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Effect of NSKE and IPM Module Treated Leaves on Rearing Performance of the Silkworm, *Bombyx mori* L.

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Abstract

An experiment was carried out by spraying NSKE (4%) on mulberry leaves at 40 and 45 days after pruning in order to know its effect on rearing performance of silkworm (PMxCSR_2) under laboratory conditions. The results revealed that, the worms fed with NSKE (4%) treated mulberry leaves on 22^{nd} days after spray recorded lowest larval mortality (2.11%) without affecting economic parameters of silkworm (PM × CSR2) when compared to other concentrations. In another experiment carried out at two different locations in the farmers' field *i.e.*, one at Chikkasadenahalli (Ramanagara district) and another one at Jangamaseegenahalli (Chikkaballapura district) the IPM module components, *i.e.,* spray of NSKE (4%), *DP*NPV (10^{-1} dilution 27.65 x 10^5 PIBs / ml) and release of egg parasitoids (*Trichogramma chilonis*) were implemented at 15, 25 and 35 days after pruning, respectively. The safety of this IPM module to the silkworm (PM × CSR_2) rearing parameters was assessed by feeding the mulberry leaves harvested at 65^{th} days after imposing of IPM to the silkworms (PM × CSR_2) and the observations on larval, cocoon, pupal and shell weights (3.08g, 1.84g, 1.53g, and 0.31g) were recorded and shell ratio (16.82%) was calculated. The leaves harvested from the mulberry plots treated with IPM module had no adverse impact on growth and productivity of silkworm (PM × CSR_2) under farm conditions.

Mulberry (*Morus* sp.) is a native of the Himalayan region. It's cultivation spread to India from China, through Tibet during 140 BC. It is now being cultivated in over 32 countries, all over the tropical, temperate and sub-tropical regions of the world. Mulberry leaves serve as the sole food source for the silkworm, *Bombyx mori* L. Scientific production of mulberry is essential for enhancing sericulture production on sound economic lines. There are several factors that influence the productivity as well as quality of mulberry leaves, among them incidence of pests and diseases acts as a major constraint in leaf production. In mulberry, 300 insect and non-insect pest species have been reported to inflict damage (Biradar, 1989; Naik, 1997), of which the leaf roller, *Diaphania pulverulentalis* (Hampson), is a major pest which causes considerable reduction in mulberry leaf yield, resulting in economic loss to sericulturists. The incidence of leaf-roller and mulberry leaf yield loss were recorded to be 70.30 and 25.20 per cent, respectively. The pest usually appears during June and persists upto February and the disappearance of this pest from March to May is attributed to pupal diapause (Rajadurai *et al*., 1999).

At present, mechanical and chemical methods have been advocated to manage this pest. In spite of which, the incidence of the pest is posing a serious threat to mulberry cultivation every year. Further, chemical means for managing the pest has the innate disadvantage of harming the health of the silkworm. Investigations on the occurrence of natural enemies of this pest led to identification of several parasitoids, predators and pathogens, including NPV. Hence, it was felt necessary to evaluate the different management strategies of the leaf-roller and develop an eco-friendly IPM module with minimal hazards to silkworm health and non-target species. However, whenever plant protection interventions are made in mulberry, the safety of such interventions to the silkworm need to be assessed before making any concrete recommendations. With this background, the present bio-assay was conducted to assess the impact of Neem Seed Kernal Extract (NSKE) (4%) and IPM module on rearing parameters of *Bombyx mori*.

Material and Methods

The effect of NSKE (4%) was studied on silkworm rearing parameters at the Department of Sericulture, UAS, GKVK, Bengaluru during 2011-12. A mulberry plot of 5 m x 5 m was selected for this purpose. The variety V_1 was chosen for the study. The NSKE (4%) was sprayed on the mulberry crop at 40 and 45 days after pruning.

