**EVALUATING THE INFLUENCE OF DIAFENTHIURON 50 % WP ON ECONOMIC TRAITS OF SILKWORM, *Bombyx mori* L.**

**ABSTRACT**

An experiment was conducted to evaluate the impact of the dual-acting molecule Diafenthiuron 50% WP, with a specific focus on its compatibility with cocoon traits in silkworms. In present investigation, mulberry leaves were sprayed with Diafenthiuron 50 % WP at a concentration of 1 g/L and after 15 and 20 days of post-spray fed to silkworms larvae of 3rd instar and onwards. The results showed that the batch of larvae fed with mulberry leaves harvested at 20 days after spraying exhibited significantly better cocoon characteristics. These included a higher percentage of good cocoons (98.12 %), increased cocoon weight (1.57 g) and shell weight (0.321 g), improved cocoon shell ratio (19.98 %), longer filament length (1031.07 m), higher filament weight (0.300 g), optimal denier (2.56) and fewer defective cocoons (1.87 %). These findings suggest that the toxicity of Diafenthiuron 50 % WP decreases over time with reduced adverse effects observed at 20 days post-application compared to 15 days after spray.

**Key words:** Diafenthiuron; Mulberry; Silkworm; Safety period; Parental breeds

**1. INTRODUCTION**

Silk has long been admired for its superior qualities such as natural sheen, softness and elegance that remain unmatched by any other textile fibre to this day. The domesticated silkworm, *Bombyx mori* L., which feeds exclusively on mulberry leaves, plays a key role in silk production. However, mulberry plants are often attacked by a variety of insect pests, necessitating the use of pesticides for their control.

While these pesticides help to protect the mulberry crop but their residues can adversely affect silkworm health and development. Ingesting contaminated leaves can impair larval growth and compromise important cocoon characteristics, ultimately impacting the quality and yield of silk. Yokoyama (1962) highlighted that pesticide residues on mulberry leaves pose a significant threat to silkworms and the silk they produce. Similarly, field studies in India have shown reduced cocoon yield and silk quality when silkworms are fed with pesticide treated mulberry leaves (Narasimhanna, 1988).

As an insect, the silkworm is highly sensitive to many of the chemical agents used for pest management. Exposure to insecticides either through direct contact or by consuming treated leaves can be detrimental, even at sub-lethal concentrations. Therefore, pesticide application in sericulture demands extreme caution with strict adherence to safety measures. It is essential to observe an adequate pre-harvest interval to ensure that mulberry leaves are safe for larval feeding. For instance, dichlorvos a commonly used contact insecticide, has been found to be highly toxic to silkworms when leaves are fed shortly after application. To avoid such toxicity, a minimum safety interval is recommended before harvesting the leaves. In light of this, the current study was initiated to evaluate the effects of diafenthiuron 50 % WP on economic traits of silkworm, *B. mori*.

**2. MATERIALS AND METHODS**

The experiment was conducted during the year 2023-2024, at the Department of Sericulture, UAS, GKVK, Bengaluru with well-established mulberry garden of V1 variety. The parental breeds namely PM, CSR2, FC1 and FC2 were reared to assess the impact of chemical used in mulberry for management of thrips and mites.

The entire rearing room and appliances were disinfected by following standard procedure (Dandin and Giridhar, 2014). The rearing room was kept air tight for 24 hours and then the room was kept open and used for rearing. The chawki silkworms were reared on the leaves harvested from control plots, from third instar onwards the larvae were fed with mulberry leaves of treatment plots harvested after 15 and 20 days of chemical post spray. A total of 150 larvae were transferred to each experimental tray in three replications after 30 minutes of initial feeding along with the mulberry leaves. In order to assess extent of toxicity of pesticide to silkworm and to determine the safe period of the chemical post spray. The impact of feeding chemical sprayed leaf on the cocoon parameters such as good cocoons (%), defective cocoons (%), cocoon weight (g), shell weight (g), cocoon shell ratio (%), average filament length (m), non-breakable filament length (m), filament weight (g) and denier of the parental breeds was observed for recording the data and that were analysed using Factorial- CRD for testing of significance by Fisher’s method of analysis of variance as outlined by Sundaraaj *et al*., 1972. The level of significance used in the F-test was P = 0.05. The critical difference (CD) values were computed to compare significance of the treatments.

