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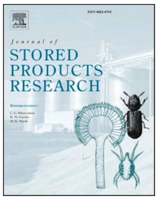

Transgenerational effects of sublethal deltamethrin exposure on development and repellency behaviour in *Callosobruchus chinensis*

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ABSTRACT

Callosobruchus chinensis, belongs to the Chrysomelidae family, is a major pest of pulse grains. It causes 60% weight loss of the pulses, making them inappropriate for planting or human consumption. *C. chinensis* is commonly controlled with insecticides; however, these insecticides have negative effects on the environment and non-target organisms. Moreover, repeated exposure to the insecticide causes the resistance in the *C. chinensis*. Initially the lethal concentration of deltamethrin (LC_{50}) was obtained by the probit analysis. Later the study aimed to examine the impact of a sublethal concentration of deltamethrin on various developmental characteristics and repellency behaviour throughout six successive generations of the *C. chinensis*. For that, two concentration were selected *viz* sublethal low concentration (1/20th) and sublethal high concentration (1/5th) of the LC_{50} compared to control. Both concentrations significantly reduce the life table parameters like egg count, total hatching, total development duration, total emergence and adult longevity in the first generation but an increasing trend in all the parameters was observed in subsequent generations (progeny). Additionally, the repellency was maximum in 1st generation but it decreases with the exposure time and successive generations which shows that it was time and generation dependent. The findings indicate that although there are predominantly adverse effects within the generation, exposure to deltamethrin at sublethal levels over the generations can lead to beneficial effects in the offspring, enhancing their ability to withstand insecticides.

1. Introduction

In the field of stored grain management, insecticides are frequently employed to manage the population of pests like beetles, weevils, and moths that infest stored grains. These pests pose risks to the quantity and quality of stored grains, necessitating the use of insecticides for effective control (Tripathi, 2018; Tesfaye et al., 2021). The main objective of insecticides is to eradicate or impede the growth and reproductive capacities of the pests. However, many insecticides may produce unintended consequences, potentially causing transgenerational effects on pest populations (Costa et al., 2023). Insecticide-induced transgenerational changes refer to the long-lasting effects of insecticide exposure on organisms, which occur across multiple generations and affect the characteristics and functioning of future offspring (Hanson and Skinner, 2016; Amiri and Bandani, 2023). These effects manifest in various ways, including altered phenotypes, physiological changes, behavioural modifications, and susceptibility to diseases or stressors (Xin et al., 2015; Castano-Sanz et al., 2022; Tamagno et al., 2023). Additionally, these alterations may affect various biological processes, including development, metabolism, reproduction, and responses to stress or toxins (Szabó et al., 2020; Wang et al., 2022; Wu et al., 2022).

Research on the transgenerational effects of insecticides on stored grain pests has gained significant attention, highlighting the crucial importance of using appropriate pesticide doses in safeguarding of stored grains. Stored insects present advantageous traits as model organisms for transgenerational investigations, given their rapid generational turnover and the ease of maintaining substantial populations in controlled laboratory settings (Mukherjee et al., 2015). Insects exhibit prompt responses to pesticides, leading to the emergence of diverse adaptive phenotypes over the course of a few generations (Dubovskiy et al., 2013; Mukherjee and Vilcinskas, 2019). Studies on the transgenerational effects of carbaryl and permethrin on aquatic insects have demonstrated that exposure to these insecticides in one generation can have a substantial impact on the survival, growth, and reproductive

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consequences of future generations (Gross and Garric, 2019; Olivares-Castro et al., 2021). Further, Jaffar et al. (2022) conducted a study to examine the transgenerational effects of imidacloprid and propoxur, on the reproductive traits like fertility and offspring growth of *Drosophila melanogaster* Meign (Diptera: Drosophilidae). Their research uncovered reduced fertility and altered offspring growth over multiple generations. Moreover, Daglish (2008) studied the transgenerational impact of methoprene, an insect growth regulator on *Sitophilus oryzae* (L.) (Coleoptera: Cruculionidae) had an effect on the reproductive output, developmental period, and population of future generations. Rösner et al. (2020) found that the deltamethrin negatively effects the reproductive performance and offspring development in subsequent generations of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Similarly, Cui et al. (2021) studied the transgenerational effects of phosphine, a commonly used fumigant, on fecundity and body weight in three stored-product insects: *T. castaneum*, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) and *S. oryzae* and have observed that phosphine exposure decreases the reproductive parameters and body weight. These studies highlight the possibility that exposure to insecticides can cause long-lasting effects over multiple generations of insects, affecting their overall health, ability to reproduce, growth patterns, and population trends (Guedes et al., 2016; Nyamukondiwa et al., 2022).

The use of insecticides can have transgenerational impacts on pests, resulting in both immediate and long-lasting implications. Over an extended period, these impacts may provide advantages to the pests, allowing them to survive and reproduce even when insecticides are present. The outcome of this situation could result in the development of insecticide resistance, which would provide significant challenges for the successful application of pest control measures (Bueno et al., 2023). Overall, the transgenerational effects of insecticides on stored grain pests highlight the need for careful and responsible insecticide use. Understanding insecticide-induced transgenerational changes is important for insecticide risk assessment, management strategies, and the development of sustainable pest control practices. Further research is needed to unravel the underlying mechanisms and assess the ecological implications of these transgenerational effects on insect populations and ecosystems. Hence, the present study was undertaken to investigate the transgenerational effects of deltamethrin on *C. chinensis* by understanding the alteration on the development and repellency behaviour. *C. chinensis* exhibits a cosmopolitan distribution pattern and has been observed in numerous countries as a result of the international trade of beans (Parish et al., 2017). The common host plants of this organisms encompass green gram, lentil, cowpea, pigeon pea, chickpea, and other pea species (Fite and Tefera, 2022). The process of oviposition takes place on the external surface of seeds. Larvae then proceed to burrow directly into the cotyledon by penetrating the seed coat after a period of 4–5 days of incubation (Broadhurst, 1996; Szentesi, 2020). Pupation occurs within the seeds under optimal environmental circumstances characterised by a temperature range of 25–30°C and a relative humidity range of 65–70% (Hariprasad, 2020; Otieno, 2020). The emergence of sexually mature adult beetles takes place during a period of 24–35 days, with the duration varying depending on the specific host seeds (Szentesi, 2020). Adult individuals reach maturity within a 24-h period following emergence, and exhibit an average lifespan ranging from 12 to 16 days (Howe and Currie, 1964; Broadhurst, 1996; Szentesi, 2020). During this temporal span, the processes of mating and oviposition occur. This unique characteristic makes it an ideal model for studying the transgenerational effects of insecticide.

2. Materials and methodology

2.1. Insect maintenance

Callosobruchus chinensis were collected from the different granaries of Vadodara, Gujarat. They were reared in laboratory conditions for at least three months before starting the final experiment, this was the stock culture. The stock culture was reared on green grams in plastic jars covered with mesh lids. The cultures were kept under 26–28°C and 60–70% relative humidity (RH), 12 h photo period (Sharma et al., 2023).

