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TOXICITY EFFECT OF CALCIUM CARBIDE ON QUANTITATIVE TRAITS OF THE SILKWORM, *BOMBYX MORI* L

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ABSTRACT
An experimental research was conducted to test the calcium carbide (CaC_2) toxic effect on six important economic traits through two different methods (toxicity parameters) in two voltine groups of the silkworm *Bombyx mori.* The calcium carbide in four sublethal concentrations were administered to V instar larvae by oral and topical methods to a multivoltine race of Nistari and a bivoltine race of NB_4D_2 race and rearing was conducted through standard procedure under equipped with good laboratory condition. The obtained data was subjected to ANOVA for statistical tests and results have indicated that, a high concentration of calcium carbide is bring about significant effect ($P<0.05$) on traits/parameters assessed. Further, more pronounced effect is observed in the batches treated with calcium carbide by oral method than compared to topical application. Hence, it is noteworthy that, the effect of calcium carbide on quantitative (economic) traits of silkworm *Bombyx mori* is harmful (loss) towards silk production and this research is being considered as pioneer work by utilizing silkworm *Bombyx mori* as a model organism are discussed in the light of its awareness to the sericulture industry.

KEY WORDS: CaC_2, Toxicity, Economic traits, *Bombyx mori.*

INTRODUCTION

India is being multi-population and diversified country, wherein excess uses of artificially fruits repining agents are occurred in plenty, such as ethylene, methionine, ethephon, ethrel and calcium carbide (CaC_2). The ethylene is being considered as a plant hormone, because it involved in regulation of several physiological responses (Abeles *et al.,* 1992; Reid, 1995; Arshad and Fragkenberger, 1998). Besides, the calcium carbide is being an established precursor of ethylene and common called as calcium carbide, actually it is improper but proper name is "calcium acetylide" and popularly called as "masala" is one of the commonly used chemical for artificial repining of various fruits, such as mango, banana, papaya, tomato, citrus fruits, *etc*. in many countries like, Pakistan, Brazil, Malaysia, Philippines Australia, South Africa, India, *etc*. (Bhuller, 1982; Medlicott *et al.,* 1987; Sudhakar, 2006; Rahman *et al.,* 2008; Siddiqui, 2008; Siddiqui and Dhua, 2009; Sy and Wainwright, 1990; Sinha, 2010 and Siddqui and Dhua, 2010). It is considered fact that, in India CaC_2 was utilizing for numerous fruits but neglecting the fact is banned under the prevention of food adulteration act 1954 and prevention of food adulteration rule 1955 (Siddiqui and Dhua, 2010). Apart, recently Food Safety and standards authority of India and Ministry of Consumer Affairs, Food and Public Distribution of India issued a notice, stating that, CaC_2 is a very harmful chemical for human health; it contains arsenic and phosphorous hydride (Delpierre, 1974), causes neurological system by inducing prolonged hypoxia (Lewis and Sax, 2004), headache, dizziness, mood disturbances, sleepiness, mental confusion, memory loss, cerebral edema and seizures, burning sensation of the eyes, chest and skin, cough, *etc* (Per *et al*. 2007 and Siddiqui and Dhua, 2010) and inhalation of 33% or 35% can cause unconsciousness within 5 and 7 minutes (Bingham *et al.* 2001). Moreover, in the field, it is indicated that applicability of CaC_2 in combination with N fertilizer increased the green pod yield as well nitrogen economy of soil and yield of Okra (Kashif, *et al.,* 2007), because, researchers have used CaC_2 as nitrification inhibitor in soil and have reported substantial improvement in N economy (Arshad and Frankenberger, 2002 and Yaseen *et al.,* 2006) and the fertilizers are widely utilized for plants to enhance the crop production, recent evidences revealed that, utility of CaC_2 not only fruits repining but also to enhance the growth parameters and yield of many plants like, significant influence on growth parameters and rice yield (Akhtar *et al.,* 2004 and Yaseen *et al.,* 2009), increases plant height of Soyabean (Arshad *et al.,* 1994), *etc*. On the other hand, silkworm, *Bombyx mori* is a monophagous insect, depends only on mulberry leaves as a food material. Apart, mulberry plant is being deep rooted, perennial and evergreen plant with the usage of various kinds of chemically modified insecticides, pesticides, fertilizers, *etc* are seen during silkworm rearing and mulberry cultivation. As a result, several pesticides in the form of fungicides, insecticides, *etc* are utilizing to control the many diseases comes across while in the silkworm as well as mulberry plant. In spite of knowing the values of CaC_2 for various proposes yet, the extent of toxic effects to the non-target organism, beside silkworm is very merge in spite of its usage in sericulture and silkworm *Bombyx mori* being an ideal bioassay material for toxicological studies. Hence, the authors in the present investigation have studied the effects of CaC_2

