

## Evaluation of oviposition deterrence and progeny inhibition activity of *Acorus calamus* linn. emulsion formulation against pulse beetle, *Callosobruchus maculatus* (fab.)

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### Abstract

Insects, rodents, microbes, improper post-harvest storage, and other factors are the leading causes of significant food grain loss. An estimated 14 million tonnes of food grain are lost annually in storage in India, primarily as a consequence of insects. Synthetic pesticides and fumigants are the only tools available for controlling these pests, which has led to numerous environmental and effectiveness problems. Phyto-insecticides have long been hailed as desirable alternatives for pest management. *Acorus calamus* Linn. is a significant Phyto-insecticide that has a wide variety of anti-insect characteristics. Their commercial application is still severely limited due to a lack of formulations. As a result, an emulsion formulation of *A. calamus* essential oil was attempted. The results clearly demonstrated the formulation's superiority in efficiently managing the pulse beetle. The highest percent oviposition deterrence (96.82), lowest percent egg hatch (44.44), and F1 progeny inhibition rate (99.59) were recorded in the treatment receiving emulsion formulation.

**Keywords:** *Acorus calamus*, *Callosobruchus maculatus*, essential oil, emulsion formulation

### Introduction

Damage caused by unscientific storage and pests are the main reasons for the post-harvest food grain loss. Among them, about 100 insect pests causing economic loss play a major role (Pimentel, 1991) [28]. In India alone, annual storage loss due to insects accounted for nearly 1,300 million rupees (Agritech, 2021) [12]. Management of these insects relies entirely on the use of synthetic fumigants, which has led to increased application costs, toxicity and environmental issues (Jembere *et al.*, 1995; Okonkwo and Okoye, 1996) [16]. Further, ban on methyl bromide and restrictions on phosphine use due to ozone depleting and resistance development respectively (EPA, 2021; Fields and White, 2002; Benhalim *et al.*, 2004) [9, 21] had dented stored product insect pest management repertoire. Hence, phyto-insecticides, long been touted as attractive alternatives for pest management, need to be revisited (Isman, 2006) [14]. However, their commercialization remains a pipe dream till the challenges in their formulation are surmounted.

*Acorus calamus* Linn. (Acoraceae), a perennial herb, having insecticidal property is used traditionally in the management of stored product pests (Koul *et al.*, 2008) [20]. Essential oil extracted from its rhizome contains  $\alpha$ ,  $\beta$  and  $\gamma$  asarone responsible for insecticidal activity (Streloke *et al.*, 1989; Juan *et al.*, 2009a; Juan *et al.*, 2009b; Liu *et al.*, 2013) [36, 17, 18, 22]. It possessed stomach, contact, and repellent actions, sterilizing effects and showed excellent potential for commercial exploitation as insecticide (El-Nahal *et al.*, 1989; Risha *et al.*, 1990; Kim *et al.*, 2003; Nandi *et al.*, 2008) [7, 32, 19, 26].

*Callosobruchus maculatus* Fab. (Bruchidae; Coleoptera), an important stored legume pest distributed in tropics and subtropics (Fatima *et al.*, 2016) [8] is known to cause cent per cent loss (Credland and Wright, 2008) [3]. Many authors revealed effectiveness of *A. calamus* essential oil against *C. maculatus*. The present study aimed to develop *A. calamus*

emulsion formulation and evaluate its anti-insect properties against *C. maculatus*.

### Materials and Methods

#### 1. Rearing of test insect Pulse beetle, *C. maculatus*

*C. maculatus* was reared as described by (Rahman and Talukder, 2006) [29] under  $28\pm 2^{\circ}\text{C}$  temperature,  $75\pm 5$  per cent relative humidity and 12h: 12h light: dark photoperiod.

#### 2. Extraction of essential oil

Rhizomes were collected from local country medicine shop, air-dried, ground into fine powder using an electric blender, sieved through 10-micron sieve and packed in an airtight container (Akinkurolere *et al.*, 2006 and Singh, 2011) [1, 35]. Essential oil was extracted from 250 grams of rhizome powder using Clevenger apparatus (2000 ml capacity). Water traces in the oil were removed by passing through anhydrous sodium sulphate (Kumar *et al.*, 2007) [11] and stored at a temperature of  $4^{\circ}\text{C}$  (Moretti *et al.*, 2002; Loni and Panahi, 2015; Ejemen, 2017a & b) [24, 23, 5].