2.2. Experimental design

A survey was conducted in different insecticide shops and ware houses to find the usage of different insecticides. The unworked or least explored insecticide was taken into the account. A semisynthetic pyrethrin insecticide, technical grade deltamethrin (98% AI, Sigma Aldrich, Saint Louis, MO) was used. To determine contact toxicity of deltamethrin against *C. chinensis*, five concentrations of deltamethrin, 6.25, 12.50, 25, 50 and 100 ppm respectively were tested. These concentrations were obtained by “dissolving 98% deltamethrin in acetone.” A stock solution of 1000 ppm was prepared from which other desired concentrations (serial dilutions) were prepared. There were three replicates for each treatment in addition to controls. One mL of each concentration was placed on the bottom of each Petri dish and spread in the entire Petri dish (9 cm diameter). After the acetone was evaporated, 10 pairs of adults of *C. chinensis* were placed into each dish. The same procedure was used for the control treated with acetone. Mortality percentages were recorded after 48 h, 72 h and 96 h of treatment. Thereafter Probit analysis was performed to obtain the LC_{50} value.

2.3. Transgenerational effect of insecticide on the development of *C. chinensis*

The experiments performed on three groups. Group I: Control (acetone); Group II: Low Concentration (L_{Lc50}) ($1/5^{th}$) and Group III: High Concentration (H_{Lc50}) ($1/20^{th}$). One mL of Control, L_{Lc50}, H_{Lc50} was evenly spread on the Petri dish (9 cm diameter). After the acetone was evaporated, 10 pairs of newly emerged (1–2 days old) *C. chinensis* (F1) were released in glass Petri dish containing 50g *Vigna mungo* (green gram) as host. These petri dishes were maintained at 26–28°C, 60–70% RH and 12h photo period, and they were allowed to mate for seven days and egg laying. The grains containing eggs were separated and observed for further development. From these eggs, F2 generation individuals were emerged out of which 10 pairs of newly emerged adults were again exposed to sublethal concentration of deltamethrin. Similarly, subsequent generations were obtained and studied, whole set up was replicated three times in all the six generations.

Statistics of the following traits were analyzed in each of the six generations:

Total number of eggs: To determine the total number of eggs, the eggs laid were counted using magnifying glass and recorded for seven days.

Total hatched eggs: Hatched eggs from the total eggs were counted.

Total development period: The period from the egg laid to adult emergence in each treatment was recorded.

Total adult emergence: Emerged adults were counted, first emergence of adult from the egg was counted as day one. The adult emergence of all the eggs were counted till day seven. Once adult emerges it was separated from the petri dish to new petri dish to avoid repeated calculation.

Adult longevity: The number of days that the emerged adult survive were recorded.

2.4. Transgenerational effect of insecticide on the repellency of *C. chinensis*

Filter papers (9 cm diameter Whatman No. 41) was cut in half and each labelled “C” for control and “L_{Lc50}” for low dose similarly it was done for Control “C” and high dose “H_{Lc50}”. The treatment half was treated with one mL of one dose and allowed to air dry for 2 min. The control half was treated with one mL of acetone only. Both halves were re-joined with clear adhesive tape and placed with the taped side down in a 9 cm petri dish. Twenty seeds of green gram were evenly distributed in the petri dish and five pairs of newly emerged adult beetles were placed in the centre of the filter paper and the dishes sealed tightly with parafilm® to prevent escape. The dispersion of the beetles on each side (treatment and control) was noted 0, 1, 2, 4, 8, 12 and 24 h. The experiment was a randomized block design with three replicates per treatment.

The percent repellency (PR) (Nerio et al., 2009) was

calculated based on the formula:

$PR = [(N_C-N_T)/(N_C + N_T)]X100$.

Nc = number of insects on control half of filter paper after required exposure interval.

N_T = number of insects on treated half of filter paper after required exposure interval.

The repellency was classified according to the following repellency scale (% repellency = class): (<0.1 = 0), (0.1–20 = I), (20.1–40.0 = II), (40.1–60.0 = III), (60.1–80.0 = IV), and (80.1–100 = V) (Patiño-Bayona et al., 2021)

The Repellent Index (RI) of Kogan and Goeden (1970) based on the formula was calculated:

$RI = 2G/(G + P)$

Where G = number of insects on treatment side.

P = Number of insects on control side.

The standard deviations (SD) of the mean values of the RI were calculated. The insecticide at two concentrations was classified based on RI as attractant ($RI > 1 + SD$), indifferent (=neutral; 1 – SD and 1 + SD) and repellent ($RI < 1–SD$).

2.5. Statistical analysis

This study was in a completely randomized design (CRD) by three replications. Statistical analysis was done by analysis of variance (ANOVA) with GraphPad prism 9.0v followed by multiple comparison test (Tukey's). Results are presented as Mean ± SEM. The level of significance was set as *$p < 0.05$, **$p < 0.01$.

3. Results

3.1. Determining LC_{50} value of deltamethrin

LC_{50} value of the deltamethrin was determined using probit analysis. Table 1 depicts the LC_{50}, Slope ± SE, Degree of freedom, chi-square value and P value. The mortality was exposure time and concentration dependent. *C. chinensis* recorded LC_{50} as 44.38 ppm, 33.77 ppm and 22.93 ppm at 48 h, 72 h and 96 h respectively. The slope values of log concentration probit (lcp) lines of deltamethrin were 4.72, 5.04 and 6.56 at 48 h, 72 h and 96 h respectively. Further, the sublethal concentrations: Low concentration (L_{Lc50})-1/20th of LC_{50}, and High concentration (H_{Lc50})-1/5th of LC_{50} were used to understand the transgenerational effects of deltamethrin on *C. chinensis*.

Table 1
Probit analyses of *C. chinensis* adult mortality for 48-h, 72-h and 96-h exposure periods.

Exposure period	LC_{50} ppm (95% CI)	Slope ± SE	P value	Degree of freedom (df)	chi-square value χ^2
48-h	44.38 (37.14–46.08)	4.72 ± 0.96	0.016	4	12.45
72-h	33.77 (25.20–36.95)	5.04 ± 1.26	0.028	4	10.86
96-h	22.93 (12.51–25.51)	6.56 ± 1.40	0.018	4	12.10

3.2. Transgenerational effect of insecticide on the development of *C. chinensis*

A statistically significant variation ($p < 0.05$ and $p < 0.01$) in the total egg count were observed across the groups throughout several generations (Fig. 1). The first generation demonstrates a noticeable impact of deltamethrin, as seen by a significant drop in the overall egg count as compared to the control group 190 ± 1.10. Specifically, the low concentration group exhibited a count of 120 ± 07 eggs, while the high concentration group had a count of 100 ± 0.4 eggs. In subsequent generations, there is a gradual increase in the total egg count compared to the first generation. By the sixth generation, the total egg count of the control group 190 ± 1.10, low concentration group 185 ± 0.6, and high concentration group 180 ± 0.7 is nearly identical.

The overall hatching has a comparable pattern to the total egg count, and it demonstrates significance ($p < 0.05$ and $p < 0.01$) among both the groups throughout several generations (Fig. 2). The first generation demonstrates a noticeable impact of deltamethrin on the hatching rate in both the sublethal concentrations. A significant reduction was observed for the low concentration group 45 ± 0.5, while the high concentration group experienced a reduction of hatching rate 30 ± 0.4 compared to the control group 120 ± 1.15. In subsequent generations, there is a gradual increase observed in the total hatching rate when compared to the first generation. By the sixth generation, the total hatching rates of the control group 120 ± 1.15, low concentration group 115 ± 1.0, and high concentration group 105 ± 0.7 were found to be in close proximity.