Effect of IPM module on silkworm rearing parameters was studied at two locations in the farmers' field, *i.e.*, one at Chikkasadenahalli village, Kanakapura taluk, Ramanagara district and also at Jangamaseegehalli village, Chintamani taluk, Chickballapura district. The sequence of imposition of IPM components was spray of NSKE (4%), followed by *DP*NPV (@27.65x10^5 PIBs / ml) and release of egg parasitoid, *T. chilonis* at 15, 25 and 35 days after pruning, respectively. The effect/safety of NSKE and IPM module to the silkworm (PM × CSR_2) as evidenced by rearing parameters was studied by feeding the worms (PM × CSR_2) with treated mulberry leaves which were harvested at 65th days after imposition. The observations on the silkworm rearing parameters *viz*., larval, cocoon, pupal and shell weights (g) were recorded and shell ratio (%) was calculated. The results were tabulated and subjected to statistical analysis for further comparisons and interpretations.

The chemical insecticide DDVP 76 EC (0.076 %) 1.00 ml / l (*i.e.,* standard check) was sprayed in each of the three replications. Observations were recorded after first, second and third weeks after spray.

Results and Discussion

Effect of NSKE on rearing parameters of silk worm (PM×CSR_2) under laboratory conditions

Larval mortality during fifth instar (%) : The worms fed with NSKE treated mulberry leaves on 22nd day after spray recorded least larval mortality (2.11%) and was significantly superior over rest of the treatments and it was also on par with untreated control (0.00%). However, silkworms reared on NSKE treated leaves which were fed on 16th, 17th, 18th, 19th, 20th and 21st days after spray, recorded significantly higher mortality of 36.24, 29.92, 26.45, 22.60, 26.15 and 8.41 per cent, respectively, which were all inferior treatments (Table I).

Fifth instar larval weight (g) : The worms fed with NSKE treated leaves on 20th, 21st and 22nd days after spray recorded higher larval weights of 2.65, 2.67 and 2.63 all of which were also on par with each other and with control (2.64 g). However, the worms fed with NSKE treated mulberry leaves at 16th, 17th, 18th and 19th days after spray recorded significantly minimum larval weight of 2.55, 2.57, 2.59 and 2.62 g, respectively, which were all on par with each other (Table I).

TABLE I

Effect of NSKE on rearing parameters of silkworm (PM × CSR_2) under laboratory conditions

No. of days after spray of 4% NSKE	Larval mortality during fifth instar (%)	Larval weight during fifth instar (g)	Fifth instar larval duration (days)
16th day	36.24 e	2.55 a	7.68
17th day	29.92 d	2.57 ab	7.74
18th day	26.45 cd	2.59 abc	7.75
19th day	22.60 c	2.62 abc	7.69
20th day	26.15 cd	2.65 cd	7.68
21st day	8.41 b	2.67 d	7.69
22nd day	2.11 a	2.63 bcd	7.73
Control	0.00 a	2.64 bcd	7.74
F – test	*	*	NS
S.Em±	1.98	0.02	-
C.D. (Pd≤0.05)	5.94	0.07	-
C.V. (%)	16.91	1.41	-

Note: *5 per cent level of significance; NS: Non-significant
Means followed by the same alphabet are not significantly different

Fifth instar larval duration (days) : No significant differences could be observed between fifth instar larval duration when worms were fed with mulberry leaves treated with NSKE after 16th, 17th, 18th, 19th, 20th, 21st and 22nd days after spray, with the corresponding fifth instar durations being 7.68, 7.74, 7.75, 7.69, 7.68 7.69 and 7.73 days, respectively, (Table I).

Effect of NSKE on cocoon parameters of silk worm (PM×CSR$_2$) under laboratory conditions

Cocoon weight (g) : The worms fed with NSKE treated mulberry leaves on 20th, 21st and 22nd days after spray recorded significantly higher cocoon weights of 1.96, 1.98 and 1.95 g, respectively and all these three treatments were on par with untreated control (1.96 g). However, worms fed with NSKE treated leaves fed at 16th, 17th, 18th and 19th days after spray recorded cocoon weights of 1.80, 1.84, 1.87 and 1.91 g, respectively, which differed significantly from that recorded in untreated control (Table II).