#### 3. Development of essential oil emulsion formulation

1, 3, 5, 7 and 10 per cent concentrations of emulsion formulation was prepared by dispersing 1, 3, 5, 7 and 10 ml of essential oil separately in an aqueous mucilage containing one per cent Tween 80 using a turbo emulsifier for 10 minutes. The prepared formulation was stored separately in glass vials at  $4^{\circ}\text{C}$  under air tight condition for further use (Moretti *et al.*, 2002) [24].

#### 4. Evaluation of Contact toxicity under laboratory conditions

50  $\mu\text{L}$ , 100 $\mu\text{L}$ , 150 $\mu\text{L}$ , 200 $\mu\text{L}$ , 250 $\mu\text{L}$ , 300 $\mu\text{L}$  and 350 $\mu\text{L}$  dose of each concentration of the prepared formulation was smeared on the inner side of plastic container (400g capacity) separately and closed air tight. 250 grams of freshly harvested un-infested green gram seeds were filled

and 20 pairs of *C. maculatus* adults were released per container. Non-formulated essential oil and emulsified water smeared containers acted as checks. Each treatment was replicated three times.

The number of eggs placed on treated (Ts) and control (Cs) seeds was recorded after one week, and the % Oviposition Deterrence (POD) was computed using the formula  $POD = [(Ts-Cs)/Cs] \times 100$ . These eggs were kept until the adults emerged. The number of adults that emerged in both the treated (Tn) and control (Cn) sets was recorded, and the % egg hatch and % inhibition rate were calculated: % egg hatch = total egg hatched / total eggs laid x 100; % inhibition rate =  $[(Cn-Tn)/Cn] \times 100$ . (Rahman and Talukder, 2006; Jayakumar, 2010 and Singh, 2011) [29, 35].

**5. Statistical analysis**

The data gathered are statistically analyzed using SPSS software

**Result and Discussion**

**1. Evaluation *A. calamus* emulsion formulation on Oviposition deterrence, Egg hatching**

The data presented in Table 1 & 2 indicated that non-formulated essential oil and *A. calamus* emulsion formulation was significantly effective in reducing the oviposition and hatching of *C. maculatus*. Further, it was found that the differences among the oviposition deterrence effects recorded in all the doses were statistically significant (p=0.000). At 50µl dose, per cent oviposition deterrence

effect recorded in non-formulated essential oil (100%) was followed by 10% (91.76%) and 7% (88.47%). Such similar results were noticed in all the other doses. These findings were supported by (Rahman and Schmidt, 1999; Shukla *et al.*, 2009; Jayakumar, 2010; Dhivya *et al.*, 2019; Saranya *et al.*, 2019 and Govindan *et al.*, 2020) [30, 1]. When the percentage increase in oviposition deterrence was determined over non-formulated essential oil treatment, it was proved that 7 and 10% concentrations offered significant deterrent at all doses tested (Fig. 1).

Hatching was reduced in non-formulated essential oil (0.08% egg hatch) followed by 10% concentration (65.07% egg hatch) of emulsion formulation at 50 µl dose. Similar results were obtained at 100 µl, 150 µl, 200 µl, 250 µl, 300 µl, and 350 µl doses also (Table 1). When the percentage decrease in egg hatch over non-formulated essential oil treatment was calculated, it was revealed that a 10% concentration clearly influenced egg hatch. It decreased egg hatch ranges from 34% to 55%, but non-formulated essential oil dropped egg hatch by 0%. (Fig. 2). The p values (p= 0.003 (200µl); p=0.001 (300µl), p=0.000 (remaining doses)) statistically supported the results. Similar reduced hatching of *C. maculatus* was evidently proved by (Mueke and Apuuli, 1986; Su, 1991; Shukla *et al.*, 2009; Juan, 2009b; Jayakumar, 2010; Regmi and Dhoj, 2011; Singh, 2011; Hafez *et al.*, 2014; Saranya *et al.*, 2019 and Govindan *et al.*, 2020) [35] and supported the present findings.

