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Research Article

Bio-efficacy of plant extracts against a major stored grain insect pest bruchid beetle F. *Callosobruchus maculatus* (Coleoptera: Bruchidae)

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Abstract

A considerable portion of crops is lost due to the use of non-selective crop protection agents. Consequently, there is growing pressure on the agrochemical industry to develop new crop protection solutions that are both environmentally friendly and safe for consumers. The current study investigates the bio-efficacy of plant extracts (*Melia azedarach* L., *Nicotiana rustica* L., *Azadirachta indica* L., *Nicotiana tabacum* L., and *Thuja orientalis* L.,) against *Callosbruchus maculatus*. The extracts of these plants were tested at six different concentrations (5, 10, 15, 20, 25, and 30%) using distilled water as a control. The experiments were replicated four times. Overall mean minimum oviposition (131.75), adult emergence (73.78%), infestations (28.14%) and host seed weight loss (12.54%) were observed with *N. tabacum*. Contact and residual methods were used to evaluate the toxic effects of the plant extract after 24-, 48-, 72- and 96-hours' exposure period. The LC50 and LC90 values were determined by probit analysis. In case of residual toxicity, out of the five plant species extract, *N. tabacum* was the most toxic against *C. maculatus* with LC50 of 0.92% and LC90 of 4.77 % respectively. In contact toxicity *N. tabacum* was found effective against *C. maculatus* with LC50 of 0.14% and LC90 of 1.59% respectively. Alkaloids, saponins, di-terphenes, phyto-sterol, flavonoids and phenols were detected in the aqueous extracts of selected plant species. The current study highlights the efficacy of *N. tabacum, N. rustica* and *A. indica* for the management of *C. maculatus*. Further investigation is therefore necessary to evaluate the potential of these plant species for the production of new bio-pesticides as safer and eco-friendlier alternative to synthetic pesticides.

Keywords: bio-pesticides; entomotoxicity; integrated pest management; phytochemicals; toxicity

Introduction

Due to increase in population of the world, the demand of food is also increased and it is essential to protect stored grains and crops from the attack of insect pests. *Callosobruchus maculatus* Fab. (Coleoptera: Bruchidae) is a multivoltine pest which causes a heavy damages to stored grains and pulses (Bibi, 2021; Khan *et al*., 2015). According to previous research of Agour *et al*. (2022), in 3 to 6 months of storage period. *C. maculatus* alone causes 90% infestations. *C. maculatus* can be managed by using fumigation with phosphine and methyl- bromide and synthetic pyrethriods (Rajendran, 2020), the complications associated with the use

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of synthetic insecticides not only poising of crops, and insect resistances but also it pollutes our environment which warning the researchers to look for new and different strategies for managing the pest infestations.

Due the over use of traditional neurotoxic insecticides which leads to the creation of insecticide-resistant, it is very difficult to control *C. maculatus* population by chemicals. Moreover, the use of synthetic insecticides damage environmental purity, and threaten the safety of food and feed (Santhoshkumar *et al*., 2024). Plant extracts is capable of replacing synthetic insecticides and conventional fumigants which was phased out after its role in ozone depletion was discovered (Stejskal *et al*., 2021). During the extreme assaults, farmers turn to risky approaches like synthetic chemical use in the circumstances when the conventional practices are useless to protect their crops and stored commodities from insect infestations. Poor farmer which has low income mostly used banned and non-appropriated insecticides who obtain them through illegal distribution networks. When the infestations of insect are low plant-based pesticides could be utilized as a best option in this situation. *C. maculatus* infest and attack cow pea both in field and storage so in both situations the managing of the pests should be considered based on the crop economic importance. When feasible, one method for managing bruchids in the latter is to apply chemical insecticides (Kedia *et al*., 2015). Mostly the insecticides are used in fumigant or in liquid form but the nonstop using of pesticides can results in severe issues developing pesticide resistance. Non chemical methods for managing *C. maculatus* populations are interesting because they have no chemical residues on the commodities and have minimal risk of insect developing resistance. Some of the methods involve put the grains or pulses in the sun regularly basis, mixing them with ash or sand or coating cooking oil have been suggested by (Kiran Babu and Rampal, 2024) . Some plants have toxic qualities that may help in pest management (Singh *et al.*, 2023). As a result, investigating an alternative insect pest management techniques that are safer for both people and the environment has become essential (Daraban *et al*., 2023).

Among the postharvest storage pests, *C. maculatus* is a major pest and loss of methyl bromide, along with possible limitations on phostoxin and increasing costs clearly highlights the need of non-chemical treatments on cowpea seeds. Moreover, the plant extracts are important because they play crucial role in pesticides testing, where their efficacy is carefully evaluated. Mostly the plant extracts consist of various mixtures of phytochemicals including aldehyde, alcohol, ester, terpenes, phenols and various other organic compounds (Faria *et al*., 2021). These phytochemicals not only contribute to the characteristic aroma, flavor, and potential biological activities of plant extracts but also play a defense role mechanism against herbivores and pathogens (Gautam *et al*., 2020). These phytochemicals are extracted from various plant parts including flowers, stem, roots, and leaves. Due to their extensive biological activity, plant extracts have been consumed for various purposes which including Toxicity, repellency, cosmetics, natural remedies and aromatherapy (Khan *et al*., 2015) (Cunha *et al.,* 2022). Plant phytochemicals have different aroma which have insecticidal properties and useful against a wide range of insect pests (Manzoor *et al*., 2025)

Synthetic insecticides are mostly used as toxicity and repellent, plant extracts are become promising and best option that could provide effective and environmentally friendly. Also including repellents and toxicity in stored pest management program can help to reduce worries about chemical residues attracting to consumers who prefer pesticide-free goods. Considering the significance of plant-based pesticides as a leading safe alternative to synthetic insecticides for managing stored product pests, recent research focused on assessing the effectiveness of five different plant extracts against *C. maculatus*.

Materials and Methods

Plants collection

We collected the plant species, including leaves of *Nicotiana rustica, Nicotiana tabacum,* seeds of *Azadirachta indica,* and fruits of *Melia azedarach* and *Thuja orientalis* from the Majmaah University campus, Al Majmaah, Saudi Arabia (Latitude: 25.6822, Longitude: 43.8650). The plant species were identified by a botanist from the Department of Biological Sciences at Majmaah University, and the specimens were subsequently deposited in the university's herbarium for further study according to the procedure described previously by Ullah *et al*. (2019).

Preparation of plant species and extraction:

The selected parts of five plant species were air washed with distilled water and then air dried under shade separately sixteen days under normal temperature 27 ± 2 °C and relative humidity of 75 ± 5%. Each plant species was then ground into fine powders with the help of electric grind and sieved using a 300 μm mesh. The plant powders 20 g were separately soaked in 200 ml of distilled water for 24 h. After 24 h we put the samples in a shaker for 50 minutes in to order to complete mixing of powders into the solvent. After shaking each concentration were filtered by using Muslin cloth and Whatman No. 1 papers. We used the aluminium foil to cover the flasks to prevent escape of solvent. The extract filtrates were then concentrated in vacuum using a Heidolph rotary evaporator, and the solvent was recovered. The concentrates were further allowed to dry to remove traces of the solvents and yield dry extracts. At 4 °C the extracts were kept in refrigerator in sample bottles.