In the first generation, the duration of complete development period (Fig. 3) was found to be 44 ± 0.43 days in the high concentration group and 36 ± 0.37 days in the low concentration group, which was substantially longer ($p < 0.01$) compared to the control group with a duration of 22.67 ± 0.33 days. In subsequent generations, there was a shift in the observed pattern, wherein the total duration of the developmental period exhibited a decreasing tendency in both the low and high concentration groups. Specifically, in the sixth generation, the low concentration group displayed a total development time of 23 ± 0.26 days, while the high concentration group exhibited a total development period of 24 ± 0.30 days, which was nearly comparable to the control group.

The duration of survival for the hatched adult individual's (longevity) (Fig. 4) exhibits a statistically significant difference ($p < 0.01$). Specifically, the first-generation the control group had a mean survival time of 16 ± 0.25 days, the low concentration group had a mean survival time of 10 ± 0.25 days, and a mean survival time of 7 ± 0.12 days in the high concentration group. The duration of survival in

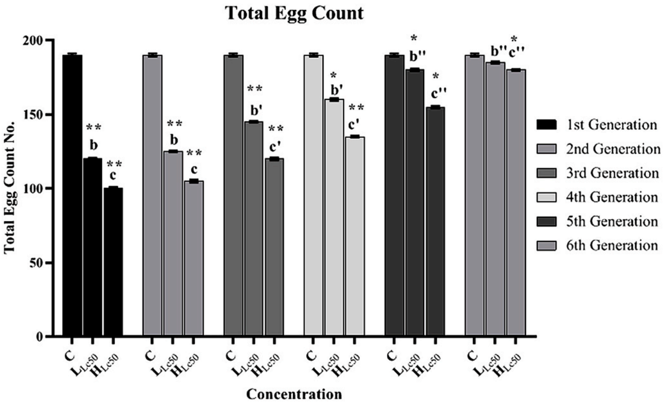

Fig. 1. Transgenerational effect of deltamethrin on the total egg count of *C. chinensis.* Significance level *($p < 0.05$); **($p < 0.01$), (same letter (b and b; b' and b'; b'' and b''; c and c; c' and c'; c'' and c'') implies no statistically significant difference and different letters (b and b'/b'' and c and c'/c'') implies that there is a statistically significant difference).

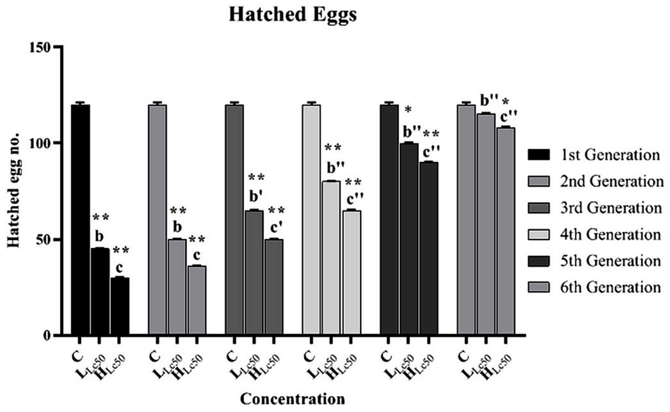

Figure: 2. Transgenerational effect of deltamethrin on the total hatching of *C. chinensis.* Significance level *($p < 0.05$); **($p < 0.01$) (same letter (b and b; b' and b'; b'' and b''; c and c; c' and c'; c'' and c'') implies no statistically significant difference and different letters (b and b'/b'' and c and c'/c'') implies that there is a statistically significant difference).

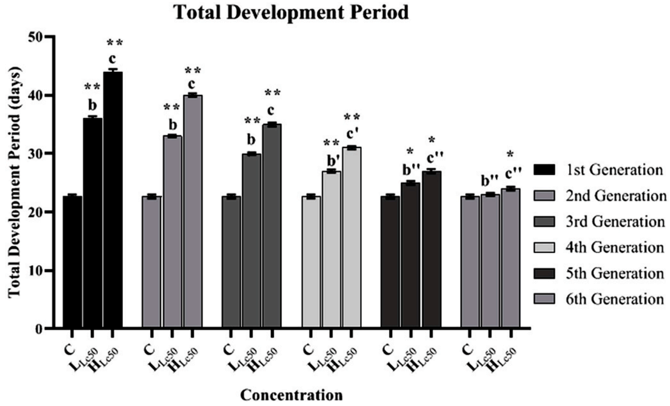

Figure: 3. Transgenerational effect of deltamethrin on the total development period of *C. chinensis.* Significance level *($p < 0.05$); **($p < 0.01$) (same letter (b and b; b' and b'; b'' and b''; c and c; c' and c'; c'' and c'') implies no statistically significant difference and different letters (b and b'/b'' and c and c'/c'') implies that there is a statistically significant difference).

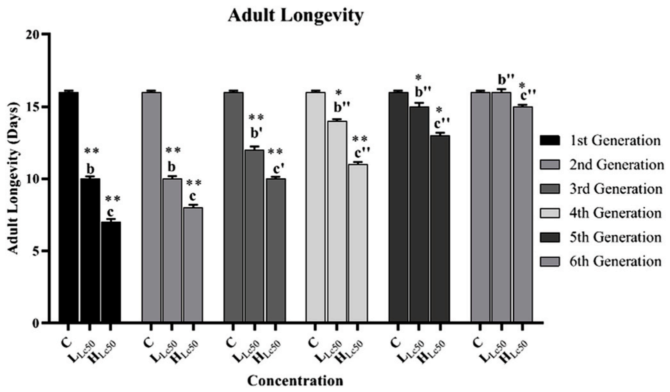

Figure: 4. Transgenerational effect of deltamethrin on the adult longevity of *C. chinensis.* Significance level *($p < 0.05$); **($p < 0.01$) (same letter (b and b; b' and b'; b'' and b''; c and c; c' and c'; c'' and c'') implies no statistically significant difference and different letters (b and b'/b'' and c and c'/c'') implies that there is a statistically significant difference).

consecutive generations exhibited an increasing trend. Specifically, at the sixth generation, the low concentration group demonstrated a survival period of 16 ± 0.18 days, while the high concentration group exhibited a survival period of 14 ± 0.22 days.

Transgenerational effects of the insecticide on the repellency behaviour of C. Chinensis

Tables 2 and 3 provide an account of the repellency percentage, while Tables 4 and 5 present the repellent index of deltamethrin against *C. chinensis.* The data reveals that *C. chinensis* exhibited the high level of repellency (80.1–100 = V) in the initial hours of 1st generation, but there was a decrease in repellency in later hours, whereas in F6 generation repellency was (20.1–40.0 = II) at initial hours and later hours decreases more (0.1–20.0 = I). This pattern was observed on both the sublethal concentrations. Similar pattern was found in the repellent index, whereby *C. chinensis* initially exhibited repellent effects (RI < 1–SD) but then transitioned to a neutral state (=neutral; 1 – SD and 1 + SD) and later on it became attractant (RI > 1 + SD). The efficacy of deltamethrin as a repellent was shown to vary depending on the duration of exposure and the specific generation being tested. The level of repellency exhibits a decline as the duration of exposure and subsequent generations progress.