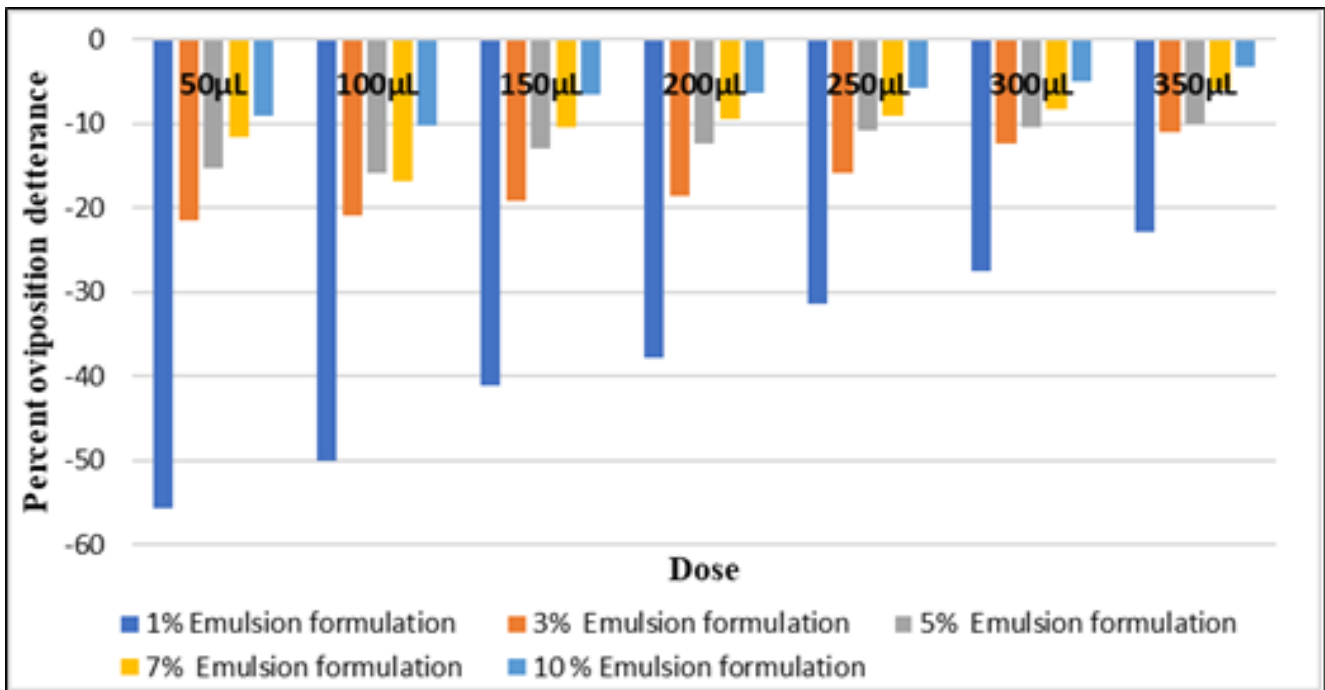


Fig 1: Effect of *A. calamus* emulsion formulation on percent increase in oviposition deterrence over non-formulated essential oil

**Table 1:** Effect of *A. calamus* emulsion formulations against oviposition and egg hatching in pulse beetle, *C. maculatus*