**Table 1: Treatment details**

|  |  |
| --- | --- |
| **Treatments** | **Description** |
| T1 | PM (diafenthiuron 50 % WP @ 1 g/l at 15 DAS) |
| T2 | CSR2 (diafenthiuron 50 % WP @ 1 g/l at 15 DAS) |
| T3 | FC1 (diafenthiuron 50 % WP @ 1 g/l at 15 DAS) |
| T4 | FC2 (diafenthiuron 50 % WP @ 1 g/l at 15 DAS) |
| T5 | PM (diafenthiuron 50 % WP @ 1 g/l at 20 DAS) |
| T6 | CSR2 (diafenthiuron 50 % WP @ 1 g/l at 20 DAS) |
| T7 | FC1 (diafenthiuron 50 % WP @ 1 g/l at 20 DAS) |
| T8 | FC2 (diafenthiuron 50 % WP @ 1 g/l at 20 DAS) |
| T9 | PM (Control) |
| T10 | CSR2 (Control) |
| T11 | FC1 (Control) |
| T12 | FC2 (Control) |

DAS: Days after spray; \*No chemical spray was used in the control treatment plots of mulberry for management of thrips and mites

**Observations recorded**

**Good cocoons (%)**

The per cent good cocoons among the cocoons spun in each treatment was calculated by using the formula,

Good cocoons (%) = × 100

**Defective cocoons (%)**

The defective cocoons percentage wascalculated by using the formula,

Defective cocoons (%) × 100

**Cocoon weight (g)**

A total of ten cocoons were selected randomly from each replication in all treatments on fifth day after spinning and weighed separately. The average weight of cocoons was computed as,

Cocoon weight (g) = × 100

**Cocoon shell weight (g)**

The cocoons were cut open, pupae and exuviae were separated and the average shell weight was computed separately for each treatment as below

Cocoon shell weight (g) = × 100

**Cocoon shell ratio (%)**

Cocoon shell ratio (CSR) is the proportion of shell weight to cocoon weight expressed in percentage and was calculated as,

Cocoon shell ratio (%) = × 100

**Average filament length (m)**

A sample of ten cocoons was randomly drawn from each replication and cooked separately in boiling water for two to three minutes to soften the sericin layer. These cooked cocoons were reeled on an eprouvette. The length of silk filament was determined using formula,

L = R ×1.125 m

Where, L = Length of the silk filament (m)

R = Number of revolutions

1.125 m = Circumference of the reel

**Non-breakable filament length (NBFL)(m)**

NBFL represents the average length of raw silk filament that can be unwound from a cocoon without any break. It was calculated by the formula,

NBFL (m) =

**Filament weight (g)**

The raw silk reeled from each cocoon was weighed separately replication wise from each treatment and the average was computed to obtain average filament weight.

**Denier**

The filament thickness was measured and expressed in terms of denier that was estimated by the formula:

Denier = × 9000

**3. RESULTS AND DISCUSSION**

**3.1 Good Cocoons (%)**

The quality of cocoons is vital factor for producing fine silk. Ideal cocoons are dense, uniform in shape and size, free from stains or damage ensuring strong and lustrous silk fibres. The percentage of good cocoons varied significantly among the different parental breeds. The multivoltine pure breed, PM exhibited the highest percentage of good cocoons (98.54 %) followed by FC1 (97.69 %). While, the bivoltine pure breed, CSR2 had lower percentage of good cocoons (96.48 %). These variations highlight that PM and FC1 are more effective at producing high-quality cocoons compared to CSR2. The highest number of good cocoons was achieved with the control (98.38 %) which was on par with the larvae fed with leaves treated with diafenthiuron 50 % WP at 20 days after spray that was 98.12 per cent and it was lowest in the larval batch fed with the leaves sprayed at 15 days after spray (95.61 %). The interaction between parental breeds and the duration of pesticide application was found to be non-significant (Table 2).