Preparation of plant species extract concentrations

Selected five plant species extract concentrated were diluted by adding distilled water a concentrations of 1 gm^{-1}, and this was labelled as stock solution (100%) as described by Gitahi *et al*., (2021) with some modifications. The concentrations were used as, 5, 10, 15, 20, 25, and 30%.

Collection of Bruchid beetle, C. maculatus

Stock culture of *C. maculatus* was introduced by collecting adult beetles from Al Majmaah city, Saudi Arabia. The infested mung bean and cow pea grain cultured in their respective food under ambient temperature of 27 ± 2 °C, relative humidity of 75 ± 5%, and suitable photoperiod (LD 12: 12). The *C. maculatus* was identified according to (Akbar et al 2024). We introduced 300 unsexed adult *C. maculatus* into six three litter glass bottles with 600 g of mung bean seed. The test beetle to oviposit for seven days and then removed the adult beetles and third generation of *C. maculatus* (usually 0 to 1 day old) of same size were used for subsequent experiment. We further maintained beetle stock culture in two-liter capacity glass bottle containing mung bean seeds. The beetles were successively reared by replacing the consumed and infested mung bean seed with fresh, uninfected and clean seed container which coved with muslin cloth to allow aerations and prevent the escape of test beetle. We used the rubber band to cover muslin cloth. *C. maculatus* breeding and the experiment was done at ambient temperature (Hajam *et al.,* 2022).

Phytochemical screening

The extracts of chosen plants were separated for the presence of various bio-active compounds such as alkaloids, phenols, phyto-sterol, terpenes and flavonoids. The detection of these compounds was carried out using standard tests as reported in literature.

<u>Maceration</u>

For maceration (for fluid extract), coarsely powdered plant was kept in a container containing extracting solvent (methanol, ethyl acetate, hexane etc.) and agitated for a defined period (Chetri *et al*., 2021).

Detection of alkaloids, phenols, phyto-sterol, terpenes and flavonoids in plant extract

This study employed a series of chemical tests to detect specific phytochemical constituents in plant extracts. Alkaloids were confirmed by the formation of dark reddish precipitates upon treatment with iodine and potassium iodide in dilute hydrochloric acid (Khanal, 2021).

Phenolic compounds were identified through the appearance of a bluish-black color after 3-4 drops of ferric chloride solution were added to plant aqueous extracts (Khanal, 2021). Similarly, the detection of phyto-sterols was achieved by the formation of a golden yellow color when extracts of the selected plants were cured with chloroform and filtered. Few drops of concentrated sulphuric acid were added into the filtered extract, and allowed undisturbed for few minutes (Abdullahi, 2018). Diterpenes were confirmed by the emergence of an emerald green color upon treatment with 3-4 drops of copper acetate solution were added into the aqueous extracts of plant species (Jou Farooqnal *et a*l., 2019). Lastly, flavonoids were detected by the bright yellow color formed with 2-3 drops of lead acetate, solutions to the plant aqueous extracts. A bright yellow color appeared which indicate the presence of flavonoids in the sample. The color vanished when few drops of t when dilute acid was added (Semwal *et al*., 2021).

Biological parameter bioassay

Oviposition

We took twenty grams (20 g) of clean mung bean seed into seven plastic jar (5 cm diameter) followed by (Singh and Swami, 2023) with little modification . Thereafter, 5, 10, 15, 20, 25, and 30% of each plant species extract was mixed with the mung bean seeds in six plastic bottles while the seventh one (control) was not treated with plant extracts. Mung bean and plant species extracts were thoroughly mixed together with a glass rod for complete mixing. Each treatment was air-dried for 2 h. Copulating 20 pairs of 24 h starved newly emerged adult *C. maculatus* were introduced into the treated and untreated bottles. There were four replications for each treatment and control and laid in a completely randomized design (CRD). The experiment was kept in the laboratory till after the death of the insects (seven days). Total number of eggs laid were counter after seven days.

$$\text{Oviposition} = \frac{\text{Total no.eggs}}{\text{female}} \qquad \text{Equation (1)}$$

Percent adult emergence:

We kept the experiment for 30 days for *C. maculatus* adults. The number of adults that emerged from each bottle was counted and recorded. The following equation was used to calculate the % adult emergence (Akbar *et al*., 2024).

$$\text{Adult emerged(\%)} = \frac{\text{Number of adult emerged}}{\text{Total number of eggs laid}} \times 100 \qquad \text{Equation (2)}$$

Percent infestation

Mung bean seed with a hole were measured as damaged or infested seeds according to (Akbar *et al*., 2024). The percentage of seed damage was calculated by using the following formula:

$$\text{Infestation (\%)} = \frac{\text{Total no.of damaged grains}}{\text{Total number of grains}} \times 100 \qquad \text{Equation (3)}$$

Percent seed weight loss

Percent seed weight loss of mung bean was calculated by obtaining initial weight and final weight after experiment. The following formula was used for % seed weight loss proposed by Sharma and Thakur (2014).

$$\text{Seed weight loss(\%)} = \frac{\text{Initial grains weight} - \text{Final grains weight}}{\text{Initial grains weigh}} \times 100 \qquad \text{Equation (4)}$$

Residual toxicity

The residual toxicity was conducted according to previous report by (Ahmady *et al*., 2024). In a typical experiment, 20 g of sterilized mung bean seeds were sprayed over plant crude extracts of five plant species extract at six different concentrations (0.50, 1.00, 1.50, 2.00, 2.50 and 3.00%). Then the seeds were air dried for 20 minutes. Treated and untreated seeds were then put on Petri plates made of plastic having a surface area of 63 cm^2. We released 10 pairs of same age and size in each treatment. *C. maculatus* mortality was assessed after 24, 48, 72 and 96 hours of exposure. The experiment was replicated four times. Dead beetles were removed from petri plates and counted on a daily basis. The following formula were employed to calculate the corrected mortality. LC50 and LC90 values were found after correcting the mortality by Abbott's equation (Abbott, 1925).

$$\text{Mortality (\%)} \frac{\text{number of dead insects}}{\text{number of insects introduced}} \times 100 \qquad \text{Equation (5)}$$

The corrections for natural mortality were done by following Abbott's formula:

$$\text{Corrected mortality (\%)} = \frac{\%\text{Mo} - \%\ \text{Mc}}{100 - \%\ \text{Mc}} \times 100 \qquad \text{Equation (6)}$$

Where:
%Mc=percentage mortality in control
%Mo= Percentage observed mortality

Topical toxicity

The insecticidal activity of six different concentrations (5,10,15,20,25 and 30%) of five plant species and control distilled water against *C. maculatus* were assessed by direct toxicity bioassay (Akbar *et al.,* 2022). All the experiment was conducted in petri plates having a surface area of 63 cm^2. I ml of each plant species extract were sprayed on the dorsal surface of adults of *C. maculatus* as fine mist by using a hand atomizer. Each concentration was replicated four times in completely randomized design. After 24-, 48-, 72- and 96-hours' exposure period insect mortality were observed After observation all the dead beetles were removed from the petri plates. LC50 and LC90 values were found after correcting the mortality by Abbott's equation as discussed in residual activity.

