 90% mortality of *C. maculatus*. Regmi and Dhoj (2011), Saranya *et al*. (2019) [34], and Govindan *et al*. (2020) [11] evidently proved that *A. calamus* essential oil effectively managed *C. maculatus*. *A. calamus* has high insecticidal activity due to active compound β-asarone (cis-2, 4, 5-trimethoxy-1- propenylbenzene), a sesquiterpenoid (Shreelaxmi *et al*., 2017). Govindan and Nelson (2007) stated that 2 per cent *A. calamus* rhizome powder showed 100 per cent mortality to *C. maculatus* two days after treatment. Saranya *et al.*, (2019) [34] concluded that *A. calamus* hexane extract at 0.1per cent resulted in cent per cent mortality of *C. maculatus*. Rathod *et al*., (2019) reported that *A. calamus* rhizome powder @ 10g/kg of green gram seeds recorded cent per cent mortality of *C. maculatus*. Several scientists tested the efficacy of sweet flag, *A. calamus* rhizome powder against the adults of *C. maculatus* and reported similar results (Chanpark *et al*., 2003; Shukla *et al.*, 2009; Chandel *et al.*, 2018; Shreelaxmi *et al*., 2019; Dhivya *et al.*, 2019) [35, 5].

Table 1: Fumigant toxicity of *A. calamus* essential oil emulsion formulations (50 µL) against pulse beetle, *C. maculatus*

Treatment	*Percent adult mortality after		
	24 hAT	48 hAT	72 hAT
1% Essential oil emulsion formulation	16 (23.57)^{d}	9^{b}	10^{b}
3% Essential oil emulsion formulation	16 (23.57)^{d}	13^{a}	11^{b}
5% Essential oil emulsion formulation	21 (27.27)^{d}	13^{a}	14^{a}

7% Essential oil emulsion formulation	17 (24.35)[c]	15[a]	15[a]
10% Essential oil emulsion formulation	24 (29.33)[b]	15[a]	15[a]
Non-formulated essential oil	96 (78.46)[a]	4[c]	0.08[c]
Untreated Control	0.08 (1.62)[e]	0.08[d]	0.08[c]
SE(d)	0.715	0.925	0.926
C.D. at P=0.05	1.534	1.984	1.986

*Mean of three replications
Values followed by different alphabets within a column differ significantly
hAT – hours after treatment

Table 2: Fumigant toxicity of *A. calamus* essential oil emulsion formulations (100 µL) against pulse beetle, *C. maculatus*

Treatment	*Percent adult mortality after		
	24 hAT	**48 hAT**	**72 hAT**
1% Essential oil emulsion formulation	15 (22.78)[c]	12[e]	13[d]
3% Essential oil emulsion formulation	15 (22.78)[c]	14[d]	16[c]
5% Essential oil emulsion formulation	16 (23.57)[c]	16[c]	17[c]
7% Essential oil emulsion formulation	29 (32.58)[b]	21[b]	22[b]
10% Essential oil emulsion formulation	31 (33.83)[b]	28[a]	25[a]
Non-formulated essential oil	100 (90)[a]	0.08[f]	0.08[e]
Untreated Control	0.08 (1.62)[d]	0.08[f]	0.08[e]
SE(d)	1.200	0.925	1.195
C.D. at P=0.05	2.574	1.984	2.563

*Mean of three replications
Values followed by different alphabets within a column differ significantly
hAT – hours after treatment

Table 3: Fumigant toxicity of *A. calamus* essential oil emulsion formulations (150 µL) against pulse beetle, *C. maculatus*

Treatment	*Percent adult mortality after		
	24 hAT	**48 hAT**	**72 hAT**
1% Essential oil emulsion formulation	18 (25.10)[e]	14[d]	9[e]
3% Essential oil emulsion formulation	21 (27.27)[de]	17[b]	13[d]
5% Essential oil emulsion formulation	22 (27.97)[d]	17[c]	17[c]
7% Essential oil emulsion formulation	28 (31.94)[c]	29[a]	26[b]
10% Essential oil emulsion formulation	36 (36.86)[b]	29[a]	29[a]
Non-formulated essential oil	100 (90)[a]	0.08[e]	0.08[f]
Untreated Control	0.08 (1.62)[f]	0.08[e]	0.08[f]
SE(d)	1.138	1.069	0.925
C.D. at P=0.05	2.441	2.293	1.984