4. Discussion

Deltamethrin is a chemical compound that mimics the properties of pyrethrins, which are naturally found in dried *Chrysanthemum* flowers (Shrivastava et al., 2011; Bhanu et al., 2011). It is widely used to combat stored-product insect pests throughout the world (Vayias et al., 2010; Trostanetsky et al., 2023). Its mode of action involves inducing paralysis in the nervous systems of insects, leading to swift incapacitation, impaired coordination, and eventual mortality (Velki et al., 2014). Deltamethrin's neurotoxic effect on insects originates from its ability to interfere with the transmission of nerve impulses along axons by modifying the ion permeability of nerve membranes (Paudyal et al., 2016, 2017). In this study, the anticipated outcome cantered on establishing the enhanced efficacy of higher doses of deltamethrin in *C. chinensis*, attributed to its rapid knockdown. Paudyal et al. (2016) observed a significant rise in death rates of adult *T. castaneum, S. oryzae,* and *R. dominica* when they were exposed to higher concentration of deltamethrin. Jacob et al. (2014) found that adult *S. zeamais* exposed to commercial grade deltamethrin at a concentration of 250 ppm had a mortality rate of 50%. In contrast, our study recorded a 50% mortality rate at a lower concentration of 22.93 ppm. The observed high mortality at low concentration in this study is likely attributed to the use of technical grade deltamethrin, also pest species differ in sensitivity to insecticides. This finding validates the susceptibility of *C. chinensis* to

Table: 2
Percent repellency of deltamethrin against *C. chinensis* on L_{Lc50} exposure.

Duration of deltamethrin exposure in hours							
Generations	0	1	2	4	8	12	24
	L_{Lc50} Mean Repellency %						
F1	100 ± 2	90 ± 5	85 ± 4	75 ± 5	70 ± 5	55 ± 2	40 ± 5
F2	100 ± 2	85 ± 5	75 ± 4	68 ± 2	60 ± 5	45 ± 5	35 ± 4
F3	95 ± 2	70 ± 5	65 ± 2	55 ± 4	45 ± 4	30 ± 4	20 ± 2
F4	90 ± 2	55 ± 4	50 ± 5	40 ± 5	35 ± 2	25 ± 5	05 ± 1
F5	86 ± 4	45 ± 2	40 ± 4	30 ± 5	22 ± 5	15 ± 4	−10 ± 2
F6	80 ± 5	35 ± 5	25 ± 5	20 ± 4	15 ± 5	05 ± 2	−20 ± 5

Table 3
Percent repellency of deltamethrin against *C. chinensis* on H_{Lc50} exposure.

Duration of deltamethrin exposure in hours							
Generations	0	1	2	4	8	12	24
	H_{Lc50} Mean Repellency %						
F1	100 ± 2	92 ± 5	90 ± 5	82 ± 4	75 ± 2	60 ± 4	50 ± 4
F2	100 ± 2	90 ± 6	84 ± 6	80 ± 5	66 ± 4	52 ± 6	40 ± 5
F3	100 ± 2	75 ± 4	72 ± 5	62 ± 6	50 ± 5	40 ± 5	30 ± 5
F4	94 ± 4	65 ± 4	55 ± 5	50 ± 5	40 ± 5	28 ± 2	15 ± 4
F5	90 ± 5	60 ± 5	50 ± 4	34 ± 4	30 ± 4	20 ± 4	10 ± 5
F6	85 ± 5	50 ± 4	35 ± 5	25 ± 5	20 ± 2	14 ± 6	−10 ± 2

Table 4
Repellent Index (RI) of deltamethrin against *C. chinensis* on L_{Lc50} exposure.

Duration of deltamethrin exposure in hours							
Generations	0	1	2	4	8	12	24
	L_{Lc50} Repellent index						
F1	–	0.10 ± 0.06	0.15 ± 0.02	0.25 ± 0.15	0.30 ± 0.10	0.45 ± 0.04	0.60 ± 0.10
F2	–	0.15 ± 0.10	0.25 ± 0.06	0.32 ± 0.10	0.40 ± 0.06	0.55 ± 0.10	0.65 ± 0.16
F3	–	0.30 ± 0.08	0.35 ± 0.12	0.45 ± 0.10	0.55 ± 0.18	0.70 ± 0.08	0.80 ± 0.22
F4	0.05 ± 0.01	0.45 ± 0.12	0.50 ± 0.15	0.60 ± 0.15	0.65 ± 0.20	0.75 ± 0.16	0.95 ± 0.12
F5	0.14 ± 0.08	0.55 ± 0.15	0.60 ± 0.10	0.70 ± 0.08	0.78 ± 0.14	0.85 ± 0.2	1.10 ± 0.10
F6	0.20 ± 0.10	0.65 ± 0.18	0.75 ± 0.15	0.80 ± 0.2	0.85 ± 0.16	0.95 ± 0.08	1.20 ± 0.23

Table 5
Repellent Index (RI) of deltamethrin against *C. chinensis* on H_{Lc50} exposure.

Duration of deltamethrin exposure in hours							
Generations	0	1	2	4	8	12	24
	H_{Lc50} Repellent index						
F1	–	0.08 ± 0.02	0.10 ± 0.0.4	0.18 ± 0.12	0.25 ± 0.14	0.40 ± 0.2	0.50 ± 0.12
F2	–	0.10 ± 0.04	0.16 ± 0.0.6	0.20 ± 0.08	0.34 ± 0.05	0.48 ± 0.12	0.60 ± 0.16
F3	–	0.25 ± 0.1	0.28 ± 0.1	0.38 ± 0.12	0.50 ± 0.08	0.60 ± 0.06	0.70 ± 0.04
F4	0.06 ± 0.02	0.35 ± 0.1	0.45 ± 0.08	0.50 ± 0.18	0.60 ± 0.12	0.72 ± 0.06	0.85 ± 0.08
F5	0.10 ± 0.06	0.40 ± 0.12	0.50 ± 0.2	0.66 ± 0.1	0.70 ± 0.06	0.80 ± 0.2	0.90 ± 0.1
F6	0.15 ± 0.06	0.50 ± 0.08	0.65 ± 0.15	0.75 ± 0.2	0.80 ± 0.2	0.86 ± 0.1	1.10 ± 0.23

technical grade deltamethrin and therefore makes it a more appropriate choice for monitoring insecticide resistance in stored grain pests (Gupta, 2019).

