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Efficacy of carbofuran against pulse beetle *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) in black gram (*Vigna mungo* L.) seeds

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Abstract
The experiment was conducted from 2013-2014 at Genetics and Molecular Biology Laboratory, Department of Zoology, University of Rajshahi in Bangladesh to evaluate the effects of carbofuran (granular) against pulse beetle *Callosbruchus maculatus* (F.). The black gram (*Vigna mungo* L.) seeds were treated with carbofuran and different reproductive potentials *viz.* fecundity, duration of immature stages, percentage of adult emergence and adult longevity of pulse beetles were investigated under laboratory conditions. Six doses of carbofuran (0, 20, 16, 12, 8 and 4 ppm) were used to pulse seeds treatment and the untreated female beetles after mating with males were released to lay eggs on treated seeds. Results revealed that the different doses of carbofuran induced significant (P<0.01) changes in the developmental period (34.76±1.00, 32.67±0.81, 32.57±0.94, 35.69±0.87, 36.06±1.70, 35.28±2.44; $F_{5,24}$=5.71) and percentage of adult emergence (67.66 ±16.40, 47.64±18.38, 67.53±15.22, 60.52±7.54, 71.92±3.68, 85.78±8.61; $F_{5,24}$=4.84) whereas fecundity (52.4±5.18, 38.8±9.04, 44.6±8.29, 40.4±6.46, 43.4±6.23, 41.2±7.66) and longevity of male (9.50±0.79, 9.44±0.85, 9.34±1.13, 9.76±0.37, 9.57±1.47, 10.25±1.81; $F_{5,24}$ = 0.38) and female (18.75±2.33, 21.37±2.27, 20.65±3.68, 21.05±1.52, 18.68±1.93, 21.03±1.60; $F_{5,24}$ =1.35) were not affected by any doses of treatments. Thus, the present findings indicated that 20 ppm carbofuran among other doses could be effective for *C. maculatus* population suppression. Further studies are still needed to determine the absolute dose levels to improve the control strategy of this pest species.

Keywords: Carbofuran, reproductive potential, *Callosobruchus maculatus,* suppression

1. Introduction

Callosobruchus maculatus (F.) (Coleoptera: Bruchidae) is a major pest of economically important leguminous grains, such as cowpeas, lentils, green gram, and black gram under storage conditions [1-4]. The damage is caused by adult female oviposition on the surface of grains and subsequent larval penetration by boring in the grains and become unsuitable for human consumption, viability for replanting, or for the production of sprouts. The whole development takes place inside seed [5] which results weight loss and reduced germination rate of seeds [6, 7]. This beetle can cause damage of legume seeds up to 100% during storage [8] or 5-10% in the temperate and 20-30% in the tropical countries [9]. To overcome the losses of grains due to bruchid infestation, various methods including plant materials and chemical controls have been applied from time to time.

The indigenous plant materials [10] even culinary spices [11] were effective as protectants of green and black gram seeds but they are ancient technologies over the world [12]. The chemical insecticide sevin (carbamate) revealed the significant reduction (lowest rate) of the adult (*C. chinensis*) emergence with keeping no effect on seed germination [13]. The effects of plant extracts are prominent but commencing a lot of problems *viz.* relatively slow action, variable efficacy, instability in the environment, disagreeable odour, poor water solubility and inconsistent availability [14].

A wide range of effective insecticides including pirimiphos-methyl, cypermethrin, carbon di-sulfide, chlorpyrifos, phosphin, methyl bromide, DDT, BHC, and malathion are using for the control of storage beetle in the genus *Callosobrucus* [15]. Furadan, the commercial name of carbofuran, is widely used to control agricultural pests such as insects, mites and nematodes [16]. Many investigators suggested to use chemical insecticides to protect pulse and cereal crops against the attack of stored pests and this is why a very few insecticides mainly carbamate group are used by the farmers [13].

A synthetic insecticide carbofuran, working on contact or ingestion is a cholinesterase inhibitor with keeping short-term effects on the nervous system [17]. It is reported that synthetic insecticides are still heavily used and considered as the most effective method of controlling stored product pests in most nations, particularly for a large scale storage [18, 19] in spite of their widespread public concern on health and environment. Therefore, the present work was undertaken to study the efficacy of carbofuran on some reproductive potentials of *C. maculatus* rearing on treated black gram seeds.