*Mean of three replications
Values followed by different alphabets within a column differ significantly
hAT – hours after treatment

Table 4: Fumigant toxicity of *A. calamus* essential oil emulsion formulations (200µL) against pulse beetle, *C. maculatus*

Treatment	*Percent adult mortality after		
	24 hAT	**48 hAT**	**72 hAT**
1% Essential oil emulsion formulation	20 (26.56)[d]	17 (24.35)[c]	17 (24.35)[a]
3% Essential oil emulsion formulation	28 (31.94)[c]	28 (31.94)[b]	26 (30.65)[b]
5% Essential oil emulsion formulation	31 (33.83)[bc]	29 (32.58)[b]	28 (31.94)[b]
7% Essential oil emulsion formulation	35 (36.27)[b]	30 (33.21)[ab]	28 (31.94)[b]
10% Essential oil emulsion formulation	35 (36.27)[b]	32 (34.44)[a]	31 (33.83)[a]
Non-formulated essential oil	100 (90)[a]	0.08 (1.62)[d]	0.08 (1.62)[d]
Untreated Control	0.08 (1.62)[e]	0.08 (1.62)[d]	0.08 (1.62)[d]
SE(d)	1.275	0.713	0.398
C.D. at P=0.05	2.734	1.529	0.853

*Mean of three replications
Values followed by different alphabets within a column differ significantly
hAT – hours after treatment

Table 5: Effect of *A. calamus* essential oil emulsion formulations against oviposition and egg hatching in pulse beetle, *C. maculatus*

Treatment	Percent oviposition Deterrence (POD)				Percent Egg Hatched			
	50 µL	100 µL	150 µL	200 µL	50 µL	100 µL	150 µL	200 µL
1% Essential oil emulsion formulation	73.40^{f}	76.24^{f}	77.64^{f}	80.95^{f}	88.73 (70.38)^{e}	79.03 (62.74)^{f}	71.92 (58.00)^{f}	68.75 (56.01)^{f}
3% Essential oil emulsion formulation	77.52^{e}	78.92^{e}	81.17^{e}	87.29^{e}	61.66 (51.74)^{d}	60 (50.76)^{e}	58.33 (49.79)^{e}	56.25 (48.59)^{e}
5% Essential oil emulsion formulation	80.14^{d}	83.13^{d}	84.70^{d}	88.48^{d}	52.83 (48.62)^{c}	56.81 (48.91)^{d}	56.41 (48.68)^{d}	37.93 (38.01)^{d}
7% Essential oil emulsion formulation	85.01^{c}	86.20^{c}	87.05^{c}	91.66^{c}	53.65 (47.09)^{c}	52.77 (46.59^{c}	48.48 (44.13)^{c}	33.33 (35.26)^{c}
10% Essential oil emulsion formulation	88.76^{b}	89.65^{b}	90.19^{b}	96.81^{b}	36.66 (37.26)^{b}	29.62 (32.97)^{b}	28 (31.75)^{b}	25 (30)^{b}
Non-formulated essential oil	100^{a}	100^{a}	100^{a}	100^{a}	0.08 (1.62)^{a}	0.08 (1.62)^{a}	0.08 (1.62)^{a}	0.08 (1.62)^{a}
Untreated Control	0.08^{g}	0.08^{g}	0.08^{g}	0.08^{g}	100 (90)^{f}	100 (90)^{g}	100 (90)^{g}	100 (90)^{g}
SE(d)	0.600	0.613	0.593	0.367	0.533	0.356	0.401	0.209
C.D. at P=0.05	1.287	1.314	1.272	0.787	1.143	0.763	0.860	0.448

*Mean of three replications
Values followed by different alphabets within a column differ significantly

Table 6: Percentage reduction in adult emergence or inhibition rate (% IR) of *A. calamus* essential oil emulsion formulations against pulse beetle, *C. maculatus*