Repellence

A choice bioassay system was used to evaluate repellence of the plant species extracts. In the repellence bioassay six different concentrations viz. 5, 10, 15, 20, 25, and 30% of aqueous plant extract were used. For a control, we used distilled water. We sliced the filter paper into two equal parts (7 cm diameter) and half portion of the filter paper were treated with plant species extracts using a micro pipette and the remaining half portion was treated with distilled water. For 30 minutes we allowed the treated filter to dry till complete evaporation of solvent and then we put in the petri plates with the help of glue at the bottom of petri plates. The filter paper which was pasted length and edge wise at the bottom of petri plates. The we release 10 pairs adults of usually of same age and size having 10 male and 10 female beetles at the middle of arena and then we covered the Petri plates with muslin cloth for aeration. After 1, 6, 12, 24-, 48-, 72- and 96-hours' exposure period we observed and counted the beetle residing on the treated and untreated side of the treated petri plates. We using the following formula as adopted by (Alharbi, & Alanazi, 2024) for percent repellence.

$$\text{Repellency (\%)} = \frac{\text{Tc}-\text{Tt}}{\text{Tc}} \times 100 \qquad \text{Equation (7)}$$

Tc= No. of insects counted in control

Tt= No. of insects counted in treated

The botanicals were then categorized into different classes according to (McDonald *et al*., 1970).

Statistical analysis

Shapiro–Wilk test (Wilk and Shapiro, 1965) test was used to assessed the normality of the samples. Given that all replicates followed a normal distribution, mean differences were examined through a one-way analysis of variance, with a 95% confidence interval. Subsequently, a Tukey HSD test was employed for post hoc analysis, utilizing STATISTIX 8.1 (Tukey and John, 1949). and (SPSS) version 20 software was used to conduct all the above statistical analysis. LC50 (Concentration for 50% adult mortality) and LC90 (Concentration for 90 percent adult mortality) of *C. maculatus* were determined by Log-Probit model (Finney *et al*., 1947).

Results

Phytochemical screening

Various phytochemicals, such as alkaloids, saponins, di-terpenes, phyto-sterols, flavonoids, and phenols, were identified in both *N. tabacum* and *N. rustica*. In contrast, *A. indica* contained only a limited number of these compounds. Notably, phyto-sterols were found in the highest concentration among the detected phytochemicals. Saponins were exclusively present in the aqueous extracts of *N. rustica* and *N. tabacum* (Table 1).

Effect of plant crude extracts treated with mung bean seeds on C. maculatus oviposition, % infestations, % adult emergence, and %host seed weight loss

Oviposition of *C. maculatus* when treated with plant crude extracts of five botanicals is shown in Figure 1(a). No. of eggs varied significantly among the treatments. *C. maculatus* oviposition significantly decreases with increase in plant concentration. *C. maculatus* oviposition were significantly higher (246.00 eggs per female) with *T. orientalis* and significantly lower (170.00 eggs/female were recorded with *A. indica* at 5% concentration. Over all oviposition of C. maculatus were significantly higher of 206.96 with *T. orientalis* and lower 131.75 with *A. indica* as shown in Table 3. With the increase in plant species concentrations *C. maculatus* % adult emergence were significantly decreased. *C. maculatus*. At 30% concentrations adult emergence were significantly higher 82.04% with *T. orientalis* and significantly lower 58.82% were recorded with *A. indica* (Figure 1(b)). From Table 3 it was cleared that mean % adult emergence was significantly maximum 82.26% with *T. orientalis* and lower of 73.78% with *A. indica*. As seen from (Figure 1(c) at 3.00% concentrations percent infestations of *C. maculatus* were significantly higher 35.74% with *T. orientalis* concentrations and lower 17.59% infestation were recorded with *A. indica*. In Table 3 over all % infestation of *C. maculatus* were significantly higher of 45.82% with *T. orientalis* and lower of 28.14% with A. indica. Mean % seed weight loss of mung bean due to infestation of *C. maculatus* significantly decreases with increase in plant concentration. % seed weight loss was significantly higher 29.00% with *T. orientalis* concentrations and significantly lower 3.70% with A. indica Figure 1(d). Over all % seed weight loss of *C. maculatus* were significantly higher of 39.32% with *T. orientalis* and lower of 12.54% with *A. indica* (Table 2).

Table 1. Phytochemical composition in aqueous extracts of various plant species

Plant species	Alkaloids	Flavonoids	Saponins	Di-terpenes	Phyto-sterol	Phenols
T. orientalis	+	+	+	+	+	+
M. azedarach	+	+	+	+	++	++
N. rustica	+	+	_	+	+	+
A. indica	+++	+++	++	++	+++	+++
N. tabacum	++	++	++	+++	++	+++

Legend: + = Low presence; ++ = Moderate presence; +++ = High presence.

Table 2. Over all mean biological parameter of *C. maculatus* when mung bean treated with five plant species extract at six concentrations

Plants species	No. of eggs/ female	% adult emergence	% infestation	% host seed weight loss
N. tabacum	131.75 e	73.78 d	28.14 e	12.54 e
N. rustica	187.96 b	78.20 c	40.44 b	30.21 b
A. indica	168.08 c	76.14 b	34.59 d	26.71 d
M. azedarach	146.75 d	77.92 b	31.03 c	18.80 c
T. orientalis	206.96 a	82.26 a	45.82 a	39.32 a
LSD	2.4158	1.4418	0.5645	1.1954
F	1330.64	38.52	1276.93	630.89
P	P < 0.05	P < 0.05	P < 0.05	P < 0.05

Means with different lowercase letters indicate that the means are significantly different from each other at p = 0.05

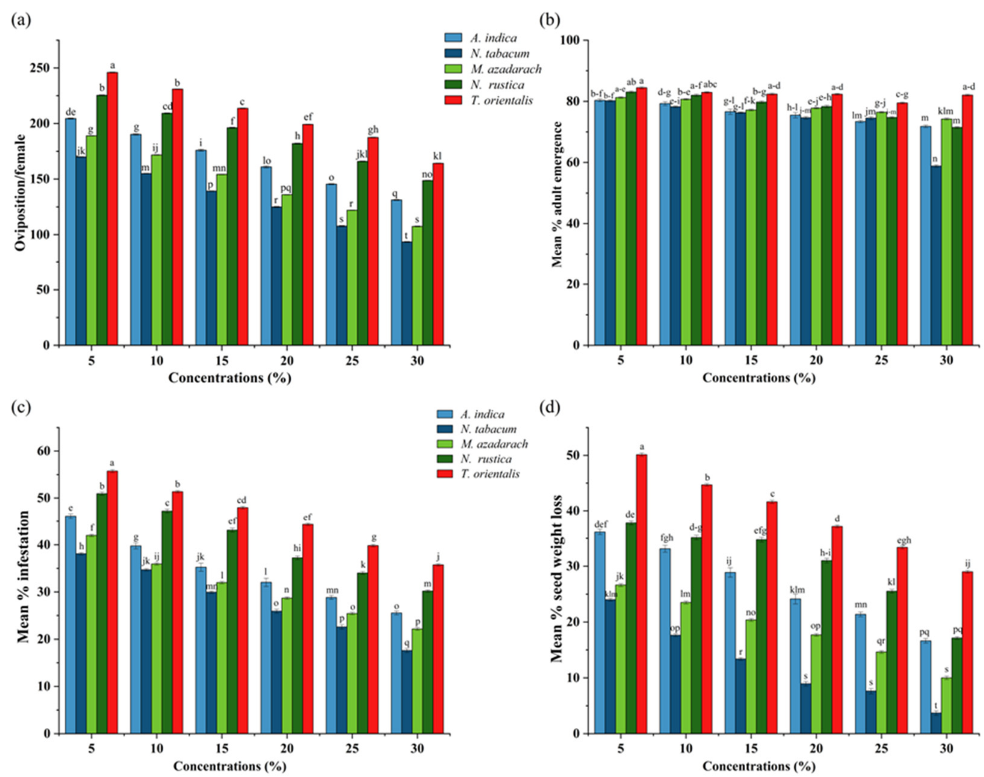

Figure 1. Biology of *C. maculatus* no. of oviposition (a), mean % adult emergence (b), mean % infestation (c), mean % host seed weight loss (d)

The bars with different lowercase letters indicate that the means are significantly different from each other at p = 0.05.