2. Materials and Methods

2.1 Carbofuran

Carbofuran is an N-methyl carbamate pesticides which was first registered in the United States in 1969 [20]. IUPAC name of carbofuran is 2, 3-dihydro-2, 2-dimethyl-7-benzofuranyl-N-methylcarbamate [21]. It is formulated as a flowable or wettable powder, while pure carbofuran is an odorless to mildly aromatic white crystalline solid [22]. It is soluble in water, methylene chloride, benzene, xylene, acetone etc. The trade name is Furadan having melting point 151˚C and boiling point 313˚C.

2.2 Collection of black gram seeds

Black gram (*Vigna mungo* L.) seeds were collected from Nawabganj District under Rajshahi Division and cleared by sieving and large dirty particles were removed by hand picking. The seeds were dried in the sun light for 3-4 days with an exposure of 2-3 h/d to minimize the microbial infestations.

2.3 Collection and rearing of beetles

Infested black gram seeds with *C. maculatus* were collected from grain stores of Nawabganj District under Rajshahi Division. The insects were transported to the laboratory in porous plastic container so that the air and moisture can come in easily. The beetles were reared in the laboratory for one successive generation in order to eliminate natural or deleterious mutations. To collect the virgins, infested seeds from the mass culture were taken in separate vials. The mouths of vial were closed by using cotton balls and kept them for 27-29 days for adult emergence. The ambient temperature and the relative humidity during the experimental period were 27.22 ± 2.41 ^{o}C and 85.32 ± 5.22%, respectively.

2.4 Doses used

There were six doses (0, 20, 16, 12, 8 and 4 ppm) of carbofuran to treat the seeds. The doses were prepared with a rate of 1 mg carbofuran solute in 1 liter water for 1 ppm concentration.

2.5 Bioassays

Carbofuran treatments were applied on fresh seeds. The calculated volume of carbofuran (dose) solution was applied on seeds in glass vials by micropipette and soaked them evenly so that the agents got mixed properly. Then they were transferred to petridishes and dried in sunlight for further use. There were 250 seeds for each dose (ppm) application and 50 seeds were for a replication. The seeds (250 for each dose having 5 replications; 1250 for 5 doses and 250 for 0 or control) were then ready for conducting the experiments.

2.6 Experimental design

On the day of emergence the unmated males were paired with virgin females separately in glass vials. Then inseminated females were allowed to oviposit on the treated seeds. After 24 hours the deposited eggs were counted and the parent beetles were removed. A total of 25 pairs of *C. maculatus* were allowed for their mating in the treated seeds. Five pairs beetle were used as control (0 ppm) line to compare the reproductive potentials with the treated lines. Observations were made on daily basis on the fecundity, egg to adult developmental period, percentage of adult emergence and adult longevity.

2.7 Statistical Analyses

Descriptive statistics was employed to ascertain mean values of reproductive potentials for different doses. One way analysis of variance (ANOVA) was carried out to compare differences in treatment means. Significant different means were separated by using Fisher's least significant difference (LSD) at $p<0.05$.

3. Results

3.1 Effect on Fecundity

The mean values (mean ± sd) for the 0, 20, 16, 12, 8 and 4 ppm treatments were 52.4 ± 5.18, 38.8 ± 9.04, 44.6 ± 8.29, 40.4 ± 6.46, 43.4 ± 6.23 and 41.2 ± 7.66 (Table 1) respectively. The fluctuations of egg laying per day by each female is illustrated in Fig. 1 and the ANOVA (P-value = 0.08) (Table 2) revealed that they were not significantly different after carbofuran treatment to reduce the pest population of *C. maculatus*.

3.2 Effect on duration of immature stages

The mean values (mean ± sd) for the 0, 20, 16, 12, 8 and 4 ppm treatments were 34.76 ± 1.00, 32.67 ± 0.81, 32.57 ± 0.94, 35.69 ± 0.87, 36.06 ± 1.70 and 35.28 ± 2.44 days (Table 1), respectively. The developmental period showed changes according to different doses (Fig. 2) and the ANOVA ($F_{5,24}$ = 5.71; $P<0.01$) (Table 2) revealed that they were significantly different.

3.3 Effect on percentage of adult emergence

The mean values (mean ± sd) for the 0, 20, 16, 12, 8, and 4 ppm treatments were 67.66 ±16.40, 47.64 ± 18.38, 67.53 ±15.22, 60.52± 7.54, 71.92± 3.68 and 85.78 ±8.61 (Table 1), respectively. The bar diagrams showed a dose dependent decline of adult emergence (Fig. 3) and the calculated 'F' value (F5,24 = 4.84; $P<0.01$) (Table 2) revealed that the applied doses were significantly effective in beetle emergence.