Treatment	Percent Inhibition rate (% IR)			
	50 µL	100 µL	150 µL	200 µL
1% Essential oil emulsion formulation	80.30^{f}	82.94^{e}	85.09^{e}	89.95^{e}
3% Essential oil emulsion formulation	89.77^{e}	92.63^{d}	92.54^{d}	93.57^{d}
5% Essential oil emulsion formulation	90.90^{d}	92.63^{d}	93.33^{d}	94.77^{c}
7% Essential oil emulsion formulation	94.31^{c}	94.18^{c}	95.29^{c}	96.38^{b}
10% Essential oil emulsion formulation	97.72^{b}	98.44^{b}	98.43^{b}	99.19^{a}
Non-formulated essential oil	100^{a}	100^{a}	100^{a}	100^{a}
Untreated Control	0.08^{g}	0.08^{f}	0.08^{f}	0.08^{f}
SE(d)	0.535	0.463	0.468	0.429
C.D. at P=0.05	1.147	0.993	1.004	0.920

*Mean of three replications
Values followed by different alphabets within a column differ significantly

Table 7: Per cent weight loss and Feeding deterrent index (After two months of storage)

Treatment	% Weight loss				Feeding deterrent index			
	50	100	150	200	50	100	150	200
1% Essential oil emulsion formulation	16.66^{e}	15.46^{f}	15.46^{f}	11.86^{e}	10.39	12.78	20	36.87
3% Essential oil emulsion formulation	11.86^{d}	12^{e}	11.86^{e}	11.86^{e}	26.74	25	32.31	36.87
5% Essential oil emulsion formulation	11.86^{d}	10^{d}	9.6^{d}	8^{d}	26.74	33.33	41.46	52.56
7% Essential oil emulsion formulation	10^{bc}	6.66^{c}	6.53^{c}	4.53^{c}	34.49	50	56.05	70.04
10% Essential oil emulsion formulation	9.6^{b}	6.13^{b}	5.46^{b}	3.6^{b}	36.28	53.06	61.86	75.45
Non-formulated essential oil	0.08^{a}	0.08^{a}	0.08^{a}	0.08^{a}	100	100	100	100
Untreated Control	20.53^{f}	20^{g}	23.20^{g}	25.73^{f}	0	0	0	0
SE(d)	0.302	0.110	0.256	0.106				
C.D. at P=0.05	0.647	0.235	0.549	0.227				

*Mean of three replications
Values followed by different alphabets within a column differ significantly
FDI – Feeding deterrent index