Residual toxicity of plant crude extracts against C. maculatus

The efficiency of the plant extracts on mortality of *C. maculatus* is shown in Table 3 after 24-hour exposure period. The value of maximum mortality differs from plant to plant. As observed in this study. Among the plant species extract *A. indica* LC50 of 30.94% and LC90 of 526.40% gave highest toxicity to *C. maculatus* and lowest toxicity was recorded with *T. orientalis* LC50 of 29.01 % and LC90 of 390.19 %. It was seen that mean % mortality of *C. maculatus* was higher 20.00 with both *N. rustica* and *N. tabacum* and significantly lower 8.00% mortality with *T. orientalis* to at 30% concentrations (Figure 2a) (df=4, F=13.10, P < 0.05=0.000).

From Table 3 it was observed that all the plant extracts caused maximum mortalities to *C. maculatus* after 48 hours' exposure period. Among the plant extract *N. tabacum* exhibited highest toxicity having LC50 0f 17.53% and LC90 of 241.89% and lowest was recorded with *T. orientalis* having LC50 of 29.01% and LC90 232.26% against *C. maculatus*. At 30% concentration mean percent mortality was maximum 33.00 % with *N. tabacum* and minimum 22.00% with *T. orientalis* against *C. maculatus* (Figure 2b) (df=4, F=10.29, P < 0.05)

After 72 hours' exposure period, the highest toxicity was recorded for extracts from *N. tabacum*, with LC50 of 4.75% and LC90 of 80.07% while *T. orientalis* were least toxic with LC50 of 8.38 % and LC90 of 91.19 % against *C. maculatus* Table 3. From Figure 2c it was cleared that at 3.00% concentration significantly mean percent highest 58.00% mortality was recorded with *N. tabacum* and lowest 42.00% with *T. orientalis* against *C. maculatus* (df=4, F=18.18, P < 0.05).

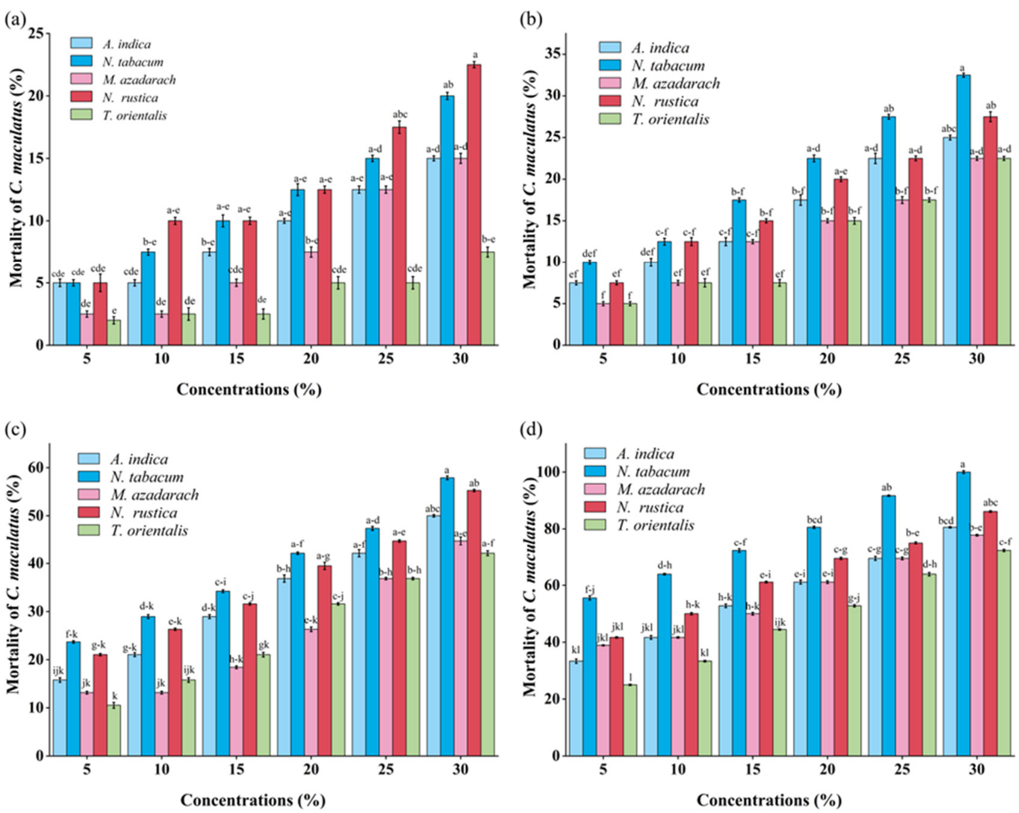

Figure 2. Mean percentage mortality of *C. maculatus* on 5 plant extracts after (a) 24 h (b) 48 h (c) 72 h and (d) 96 h in residual toxicity test

Statistically significant difference among the various treatments is indicated by the error bars at 0.5% Significance level

Toxicity of five different plant extracts increased with increasing in exposure time as shown in Table 3. After 96 hour's exposure period plant extract exhibit highest toxicity to *C. maculatus*. Among the plant extracts, the highest toxicity was recorded for *N. tabacum* with LC50 of 0.92% and LC90 of 4.77%. on the other hand, *T. orientalis* were less toxic with LC50 of 2.55% and LC90 of 20.49% against *C. maculatus*. From Figure 2d at 3.00% concentration 100.00% mortality to *C. maculatus* was observed with *N. tabacum* and minimum 72.00% with *T. orientalis* (df=4, F=62.97, P < 0.05)