Table 1: Showing the descriptive statistics of carbofuran induced changes in the reproductive potentials of *C. maculatus*.

Treatments	Fecundity Mean± Sd	Duration of immature stage (days) Mean ± Sd	Percentage of adult emergence Mean ± Sd	longevity (days) of Male Mean ± Sd	longevity (days) of female Mean ± Sd
0 ppm	52.4 ± 5.18^{a}	34.76 ± 1.00^{a}	67.66 ±16.40^{b}	9.50 ± 0.79^{a}	18.75 ± 2.33^{a}
20 ppm	38.8 ± 9.04^{a}	32.67 ± 0.81^{b}	47.64 ± 18.38^{c}	9.44 ± 0.85^{a}	21.37 ± 2.27^{a}
16 ppm	44.6 ± 8.29^{a}	32.57 ± 0.94^{b}	67.53 ±15.22^{b}	9.34 ± 1.13^{a}	20.65 ± 3.68^{a}
12 ppm	40.4 ± 6.46^{a}	35.69 ± 0.87^{a}	60.52± 7.54^{bc}	9.76 ± 0.37^{a}	21.05 ± 1.52^{a}
8 ppm	43.4 ± 6.23^{a}	36.06 ± 1.70^{a}	71.92± 3.68^{b}	9.57 ± 1.47^{a}	18.68 ± 1.93^{a}
4 ppm	41.2 ± 7.66^{a}	35.28 ± 2.44^{a}	85.78 ±8.61^{a}	10.25 ± 1.81^{a}	21.03 ± 1.60^{a}

Means ± Sd within the column followed by the same small letter are not significantly different ($p \geq 0.05$).

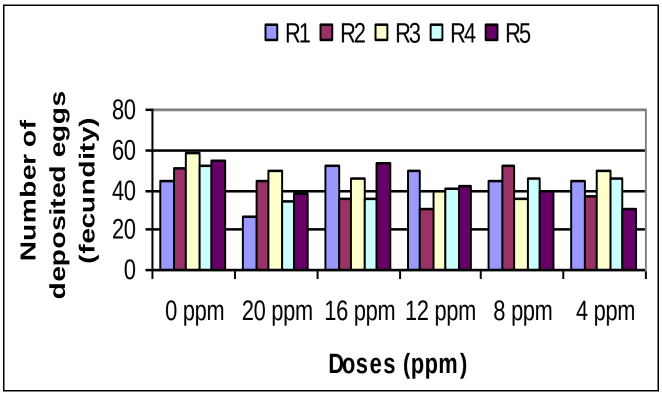

Fig 1. Showing the fecundity of *C. maculatus* after carbofuran treatment. R stands for replication.

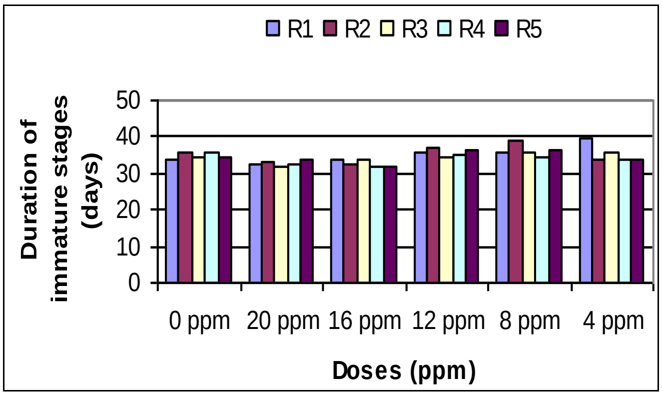

Fig 2: Showing the duration of immature stages of *C. maculatus* after carbofuran treatment. R stands for replication.

Table 2: Statistical analyis (F value) showing the effects of carbofuran on reproductive potentials of *C. maculatus*.