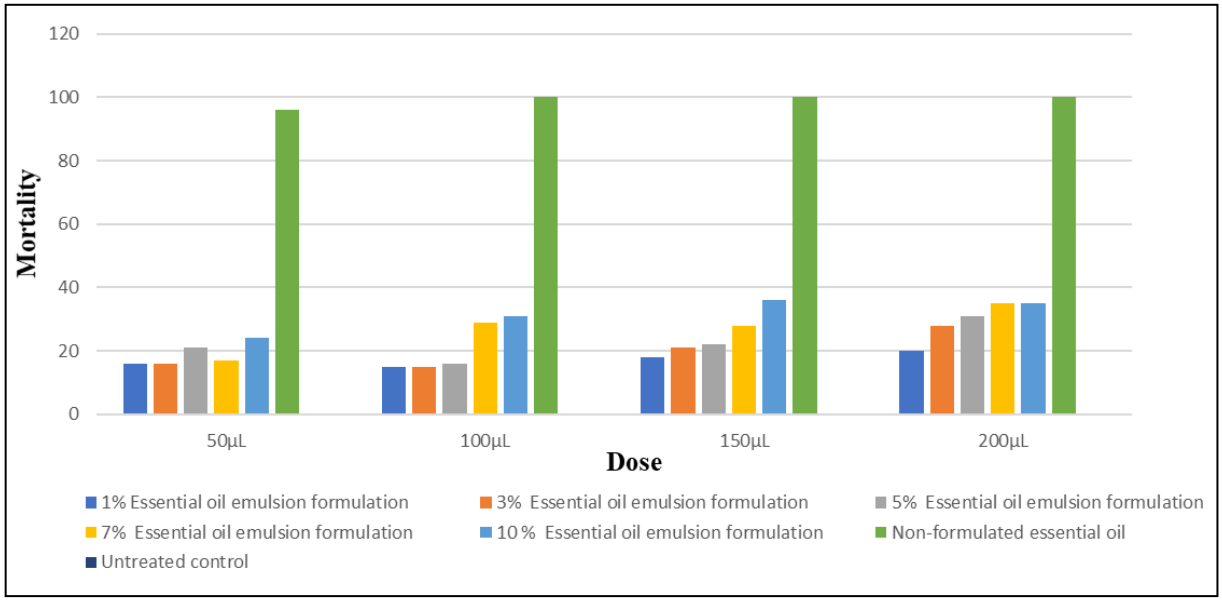

Fig 1: Effect of *A. calamus* essential oil emulsion formulation on percent adult mortality 24 hours after treatment

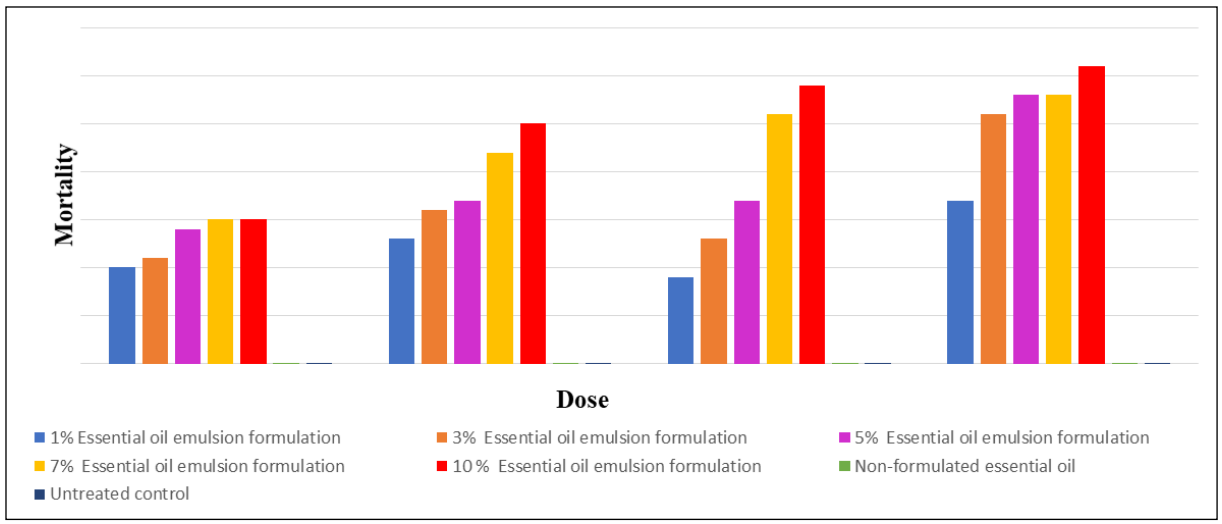

Fig 2: Effect of *A. calamus* essential oil emulsion formulation on percent adult mortality 72 hours after treatment