Table 3. Residual toxicity of plant extracts against *C. maculatus*

Time	Plant species	LC50 (95% CLs)	LC90 (95% CLs)	χ^2	P	Slope ±SE
24 h	*A. indica*	30.94 (14.26-308.28)	526.40 (95.25- 950.38)	0.54	0.96	1.08±0.26
	N. rustica	37.19 (16.03-495.66)	582.35 (99.72-148.97)	1.59	0.80	1.07±0.28
	M. azedarach	47.63 (18.12-130.62)	843.89 (116.75-838.64)	0.69	0.95	1.02±0.29
	N. tabacum	34.73 (16.01-359.42)	269.36(62.91-234.52)	2.28	0.68	1.44±0.37
	T. orientalis	54.85 (18.42-189.93)	390.19 (59.42-105.58)	1.37	0.84	1.05±0.53
48 h	*A. indica*	28.36 (13.71-217.70)	453.05 (90.17-470.43)	1.17	0.88	1.06±0.26
	N. rustica	24.39 (12.49-148.61)	388.83 (83.53-278.02)	0.40	0.98	1.06±0.25
	M. azedarach	29.57 (14.34-220.22)	370.16 (80.32-282.43)	1.10	0.89	1.22±0.28
	N. tabacum	17.53 (10.27-62.95)	241.89 (66.02-608.88)	1.39	0.84	1.12±0.24
	T. orientalis	29.01 (14.35-194.43)	232.26 (75.12-182.97)	2.37	0.66	1.18±0.29
72 h	*A. indica*	6.39 (4.93-10.16)	80.07 (33.60-92.03)	1.61	0.80	1.16±0.21
	N. rustica	5.44 (4.23-8.48)	87.86 (34.25-704.26)	3.06	0.54	1.06±0.20
	M. azedarach	9.15 (5.40-101.82)	109.95 (25.29-289.35)	7.04	0.13	1.03±0.22
	N. tabacum	4.75 (3.74-7.01)	80.51 (32.12-648.77)	3.16	0.53	1.18±0.20
	T. orientalis	8.39 (6.20-14.91)	91.19 (37.50-577.97)	1.76	0.77	1.03±0.22
96 h	*A. indica*	1.86 (1.43-2.24)	15.17 (10.20-30.14)	4.94	0.29	1.40±0.20
	N. rustica	1.34 (0.93-1.69)	11.27 (7.94-20.68)	4.83	0.30	1.39±0.20
	M. azedarach	1.72 (1.21-2.15)	20.33 (12.16-54.71)	6.58	0.16	1.19±0.20
	N. tabacum	0.92 (0.04-1.61)	4.77 (2.97-36.93)	17.36	0.00	1.79±0.23
	T. orientalis	2.55 (2.10-3.02)	20.49 (13.08-44.75)	3.49	0.41	1.41±0.20

Lethal concentrations (LCs), along with their 95% confidence limits (CLs), are reported for plant extracts, including LC50 and LC90 values.

Toxicity (topical) of plant crude extracts against C. maculatus

Toxicity varied with the plant extract after 24 hours' exposure period, the highest toxicity was recorded for extracts from *N. tabacum* with LC50 of 26.98% and LC90 of 1034.44 %. *T. orientalis* exhibited least toxicity having LC50 of 78.99 % and LC90 of 3405.59 % against *C. maculatus*. From Figure 3a it was cleared that mean percent mortality was significantly higher 25.00% with *N. tabacum* and minimum of 13.00% with *T. orientalis* against *C. maculatus* at 3.00% higher concentrations (df=4, F=6.46, P < 0.05). The response of *C. maculatus* when exposed to plant extract for 48 hr. Table 4 indicated that among the plant species extracts *N. tabacum* having LC50 of 7.73 % and LC90 of 125.37 % were the most effective plant extract, while *T. orientalis* showed lower toxicity effect having LC50 of 78.99 % and LC90 of 3405.59 %. In case of mean percent mortality at 30% higher concentrations *N. tabacum* exhibited significantly highest 47.00 % and lowest 26.00 % was seen with *T. orientalis* against *C. maculatus* as shown in Figure 3 b (df=4, F=7.11, P < 0.05). After 72 hours' exposure period *N. tabacum* showed highest toxicity with LC50 of 1.66% and LC90 10.08 % and *T. orientalis* showed lowest toxicity having LC50 of 4.25 % and LC90 of 80.78 % against *C. maculatus* as shown in Table 4. From Figure 2c it was clearly observed that mean percent mortality to *C. maculatus* was significantly higher 89.00% with *N. tabacum* and lower 57.00 % with *T. orientalis* (df=4, F=20.30, $P < 0.05$). Toxicity of five different plant extracts increased with increasing in exposure time as shown in Table 4. After 96 hour's exposure period plant extract exhibit highest toxicity to *C. maculatus*. The highest toxicity was recorded for extracts from *N. tabacum* with LC50 of 0.14% and LC90 of 1.59% and *T. orientalis* were less toxic with LC50 of 0.41 % and LC90 19.27 % against *C. maculatus*. Mean percent mortality to *C. maculatus* at maximum 30% concentration was significantly higher 91.67% for *N. tabacum* and 63.10 % with *T. orientalis* as shown in Figure 3d (df=4, F=33.80, $P < 0.05$) as shown in Figure 3.

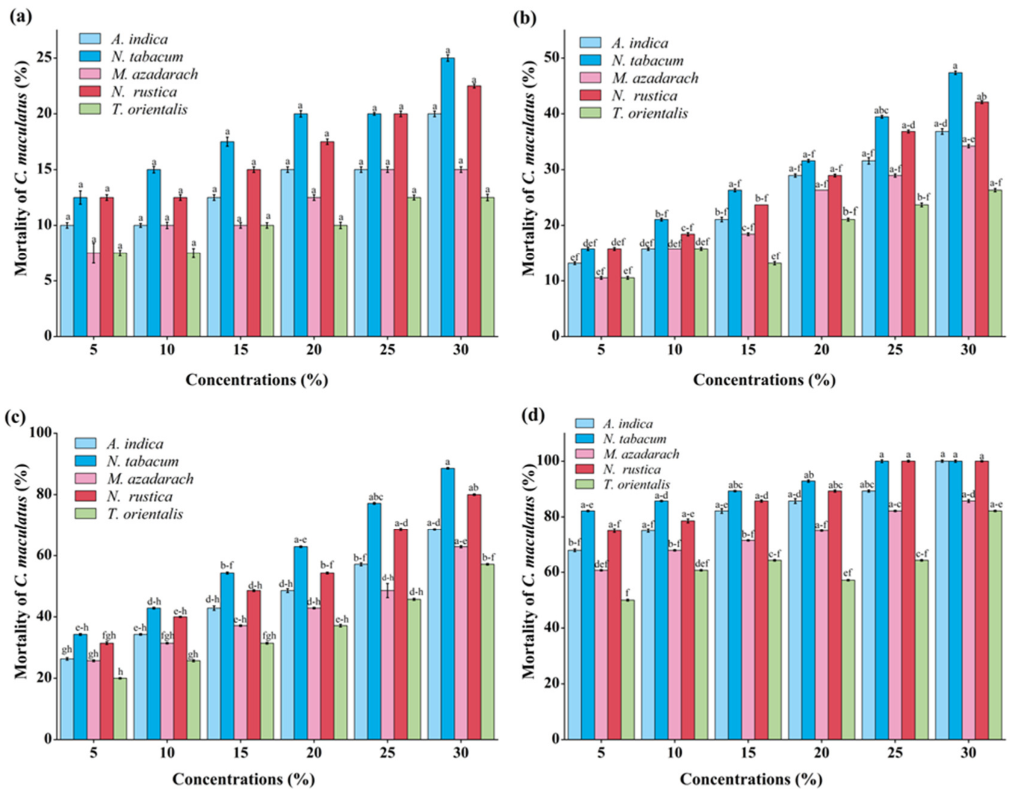

Figure 3. Mean percentage mortality of *C. maculatus* on 5 plant extracts after (a) 24 h (b) 48 h (c) 72 h and (d) 96 h in topical(direct) toxicity test

Statistically significant difference among the various treatments is indicated by the error bars at 0.5% significance level