Reproductive potentials		One-way ANOVA						
		Source of Variation	SS	df	MS	F	P-value	F crit
Fecundity		Between Groups	587.0667	5	117.4133	2.225142	0.084787	2.620654
		Within Groups	1266.4	24	52.76667			
Duration of immature stage (day)		Between Groups	57.94695	5	11.58939	5.718872**	0.001303	2.620654
		Within Groups	48.6364	24	2.026517			
Adult emergence (%)		Between Groups	3969.894	5	793.9789	4.844702**	0.003334	2.620654
		Within Groups	3933.264	24	163.886			
Adult longevity (day)	♂	Between Groups	2.67547	5	0.535094	0.38912	0.851321	2.620654
		Within Groups	33.00336	24	1.37514			
	♀	Between Groups	37.08398	5	7.416795	1.358835	0.274517	2.620654
		Within Groups	130.9968	24	5.458202			

** (P<0.01)

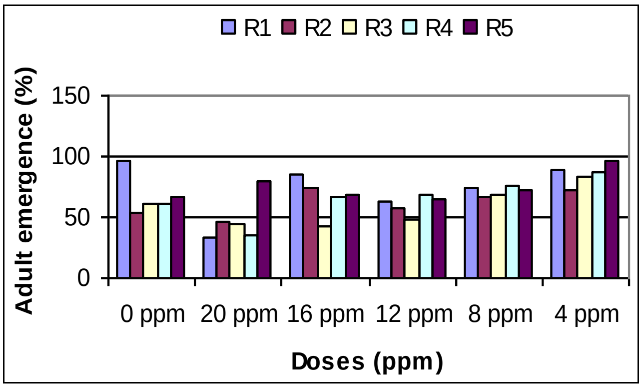

Fig 3: Showing the percentage (%) of adult emergence of *C. maculatus* after carbofuran treatment. R stands for replication.

3.4 Effect on longevity

The mean male longevity (mean ± sd) for the 0, 20, 16, 12, 8 and 4 ppm treatments were 9.50 ± 0.79, 9.44 ± 0.85, 9.34 ± 1.13, 9.76 ± 0.37, 9.57 ± 1.47 and 10.25 ± 1.81 (Table 1), respectively. Fig. 4 showed no effect of carbofuran with different doses on longevity of male insects ($F_{5,24}$ = 0.38) (Table 2).

On the contrary, the mean values (mean ± sd) for female beetle for the 0, 20, 16, 12, 8 and 4 ppm treatments were 18.75 ± 2.33, 21.37 ± 2.27, 20.65 ± 3.68, 21.05 ± 1.52, 18.68 ± 1.93 and 21.03 ± 1.60 (Table 1) and the results also showed no significant effect ($F_{5,24}$ = 1.35; Table 2) (Fig. 5).

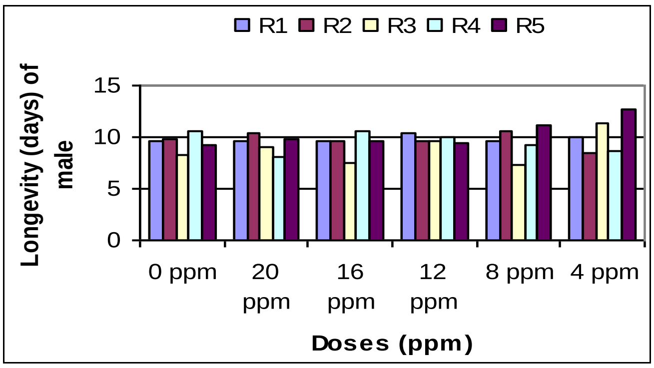

Fig 4: Showing the adult longevity of *C. maculatus* (male) after carbofuran treatment. R stands for replication.

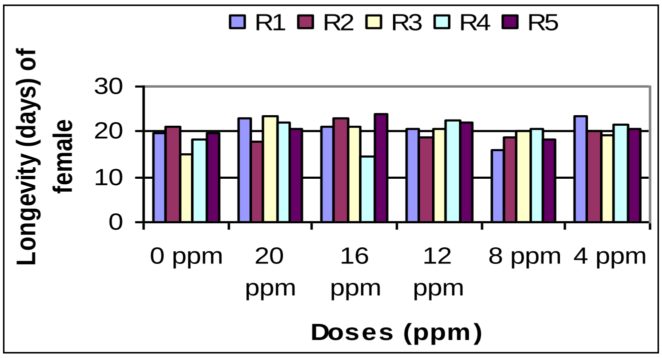

Fig 5: Showing the adult longevity of *C. maculatus* (female) after carbofuran treatment. R stands for replication.