Treatment	Percent oviposition Deterrence (POD)							Percent Egg Hatched						
	50 $\mu$ L	100 $\mu$ L	150 $\mu$ L	200 $\mu$ L	250 $\mu$ L	300 $\mu$ L	350 $\mu$ L	50 $\mu$ L	100 $\mu$ L	150 $\mu$ L	200 $\mu$ L	250 $\mu$ L	300 $\mu$ L	350 $\mu$ L
1% Emulsion formulation	64.19 (53.24) <sup>f</sup>	66.66 (54.73) <sup>e</sup>	70.87 (57.33) <sup>f</sup>	(72.55) <sup>f</sup>	(76.07) <sup>e</sup>	(78.43) <sup>f</sup>	81.35 (64.41) <sup>e</sup>	80.45 (63.75) <sup>d</sup>	80.01 (63.44) <sup>e</sup>	78.97 (62.72) <sup>d</sup>	81.46 (64.49) <sup>d</sup>	90.24 (71.79) <sup>f</sup>	80.02 (63.45) <sup>d</sup>	72.36 (58.28) <sup>d</sup>
3% Emulsion formulation	82.3 (65.12) <sup>e</sup>	82.73 (65.44) <sup>c</sup>	83.91 (66.35) <sup>e</sup>	(84.32) <sup>e</sup>	(86.27) <sup>d</sup>	(89.02) <sup>e</sup>	90.07 (71.63) <sup>d</sup>	86.19 (68.18) <sup>e</sup>	80.22 (63.59) <sup>e</sup>	71.43 (57.68) <sup>c</sup>	70.33 (56.99) <sup>c</sup>	71.46 (57.71) <sup>c</sup>	82.59 (65.34) <sup>e</sup>	80.09 (63.49) <sup>e</sup>
5% Emulsion formulation	86.82 (68.71) <sup>d</sup>	86.34 (68.31) <sup>c</sup>	88.51 (70.17) <sup>d</sup>	(89.01) <sup>d</sup>	(90.19) <sup>c</sup>	(90.58) <sup>d</sup>	90.87 (72.41) <sup>d</sup>	87.57 (69.35) <sup>f</sup>	96.96 (79.95) <sup>d</sup>	93.33 (75.03) <sup>e</sup>	90 (71.56) <sup>f</sup>	76.38 (60.92) <sup>d</sup>	84.72 (66.98) <sup>f</sup>	73.81 (59.21) <sup>d</sup>
7% Emulsion formulation	88.47 (70.15) <sup>c</sup>	83.13 (65.75) <sup>c</sup>	89.66 (71.23) <sup>c</sup>	(90.58) <sup>c</sup>	(90.98) <sup>c</sup>	(91.76) <sup>c</sup>	94.05 (75.88) <sup>c</sup>	71.11 (57.48) <sup>c</sup>	77.54 (61.71) <sup>c</sup>	100 (90.00) <sup>f</sup>	80.55 (63.83) <sup>e</sup>	78.57 (62.42) <sup>e</sup>	76.19 (60.79) <sup>c</sup>	52.22 (46.27) <sup>c</sup>
10% Emulsion formulation	91.76 (73.32) <sup>b</sup>	90.76 (72.3) <sup>b</sup>	93.86 (75.65) <sup>b</sup>	(94.12) <sup>b</sup>	(94.51) <sup>b</sup>	(95.29) <sup>b</sup>	96.82 (79.73) <sup>b</sup>	65.07 (53.77) <sup>b</sup>	53.33 (46.91) <sup>b</sup>	50 (45.00) <sup>b</sup>	53.33 (46.91) <sup>b</sup>	50 (45.00) <sup>b</sup>	52.22 (46.27) <sup>b</sup>	44.44 (41.18) <sup>b</sup>
Non-formulated essential oil	100 (90) <sup>a</sup>	100 (90) <sup>a</sup>	100 (90) <sup>a</sup>	(100) <sup>a</sup>	(100) <sup>a</sup>	(100) <sup>a</sup>	100 (90) <sup>a</sup>	0.08 (1.62) <sup>a</sup>	0.08 (1.62) <sup>a</sup>	0.08 (1.62) <sup>a</sup>	0.08 (1.62) <sup>a</sup>	0.08 (1.62) <sup>a</sup>	0.08 (1.62) <sup>a</sup>	0.08 (1.62) <sup>a</sup>
Untreated Control	0.08 (1.62) <sup>g</sup>	0.08 (1.62) <sup>f</sup>	0.08 (1.62) <sup>g</sup>	(0.08) <sup>g</sup>	(0.08) <sup>f</sup>	(0.08) <sup>g</sup>	0.08 (1.62) <sup>f</sup>	100 (90.00) <sup>g</sup>	100 (90.00) <sup>f</sup>	100 (90.00) <sup>f</sup>	100 (90.00) <sup>g</sup>	100 (90.00) <sup>g</sup>	100 (90.00) <sup>g</sup>	100 (90.00) <sup>g</sup>
SE(d)	0.548	1.475	0.458	0.662	0.512	0.525	0.955	0.339	0.088	0.433	0.278	0.245	0.158	0.055
C.D. at P=0.05	1.175	3.165	0.983	1.419	1.098	1.126	2.048	0.727	0.188	0.928	0.596	0.525	0.338	0.117