**3.2 Defective Cocoons (%)**

Among the parental breeds used in the present experiment a significant difference was observed regarding percentage of defective cocoons. The bivoltine pure breed, CSR2 recorded highest percentage of defective cocoons (3.51 %) while, PM had the lowest percentage of defective cocoons (1.45 %). The duration after spray also significantly affects the defective cocooning percentage, when the silkworms were fed with diafenthiuron 50 % WP sprayed mulberry leaves. The highest percentage of defective cocoons was recorded in 15 DAS (4.38 %) and lowest was found in 20 DAS (1.87 %) which is on par with control (1.61 %). The interaction between breeds and duration of the spray showed no significant difference concerning to percentage of defective cocoons, indicating that the effect of the spray duration on per cent good cocoon was consistent across all breeds (Table 2).

**Table 2: Effect of feeding mulberry leaves treated with diafenthiuron 50 % WP at different days after spray on good and defective cocoons of parental breeds of silkworm, *B. mori***

|  |  |  |
| --- | --- | --- |
| **Treatments** | **Good cocoons (%)** | **Defective cocoons (%)** |
| **Breeds (B)** | | |
| B1: PM | 98.54 | 1.45 |
| B2: CSR2 | 96.48 | 3.51 |
| B3: FC1 | 97.69 | 2.30 |
| B4: FC2 | 96.77 | 3.22 |
| F-test | \* | \* |
| S.E. m± | 0.367 | 0.367 |
| C.D. 0.05 | 1.079 | 1.072 |
| **Safety period (S)** | | |
| S1: 15 DAS | 95.61 | 4.38 |
| S2: 20 DAS | 98.12 | 1.87 |
| S3: Control | 98.38 | 1.61 |
| F-test | \* | \* |
| S.E. m± | 0.318 | 0.318 |
| C.D. 0.05 | 0.934 | 0.920 |
| **Interaction (B×S)** | | |
| B1S1 | 97.97 | 2.02 |
| B1S2 | 98.66 | 1.33 |
| B1S3 | 98.99 | 1.00 |
| B2S1 | 93.59 | 6.40 |
| B2S2 | 97.91 | 2.08 |
| B2S3 | 97.93 | 2.06 |
| B3S1 | 95.81 | 4.18 |
| B3S2 | 98.63 | 1.36 |
| B3S3 | 98.65 | 1.34 |
| B4S1 | 95.09 | 4.90 |
| B4S2 | 97.27 | 2.72 |
| B4S3 | 97.95 | 2.04 |
| F-test | NS | NS |
| S.E. m± | - | - |
| C.D. 0.05 | - | - |

\*Significant at 0.05; NS: Non-significant; DAS: Days after spray; The mentioned values represent the average of two rearing.

**3.3 Cocoon weight (g)**

Cocoon is the economically valuable part in silkworm rearing,Significant differences in cocoon weight were observed among the all the parental breeds used in the study. The heaviest cocoons were found in FC2 (1.71g) followed by FC1 (1.57 g). While, PM had the lightest cocoons, weighing 1.22 g. These difference in cocoon weight was attributed to the distinct characteristics of each breed. Cocoon weight also recorded significant difference when silkworms were fed with diafenthiuron 50 % WP sprayed mulberry leaves harvested at 15 and 20 DAS. The maximum cocoon weight of 1.57 g was observed at 20 days after spray followed by 15 DAS and the control group recorded least cocoon weight of 1.44 g. The interaction between parental breeds and duration of spray on cocoon weight showed no significant difference indicating the consistency of residual toxicity of the chemical across the parental breeds of *B. mori* silkworm with respect to cocoon weight (Table 03).

The multivoltine though less productive, are known to have high intensity of sturdiness and can withstand the adverse rearing conditions compared to bivoltine (Kumaresan *et al*., 2012). The same was observed in the present study where the residual toxicity of the chemical had less effect on multivoltine, PM than the bivoltine breeds. The extended safety period resulted in the improved cocoons traits. Hence, following a safe waiting period is must before feeding the silkworms with mulberry leaves from chemical sprayed gardens is imminent as observed in the study conducted by Kalpana *et al*. (2022) documented a significant improvement in larval and cocoon weight upon feeding silkworms on mulberry leaves treated with abamectin 1.9 % EC and diafenthiuron 50 % WP. Misra *et al*. (2003) also found improved cocoon weight when larvae were fed with mulberry leaves treated with dimethoate (0.2 %) and methyl parathion (0.2 %), after a notable reduction in residual effects.