4. Discussion

Botanicals and synthetic insecticides are applied to pulse seeds to protect them from the infestation of pulse beetles. We are here to first time describe the changes occurred in the reproductive potentials of *C. maculatus* after carbofuran treatment in black gram seeds. Previous studies have reported on the toxicity of different bioactive plants compounds mixed with different types of cowpea seeds to evaluate the fecundity of *Callosobruchus maculatus* (F.) in storage condition. The plant products (garlic, chilies, peppermint) significantly reduced the number of eggs laid by cowpea weevils (*C. maculatus*) on the cowpea seeds [23], oil at a rate of 0, 2.5, 5.0, 7.5 and 10 ml/kg to cowpea seeds gave lower mean oviposition (10.3%) than the untreated control (96.3%) [24]. Plant derivatives at 0.025, 0.50, 0.75 and 1.00% to black gram where 1.00% neem oil decreased fecundity (33) which was lowest for a 7 days trial [25]. Similarly, 5 doses at a rate of 0.5, 1.0, 2.0, 3.0 and 4.0 ml to cowpea seeds (*Vigna unguiculata* L. WaIp) showed a dose dependent decrease in fecundity of *C. maculatus* and highest dose of neem (4 ml/50g seeds) was absolutely efficient (0 fecundity) [26]. Fecundity of *C. chinensis* was 0 with a treatment of 30 µl of *Artemisia herba-alba*, *Salvia verbenaca*, and *Scilla maritima* plant oils to chickpea (*Cicer arietinum* L) [27]. *Zingiber officinale* (L.) at 1.151 µl/l air killed 50% of the population (LC_{50}) for egg of *C. maculatus* whereas 700 µl/ml yielding 100% repellency in cowpea seeds [28, 29] and 0.072 µl/l air was for *Mentha pulegium* (L.) [28]. On the other hand, chemical insecticide carbofuran used to control the coleopteran beetles, *Heteroligus meles* (Bilb.) [24]. The chickpea with Curaterr 5GR-a 5% granular formulation with a 30% liquid concentrate of carbofuran at 0.5 to 80 mg a.i. inhibited the beetle infestation resulting enhanced germination [30]. Gram seed treatment with permethrin @ 60 ppm was effective because least number of eggs were laid by *C. maculatus* [31]. Adult females laid the least number of eggs (3.16) at 4% of ethanol extract comparing to control (58.50) [32]. The carbaryl compound causes reduction in fecundity of *Musca domestica* [33] and increase in the western corn root worm (*Diabrotica virgifera)* [34] and pirimiphos methyl reduces oviposition and causing mortality of *C. maculatus* [35]. It is evident from these studies that either chemical insecticides or botanicals decreased the fecundity with the increase of dose levels along with trials more than 3 days or 24 hours. In our experiments, the fresh pulse beetles (female) laid eggs on treated black gram seeds smoothly for 24 hours and carbofuran induced toxicity did not significantly change *C. maculatus* fecundity with comparing to control (0 ppm). Thus, the present results are not in agreement with above mentioned reports. The applied doses might not be effective enough with short duration of trial conduction to destroy the viability of eggs inside reproductive system of bruchid weevils or kill the gravid females from laying eggs on the treated seeds.

Development period of *C. maculatus* was shorter in green-gram with softer seeds and was prolonged in black-gram with harder seeds [36], temperature (28ºC; with 60±10% R.H) brought 27.7 days as total developmental period in cowpea seeds [37]. Neem, castor, eucalyptus and sunflower oils @ 1 ml/kg to pigeon pea seeds resulted the longer developmental period (41.33, 41.66, 42.50 and 37.35 days; control, 29.33 days) of *C. maculatus* while no adult emerged (0) after 3 ml/kg of neem, castor and eucalyptus oil application and sunflower had taken longer (38.00 days) than the control (29.66 days) [38]. The biopesticides or botanicals treatment with 0, 0.2, 0.4, 0.6, 0.8, 1.0 g per 20 g of each grain and a synthetic chemical (pirimiphos methyl) was 0.1 g per 20 g of each grain revealed that the highest delay in egg hatch in *C. maculatus* recorded with pirimiphos methyl (28, 26, 24 days) in all grains. The developmental period (egg-adult) was gradually increased with the dose level increase i.e. highest 40 days was for *Vigna unguiculata*, 38 for *Cajanus cajan* and 37 for *V. subterranea* after each botanicals [39]. In the present study we have observed that the larvae hatched from egg after three or four days from egg lay and made burrow on the seed coat and started to penetrate by eating the endosperm of seed. Thus the grub experienced with toxic carbofuran. The lower dose levels showed delayed developmental periods whereas higher doses (20 ppm and 16 ppm) revealed advance emergence of adults comparing to control (0 ppm) (Table 1) which is yet to be elucidated. So, the present results do not confirm the above mentioned reports.