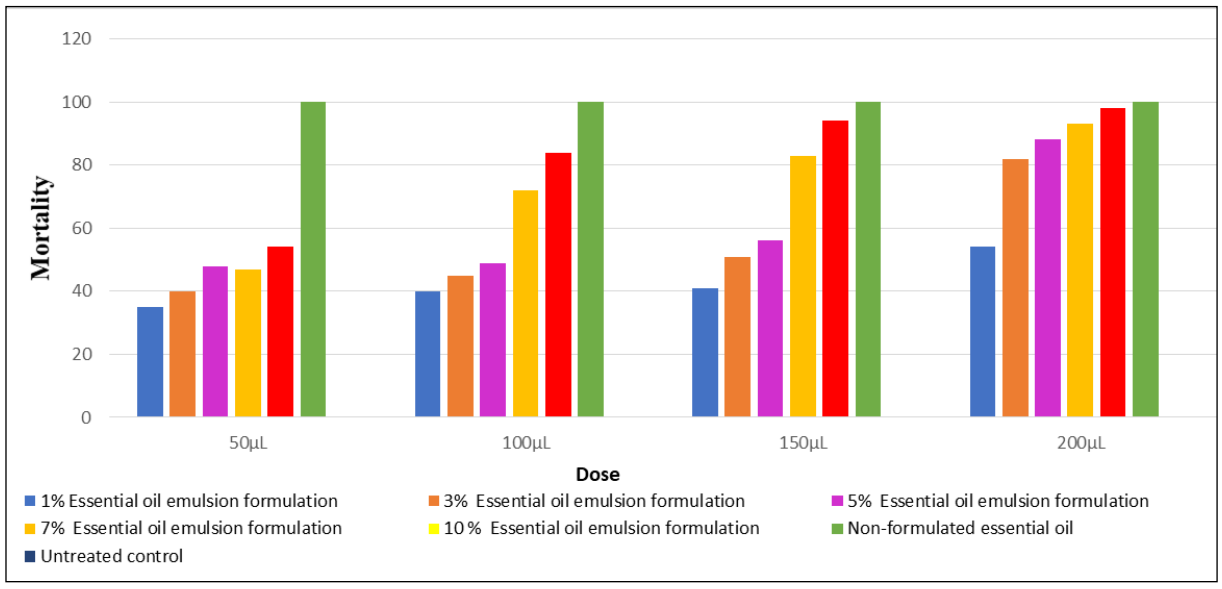

Fig 3: Effect of *A. calamus* essential oil emulsion formulation on cumulative mortality

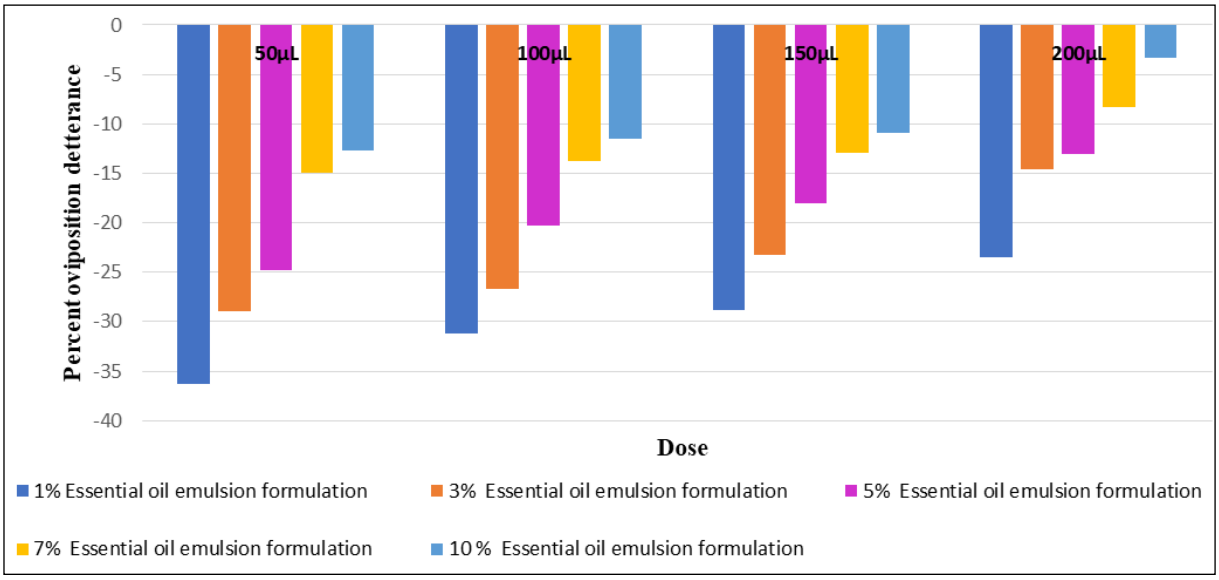

Fig 4: Effect of *A. calamus* essential oil emulsion formulation on percent increase in oviposition deterrence over non-formulated essential oil