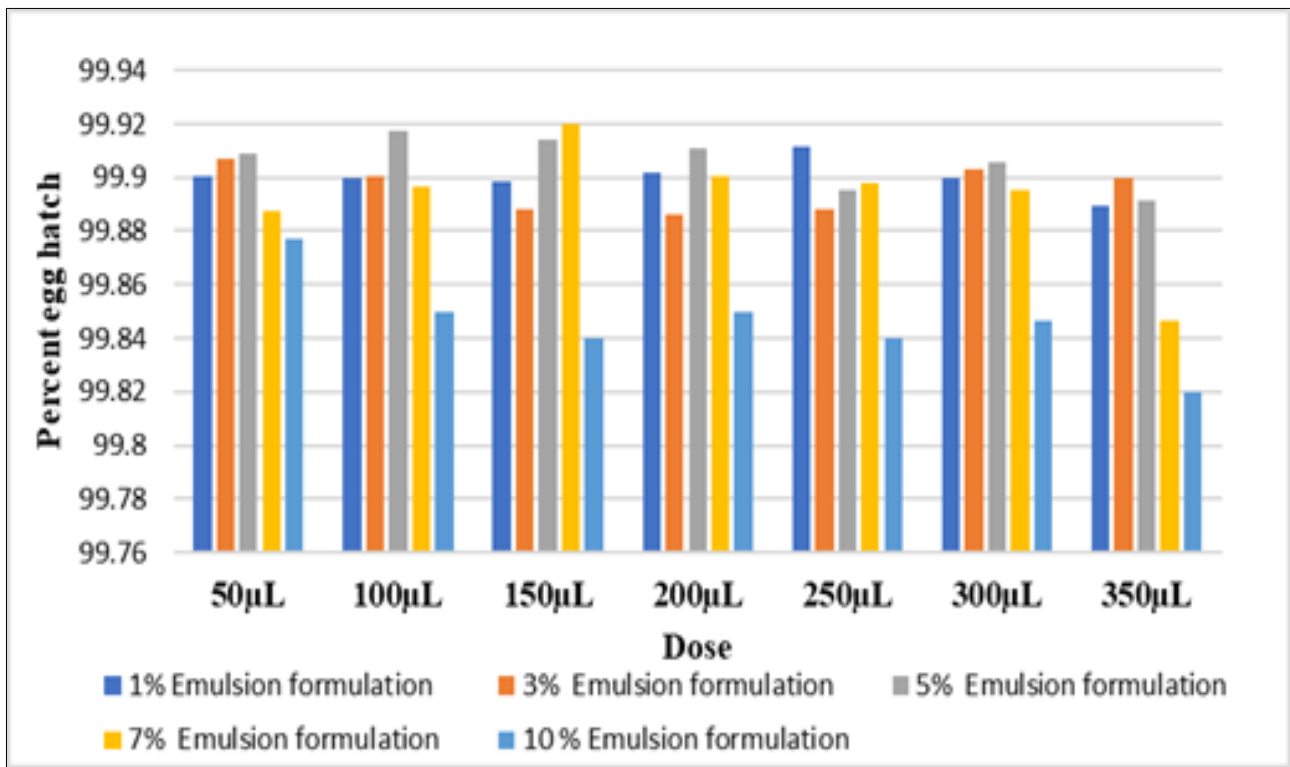
**Table 2:** Percentage reduction in adult emergence or inhibition rate (% IR) of *A. calamus* emulsion formulations against pulse beetle, *C. maculatus*