The extent to which insecticide effects persist from one generation to the next remains unexplored, however it carries significant implications. Margus et al. (2019) emphasized that research mostly focuses on the effects within and between generations, rather than the implications that span at multiple generations. The present study addresses the transgenerational impact of a sublethal concentration of deltamethrin *viz*. sublethal low concentration and sublethal high concentration on various developmental parameters, including total egg count, total hatching, hatching percentage, total development period, and adult longevity. Our findings show a significant negative effect on the development parameters of the initial generations with respect to the control. A comprehensive analysis of the subsequent generations reveals a notable decline in the adverse impact caused by the deltamethrin sublethal concentrations on the developmental parameters. This suggests that the transgenerational effect of deltamethrin exhibits a discernible trend wherein the insects gradually develop a greater tolerance towards it (Brevik et al., 2018). The present study evaluated the transgenerational effects of the sublethal concentrations of deltamethrin on the overall egg count and hatching of *C. chinensis*. The findings demonstrate that both concentrations of deltamethrin significantly reduced the total number of eggs and the hatching rate in the initial generation, as compared to the control group. However, in subsequent generations, the observed effects were not as pronounced and were almost found to be similar to that of the control group. Various studies on effect of deltamethrin on egg laying, or other development parameter has been explored like Toumi et al. (2013) studied the effects of deltamethrin on daphnid reproduction where they recorded reduced egg number, longevity and decrease in overall length. Similarly, Montaño-Campaz et al. (2022) observed that deltamethrin sublethal exposure reduced the fecundity in *Chironomus columbiensis* (Diptera: Chironomidae). Our results are in accordance with the existing literature, which have provided evidence that the egg laying and hatching of *Aphis gossypii* Glover (Hemiptera: Aphididae) and *Plutella xylostella* (L.) (Lipdoptera: Plutellidae) are affected by the insecticides cycloxaprid and spinetoram (Qu et al., 2017; Tamilselvan et al., 2021); spinetoram inhibitory effect on the reproductive capacity of *R. dominica*, *Prostephanus truncatus* (Horn), and *Sitophilus granaries* (L.) (Coleoptera: Curculionidae) (Vassilakos et al., 2012, 2015; Rumbos et al., 2018); cyantraniliprole had adverse effects on the fecundity of *Helicoverpa assulta* (Guenée) (Lepidoptera: Noctuidae) (Dong et al., 2017); flupyradifurone adversely impacted the reproductive capacity of *A. gossypii* (Liang et al., 2019) as well as studies by Ali et al. (2017) had shown significant reduction in the fecundity and hatchability for generations of *Sogatella furcifera* (Horváth) (Hemipetra: Delphacidae) after buprofezin treatment.

According to the findings of our research, exposure of deltamethrin on the F1 generation has a negative impact but gradually this impact decline in subsequent generations. In the F1 generation, the total development period was significantly increased. These findings are in agreement with the earlier reported work where, exposure of spinetoram and cyantraniliprole prolong the developmental duration of *P. xylostella* and *Agrotis ipsilon* first generation (Guo et al., 2013; Xu et al., 2016). Similarly, sublethal concentration of spinosad and chlorantraniliprole significantly increase the development period of *P. xylostella* and *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) (Yin et al., 2008; Zhang et al., 2013). The adult longevity significantly decreases in the F1 generation but in subsequent generations the adult longevity shows an increasing trend and in F6 generation it was at par with control. This shows that with time, the insects are developing tolerance towards the insecticide especially in later generations F5 and F6 where it was observed that the *C. chinensis* were able to survive normal duration. The current study is in accordance with the studies of Ali et al. (2017) who has reported that adult longevity significantly decreased for generations of *S. furcifera* after buprofezin exposure. Similarly, Deng et al.

(2019) has reported that the sublethal concentration of dinotefuran on *Rhopalosiphum padi* (L.) (Hemiptera: Aphididae) adults of the F1 generation reduced adult longevity.

Another approach to examine the impact of the insecticide is to understand the effect of insecticide on repellency behaviour. These might be linked to the resistance or susceptibility of insects to insecticides and might potentially influence the interaction between deltamethrin and insects (Ngom et al., 2021; Tenrirawe et al., 2023). Based on the current literature, there appears to be a noticeable gap in the research pertaining to the transgenerational repellency effects of insecticide on the store grain pests. The understanding of the potential transgenerational consequences resulting from exposure to insecticide is currently limited and lacks comprehensive knowledge of the mechanisms like histone acetylation and deacetylation processes involved controlling gene expression leading to resistance. The current study is the first of its kind which reports deltamethrin induced transgenerational effect on the repellency in *C. chinensis.* The findings of this study indicate that the efficacy of deltamethrin as a repellent exhibited variability in relation to both time of exposure and generation. The degree of repellency diminishes as the time of exposure lengthens, and this pattern persists in following generations. In a study conducted by Muntaha et al. (2017) the repellent properties of pyrethroids on *C. chinensis* were investigated. The results indicated that deltamethrin exhibited the maximum repellency followed by cypermethrin and bifenthrin. In the current study, it was found that the initial generations exhibited a high repellency rate 85%. This observation implies that the insects initially may possess limited tolerance towards the insecticide. However, in subsequent generations, a declining trend in the repellency rate was observed. Deng et al. (2019) has reported that when insects are exposed to sublethal concentrations of insecticide over multiple generations, it may develop resistance. The aforementioned finding also indicates that the *C. chinensis* may have gradually acquired an enhanced capacity to withstand the effects of the deltamethrin. Other possible reason for high repellency in F1 generation and tolerance in F6 generation could be related with the olfactory receptors as Yan et al. (2021) has worked on the pyrethrins activate olfactory-receptor neurons and elicit spatial repellency in *Aedes albopictus* (Diptera: Culicidae). Similarly, Valbon et al. (2022) has also reported that bioallethrin activates specific olfactory sensory neurons and elicits spatial repellency in *Aedes aegypti* (Diptera: Culicidae). Most of the repellency based studied has been performed on mosquitoes as the model organism, there is gap as far as stored grain pest are considered. However, to confirm the findings comparative transcriptome analysis between the treatment and control group can be performed which will elucidated to understand the exact mechanism of resistance.

In recent times, transcriptome has been an important way to study the insecticide resistance mechanism which helps to understand the exact role and mechanisms of differentially expressed genes in xenobiotic metabolism or cuticle thickness. Such studies have been explored on different insect like, Lv Yuan et al. (2016) has done the comparative transcriptome analyses of deltamethrin-susceptible and -resistant *Culex pipiens pallens* (Diptera: Culicidae) by RNA-seq and has reported the association between the *CYP6AA9* gene and deltamethrin resistance. Zhou et al. (2018) has performed a comparative transcriptome analysis of *S. furcifera* (Horváth) exposed to different insecticides like imidacloprid, deltamethrin, and triazophos and observed that P450s, GSTs, COEs, Hsps, and ABC transporters were highly expressed at different levels under insecticide stress. These proteins might be involved in the metabolism and translocation of insecticides in *S. furcifera.* Similarly, Traverso et al. (2022) has performed transcriptomic modulation in response to an intoxication with deltamethrin in a population of *Triatoma infestans* (Hemiptera: Reduviidae) with low resistance to pyrethroids and characterized the repertoire of previously uncharacterized detoxification-related gene families in *T. infestans* and *Rhodnius prolixus* (Hemiptera: Reduviidae). Chitin also has a vital role in maintaining the structural integrity of insects and protecting them from stresses from the outside (Merzendorfer, 2011; Wang et al., 2012). The enzyme known as CHS plays a pivotal part in the intricate process in chitin formation and is an essential need for the growth and development of insects (Van Leeuwen et al., 2012). Nevertheless, the verification of the majority of such genes contributions necessitates substantial investigation and this can be done through comparative transcriptomic studies.

5. Conclusion

The transgenerational effects of deltamethrin suggests that sublethal concentration has influenced the development of *C. chinensis*, where significant decrease in the egg count and total hatching with prolonged development period, as well as shortening of the longevity was observed in adults of the F1 generation. However, in F5 and F6 generations' *C. chinensis* were able to overcome these effects. Similarly, in the F1 generation high repellency was recorded compared to F5 and F6 generations. It was concluded that the repellency depends upon time and generation. The accumulation of all the findings have suggested that F6 generation have developed tolerance and lead to resistance against deltamethrin. Further studies will direct us to the new findings on how the *C. chinensis* is acquiring resistance and hence, it will open up new avenues for target-based research for developing new generation insecticides.