Table 4. Toxicological direct effect of plant extracts against *C. maculatus*

Time interval	Plant Species	LC50 (95% CLs)	LC90 (95% CLs)	χ^2	P	Slope ±SE
24 h	*A. indica*	47.35 (17.17-240.61)	1531.88 (154.06-116.55)	0.75	0.78	0.43±0.36
	N. rustica	47.30 (16.01-546.51)	2729.31 (195.15-380.14)	0.84	0.93	0.72±0.23
	M. azedarach	40.86 (16.18-961.56)	997.60 (128.62-125.91)	0.25	0.99	0.92±0.26
	N. tabacum	26.98 (12.07-419.44)	1034.44(128.39-162.64)	0.03	1.00	0.80±0.23
	T. orientalis	78.99 (21.62-415.54)	3405.59 (211.58-289.28)	0.23	0.99	0.78±0.26
48 h	*A. indica*	13.08 (7.92-47.22)	321.4 (72.97-183.51)	1.43	0.83	0.92±0.21
	N. rustica	10.21 (6.70-27.58)	230.37 (60.64-717.18)	2.43	0.65	0.94±0.21
	M. azedarach	15.13 (8.78-63.23)	360.17 (78.15-247.79)	0.91	0.92	0.93±0.22
	N. tabacum	7.73 (5.61-14.80)	125.37 (43.51-140.28)	2.37	0.66	1.05±0.21
	T. orientalis	23.97 (11.25-287.51)	921.13 (122.20-901.35)	2.96	0.56	0.80±0.22

72 h	*A. indica*	2.73 (2.24-3.27)	15.39(15.13-65.23)	2.12	0.71	1.32±0.20
	N. rustica	1.94 (0.89-2.76)	17.08 (8.39-217.30)	7.90	0.09	1.35±0.20
	M. azedarach	3.24 (2.50-4.32)	73.13 (28.15-705.65)	4.33	0.36	0.94±0.20
	N. tabacum	1.66 (0.66-2.40)	10.08 (5.80-66.94)	11.09	0.02	1.64±0.20
	T. orientalis	4.25 (3.35-6.09)	80.78 (31.28-703.12)	3.84	0.42	1.00±0.20
96 h	*A. indica*	0.31 (0.08-0.57)	3.07 (2.41-4.23)	4.37	0.35	1.29±0.25
	N. rustica	0.32 (0.00-0.89)	2.15 (0.38-7.65)	12.85	0.01	1.55±0.28
	M. azedarach	0.20 (0.00-0.54)	8.45 (5.19-36.20)	1.46	0.83	0.79±0.22
	N. tabacum	0.14 (0.01-0.35)	1.59 (0.93-2.17)	4.87	0.30	1.23±0.30
	T. orientalis	0.41 (0.04-0.84)	19.27 (9.38- 183.44)	4.17	0.38	0.76±0.20

Lethal concentrations (LCs), along with their 95% confidence limits (CLs), are reported for plant extracts, including LC50 and LC90 values

Repellence

The settling response of *C. maculatus* was significantly (P<0.05) affected by concentration. As compared to treated arena adults of *C. maculatus* preferred the untreated arena (control). As the concentrations of plant species extracts increased, the preference response of tested insects significantly declined. The results revealed that after one hour of exposure period at 30% concentration, the highest repellence of *C. maculatus* was observed with *A. indica (*57.50%) while significantly lowest with *T. orientalis* (41.25%) (Figure 4a). According to Table 5, the overall mean % repellence against *C. maculatus* was significantly higher with *A. indica* 43.87% (Class III repellency) compared to *T. orientalis*, which exhibited a lower repellence of 34.12% (Class II repellence) (df=4, F=9.25, P < 0.05). From Figure 4b, after 6 hours' exposure period, the results revealed that the highest repellence of *C. maculatus* was seen with *A. indica* (60.00%) and significantly lower (51.50%) with *T. orientalis* at 30% concentrations. Among the plant species, the overall mean repellence against *C. maculatus* was significantly higher with *A. indica* (48.25%, class III repellence) and lower with *T. orientalis* (39.95%, class II repellency). (df=4, F=10.96, P < 0.05) as shown in Table 6. After 12 h exposure period, significantly higher (66.00%) repellence with 30% concentrations of *A. indica* and significantly lower (51.25%) with *T. orientalis* (Figure 4c) against *C. maculatus*. Significantly higher mean % repellence of *C. maculatus* was higher 54.16% with A. indica (Class III repellency), while it was lower 42.16% with *T. orientalis* (also Class III repellency) (df=4, F=31.08, P < 0.05). The results showed that after 24 hours' exposure period (Figure 4d), the highest repellency was noted for *A. indica* (72.51%) and significantly lower (60.25%) for *T. orientalis* at 30% concentrations. The overall mean repellence of *C. maculatus* was significantly higher with *A. indica*, reaching 61.14% (Class IV repellence), while it was lower with *T. orientalis* at 49.42% (Class III repellency) (df=4, F=21.55, P < 0.05) as shown in Table 5. From Figure 4e it was cleared that C. maculatus repellency was significantly higher (85.00%) with A. indica concentrations and significantly lower (70.00%) with *T. orientalis* at 30% concentrations after 48 hours' exposure period. The overall mean repellence of *C. maculatus* was significantly higher with *A. indica* (68.29%, Class IV) compared to *T. orientalis* (57.46%, Class III) (df=4, F=21.29, P < 0.05) as shown in Table 5. At a 30% concentration, Figure 4f demonstrated that 72 hours' post-treatment, A. indica showed significantly higher repellence against *C. maculatus* (91.25%) compared to other treatments, while *T. orientalis* exhibited significantly lower repellence (75.00%). According to Table 6, the overall mean repellence (%) of *C. maculatus* was significantly higher with A. indica (71.92%, class-IV repellence) and lower with *T. orientalis* (59.042%, class-III repellence) (df=4, F=25.49, P < 0.05). The results depicted in Figure 4g demonstrates that after 96 hours of exposure, *C. maculatus* repellence (%) was significantly higher at 86.25% with A. indica and notably lower at 57.50% with *T. orientalis*, both at 30% concentrations. Repellence increased proportionally with the concentration of all five plant species tested. Significantly an average *C. maculatus* % repellence was maximum higher with *A. indica* (67.54%, class IV repellency) and lowest with *T. orientalis* (45.87%, class III repellence) (df=4, F=116.15, P< 0.05) as presented in Table 5.