both on the rearing performance and toxicity parameters is being pioneer work by utilizing silkworm *Bombyx mori* as a model organism. Apart, the evaluation of the economic traits is being considered as an additional support for the toxicological studies.

MATERIALS & METHODS

The disease free layings of two aboriginal pure races *viz*., a multivoltine of Nistari race and a bivoltine of NB_4D_2 race were maintained in the germplasm bank of Department of Studies in Sericulture Science, University of Mysore, Karnataka, India formed the biological material for the present research investigation. The CaC_2 was procured from the Loba Chemie, Pvt. Ltd, India, with the molecular weight of 64.10. The layings were prepared through the standard procedure of Tazima, (1978) and after hatching, the silkworm larvae of said two races were maintained up to IV moult by following standard rearing procedures suggested by Krishnaswami *et al.*, (1978). The larvae of each race after resuming from the IV moult were made into groups of larvae comprising 100 each in three replicates. The CaC_2 was administered to both early and late V instar larvae of selected races by following the two popular methods of pesticide administration *viz.*, oral and topical methods. The four sublethal concentrations derived from the pilot toxicity experiments of Deepak, (1998) were selected and the method followed is described below.

Oral method: The four sublethal concentrations of CaC_2 ranging from 5 to 20 x 10^2 ppm were prepared in distilled water and the volume of solution was 100 ml (Plate-1). The M_5/Kanva-2 variety mulberry leaves, which are suitable to this larval stadium, were collected and dipped in the four sublethal concentrations of CaC_2 for 30 minutes and simultaneously treated leaves were surfaces air dried under hygienic laboratory condition and stored in labeled polythene covers having perforations and fed to larvae in groups of 100 each in three replications for a day (four feedings). Parallel to this, a control batch was setup by feeding the leaves dipped in distilled water. Subsequently, both treated and control batches were fed with normal leaves from next day onwards.

Topical method: The sublethal concentrations of CaC_2 ranging from 5 to 20 grams/100 larvae was dusted evenly over the larvae of the races selected in groups comprising 100 individuals in each stages mentioned above using a muslin cloth. On the other hand, one hundred larvae without such treatment served as control.

Moreover, after the administration of CaC_2 by oral and topical methods, both control and treated batches were reared following the standard procedures till spinning. The experimental batches were subjected to the assessment of toxicity as well as the six economic important traits such as, V age 10 larval weights (g), larval duration (hrs), single cocoon weight (g), single shell weight (g), shell ratio (%) and filament length (mtr). The results obtained from the experiment thereby were statistically analyzed through ANOVA test.