Pupal weight (g) : The worms fed with NSKE treated leaves on 20th, 21st and 22nd days after spray recorded significantly maximum pupal weights of 1.57, 1.59 and 1.58 g, respectively and these three treatments were on par with untreated control (1.58 g). However, worms fed with NSKE treated leaves on 16th, 17th, 18th and 19th day after spray recorded significantly lower pupal weights of 1.50, 1.52, 1.54 and 1.56 g, respectively. Notably, worms fed with leaves treated after 19th and 20th days after spray were also found to be on par with each other (Table II).

Shell weight (g) : The worms fed with NSKE treated leaves on 20th, 21st and 22nd days after spray recorded significantly higher shell weight of 0.38, 0.39 and 0.38 g, respectively as compared to worms fed with NSKE treated leaves after 16th, 17th, 18th, 19th day after spray which recorded significantly lower shell weights of 0.30, 0.32, 0.33 and 0.35 g, respectively. However, shell weights in worms fed with treated leaves on 19th and 20th days after spray were found to be on par with each other (Table II).

Shell ratio (%) : The worms fed with NSKE treated leaves on 18th, 19th, 20th, 21st and 22nd day after spray recorded significantly higher shell ratios of 17.66, 18.14, 19.58, 19.58 and 19.31 per cent, respectively and these five treatments were found to be on par with untreated control (19.38%). Further, feeding the worms with NSKE treated leaves on 16th day (16.62%), 17th day (17.28%), 18th day (17.66%) and

TABLE II

Effect of NSKE on cocoon parameters of silkworm (PM × CSR$_2$) under laboratory conditions

No. of days after spray of 4% NSKE	Cocoon weight (g)	Pupal weight (g)	Shell weight (g)	Shell ratio (%)
16th day	1.80 e	1.50 e	0.30 c	16.62 c
17th day	1.84 de	1.52 de	0.32 c	17.28 bc
18th day	1.87 cd	1.54 cd	0.33 bc	17.66 abc
19th day	1.91 bc	1.56 bc	0.35 abc	18.14 abc
20th day	1.96 a	1.57 ab	0.38 ab	19.58 a
21st day	1.98 a	1.59 a	0.39 a	19.58 a
22nd day	1.95 ab	1.58 ab	0.38 ab	19.31 ab
Control	1.96 a	1.58 ab	0.38 ab	19.38 ab
F – test	*	*	*	*
S.Em±	0.01	0.01	0.02	0.65
C.D. (Pd≤0.05)	0.04	0.02	0.05	1.96
C.V. (%)	1.26	0.81	7.90	6.16

Note: *5 per cent level of significance; Means followed by the same alphabet are not significantly different

19^{th} day (18.14%) were also found to be on par with each other (Table II) as far as their shell ratios were concerned.

The study thus demonstrated that the worms fed with NSKE 4 per cent treated mulberry leaves on 22^{nd} day after spray was the safest to the silkworm health by virtue of registering significantly lowest silkworm mortality, significantly higher cocoon weight, pupal weight, shell weight and shell ratio. On the contrary, feeding the silkworm with NSKE treated leaves after 16, 17, 18 and 19 days after spray were found to be unsafe for most of the silkworm rearing parameters.