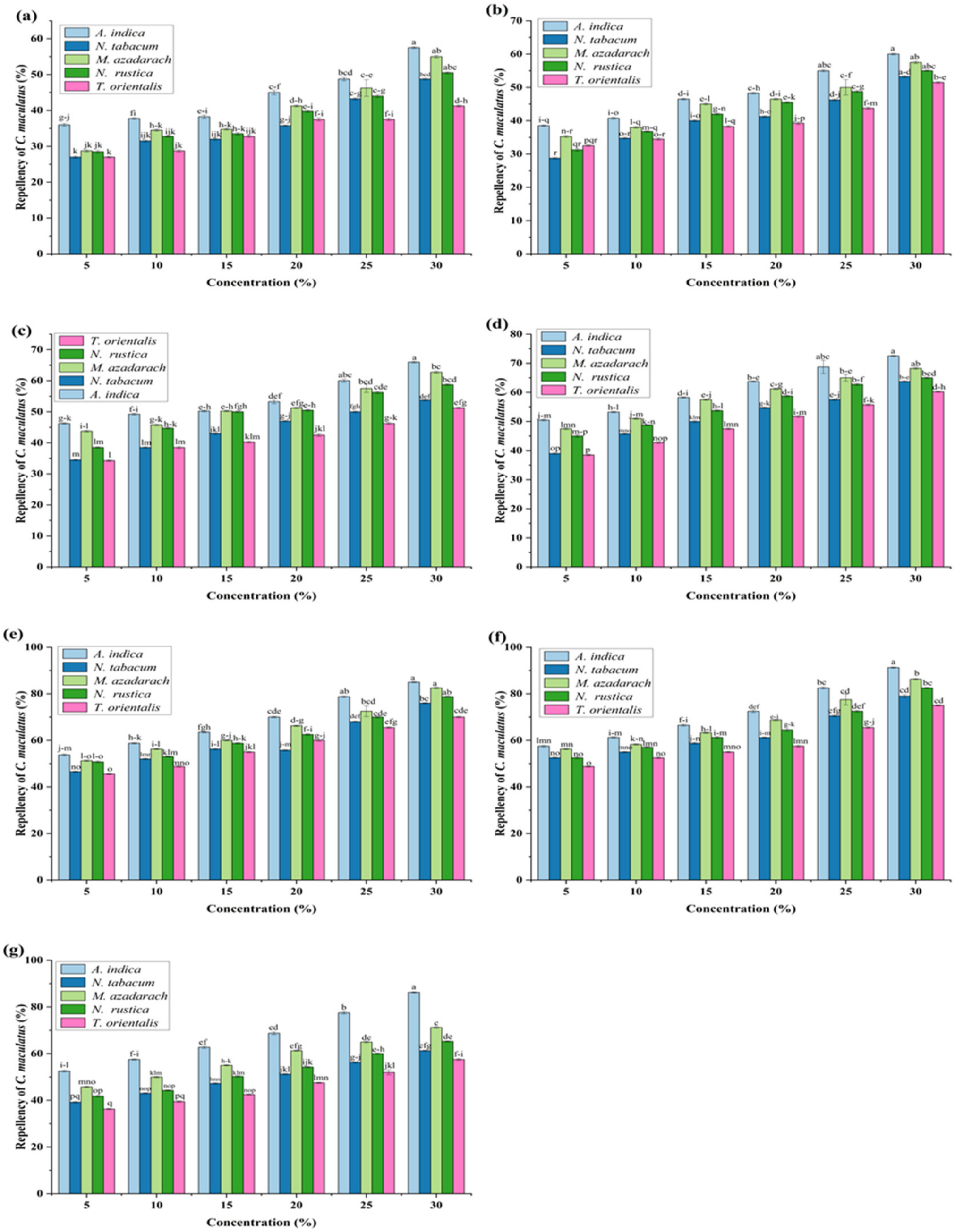

Figure 4. Mean percentage repellence of *C. maculatus* (a) 1 h, (b) 6 h, (c) 12 h, (d) 24 h, (e) 48 h, (f) 72 h exposure (g) 96 h to aqueous extracts of five plant species at six different concentrations

The bars with different lowercase letters indicate that the means are significantly different from each other at p = 0.05

Table 5. Over all mean % repellence of five plant species aqueous e against *C. maculatus*

Plant species	1h	6 h	12 h	24 h	48 h	72 h	96 h	Repellence class
A. indica	43.87 a	48.25 a	54.16 a	61.14 a	68.29 a	71.92 a	67.54 a	Class-III-IV
N. rustica	36.37 cd	40.70 cd	51.87 ab	51.79 c	59.08 c	62.83 c	58.04 b	Class-II-IV
M. azedarach	40.08 b	45.37 ab	49.79 b	58.42 ab	64.79 b	68.37 b	52.62 c	Class-III-IV
N. tabacum	38.16 bc	43.20 bc	44.45 c	55.67 b	62.29 b	65.04 c	49.70 d	Class-III-IV
T. orientalis	34.12 d	39.95 d	42.16 c	49.42 c	57.46 c	59.04 d	45.87 e	Class-II-III
LSD	3.43	2.89	2.54	2.89	2.65	2.76	2.194	

Means with different lowercase letters indicate that the means are significantly different from each other at p = 0.05

Discussion

This study aimed to evaluate the biological parameters, repellence, and toxic effects of plant species extracts on *C. maculatus*. Phytochemical analysis of N. tabacum revealed a high concentration of alkaloids, along with the presence of phenolic compounds, flavonoids, tannins, saponins, terpenoids, proteins, and carbohydrates. The primary active compound present in *N. tabacum* nicotine is known to cause stomach, contact, and respiratory poisoning, as previously documented by (Alamgir, 2017). The findings of this study are consistent with earlier research by pests, who reported that (Farhan *et al*., 2024), saponins, alkaloids, flavonoids, tannins, and cyanogenic glucosides are found in the crude extracts of A. indica. The natural phytochemicals present in plants offer an eco-friendly alternative to synthetic pesticides for managing insect pests (Farhan *et al*., 2024). The current experiments demonstrated a significant reduction in *C. maculatus* oviposition across all treatments, with oviposition decreasing as extract concentrations increased. Similar findings were reported by Idoko and Adesina (2012), where higher concentrations of *Piper guineense* extract significantly reduced *C. maculatus* oviposition. The highest concentration of crude plant extracts (3.00%) was the most effective in reducing the egg-laying capacity of the beetles compared to the lowest concentration (0.5%). These extracts displayed oviposition deterrent activity at the 3% concentration, possibly due to their repellent properties. The deterrent effect on oviposition may result from physiological and behavioral changes in adult C. maculatus, as evidenced by their reduced egg-laying capacity (Chauhan and Jindal, 2016). Several plants have demonstrated effectiveness against stored product pests, including *Acorus calamus*, *N. tabacum, Allium sativum, A. indica*, red seaweeds, and *Cascabela thevetia* (Akbar *et al*., 2024; Tesfaye *et al*., 2021). Among these, *Azadirachta indica* Juss. is particularly notable for its high efficacy against insects (Lin *et al*., 2021). This plant affects the biology of the rice weevil, reducing its damage potential. Azadirachtin, the active compound in Neem, adversely impacts female fecundity by decreasing the number of eggs laid (Ferdenache *et al*., 2019). Additionally, in females, azadirachtin inhibits oocyte growth and ovarian ecdysteroid synthesis, leading to the destruction of follicular cells and mitochondrial disruption in the ovaries (Ghazawi *et al*., 2007). It may also impair yolk protein synthesis and/or its incorporation into oocytes (Boulahbel *et al*., 2015). In males, azadirachtin significantly reduces the number of cysts and disrupts the meiotic process responsible for sperm production (Oulhaci et al., 2018). Furthermore, adult emergence of *C. maculatus* was significantly reduced by the concentrations of all the tested plant species. These findings are consistent with earlier research (Vasudev and Sohal, 2016) which reported that the aqueous extract of tobacco significantly suppressed adult emergence of *C. maculatus* in cowpea.

Akbar *et al*. (2024) observed that elevated concentrations of *A. indica* led to a significant reduction in the emergence of adult *C. maculatus*. Other studies have similarly noted that botanical treatments can inhibit adult emergence in *C. maculatus* (Manju *et al*., 2019). Akbar *et al*. (2024) also demonstrated that plant extracts notably impact the postembryonic survival of insects, resulting in reduced adult emergence across various plant concentrations. Our findings revealed that seeds treated with *T. orientalis* experienced the highest infestation by *C. maculatus*, while the lowest infestation was recorded in seeds treated with A. indica. Our findings align with those of (Mounika *et al*., 2021) who reported that *M. azedarach* significantly reduced *C. chinensis* infestation in chickpea. The observed weight loss in mung beans was attributed to larval feeding by *C. maculatus*, with a direct correlation between the number of emerged beetles and the extent of seed weight loss (Saleem *et al*., 2020). Seeds treated with *T. orientalis* exhibited the highest percentage of seed weight loss, while those treated with *N. tabacum*, *A. indica*, and M. azedarach showed lower weight losses. These findings are supported by (Adesina and Mobolade-Adesina, 2020) who found that neem seed extract significantly inhibited the emergence of F1 adults of *C. maculatus* and minimized weight loss caused by the pest. Additionally, *N. tabacum* was reported to suppress adult emergence, leading to reduced seed weight loss (Vasudev and Sohal, 2016).