RESULTS & DISCUSSION

The data pertaining to the oral as well as topical application of calcium carbide to the early V instar larvae of two different voltine groups of the silkworm *Bombyx mori* are presented in tables-1 and 2 (Same are represented as figures from 1-12) respectively. The significant differential effect was noticed in all the races selected for the experiment to almost all the commercial traits and two toxicity parameters analyzed. The important traits of larval weight and cocoon weight were exhibited highest of 24.253 ±0.04g, 41.117 ±0.07g and 0.127 ±0.003g, 0.341 ±0.010g in Nistari and NB_4D_2 races respectively at 5% among the treated batches with CaC_2 through oral method against control, while both races have shown the minimum effect for these traits with increases the concentration of CaC_2. Further, there is a statistical significant difference (*) and highly significant difference (**) decreases ($P<0.05$) in the larval weight and cocoon weight after the concentration of CaC_2 treatments decreases in all the two races tested. For the traits larval duration, it is observed that, the reduction of larval duration occurred in the both races selected for the experiment from 503.67±4.63h (control) to 486.00 ±10.5 (20% CaC_2) and 575.67 ±2.40 (control) to 565.33 ±3.18 (20% CaC_2) in Nistari and NB_4D_2 races respectively through oral feeding and trait shown statistically non-significant ($P>0.05$) by both toxicity parameters except NB_4D_2 race exhibited highly significant difference ($P<0.05$) through oral feeding method. For the post cocoon parameters namely, shell weight (Figs.4&10), Nistari revealed highly significant difference ($P<0.05$) in oral feeding compared to topical (non-significant), but in case of NB_4D_2 race showed highly significant through both toxicity parameters observed in Tables 1 and 2 (Figs 4&10) with compared to control. On the other hand, it is very interesting that, for the trait shell ratio (Figs. 5 & 11) statistically shown non-significant difference ($P>0.05$) of toxicity effect through both toxicity parameters (oral & topical methods) among all the races (Tables 1&2). Moreover, it is important to note that, the trait filament length reeled showed lowest of 332.33±4.91m filament length (Table-1&Fig.6) treated with CaC_2 at 20% by oral method than compared to topical (361.00 ±5.51m) at the same concentration (Table-2 & Fig.12) in Nistari race. On other hand, the same pattern of CaC_2 toxicity effect observed for said traits in NB_4D_2 race. In addition to this, it is noteworthy that, the two races of same trait statistically exhibited highly significant difference ($P<0.05$) through both toxicity parameters except NB_4D_2 race shown non-significant difference ($P>0.05$) administered CaC_2 with orally.

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TABLE 1: Effect of toxicity parameters (@) and economic traits after oral feeding of CaC_2 to early V instar larvae of the Silkworm, *Bombyx mori*