**3.4 Shell weight (g)**

The parental breeds showed significant variation for shell weight also. The maximum shell weight was recorded in FC2, which had shell weight of 0.384 g and the multivoltine breed, PM recorded the minimum shell weight of 0.142 g, respectively (Table 03). The shell weight also varied significantly for different duration after spray. The shell weighed heavier when the

**Table 3: Effect of feeding mulberry leaves treated with diafenthiuron 50 % WP at different days after spray on cocoon weight, shell weight and cocoon shell ratio of parental breeds of silkworm, *B. mori***

|  |  |  |  |
| --- | --- | --- | --- |
| **Treatments** | **Cocoon weight (g)** | **Shell weight (g)** | **Cocoon shell ratio (%)** |
| **Breeds (B)** | | | |
| B1: PM | 1.22 | 0.142 | 11.62 |
| B2: CSR2 | 1.47 | 0.329 | 22.32 |
| B3: FC1 | 1.57 | 0.334 | 21.28 |
| B4: FC2 | 1.71 | 0.384 | 22.45 |
| F-test | \* | \* | \* |
| S.E. m± | 0.012 | 0.003 | 0.202 |
| C.D. 0.05 | 0.035 | 0.009 | 0.595 |
| **Safety period (S)** | | | |
| S1: 15 DAS | 1.47 | 0.291 | 19.36 |
| S2: 20 DAS | 1.57 | 0.321 | 19.98 |
| S3: Control | 1.44 | 0.280 | 18.90 |
| F-test | \* | \* | \* |
| S.E. m± | 0.010 | 0.003 | 0.175 |
| C.D. 0.05 | 0.030 | 0.007 | 0.561 |
| **Interaction (B×S)** | | | |
| B1S1 | 1.19 | 0.138 | 11.55 |
| B1S2 | 1.29 | 0.156 | 12.02 |
| B1S3 | 1.19 | 0.134 | 11.31 |
| B2S1 | 1.44 | 0.320 | 22.24 |
| B2S2 | 1.55 | 0.355 | 22.89 |
| B2S3 | 1.42 | 0.310 | 21.83 |
| B3S1 | 1.54 | 0.328 | 21.28 |
| B3S2 | 1.65 | 0.364 | 22.01 |
| B3S3 | 1.51 | 0.311 | 20.51 |
| B4S1 | 1.69 | 0.379 | 22.39 |
| B4S2 | 1.77 | 0.407 | 22.98 |
| B4S3 | 1.66 | 0.366 | 21.96 |
| F-test | NS | NS | NS |
| S.E. m± | - | - | - |
| C.D. 0.05 | - | - | - |

\*Significant at 0.05; NS: Non-significant; DAS: Days after spray; The mentioned values represent the average of two rearing.

silkworms were fed with diafenthiuron 50 % WP sprayed leaves at 20 days after spray (0.321 g) followed by 15 DAS. The lowest shell weight of 0.280 g was recorded in control group where treatment was not imposed (Table 02).There was no significant difference found among the interaction between breeds and the duration of spray on shell weight, indicating that the influence of spray timing on shell weight does not vary across different breeds (Table 03).

The cocoon shell weight is a component of total cocoon weight. Hence any variation in cocoon weight results in variation in the shell weight also. Further, varied toxicity level in respective treatments influence the quality of leaf and hence the cocoons spun by the worms feeding on such leaves. These findings are in accordance with the observations reported by Muthuswami *et al*. (2010). Feeding silkworm with buprofezin 25 SC (@ 1 ml/l) treated leaves harvested at 20, 30 and 40 DAS resulted in decline in the shell weight (Maria *et al.,* 2000). However, feeding of silkworms with insecticide sprayed mulberry harvested after safe waiting period showed a significant improvement with respect to larval weight, cocoon weight and shell weight (Kariappa and Narasimhanna, 1978).