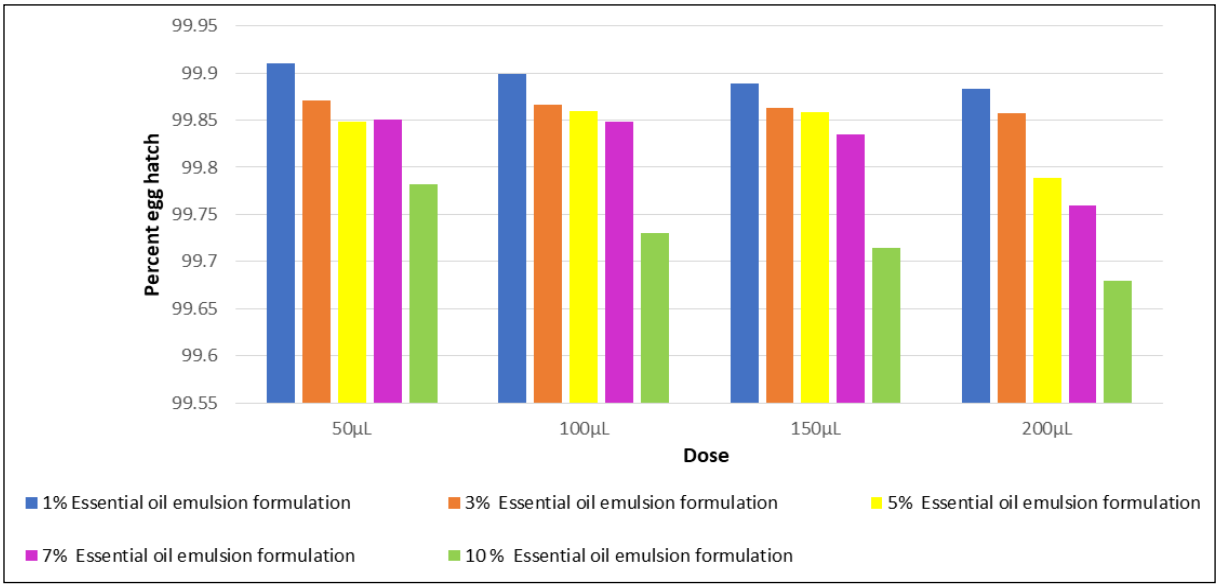

Fig 5: Effect of *A. calamus* essential oil emulsion formulation on percent decrease in egg hatch over non-formulated essential oil

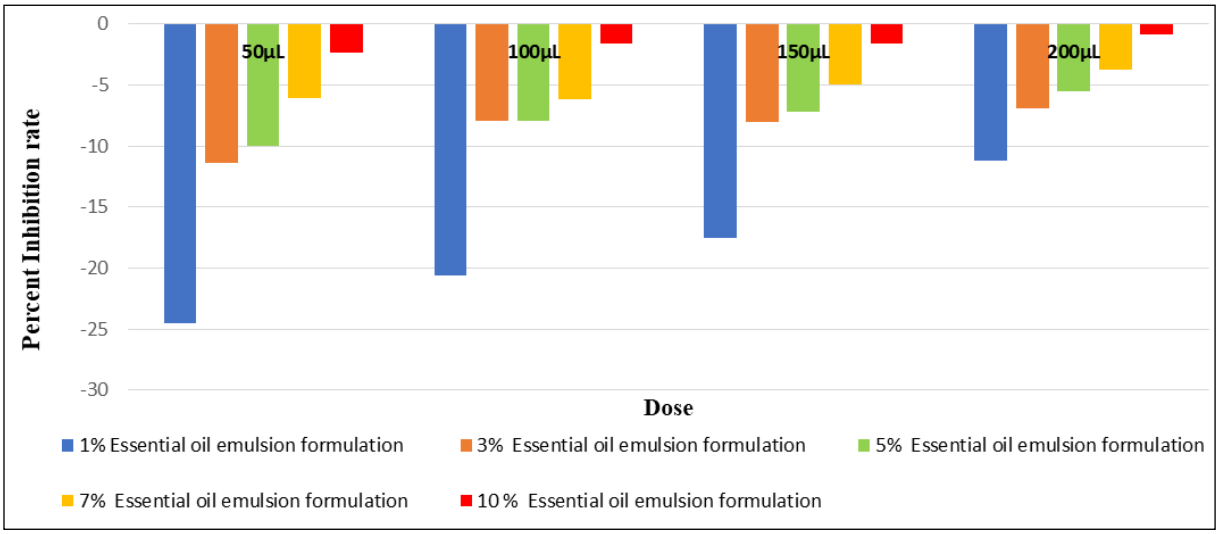

Fig 6: Effect of *A. calamus* essential oil emulsion formulation on percent increase in inhibition rate over non-formulated essential oil