Previous study showed that the adult emergence was reduced by the plant products [23]. Treatment at 0, 2.5, 5.00, 7.5, and 10 ml/kg of different plant oils showed lower adult weevils` (*C. maculatus*) mean emergence (6.3%) against untreated grains (88.2%) [24]. There were two F_1 adults emerged out of 33 eggs after 1.00% neem oil treatment in black gram [25]. Cypermethrin @ 25 ppm, deltamethrin @ 3 ppm and dichlorvos @ 80 and 100 ppm recorded no emergence of adult. Malathion with 20 ppm recorded highest adult emergence of 61.33 followed by spinosad @ 0.5 ppm (67.00) [40] and with different dosage of malathion @ 20 and @ 30 ppm and cypermethrin @ 15 ppm proved to be highly effective to control *C. maculatus* [41]. Direct application of malathion @ 10 ppm to grain prior to storage which is safe and effective in preventing damage from pulse beetles [42]. Phosphine @ 2 tablets per bag or pirimphos-methyl 2 per cent dust @ 4 ppm afforded protection to cowpea against *C. maculatus* [43]. Seed treatment with permethrin @ 60 ppm was proved to be the most effective treatment giving absolute protection to gram seed with no adult emergence [31]. Indoxocarb @ 2 ppm treatment on cowpea seeds showed 16.67%, 43.33%, and 91.67% adult mortality (*C. maculatus*) after 3, 7 and 10 days [44]. 100% mortality of *C. chinensis* (L.) was noted with the use of Artemisia oil (20 µl) in chickpea (*Cicer arietinum* L.) [27], LC_{50} for larvae of *C. maculatus* was 2.336 µl/l air of *Z. officinale* followed by 0.113 µl/l air essential oil of *M. pulegium* [28]. The mean adult emergence was 0.0 for 700 µl/ml, 350 µl/ml, 175 µl/ml and 87.5 µl/ml of the *Z. officinale* oil extract used against *C. maculatus* [29]. Pirimiphase methyl 2% at 1 g/kg seeds and k-othrin 2% at 0.5 g/kg seeds revealed good storage of seeds for sowing [45] but no adults emerged from the treated seeds with pirimiphos methyl 0.1 g per 20 g of each grain [39]. Spinosad at the rate of 0.3 g/kg on split pea and cowpea reduced progeny production (94.33 and 94.21%, respectively) of *C. maculatus* [46]. So, suppression of the subsequent generations is one of the basic characteristics of a successful grain protectant [47]. The results obtained from the present study demonstrated that the progeny production (percentage of adult emergence) of *C. maculatus* significantly decreased (<50%) by carbofuran (20 ppm) (Table 1, Fig. 3) in black gram seeds which is relevant to the above mentioned reports i.e. it can suppress the pest populations.

The tested plant part powders of *Alstonia boonei* significantly ($P<0.05$) reduce the longevity of adult *C. maculatus* on treated cowpea seeds [48]. The longevity of adults (male and female) decreased with increasing the concentration of the plant extracts [32]. Four concentrations (0.125, 0.25, 0.5 and 1.0%) of two vegetable oils, namely ethyl oleate and *Acorus calamus* were tested and found an inconsiderable latent effect on the immature stages of the *C. maculatus* beetle [49]. Low lethal concentration (LC_{20}) negatively affected the longevity [50]. All the leaf and stem powders reduced only the longevity of males and showed strongly repellent activities against females [51]. Male and female adults of *C. maculatus* in contact the different concentrations of garlic essential oils lived only 1 to 3 days following treatment, while longevity of control male and female adults varied from 1 to 13days [52]. Here, the carbofuran toxicity did not change the adults (progeny) longevity (Table 1 & 2) which is not in agreement with previous studies.

5. Conclusion

The insecticidal activity of carbofuran indicated its potentials for the suppression of *C. maculatus* population in black gram (*Vigna mungo* L.). Here, the delayed developmental periods were found to be 35, 36 and 35 days in lower dose levels (12, 8, and 4 ppm) whereas 20 and 16 ppm showed advance emergence (32 days) comparing to control (0 ppm; 34 days). The present study also revealed that 20 ppm reduced the adult emergence by 53% and therefore, it can be concluded that carbofuran could be used as an alternative of black gram seed protectant for the management of pulse beetle *C. maculatus* in storage condition. Further studies are necessary to determine the absolute dose level of carbofuran to improve the control strategy of this species and other pest species of Leguminosae family.

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