**Safety Assessment of Diafenthiuron 50% WP on the Reproductive Traits of *Bombyx mori* L.**

**Abstract**

The impact of pesticides on beneficial insects like silkworms has raised concerns in sericulture, as even sublethal doses of toxic substances can negatively affect silkworm reproduction. Consequently, it is crucial to evaluate the compatibility of chemicals for pest management in mulberry cultivation. In this study, the impact of diafenthiuron 50% WP-sprayed mulberry leaves on the reproductive performance of *Bombyx mori* was assessed. The results demonstrated that diafenthiuron, when applied to mulberry leaves at 20 days after spraying (DAS), was found safer to the silkworm’s parental generation, as evidenced by enhanced reproductive traits, including maximum fecundity (530.13 No./moth), hatching rate (97.03%), weight of 100 eggs (57.73 mg), egg recovery (54.66 g/kg of cocoons), and minimal egg retention in the ovary (4.11%), dead eggs (2.32%), and unfertilized eggs (0.64%).

Key words: Diafenthiuron, Mulberry, Silkworm, Parental breeds

1. **Introduction**

Sericulture is an integral part of the rural economy in an agrarian country like India. Globally, India stands second in silk production next only to China, of the four kinds of silks produced in India, mulberry silk comprises 70 % (Awquib *et al*., 2016) and the silkworm, *Bombyx mori* a completely domesticated species depends solely on mulberry leaf for its growth and development. The cocoon productivity and profitability mainly depend on quality and quantity of mulberry foliage. However, mulberry is infested by several pests and pathogens due to presence of evergreen lush foliage. These pests affect the growth of mulberry and cause considerable damage to the plant and loss in the yield. Management of these pests is imperative for sustained productivity of quality silk, which necessitates the use of pesticides. But the pesticides leave residues on the leaf, which adversely affects silkworms impairing their growth, productivity, cocoon quality and reproductive traits (Bhosale & Kallapur, 1988). Hence, it is imperative to handle pesticides with care and ensure their responsible use that would ensure safeguarding the health and well-being of the silkworms while effectively managing pests in mulberry plantations. Exploring alternative pest control measures and embracing integrated pest management practices will empower the industry to cultivate mulberry sustainably, safeguarding cocoon crop and ecological balance in the process. In the light of the above, a study was conducted to know the effect of feeding diafenthiuron 50 % WP sprayed mulberry leaves on reproductive performance of *B. mori*.

1. **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 with V1 variety. The parental breeds, namely PM, CSR2, FC1 and FC2 were reared to assess the impact of the chemical used in mulberry for management of thrips and mites.

The entire rearing room and appliances were disinfected by following standard procedure (Dandin & Giridhar, 2014). The chemical diafenthiuron 50 % WP was sprayed to mulberry on 25th and 30th day after pruning and the leaves were harvested from treatment plots at 15 and 20 days for feeding silkworms from second feed of the third instar. 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 impact of pesticide toxicity and to determine the post spray safety period of the chemical on the reproductive performance of *B.* *mori* silk moth. The traitssuch as fecundity, egg retention in ovary, weight of 100 eggs, egg recovery, hatching, dead and unfertilized eggs in the parental breeds were observed and the data were analysed using Factorial- CRD for testing of significance by Fisher’s method of analysis of variance (Sundararaj *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.

* 1. **Observations Recorded**

**Fecundity (No.):**

A total of five disease free laying’s (DFls) were randomly selected from each replication of all treatments and the number of eggs in each laying was counted and recorded as fecundity of individual moth.

**Weight of 100 eggs (mg):**

 A total of 100 eggs were collected from each replication of all treatments and weighed separately and recorded as weight of 100 eggs (mg).