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Availability of data and material

The data can be available within the article.

Ethics approval

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Consent for publication

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CRediT authorship contribution statement

Pankaj Sharma: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ankita Salunke:** Methodology, Formal analysis, Data curation. **Nishi Pandya:** Methodology, Formal analysis, Data curation. **Parth Pandya:** Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization. **Pragna Parikh:** Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used Grammarly in order to check the grammer and language. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jspr.2024.102379.

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ELUCIDATING THE HOST PREFERENCE BY THE PULSE BEETLE *CALLOSOBRUCHUS CHINENSIS* (L)

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ABSTRACT

***Callosobruchus chinensis* (Bruchidae: Coleoptera) known as pulse beetle is a serious pest of pulses causing economic losses. The present work reports the effect of pulses on life cycle parameters (oviposition, emergence, longevity, total development period) of *Callosobruchus chinensis* (L.), and also on the nutritional loss incurred by it. Grains of seven pulses viz. *Vigna radiata* (green gram), *Vigna aconitifolia* (moth bean), *Cicer arietinum* (desi chickpea), *Vigna unguiculata* (cowpea), *Cajanus cajan* (pigeon pea), *Vigna mungo* (black gram) and *Pisum sativum* (yellow pea) were used as host with three replications. The results exhibit alterations in the developmental period as it was recorded least in green gram (23± 0.58 days) and maximum in peas (42± 1 days). The number of eggs were maximum on pea (310± 2) and least in moth (180± 2). Maximum longevity was recorded on green grams (16 days) and least on pea (9± 1 days). A significant correlation between weight loss and adult emergence was observed. Loss of nutritional content like protein and carbohydrate was significant.**

Key words: *Callosobruchus chinensis, Vigna radiata, Vigna aconitifolia, Cicer arietinum, Vigna unguiculata, Cajanus cajan, Vigna mungo, Pisum sativum,* oviposition, developmental period, emergence, longevity, nutrition, protein, carbohydrate

Agriculture contributes about 14% to the GDP of India and about 11% of its total exports (Jha et al., 2015). A large-scale loss is occurring in agriculture at post-harvest such as storage transport, retailing and processing. A study measuring crop losses has revealed a loss of cereals (3.9% - 6%), pulses (4.3%-6.1%), oilseeds (2.8%-10.1%), Fruits (5.8%-18.1%) and vegetables (6.9%-13%) during harvesting, post-harvest activities, handling and storage (Jha et al., 2015). Insects are accountable for the deterioration of stored grains, and have been reported for about a yearly loss of 30% (Adu et al., 2014; Kumar and Kalita, 2017). *Callosobruchus chinensis* i.e., pulse beetle is a cosmopolitan and a serious pest of green gram, black gram, cowpea, red gram and chickpea and 32-64% loss in cowpea is due to *C. chinensis* (Duan et al. 2014). Jaiswal et al. (2018 and 2019) reported around 60% loss in weight of the pulses due to pulse beetle. Till date the biology of *C. chinensis* has been explored on chick pea (Chandel and Bhaudaria, 2015; Rana et al., 2020), green gram (Devi and Devi, 2014; Kumari et al., 2020; Gopi and Singh 2020), black gram (Dalal et al., 2020), cowpea (Augustine and Balikai, 2018), moth bean (Meghwal and Singh 2005) and multiple hosts (Patel et al., 2005; Hosamani et al., 2018; Jaiswal et al., 2018 & 2019; Mehta and Negi, 2020). Due to differences in the physical characteristics of the host, the type of host has a considerable impact on the insect's development. The present study aims to decipher some hosts on the development preference by *C. chinensis* under laboratory conditions.

MATERIALS AND METHODS

Pulse beetle, *C. chinensis* for its biology was studied under laboratory conditions at the Zoology Department, The Maharaja Sayajirao University of Baroda. The stock cultures of *C. chinensis* was collected from the warehouses of Vadodara, and culture was acclimatized in the laboratory conditions. Identity of species was confirmed using standard taxonomic keys (Raina, 1970; Harde, 1984). From the stock, 50 pairs of adults of length 3-4 mm were introduced into 250 g of different pulses in plastic jars covered with mesh lids and were allowed to mate, and oviposit. The cultures were kept under 26^0-28^0C and 60-70% RH, and 12-hour photo period. Cultures were observed daily until new progenies emerged. The pure culture from the fifth generation was removed and was used in all further experiments. Ten pairs freshly emerged (up to 24 hrs) adults were introduced in 50 g pulses viz. green gram, moth bean, desi chickpea, cowpea, pigeon pea, black gram and yellow pea kept in plastic jars covered with mesh lids. These were compared with the control group having only grains for each group. These jars were maintained at 26^0-28^0C, 60-70% RH and 12-hour photo period, and the adults were allowed to mate till the ten pairs died, whole set up was replicated three times (Jaiswal et al., 2018; 2019; Nisar et al., 2021). Cultures were monitored and the developmental period i.e., from eggs to adults was recorded with the overall emergence period of the adults and their longevity. Difference between the loss in weight and nutritional content of the grains exposed to pulse beetle and its control group was calculated. Total carbohydrates estimation was determined by DNSA method, while the total protein content was estimated by Bradford method (1976). The data was analysed using one-way ANOVA (α=0.05 and *p<0.05) using Graph Pad Prism software version 6.

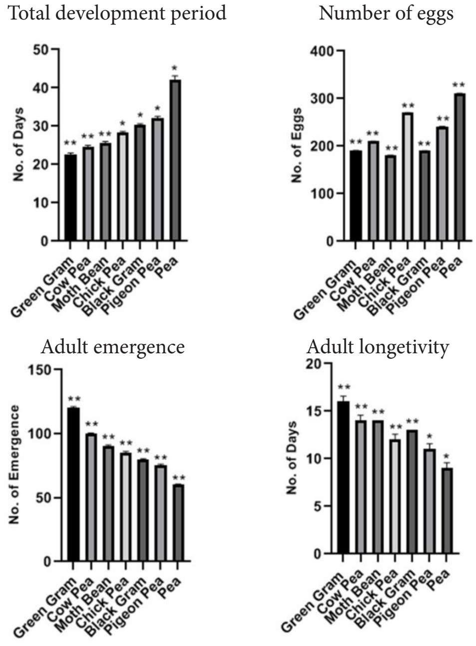

Fig. 1. Life cycle parameters of *C. chinensis* on different host

RESULTS AND DISCUSSION

Life cycle of insects in general and pest in particular depends on the type of food (Singh et al., 2013; Mason et al., 2016). In the present study the life cycle was found to vary according to the host. However, the life cycle was found to be in the range of 25-32 days. Numbers of eggs were maximum on pea 310± 2 (p<0.01) and least on moth bean 180± 2 (p<0.05), Mebarkia et al. (2009) and Padmasri et al. (2017) reported that egg laying depends on softness or hardness of the grain. Adebayo and Ogunleke (2016) reported that increase in the length and width leads to high oviposition activity as observed in the present study (Fig.1); maximum egg laying activity was observed on pea which have more hardness and surface area. The minimum egg laying activity was recorded on moth bean followed by green gram, as these both grains are almost similar in size so much difference was not observed. Total development period (p<0.01) was in the range of 23 to 32 days; minimum development period was reported in green gram followed by cow pea. Similar finding was reported by Radha and Sushila (2014), Hosamani et al., (2018) and Jaiswal et al. (2019). Development period in chick pea was in a range of 28± 0.35 days and this was almost in the range given by Swella and Mushobozy (2009) Kamble et al. (2016) and Ahmad et al. (2017). Maximum adult emergence was observed in green gram 120± 2 followed by cow pea and least in pea 60± 1, and those finding are in accordance with those of Deeba et al. (2006) and Chandel and Bhaudaria (2015). The drop in adult emergence in pea could be because of the low hatchability of eggs due to hard seed coat (Padmasri et al., 2017). The significant difference (p<0.01) was recorded in the longevity of adult *C. chinensis* reared on different host where maximum longevity was recorded in green grams (Fig.1). The present findings are in agreement with Hosamani et al., (2018) and Mehta and Negi (2020).