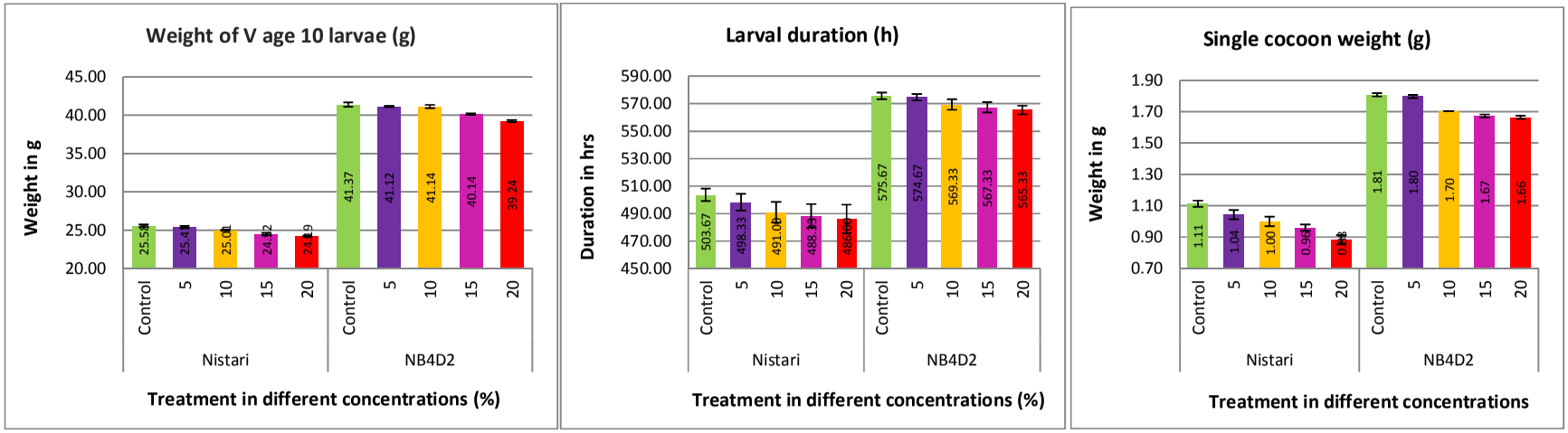
Races \ Traits	CaC_2 treatment in different concentrations (%)	Weight of V age 10 larvae (g)	Larval duration (h)	Single cocoon weight (g)	Single shell weight (g)	Shell ratio (%)	Length of the filament (m)
		Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE
Nistari	Control	24.917±0.05	503.67±4.63	1.113±0.02	0.138±0.004	12.417±0.15	434.00±4.36
	5	24.253±0.04	498.33±6.12	1.043±0.03	0.127±0.003	12.203±0.10	409.67±4.91
	10	23.327±0.27	491.00±7.51	1.000±0.03	0.122±0.002	12.173±0.13	401.00±5.51
	15	22.143±0.04	488.33±8.67	0.960±0.02	0.114±0.003	11.867±0.08	353.67±4.26
	20	21.353±0.08	486.00±10.5	0.883±0.03	0.104±0.003	11.817±0.26	332.33±4.91
	F-test	12.672*	0.898 N/S	10.394*	15.330**	2.591 N/S	75.806**
NB_4D_2	Control	41.370±0.29	575.67±2.40	1.807±0.01	0.352±0.011	19.473±0.53	913.33±3.53
	5	41.117±0.07	574.67±2.33	1.797±0.01	0.341±0.010	18.913±0.49	902.00±6.08
	10	41.140±0.21	569.33±3.85	1.703±0.00	0.332±0.004	19.447±0.20	898.33±7.27
	15	40.140±0.09	567.33±3.71	1.673±0.01	0.325±0.004	19.333±0.14	891.67±5.46
	20	39.240±0.12	565.33±3.18	1.663±0.01	0.317±0.003	18.993±0.10	889.33±5.55
	F-test	25.087 N/S	2.058 **	105.075**	3.770*	0.483 N/S	2.781 N/S
	SE(m)±	0.158	5.921	0.020	0.006	0.267	5.28
	SE(d) ±	0.224	8.373	0.028	0.008	0.377	7.46
	C.D. at 5%	0.470	17.58	0.058	0.016	0.793	15.68
	C.V. (%)	0.788	1.928	2.482	4.219	2.950	1.42

* = Significant (P <0.05), ** = Highly significant (P <0.05), N/S = Non-significant (P >0.05)