At all tested concentrations, *A. indica, M. azedarach, N. tabacum* and *N. rustica* exhibited significant residual toxicity against tested beetle. This observation aligns with the findings of Tavares *et al.* (2021) who reported that *N. tabacum* crude extracts demonstrated the highest residual toxicity, achieving 89.00% against *Tribolium castaneum* at 3% concentration. Botanical insecticides are known to contain diverse toxic compounds that interfere with insect physiology and behavior, influencing processes such as feeding, mating, mortality, and oviposition (Jumbo *et al*., 2018). Secondary metabolites in *N. tabacum* and *N. rustica*, such as alkaloids, flavonoids, saponins, and diterpenes, are particularly effective. Nicotine, an alkaloid found in tobacco, functions as a stomach poison. The physiologically active compounds in tobacco leaves disrupt the central nervous system of insects, ultimately leading to their paralysis and death (Nwachukwu, 2017). Additionally, recent research by our group has shown that A. indica exhibits persistent toxicity against *C. maculatus* at higher doses (Akbar and Khan, 2021). The primary mechanism of action for azadirachtin, a key component of A. indica, includes reducing the release of neurosecretory materials from the *Corpora cardiaca* and altering the activity of prothoracicotropic hormone (PTTH) in brain neurosecretory cells (Sarwar, 2020).

A similar study indicated by (Ashamo *et al*., 2021) that bablah wood ash showed maximum residual toxicity among the tested plant species against *C. maculatus*. Among the tested species, *N. tabacum* followed by *N. rustica*, *A. indica*, and *M. azedarach* showed the highest cumulative mortality, while *T. orientalis* resulted in the lowest mortality. The variation in the lethality of these extracts may be due to the differences in how the active components penetrate the insect system. The bioactive compounds in the extracts are believed to enter the insect through the integumentary system (Kopit *et al.,* 2018). Specifically, toxins are reported to penetrate target sites via the cuticle's lipophilic and hydrophilic layers, employing various mechanisms to exert their effects. The structure of the cuticle, with its hydrophilic and hydrophobic properties, influences the penetration and effectiveness of pesticides (Kopit *et al.,* 2018). The chemical properties and polarity of the active compounds also affect their passage through the cuticle. The lipophilic outer layer of the cuticle favours the movement of nonpolar molecules, allowing only those toxins with suitable polarity and chemical composition to reach the target site, leading to varied mortality effects facilitated by crude extracts. According to the previous findings of Alghamdi *et al*. (2018) that there is a relationships between the concentration of a given crude plant extract and the corresponding percent mortality. The toxic effects of the compounds found in the studied plant species likely contributed to the observed high mortality rates of C. maculatus in this study, which aligns with findings reported in other similar studies (Trivedi *et al*., 2018). Although all the plants demonstrated potential as insecticides, their toxicity against *C. maculatus* varied, probably due to differences in their phytochemical composition. Topical toxicity refers to the direct contact of the botanical substances with the *C. maculatus* population. In this study, N. tabacum showed the highest mortality rate at 3% concentration against *C. maculatus*. Similar results have been confirmed by (Mamoon *et al*., 2018), who also reported high mortality of the pulse beetle at the same concentration of N. tabacum. The presence of secondary metabolites in these plants may hinder the emergence of adult insects, as suggested by Ashamo *et al*. (2021), who found that the phytochemicals present in the plant species not only disrupt the life cycle of insect but also affect their growth and larval survival. The findings of this study are consistent with those of previous research, which also observed the highest repellence of *A. indica* (Neem) against *Tribolium castaneum*, with the repellence effect diminishing over time (Berhe *et al*., 2021) Our results align with earlier studies of (Atawodi and Atawodi, 2009) that demonstrate the insect-repellent properties of A. indica, which also inhibits insect feeding. The active compounds in Neem, such as azadirachtin and salannin, are known to act as feeding deterrents for insects (Ngegba *et al*., 2022). Similarly, *A. indica* has been reported to be effective in controlling various foliage pests (Manzoor *et al*., 2011). Regarding *M. azedarach*, our results support previous findings (Naushad and Raziuddin, 2006) which showed that its repellence effect decreases after 72 hours of exposure. Research conducted globally over the past three decades has consistently highlighted the significant repellence effects of *M. azedarach* in the management of stored-product pests (Naimi *et al*., 2022).

Tobacco species (*N. tabacum* and *N. rustica*) have long been recognized for their natural insecticidal properties (Yasir, 2018). In our study, *N. tabacum* demonstrated 82% repellence, while *N. rustica* showed 76% repellence against *C. maculatus* at a 3% concentration. These findings align with the results reported by (Sagheer *et al*., 2013) who observed a similar trend of increasing repellence with higher concentrations of plant extracts.. Similarly, Souto *et al*. (2021) also documented maximum repellence for *N. tabacum* against *Tribolium castaneum* at elevated concentrations. The insecticidal activity of *Nicotiana* species is attributed to nicotine, an alkaloid that binds to acetylcholine receptors, disrupting nerve transmission and acting as a potent feeding deterrent. Among the botanicals tested in our study, *T. orientalis* exhibited the lowest repellence against *C. maculatus*. Our findings contradict those of (Amoura *et al*., 2021) who reported a high repellence rate (92%) of *T. orientalis* against *Tribolium confusum*. This discrepancy may stem from differences in the plant parts used for extraction, which can lead to variations in the chemical composition of active ingredients (Molapour *et al*., 2020). Many plant extracts and essential oils contain insecticidal compounds, primarily monoterpenoids (Akbar *et al*., 2024). These compounds are generally volatile and lipophilic, allowing them to rapidly penetrate insects and disrupt physiological functions (Akbar *et al*., 2024). he high volatility of monoterpenoids also grants them fumigant properties, making them potentially useful for managing stored-product insect pest. Over the past three decades, research has greatly expanded our understanding of botanical pesticides. Numerous plant-derived natural products with insecticidal activity can be sourced from locally available materials, potentially reducing costs by enabling production at the site of application (Akbar *et al*., 2024).

Conclusions

The usage of synthetic insecticides for stored product insect pests leads a serious threat for environment. Due to this regard, we tested plant aqueous extracts as alternative and sustainable biopesticide against *C. maculatus*. Among the tested plant species extract when treated with mung bean seed, *N. tabacum* gave promising results having less Oviposition, % adult emergence, % infestations and % seed weight loss. In case of toxicity experiment *N. tabacum* exhibited the highest toxicity in residual and direct assays followed by *N. rustica* and *A. indica*. This research concluded that that *N. tabacum* and *N. rustica* are particularly promising as biological control agents due to their high concentrations of toxic phytochemicals. The plant extracts are safer for consumer because they have toxic residues on food commodities or the environment. Hence it can be considered as a green pesticide from plant species and can incorporated as a potential compound in the integrated pest management strategies for controlling storage insect pests

Authors' Contributions

The author read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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