Additionally, loss in weight of grains also altered significantly (Fig. 2); where the highest was seen in green gram 11.4± 0.5g (p<0.01), and least with pea 6.5± 0.5g (p<0.05). Gupta and Apte (2016) and Bharathi et al. (2017) reported maximum weight loss due to *C. maculatus* also Jaiswal et al. (2019) reported maximum loss in chick pea and green gram by *C. chinensis*. The nutrition content was also analyzed and the results obtained revealed that there was a significant (p<0.01)

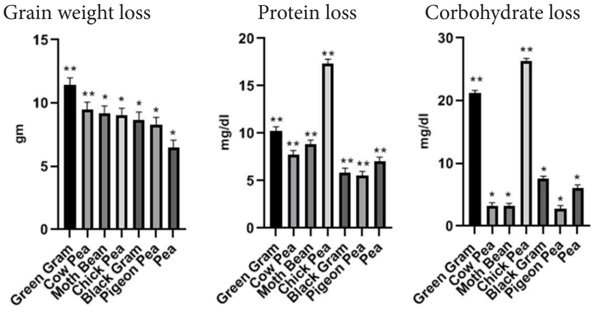

Fig. 2: Quantity and Quality losses by *C. chinensis* on different host (Grain loss, Carbohydrate loss, Protein loss) Significant level *(p<0.05); ** (p<0.01)

decrease of carbohydrates and protein in infested grain. The loss of carbohydrates was highest in chickpea 27.29± 0.82 mg/ dl and least 3.3± 0.4 mg/ dl in pigeon pea ($p<0.05$). The reduction in carbohydrates observed in the chickpea followed by green gram and least in pigeon pea, is almost parallel with the rate of infestation. It was observed that protein content also got reduced, where maximum loss was observed in chick pea (17.48 ± 0.8 mg/dl) and least loss in pigeon pea (4.84± 0.5 mg/dl) ($p<0.05$). Losses in nutritional values, such as protein content, are mainly attributed to storage insect pests, which preferentially feed on grain embryos (Taddese et al., 2020). These observations are in agreement of previous studies by Thakkar and Parikh (2018) who reported nutritional loss by *Sitophilus oryzae* when exposed to different stored grains.

The present study on elucidating the host preference by *C. chinensis* reports that egg count, total development period, adult emergence, adult longevity, weight loss was maximum in green gram also a good amount of nutritional loss was recorded in green grams. Thus, from the present study it can be concluded that for laboratory work green grams are the suitable host for mass rearing of pulses beetle.

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AUTHOR CONTRIBUTION STATEMENT

PS, PP and PP* designed and conceptualized the study. PS performed the experimental work. PS and PP analyzed the data. PS and PP wrote the draft manuscript. PS, PP and PP* reviewed and revised the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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BIOTECHNOLOGICAL APPROACHES IN INSECT PEST MANAGEMENT

Abstract

The global population is projected to reach almost 10 billion by 2050, demanding a 50% increase in agricultural production. In light of the increasing occurrence of pest and disease outbreaks, which jeopardize food security, it is crucial to adopt innovative approaches. Conventional approaches such as chemical pesticides have been found to be inadequate, prompting a shift towards biotechnology alternatives. Biotechnological interventions, such as gene transformation and genetic engineering, provide innovative approaches for controlling insect pests. The advancements in gene editing technologies, such as CRISPR-Cas9, offer possibilities for managing insect pests. RNA interference (RNAi) methods, including double-stranded RNA (dsRNA), have demonstrated potential in specifically eliminating pest species while leaving non-target species. The gene-drive approach modifies the inheritance of specific genes, providing a potent tool for managing insect pests. The book chapter explores the diverse applications of biotechnology in insect pest management, covering gene editing, RNAi, and gene-drive technologies. It highlights successful cases of gene editing in various insect species, such as fruit flies and the migratory locust, and discusses the potential for CRISPR-Cas9 to modify plants for insect resistance. In summary, the incorporation of biotechnology in agriculture provides inventive remedies to tackle the difficulties presented by rising insect prevalence, thereby promoting sustainable and robust food supply for the growing global population.

Keywords: Agricultural Production, Biotechnological Interventions, Genetic Engineering, CRISPR-Cas9

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INSECTICIDAL ACTIVITY OF ESSENTIAL OILS FROM MINT AND AJWAIN AGAINST PULSE BEETLE *CALLOSOBRUCHUS CHINENSIS* (L)

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ABSTRACT

The present study evaluates the insecticidal activity of two essential Oil (EOs) mint (*Mentha arvensis*), and ajwain (*Carum capyicum*) against pulse beetle (*Callosobruchus chinensis)* (L). Contact toxicities of these were evaluated using parameters of lifecycle like total development period, numbers of eggs laid, adult emergence and adult longevity. Along with these detoxification enzyme inhibition activities of acetyl cholinesterase (AChE), alkaline phosphatase (ALP), transaminases enzymes- aspartate aminotransferases (AST) and alanine aminotransferases (ALT) and total protein were estimated. EOs were observed showing toxicity (mint LC_{50} = 5.9 µl/ ml and ajwain LC_{50}= 7.02 µl/ ml). Exposure of EOs altered the lifecycle parameters significantly ($p<0.01$). The detoxification enzyme inhibition activities were also significant ($p<0.01$). Thus, it is concluded that these EOs can be recommended as safe and ecofriendly alternatives.