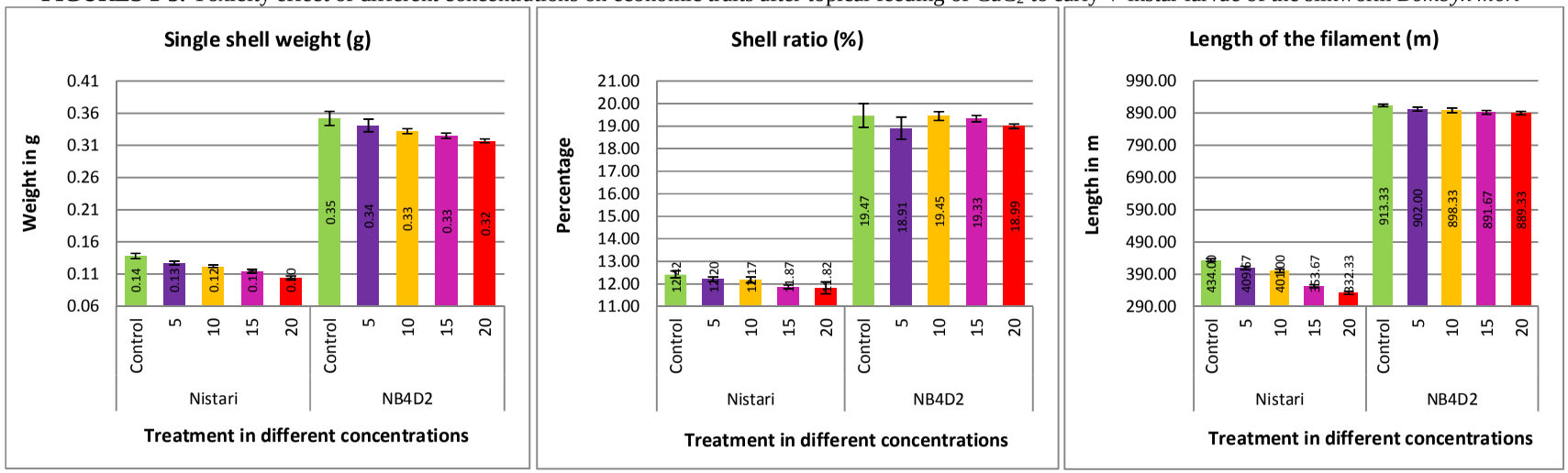
TABLE 2: Effect of toxicity parameters (@) and economic traits after topical feeding of CaC_2 to early V instar larvae of the silkworm, *Bombyx mori*

Races \ Traits	CaC_2 treatment in different Concentrations(%)	Weight of V age 10 larvae (g)	Larval duration (h)	Single cocoon weight (g)	Single shell weight (g)	Shell ratio (%)	Length of the filament (m)
		Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE
Nistari	Control	25.583±0.17	505.67±3.18	1.080±0.04	0.136 ±0.003	12.640 ±0.21	435.33±3.18
	5	25.410±0.15	502.00±4.36	1.060±0.03	0.134 ±0.003	12.643 ±0.08	420.00±5.20
	10	24.340±0.27	498.33±3.84	1.033±0.04	0.130 ±0.003	12.563 ±0.23	403.33±5.61
	15	24.523±0.14	494.33±3.84	0.997±0.05	0.127 ±0.004	12.587 ±0.25	386.67±3.18
	20	24.190±0.09	489.00±4.16	0.943±0.05	0.120 ±0.006	12.760 ±0.26	361.00±5.51
	F-test	124.282**	2.784 N/S	1.671 N/S	2.440 N/S	0.123 N/S	38.421**
NB_4D_2	Control	41.437±0.11	580.33±6.39	1.997±0.04	0.384 ±0.007	19.250 ±0.11	917.33±4.98
	5	41.383±0.10	574.33±4.98	1.807±0.00	0.344 ±0.006	19.043 ±0.31	901.67±5.78
	10	40.127±0.08	571.00±5.86	1.750±0.03	0.331 ±0.006	18.927 ±0.16	884.00±4.36
	15	39.237±0.08	570.33±5.49	1.723±0.01	0.328 ±0.004	18.933 ±0.18	853.67±5.24
	20	38.633±0.13	563.33±4.98	1.714±0.01	0.324 ±0.003	18.877 ±0.13	798.00±5.69
	F-test	154.305**	1.241 N/S	24.062 **	21.002**	0.618 N/S	81.073**
	SE(m)±	0.082	4.804	0.034	0.005	0.204	4.96
	SE(d) ±	0.116	6.794	0.048	0.007	0.288	7.014
	C.D. at 5%	0.244	14.271	0.102	0.014	0.606	14.735
	C.V. (%)	0.413	1.556	4.207	3.49	2.232	1.351