**3.5 Cocoon shell ratio (%)**

A good cocoon shell ratio represents good quality cocoons. The cocoon shell ratio, significantly differ among the parental breeds. The cocoons shell ratio recorded maximum in bivoltine double hybrid, FC2 (22.45 %) and the multivoltine pure breed PM had the lowest cocoon shell ratio of 11.62 per cent (Table 02).The duration of pesticide spray also had a significant impact on the cocoon shell ratio. The highest cocoon shell ratio was observed at the duration of 20 days after spray (19.98 %) and the lowest cocoon shell ratio of 18.90 per cent was recorded in untreated control (Table 03).The interaction effects were non-significant between duration of spray and parental breeds, indicating that the effect of spray timing on the cocoon shell ratio was consistent across all the parental breeds used in the experiment (Table 3).

Anitha *et al*. (2016) recorded the minimum shell ratio of 13.73 and 13.70 g during first and second rearing when silkworms fed with chlorantraniliprole sprayed leaves at 10 DAS. Similar observations also made by Roxelle *et al*. (2013). Sunil Kumar *et al*. (2016) reported that the novel insecticide chlorantraniliprole was found to have a residual effect on silkworms even after 15 days after spraying (DAS), leading to the production of poor-quality cocoons. However, previous studies by Kalpana *et al*. (2022) and the current research also shown that cocoon parameters such as cocoon weight, shell weight and cocoon shell ratio were higher at 20 DAS compared to 15 DAS. This suggests a significant reduction in residual toxicity with an extended safety period, resulting in improved larval and cocoon characteristics.

**3.6 Average filament length (m)**

The average filament length showed significant difference among parental breeds of silkworm. The longest filament length was recorded in the bivoltine hybrid, FC2 (1225.03 m) and the multivoltine pure breed, PM had the shortest filament length (533.21m) (Fig.01). Irrespective of parental breeds, the duration of the spray also significantly affected the average filament length when the silkworms were fed with diafenthiuron 50 % WP sprayed mulberry leaves during different intervals of spray. The longest filament length was observed at 20 DAS (1031.07 m) followed by 15 DAS (1001.43 m). The control group had shorter filament length of 978.44 m (Fig. 01).There was no significant difference found among the interaction between breeds and duration of the spray concerning to the filament length (Fig .01).

The residual effects of insecticides led to the construction of poor-quality cocoons, particularly thin-shelled ones, thereby reducing silk production and causing a noticeable decrease in the silk filament length when silkworms were fed with pesticide-sprayed mulberry leaves (Roxelle *et al.,* 2013; Anitha, 2015; Sunil Kumar *et al.,* 2016). However, the filament length showed a significant increase when the toxicity levels were reduced, highlighting the importance of careful harvesting of leaves after the specified safety period, as evidenced in the present study.

**3.7 Non-breakable filament length (m)**

A significantdifference was noticed for non-breakable filament length among the parental breeds used in the study. The bivoltine hybrid, FC2 exhibited the longest non- breakable filament length of 1159.72 m. The multivoltine pure breed, PM had the shortest non-breakable filament length (492.97 m) (Fig. 01). The observations regarding Non-breakable filament length (NBFL) differed significantly at 15 and 20 DAS. The longest non-breakable filament length was observed at 20 days after spray, which was about 988.20 m followed by the duration of 15 days after spray. The shortest non-breakable filament length was recorded in control (918.64 m) (Fig. 1).

The interaction between the parental breeds and duration of spray regarding non-breakable filament length was found to be non-significant, indicating that the influence of safety duration on filament length was consistent across silkworm breeds.

**Figure 1: Effect of feeding mulberry leaves treated with diafenthiuron 50 % WP at different days after spray on average and non-breakable filament length of parental breeds of silkworm, *B. mori***

**B1**:PM, **B2**:CSR2, **B3**:FC1, **B4**:FC2

**S1**: 15 DAS, **S2**: 20 DAS, **S3**: Control

**B1S1**:PM (15 DAS), **B1S2**:PM (20 DAS), **B1S3**:PM (Control)

**B2S1**:CSR2 (15 DAS), **B2S2**:CSR2 (20 DAS), **B2S3**:CSR2 (Control)

**B3S1**:FC1 (15 DAS), **B3S2**:FC1 (20 DAS), **B3S3**:FC1 (Control)

**B4S1**:FC2 (15 DAS), **B4S2**:FC2 (20 DAS), **B4S3**:FC2 (control)

**DAS**: days after spray

**Table 4: Effect of feeding mulberry leaves treated with diafenthiuron 50 % WP at different days after spray on filament weight and denier of parental breeds of silkworm, *B. mori***