Key words: *Callosobruchus chinensis*, essential oils, *Mentha arvensis*, *Carum capyicum*, lifecycle, acetyl cholinesterase, alkaline phosphatase, transaminases enzymes, inhibition

India is one of the leading producers of food in the world and it produces more than a billion tonnes of agricultural product. 58% of India's population is dependent on agriculture as its primary source of livelihood. In India, advancement of technology has increased the production of grains; however, improper storage has resulted in huge loss and has been reported to be around INR 926 billion loss annually (Singh and Khanna, 2019; Sirohi et al., 2021). Infestation of stored grain by many insects, mite and fungi degrade the quality and quantity of grains (Lal et al., 2017; Jerbi et al., 2021). The total productivity of agricultural crops of India is 3 tonnes/ha; out of which loss due to insect pest is about 26 % (Lal et al., 2017), like the lesser grain borer, *R. dominica*'s larva and adult infests the grains and declines its quality (Jerbi et al., 2021). Rice pest *S. oryzae*, causes qualitative and quantitative loss (Saad et al., 2018). *C. chinensis* a major pest of stored pulses and is reported to cause 32-64% loss under storage condition (Femeena et al., 2018). After discovery of DDT, Insect pests are mainly controlled by synthetic pesticides (Lal et al., 2017; Demeter et al., 2021). WHO has reported that every year two lakhs people die due to pesticide poisoning owing to its carcinogenic and teratogenic properties (Sarwar, 2016). Use of synthetic pesticide is a easy and quick solution for controlling insect pests but pose a potential risk not only to humans but also to the environment as their residues have been reported to be present in soil, air and water (Said and Pashte., 2015; Lal et al., 2017). The repeated uses of synthetic insecticide for decades has disrupted biological control by natural enemies and has led to outbreaks of other insect species and at times have resulted in resistance of pesticides in insect pest (Hill et al., 2017; Hawkins et al., 2019). Hence, there is need for alternative solution which environment friendly does not harm other non-target species. Plants and their derivatives have been proved to be a viable alternative as more than 2000 plant species have been recorded to possess insecticidal properties and possess low health risks (Pavela, 2016; Jerbi et al., 2021). EOs are naturally produced by plants as secondary compounds which are volatile, but as natural products protects the stored grains from pest attack (Omar, 2020). EOs has multiple components mixture and causes toxicity by interfering with various aspects of insect's physiology and biochemistry (Kiran et al., 2017). Present work evaluates the insecticidal potential of the two EOs *M. arvensis* and *C. capyicum* against *C. chinensis* (pulse beetle) adults.

MATERIAL AND METHODS

The adult insects were collected from the infested grains from the granary and were reared on 500 g green gram (variety - Sabarmati PS 16) maintained in laboratory at Department of Zoology, The Maharaja Sayajirao University of Baroda. A culture of *C. chinensis*

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ECOLOGICAL ROLE OF *ONTHOPHAGUS TAURUS* (SCHREBER) IN SOIL NUTRIENT MOBILIZATION

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ABSTRACT

Dung beetles play a major role in the pasture ecosystem. The manure recycling activity of dung beetles is linked to their tunneling behavior. The present study was designed to analyze the tunnel pattern and nutrient mobilization by dung beetles, *Onthophagus taurus* (Schereber, 1759) in different soil types. A simple type of tunnel pattern was observed in all the four types of soil after 30th day of their introduction (10 pairs of male and female) into the experimental setup. However, the maximum number of tunnels was observed in the sandy and sandy clay loam (no. of openings- 15), followed by loamy soil (no. of openings- 13). The physical (texture, water holding capacity, porosity, moisture content) and chemical parameters (pH and nutrients) of all the four types of soils were evaluated. Soil texture analysis revealed the texture to be of sandy (yellow soil), sandy clay loam (red and black soil), and loamy sand (brown soil) types. Water holding capacity and the soil porosity were recorded highest in the sandy soil, whereas moisture content was found maximum in the sandy clay loam. Soil nutrient analysis illustrated a significant increase in the amount of nitrogen (N), phosphorus (P), calcium (Ca), sulfur (S), sodium (Na), potassium (K), organic carbon and organic matter. Thus, the present study confirms that tunneling activity of *O. taurus* enhances the soil nutrients by carrying out dung decomposition.

Key words: Dung beetle, *Onthophagus taurus*, nesting, tunneling, nutrients, soil parameters, texture, water holding capacity, porosity, moisture, nutrients, sandy, clay, loam, red and black soils

Arthropods are one of the most successful and cosmopolitan group of animals on earth. Their ability to adapt to the changing environment makes them the most successful and diverse group of animals (Giribet, 2019). Among the arthropods, class Insecta is the largest group and the order Coleoptera is the leading order of the animal kingdom constituting almost 25% of all the living organisms and it includes around 3,50,000 species worldwide and among these around 15,088 species are present in India. Among 25% of insect species, 40% are beetles (Thakkar, 2016). Scarab beetles commonly known as dung beetles of the family Scarabaeidae have approximately 30,000 species of beetles (Cajaiba et al., 2017). They exhibit a wide range of ecological, morphological as well as behavioral adaptations which makes them universally distributed. Mostly dung beetles prefer to be omnivore, than herbivore dung, and the least preferred is carnivore dung (Frank et al., 2017, unpublished data). Mandibles and maxillae of adult dung beetles have a fine outer edge which helps in modifying and filtering out the content of dung (Shukla et al., 2016). Further, tibia of forelegs have spines and spurs which helps them in digging and forming the tunnel. Tibial spur number varies among the species which helps taxonomist to classify the dung beetles (Linz et al., 2019). In addition, head of the dung beetles has a hard, scoop like structure which helps in rolling the dung balls for their nesting (Ix-Balam et al., 2018). *Onthophagus taurus* (Schreber), as a tunneler makes “multimedia galleries” (tunnels) deep into the soil for laying eggs in the brood balls. These tunnels can be formed by both male and female or only by single parent. Brood balls are placed into the blind end of the tunnel. Single branch of these complex tunnels may contain one or multiple brood balls (Tonelli, 2021). This behavioral aspect enhances their ecological efficiency for dung decomposition, bioturbation, seed dispersal, parasite suppression, fly control and nutrient recycling (Shahabuddin et al., 2017). Further, tunneling activity makes the continuous movement of the soil and thereby increases soil aeration and its water holding capacity (Nichols et al., 2008; Doube, 2018). Dung produced by livestock are source of many greenhouse gases such as nitrous oxide (N_2O), methane (CH_4), and carbon dioxide (CO_2) which is reduced by dung beetles by reducing organic matter from the dung by their relocation into the soil (Piccini et al., 2017).

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Chapter

1

Molecular Characterization of Lepidoptera and Hemiptera Pests of Vadodara

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ABSTRACT

Insect pestswere collected from various agriculture fields belonging to order Lepidoptera, and Hemipters of Vadodara, Gujarat. DNA isolation and PCR amplification of the Cytochrome oxidase I (COI) gene were carried out (Amplicone size 720 bp). A total of 29 species representing 13 families of Hemiptera orders and of 33 species representing 14 families ofLepidoptera were reported and barcodes of 10 species of each order morphologically identified species were successfully obtained. The present study shows the importance of barcode data obtained from COI and 16S rRNA and helped us to identify Hemiptera and Lepidoptera species in a more precise manner. The sequence uploaded on the NCBI can be reliably used further for developing reference libraries for the species identification and also can be used to find the genetic distance among the species by Neighbor-Joining cluster, Maximum Likelihood analysis. Phylogenetic information could be beneficial in predicting the expected status of agricultural pests, and will be helpful in the development of more effective pest management options for regulating pest species.

Keywords: Hemiptera, Lepidoptera, DNA Barcoding, Cytochrome Oxidase.

INTRODUCTION

The roots of insect systematics go back to the sixteenth, seventeenth, and eighteenth centuries. Influential pioneers of entomology were the Italian naturalist Ulisse Aldrovandi (1522–1605), the Dutch doctor, and microscopist Jan Swammerdam (1637–1680), and the German naturalist August Johann Rösel von Rosenhof (1705–1759). Insects are ancient (>450 million years ago) and taxonomically diverse group having a worldwide distribution and a complex evolutionary history (Sahney *et al.*, 2010). Insects are important because of their