Treatment	Percent Inhibition rate (% IR)						
	50 µL	100 µL	150 µL	200 µL	250 µL	300 µL	350 µL
1% Essential oil emulsion formulation	78.33 (62.25) <sup>d</sup>	81.12 (64.24) <sup>e</sup>	78.93 (62.67) <sup>f</sup>	80.78 (63.99) <sup>e</sup>	81.18 (64.28) <sup>f</sup>	85.10 (67.28) <sup>f</sup>	(89.55) <sup>d</sup>
3% Essential oil emulsion formulation	88.75 (70.4) <sup>c</sup>	88.75 (70.4) <sup>d</sup>	89.65 (71.23) <sup>e</sup>	90.19 (71.75) <sup>d</sup>	91.37 (72.95) <sup>e</sup>	92.55 (74.16) <sup>e</sup>	(95.58) <sup>c</sup>
5% Essential oil emulsion formulation	90.00 (71.56) <sup>c</sup>	89.15 (70.76) <sup>d</sup>	90.8 (72.34) <sup>d</sup>	90.98 (72.52) <sup>d</sup>	93.33 (75.03) <sup>d</sup>	93.73 (75.48) <sup>d</sup>	(97.18) <sup>b</sup>
7% Essential oil emulsion formulation	96.66 (79.46) <sup>b</sup>	95.98 (78.43) <sup>c</sup>	92.72 (74.34) <sup>c</sup>	93.73 (75.48) <sup>c</sup>	94.12 (75.96) <sup>c</sup>	95.29 (77.46) <sup>c</sup>	(97.99) <sup>b</sup>
10% Essential oil emulsion formulation	96.66 (79.46) <sup>b</sup>	97.99 (81.85) <sup>b</sup>	98.46 (82.87) <sup>b</sup>	98.04 (81.95) <sup>b</sup>	98.04 (81.95) <sup>b</sup>	98.82 (83.76) <sup>b</sup>	(99.59) <sup>a</sup>
Non-formulated essential oil	100 (90) <sup>a</sup>	100 (90) <sup>a</sup>	100 (90) <sup>a</sup>	100 (90) <sup>a</sup>	100 (90) <sup>a</sup>	100 (90) <sup>a</sup>	100 <sup>a</sup>
Untreated Control	0.08 (1.62) <sup>e</sup>	0.8 (1.62) <sup>f</sup>	0.08 (1.62) <sup>g</sup>	0.08 (1.62) <sup>f</sup>	0.08 (1.62) <sup>g</sup>	0.08 (1.62) <sup>g</sup>	(0.08) <sup>e</sup>
SE(d)	0.704	0.486	0.501	0.513	0.362	0.363	0.743
C.D. at P=0.05	1.510	1.043	1.075	1.101	0.776	0.778	1.594