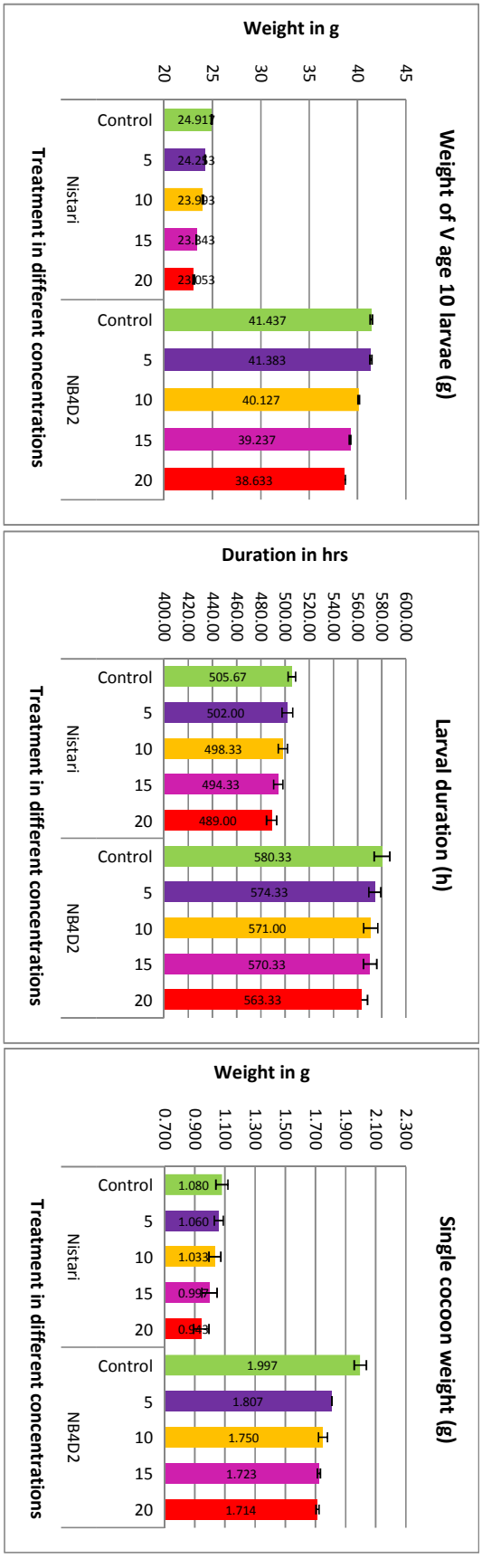
* = Significant (P <0.05), ** = Highly significant (P <0.05), N/S = Non-significant (P >0.05)



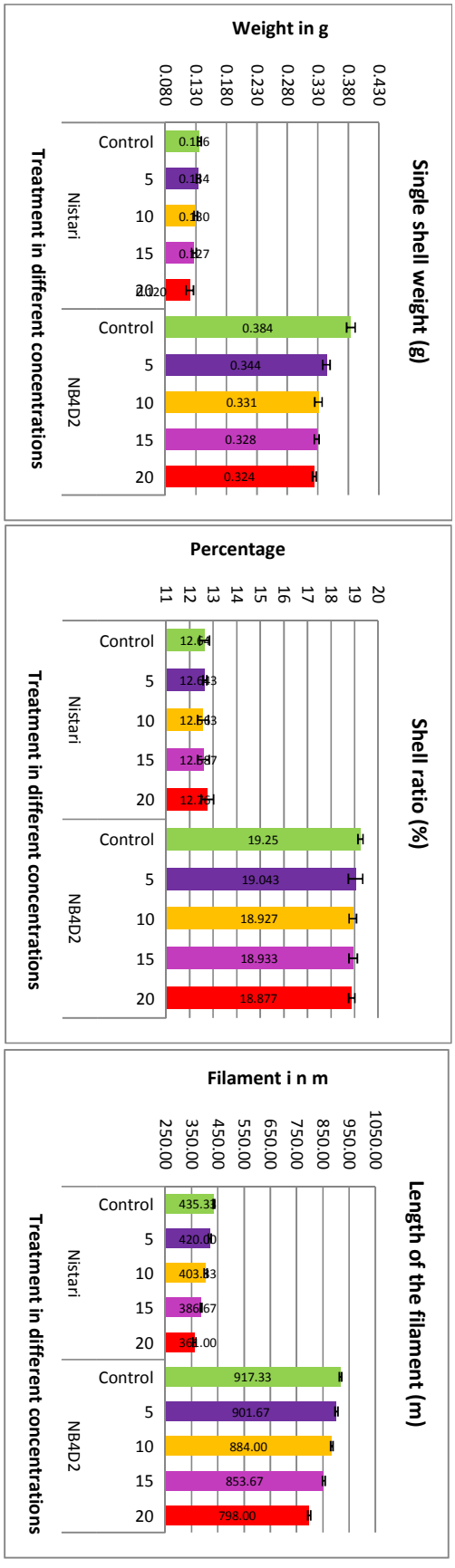
FIGURES 1-3: Toxicity effect of different concentrations on economic traits after topical feeding of CaC_2 to early V instar larvae of the silkworm *Bombyx mori*