Influence of *A. calamus* essential oil emulsion formulation on Oviposition deterrence and Egg hatching

The data presented in Table 5 indicated that the non-formulated essential oil and *A. calamus* essential oil emulsion formulation at all concentrations and doses tested were significantly effective in reducing oviposition and progeny production of *C. maculatus*. The oviposition deterrent effect recorded clearly indicated supremacy of non-formulated essential oil ((At 50-200µl - 100%) followed by 10 and 7 % concentrations of essential oil emulsion formulation at all doses (At 50µl - 10% (88.76), 7% (85.01); At 100µl - 10% (89.65), 7% (86.20); At 150 µl - 10% (90.19), 7% (87.05); At 200µl - 10% (96.81), 7% (91.66)). They were found significantly different (p=0.000) when compared with control. These findings were supported by Rahman and Schmidt (1999) [31], Shukla *et al*. (2009) [35] and Jayakumar (2010) [16].

When the percent increase in oviposition deterrence over non-formulated essential oil treatment was calculated, it was evident that 7 and 10 % concentrations provided increased deterrence at all the doses tested (Fig 4). Hatching was reduced at 10% (36.66% egg hatch) and 7% (53.65% egg hatch) concentrations when compared with the untreated control (100% egg hatch) at 50 µl dose. Similar results obtained at 100 µl, 150 µl, and 200 µl doses also. When percent decrease in egg hatch over non-formulated essential oil treatment was computed, it was found that 10 % concentration clearly impacted the egg hatch. It reduced the egg hatch from 64-75% whereas the non-formulated essential oil reduced egg hatch by cent percent (Fig 5). Reduced hatching of *C. maculatus* was evidently proved by Shukla *et al*. (2009) [35], Jayakumar (2010) [16], Regmi and Dhoj (2011), Saranya *et al*., (2019) [34] and Govindan *et al*. (2020) [11] and supported the present studies.

Influence of *A. calamus* Essential Oil Emulsion Formulation on F1 Progeny Inhibition

The percent reduction in adult emergence / Inhibition rate illustrated the effectiveness of non-formulated essential oil followed by essential oil emulsion formulation at all the concentrations tested in all the doses. The minimum percent inhibition rate recorded in the essential oil emulsion formulation itself was 80.30 percent at the lowest dose concentration (1% of 50µl) tested, indicated the effectiveness of the formulation. The percent increase in inhibition of F1 progeny over non-formulated essential oil illustrated that 10 and 7% concentrations inhibited nearly all the F1 progenies at all the doses tested (Fig 6). Shukla *et al*. (2009) [35] and Saranya *et al*. (2019) [34] reported that *A. calamus* rhizome imparted cent percent protection on emergence of *C. maculatus*.

Influence of *A. calamus* Essential Oil Emulsion Formulation on Weight Loss

Differences among weight loss recorded at all the doses were significant (p=0.000). Among the concentrations at 50µl dose, weight loss recorded in non-formulated essential oil (0.08) was the least followed by 10% (9.60) and 7% (10.00). Such similar results were noticed in all other doses. The highest FDI was observed in non-formulated essential oil (100) followed by 200µl dose at 10% concentration (75.45) and 7% (70.04). The p value (p= 0.000) statistically supported the results. These results were supported by Regmi and Dhoj (2011), Dhivya *et al*. (2019), Saranya *et al*. (2019) [34] and Govindan *et al*. (2020) [11].

Conclusion

The present study revealed that *A. calamus* essential oil formulated as Essential oil emulsion formulation effectively managed the *C. maculatus.* Among all the treatments tested, non-formulated essential oil followed by 10% Essential oil emulsion formulation at 200 µL dose was the most effective treatment against *C. maculatus*.

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