\*Mean of three replications

Values followed by different alphabets within a column differ significantly

hAT – hours After Treatment



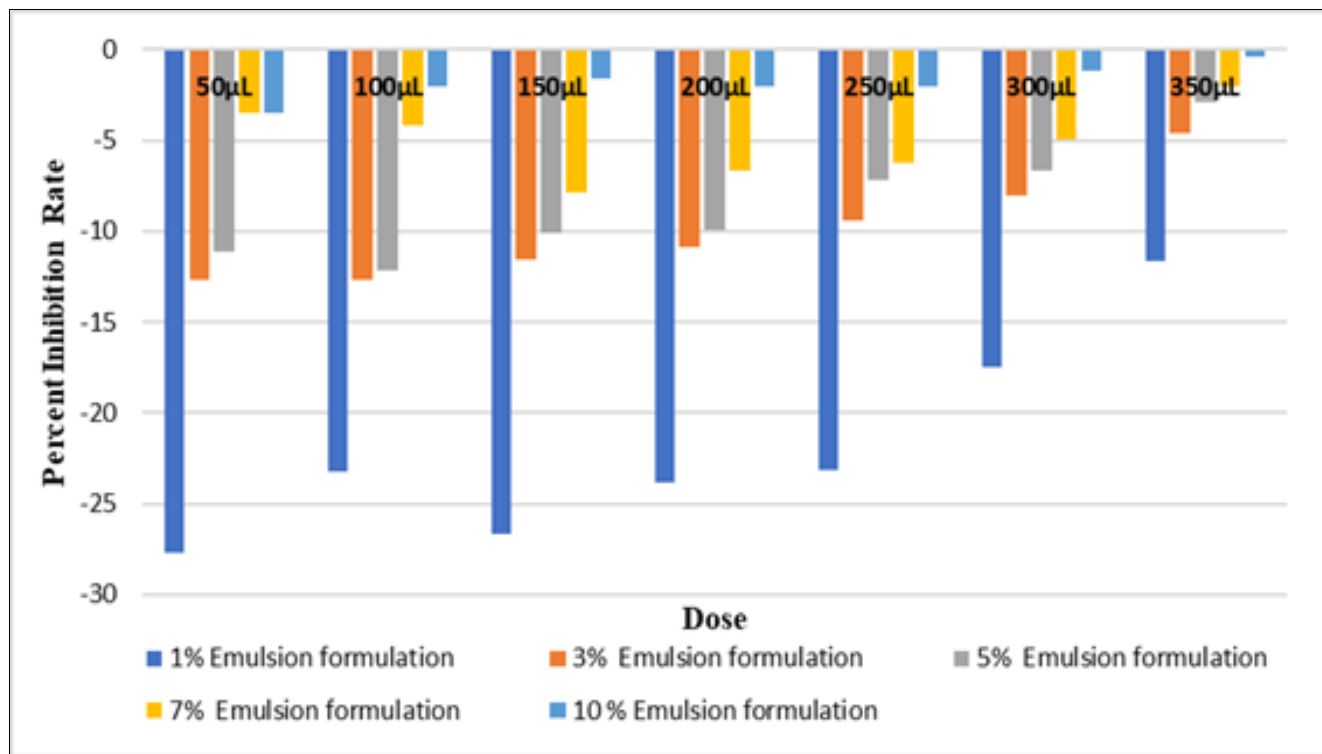
**Fig 2:** Effect of *A. calamus* emulsion formulation on percent decrease in egg hatch over non-formulated essential oil

**2. Evaluation of *A. calamus* emulsion formulation on F1 progeny inhibition**

The data presented in Table 2 indicated that non-formulated essential oil and *A. calamus* emulsion formulation was significantly effective in reducing the *C. maculatus* progeny. The percentage reduction in adult emergence/inhibition rate proved the efficacy of non-formulated essential oil followed by emulsion formulation at all concentrations evaluated in all dosages. The results were statistically confirmed by the p value (p= 0.000). The minimum percent inhibition rate recorded in the emulsion formulation was 78.33 percent at the lowest dose concentration tested (50µl of 1%), proving

the formulation's efficacy. The percentage increase in F1 progeny inhibition over non-formulated essential oil revealed that 10% and 7% concentrations inhibited almost all F1 progenies at all doses tested (Fig. 3).

According to Shukla *et al.*, 2009 [34] and Saranya *et al.*, 2019 [33], *A. calamus* rhizome provided 100% protection against the emergence of *C. maculatus*. Similar results were revealed by (Mueke and Apuuli, 1986; Su, 1991; Juan, 2009b; Jayakumar, 2010; Regmi and Dhoj, 2011; Singh, 2011; Hafez *et al.*, 2014 and Govindan *et al.*, 2020) [25, 37, 18, 15, 31, 35, 11, 10].



**Fig 3:** Effect of *A. calamus* emulsion formulation on percent increase in inhibition rate over non-formulated essential oil

### Conclusion

*A. calamus* essential oil formulated as controlled-release Emulsion formulation was found to effectively managing *C. maculatus*. Among the doses evaluated, 10 and 7 percentage concentrations of emulsion formulation at 350µl dose managed the *C. maculatus* successfully. In future, it could be used as commercial formulations after developing proper delivery systems.

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