FIGURES 4-6: Toxicity effect of different concentrations on economic traits after topical feeding of CaC_2 to early V instar larvae of the silkworm *Bombyx mori*



FIGURES 7-9: Toxicity effect of different concentrations on economic traits after oral feeding of CaC_2 to early V instar larvae of the silkworm *Bombyx mori*



FIGURES 10-12: Toxicity effect of different concentrations on economic traits after oral feeding of CaC_2 to early V instar larvae of the silkworm *Bombyx mori*

Under topical application in both the selected Nistari and NB_4D_2 races showed insignificant difference ($P>0.05$), CaC_2 has induced larval weight in the highest concentration tested, which is statistically significant ($P<0.05$) while, there is insignificant difference ($P>0.05$) between treated and control batches for the trait. Further, for larval duration results was statistically insignificant (non-significant) in all concentrations treated with CaC_2 for the all races against the control batches. With regard to all traits assessed, it is noteworthy that, CaC_2 has induced significant effect ($P<0.05$) except shell ratio trait in both the races (oral & topical methods) and larval duration through topical administration respectively.

In the present investigation, the orally administered batches of silkworm larvae with CaC_2 have responded by tending to show reduction in the larval weight, cocoon weight, filament length and larval duration. For instance, a decrease in larval weight by 3.564g & 2.13g, cocoon weight by 0.23g & 0.144g and filament length by 101.67m & 24.00m in Nistari and NB_4D_2 races respectively through oral method, while same pattern was observed through topical application. On the other hand, the trait larval duration showed reduction by 17.67h (Nistari) & 10.34h (NB_4D_2) through oral toxicity method and by 16.67h & 17.00h in Nistari and NB_4D_2 races respectively through topical administration.

It is understood from research literatures survey, revealed retardation in larval growth resulting automatically decreases the post cocoon parameters and prolongation of larval duration when silkworms and drosophila were treated with several kinds of pesticides (Kuribayashi, 1981; Surendranath, 1993; Hiriyanna, 1997; Venkatareddy *et al.*, 1989 and Deepak, 1998). The authors observed a similar finding with calcium carbide agrees with the above results.

The results have indicated that the oral administration of CaC_2 has produced more pronounced effect on rearing parameters than the topical application as evidenced by lower larval weight subsequently reducing larval duration as well as cocoon and post cocoon characters. The similar results were investigated by using different chemicals by utilizing silkworm *Bombyx mori* (Kuwana *et al.*, 1967 and pai *et al.*, 1989). Moreover, it digested fact that, a trend of decrease noticed for the five out of six traits selected for the study (Tables 1&2). Hence, it is clear from the present studies have revealed various toxicity aspects of CaC_2, which may indicated the possible outcome of its usage in sericulture and warns us against the indiscriminate use of this harmful chemical.

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