Similar findings have been reported by Seelan (1999) who studied the residual toxicity of insecticides and botanicals against *B. mori* by drawing leaves from the plots on 1^{st}, 3^{rd}, 5^{th}, 7^{th} and 10^{th} day after spray and found that Anona Seed Kernel Extract (ASKE), *Vitex negundo* leaf extract and untreated leaves fed worms showed maximum (90%) effective rate of rearing (ERR), followed by Neem oil, Pongamia oil and NSKE (75 to 85%) and the lowest (50%) was in Dichlorvos and Malathion treated leaves. However, when the silkworms fed with leaves treated with plant products, moderate antifeedant effect was observed and the larvae consumed less quantity of leaves. But, it did not register higher antifeedant effect on tenth day after spray when compared to Dichlorvos and Malathion. The ERR was also significantly higher in ASKE, followed by *Vitex negundo* leaf extract and untreated control. The phosalone treated leaves caused cent per cent mortality of the silkworm. The ERR was less than 50 per cent in Dichlorvos and Malathion treatments. Neem products (Neem oil 3.0 %, NSKE 5 %) recorded the ERR of 80 per cent. Similarly, Gandhi Gracy (2000) reported that all the plant products, *Vinca rosea* leaf extract (10%), Illupai oil (3%), Neem oil (3.0%) and Notchi leaf extract (10%) were safer to *B. mori* from seventh day after spray. However, Baskar (1997) reported that Dichlorvos and Malathion were found to be non-toxic to silkworm on tenth day after spraying, whereas, larval mortality due to botanicals (NSKE, Neem oil, Pongam oil) were not observed even on the fifth day after spray. The total mortality of the silkworms were not observed in the NSKE 4 per cent sprayed leaves. Gandhi Gracy (2000) reported that single cocoon weight was higher in healthy leaves fed worms (1.466 g) and this was followed by *Vinca rosea* (1.398 g), Notchi (1.352 g), Illupai oil (1.337), Propoxur (1.145), Neem oil (1.272 g), Dichlorvos (0.847 g) and Carbaryl (0.626 g) treated leaves, respectively. Shell weight was recorded higher in healthy leaves fed silkworms(control) (0.246 g) and this was followed by *Vinca rosea*, Illupai oil, Notchi oil, Neem oil, Propoxur, Dichlorvos and Carbaxyl with mean shell weight of 0.221, 0.215, 0.215, 0.192, 0.140, 0.108 and 0.072 g, respectively.

Effect of imposing IPM module under field conditions on rearing parameters of silkworm (PM×CSR$_2$) under laboratory conditions

Fifth instar larval weight (g) : The results revealed that there were no significant differences in the larval weights when worms were fed with mulberry leaves harvested from plots in which the IPM module and chemical control treatments were imposed. However, larval weight was found to be higher when fed with leaves from chemical control plot (3.20 g), followed by those fed with leaves from absolute control (3.18 g) and it was least in case of IPM module imposed plots (3.08 g) (Table III).

Cocoon weight (g) : Significant differences were observed between the cocoon weights of worms fed with mulberry leaves harvested from plots in which the different treatments were imposed. Cocoon weight was significantly higher in chemical control treatment (1.89 g), followed by absolute control (1.88 g), both of which were on par with each other. However, least cocoon weight was recorded when worms were fed with leaves from IPM module plot (1.84 g) (Table III).

Pupal weight (g) : The results revealed that the worms fed with mulberry leaves from different treatments recorded significant differences in the pupal weight. Maximum pupal weight was recorded in case of chemical control treatment (1.56 g), followed by absolute control (1.55 g), while, significantly minimum pupal weight (1.53g) was recorded in IPM module treatment (Table III). Moreover, all these three treatments were statistically on par with each other.

Shell weight (g) : Significant differences were observed in the shell weight of the worms when they

TABLE III

Effect of imposing IPM module under field conditions on rearing parameters of silkworm ($PM \times CSR_2$) under laboratory conditions

Treatment	Fifth instar larval weight (g)	Cocoon weight (g)	Pupal weight (g)	Shell weight (g)	Shell ratio (%)
IPM module	3.08	1.84 [a]	1.53 [a]	0.31 [a]	16.82 [a]
Chemical control	3.20	1.89 [b]	1.56 [a]	0.34 [a]	17.96 [a]
Absolute Control	3.18	1.88 [b]	1.55 [a]	0.33 [a]	17.41 [a]
F-test	NS	*	*	*	*
S.Em±	-	0.01	0.01	0.02	0.01
C.D. ($Pd \leq 0.05$)	-	0.04	0.04	0.04	1.69
C.V. (%)	-	0.27	0.78	0.81	5.89

Note: *5 per cent level of significance; NS: Non-significant;
Means followed by the same alphabet are not significantly different

were fed with mulberry leaves harvested from treated plots. Maximum shell weight was recorded in case of chemical control (0.34 g) followed by absolute control (0.33 g) and minimum shell weight was recorded in case of IPM module (0.31 g) (Table III). Further, all these three treatments were statistically on par with each other.