|  |  |  |
| --- | --- | --- |
| **Treatments** | **Filament weight (g)** | **Denier** |
| **Breeds (B)** | | |
| B1: PM | 0.123 | 2.08 |
| B2: CSR2 | 0.327 | 2.52 |
| B3: FC1 | 0.319 | 2.63 |
| B4: FC2 | 0.363 | 2.67 |
| F-test | \* | \* |
| S.E. m± | 0.002 | 0.024 |
| C.D. 0.05 | 0.014 | 0.060 |
| **Safety period (S)** | | |
| S1: 15 DAS | 0.281 | 2.46 |
| S2: 20 DAS | 0.300 | 2.56 |
| S3: Control | 0.269 | 2.41 |
| F-test | \* | \* |
| S.E. m± | 0.002 | 0.020 |
| C.D. 0.05 | 0.006 | 0.060 |
| **Interaction (B×S)** | | |
| B1S1 | 0.121 | 2.06 |
| B1S2 | 0.133 | 2.19 |
| B1S3 | 0.116 | 2.00 |
| B2S1 | 0.328 | 2.52 |
| B2S2 | 0.344 | 2.58 |
| B2S3 | 0.310 | 2.46 |
| B3S1 | 0.316 | 2.61 |
| B3S2 | 0.337 | 2.70 |
| B3S3 | 0.304 | 2.60 |
| B4S1 | 0.358 | 2.65 |
| B4S2 | 0.385 | 2.77 |
| B4S3 | 0.346 | 2.58 |
| F-test | NS | NS |
| S.E. m± | - | - |
| C.D. 0.05 | - | - |

\*Significant at 0.05; NS: Non-significant; DAS: Days after spray; The mentioned values represent the average of two rearing.

**3.8 Filament weight (g)**

The filament weight varied significantly among the parental breeds used in the experiment. The highest filament weight was recorded in the bivoltine hybrid, FC2 (0.363 g) followed by bivoltine pure breed, CSR2 (0.327 g) while it was least in multivoltine pure breed, (0.123 g) (Table 4). In spite of parental breeds, the duration of spray also had a significant effect on filament weight. When the silkworms were fed with diafenthiuron 50 % WP sprayed leaves at 20 DAS had constructed quality cocoons with maximum filament weight of 0.300 g followed by 15 DAS (0.281 g) and least filament weight was recorded in untreated control that was 0.269 g (Table 4). The interaction between parental breeds and duration after spray regarding filament weight was found to be non-significant. However, the highest filament weight was recorded in the bivoltine hybrid, FC2 at 20 DAS (0.385 g) and it was recorded least in control of PM (0.116 g) (Table 04).

**3.9 Denier**

The result on denier of silkworm as influenced by duration of spray and their interaction during successive rearing period are presented in the (Table 4).

For denier, significant difference was observed across the parental breeds used in the study. The lowest denier was observed in the multivoltine pure breed, PM (2.08) and the highest was recorded in the bivoltine hybrid, FC2 (2.67) followed by FC1 (2.63) (Table 04).The duration after spray also had a significant impact on denier. The highest denier was recorded in 20 days after spray (2.56) followed by 15 DAS (2.46). The lowest denier was observed in control (2.41) (Table 4).

**4. CONCLUSION**

The study on effect of Diafenthiuron 50 % WP sprayed mulberry leaves on the economic traits of the silkworm, *Bombyx mori* L., reveals a clear correlation between the post-spray interval and silkworm performance. When the 3rd instar onwards larvae were fed with the mulberry leaves harvested at 15 days after spray, a noticeable negative impact on cocoon traits was observed. This suggests that residual toxicity from Diafenthiuron 50 % WP persists in the leaves at this stage, adversely affecting larval development and cocoon formation. However, when the same pesticide-treated leaves were harvested after a 20 days post-spray interval, the cocoon traits improved significantly, indicating that the harmful residues had likely degraded to non-toxic levels by this time. This demonstrates that a minimum waiting period of 20 days post-application is necessary to ensure the leaves are safe for silkworm consumption and do not compromise cocoon quality.

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