Shell ratio (%) : There was significant differences between the treatments with respect to shell ratio of cocoons obtained from worms fed with mulberry leaves from the three different treatments. However, maximum shell ratio was recorded in chemical insecticide (DDVP) treated plots (17.96%), followed by those obtained in absolute control treatment (17.41%). IPM module treated plots recorded minimum shell ratio (16.82%) (Table III).

The earlier work by Harish Babu (2015) has revealed that the cost of IPM intervention against *D. pulverulentalis* in mulberry (*i.e.,* spray of NSKE (4%), followed by *DP*NPV (@27.65×10^5 PIBs/ml) and release of egg parasitoid, *T. chilonis* at 15, 25 and 35 days after pruning, respectively was found to be ₹ 350 per acre/crop in comparison with the chemical control (₹ 500 per acre / crop). The net gain in case of IPM module was found to be ₹ 2,500 per acre / crop as compared to the chemical control (₹ 2,350 per acre / crop). Therefore, the cost : benefit ratio was found to be maximum in case of IPM module (1:7.14) as compared to the chemical control (1:4.70). In continuation of these findings, the present results reveal that IPM module was more or less on par with both chemical control and untreated control, implying that it did not have any adverse effects on the health of *B. mori*.

Likewise, earlier workers like Muthuswami (2004) and Anonymous (2008) have also reported that an IPM module consisting of irrigation of mulberry garden (on the day of pruning), release of *Tetrastichus howardii* @ 50,000 / ha (one day after pruning) and *T. chilonis* @ 5cc / ha (10 days after pruning), spraying of dichlorvos @ 1ml / l (30 days after pruning), mechanical clipping and burning of affected shoots (40 days after pruning) was effective in leaf roller management). Similarly, demonstration of IPM against *D. pulverulentalis* was taken up in the farms of Karnataka by adopting the IPM package *viz*., spray of DDVP, release of egg parasitoid and pupal parasitoid resulting in suppression of pest incidence by 47 to 53 per cent over control. On-farm trails of IPM against leaf webber by Regional Sericultural Research Station, Chamarajanagara and Regional Sericultural Research Station, Salem indicated 2.54 to 7.35 per cent and 3.00 to 16.22 per cent reduction in the pest incidence, respectively (Anonymous, 2000). Morever, Gururaj and Choudhury (2001) reported that percentage of reduction was significantly highest in IPM (79 %) followed by chemical control (67 %) and least in physical control (35 %) and they opined that IPM module can be advocated for management of leaf roller of mulberry.

Velavan *et al*. (2001) reported that when the parasitoids, *Tetrastichus howardii* and *Trichogramma chilonis* were integrated with spray of propoxur (0.1%), the larval population of *D. pulverulentalis* was reduced to 2.46 larvae per plant as against 60.37 in the control. Integration of natural enemies, egg parasitoids at 5 cubic centimeter per ha (30 days after pruning) and pupal parasitoid at 0.25 million adults per ha (53 days after pruning) with chemical propoxur 20 EC at one per cent (45 days after pruning) showed significant reduction in leaf webber larval population by 61.38 per cent as well as shoot damage by 41.39 per cent over control. Similarly, Manjunath Gowda *et al*. (2005) reported that clipping and burning of affected plant parts and spraying 0.2% Dichlorvos (76% EC) reduces the larval population of leaf roller. Installation of light traps and providing bird perches in mulberry garden has been recommended for the suppression of the pest. Other insecticides like Monocrotophos (0.072%), NSKE and Neem oil have also been found to be effective against the pest.

None of these earlier reports have brought the comparison between IPM module and chemical control methods of *D. pulverulentalis* on the rearing performance of *B. mori* in relation to their safety. The present findings revealed that NSKE (4%) treated mulberry leaves can be fed to silkworm by adopting a safety period of 22 days. Similarly, IPM module treated mulberry leaves also have no adverse impact on the pupal weight, shell weight and shell ratio of *B. mori*.

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