**Egg retention in ovary (%)**

The eggs retained in the ovary after 24 hours of oviposition were counted from all the dissected silkmoths in each replication of different treatments and per cent egg retention was calculated using the formula,

Egg retention in ovary (%) = $\frac{Number of eggs retained in the ovary}{ Total number of eggs laid by moth+Number of eggs in ovary}$

**Hatching percentage (%)**

The disease-free laying’s that were used to record fecundity were considered and observing hatching percentage by using the formula,

Hatching (%) = $\frac{Number of eggs hatched}{ Total number of eggs/laying}$ × 100

**Dead eggs (%)**

The number of eggs that did not hatch and showed depression at the centre were counted in the same DFLs and recorded as dead eggs percentage by using the formula,

Dead eggs (%) = $\frac{Number of dead eggs }{ Total number of eggs/laying }$ × 100

**Unfertilized eggs (%)**

The unfertilized eggs will turn white colour and such eggs were counted separately in each laying to record per cent unfertilized eggs, which was computed as,

Unfertilized eggs (%) = $\frac{Number of unfertilized eggs }{ Total number of eggs/laying }$ × 100

**Egg recovery (g/kg cocoons)**

The cocoons were stored in trays for moth emergence and the moths were allowed for selfing. The gravid females from each replication were collected and allowed for oviposition on loose egg sheets. The total number of eggs obtained from each replication among different treatments were weighed separately as per treatment and recorded as egg recovery.

**3. Results and Discussion**

**3.1 Fecundity (No.)**

The moths of respective treatments were allowed for pairing replication wise and decoupled after 4 hours. The mated females were allowed to lay eggs for 24 hours to count the fecundity.

The fecundity showed a significant difference among the parental breeds reared in the experiment, among which FC2, the bivoltine hybrid recorded highest fecundity of 547.77 No./moth and least fecundity was reported in multivoltine pure breed, PM (493.45 No./moth). The residual toxicity of chemical sprayed to the host plant found to exhibit significant influence on the fecundity in different parental breeds of the silkworm, *B. mori*. The highest number of eggs were laid by the moths where the larvae were fed with diafenthiuron 50 % WP sprayed mulberry leaves harvested after 20 days (530.13 No./moth) which was comparable with control (537.59 No./moth) and minimum number of eggs were observed at 15 DAS (502.75 eggs/moth). The combined effect of spray duration and parental breeds did not show a significant variation in relation to fecundity (Table 2)

Kumutha *et* *al*. (2013) found that dichlorvos at a higher concentration of 0.0005 % significantly reduced moth fecundity to 485±4.71, whereas at a lower concentration of 0.0001 %, fecundity (546±1.43) was similar to that of the control group (547±4.12) in the breed (LxCSR2). Similarly, the fecundity of adults emerged from silkworms fed with leaves treated with 0.025 per cent malathion was 542 eggs/moth, whereas it decreased to 319 eggs/moth at a concentration of 0.05 %, compared to the control group (549 eggs/moth) (Kuribayashi, 1981).

The negative impact of pesticides can be mitigated by using the correct dosage and adhering to the proper spray duration, which may prevent any adverse effects on the reproductive performance of *B. mori*. Exposure to trace amount of acetamiprid can affect the development of the oviduct in the silkworm (Jingsong X Haoyue), leading to decreased egg production (Cheng *et al*., 2019). Santorum *et* *al*., (2021) also observed significant reduction in the fecundity upon exposure to novaluron where the treated group recorded 597 eggs/moth compared to 796 eggs/moth in control group. Novaluron is an insect growth regulator, it may affect the balance of hormones like juvenile hormone and ecdysteroids, essential for egg production and maturation, leading to a decrease in the number of eggs laid by the silkmoths.

Patnaik *et* *al*. (2011) reported the fecundity of 426 eggs per moth in the untreated control group while the moths emerged from the cocoons spun by the silkworms fed on the leaves sprayed with a neonicotinoid insecticide *viz.*, thiamethoxam @ 0.015% (388 and 430 eggs/moth) and clothianidin @ 0.0047 % (387 and 433 eggs/moth) at 14 and 21 days after treatment, respectively. Similar observation has been documented in the present study with respect to the

**Table 2: Effect of feeding mulberry leaves treated with diafenthiuron 50 % WP at different days after spray on fecundity, egg retention in the ovary and weight of 100 eggs of parental breeds of *B. mori***

|  |  |  |  |
| --- | --- | --- | --- |
| **Treatments** | **Fecundity (No.)** | **Egg retention in ovary (%)** | **Weight of 100 eggs (mg)** |
| **Breeds (B)** |
| B1: PM | 493.45 | 3.76 | 54.70 |
| B2: CSR2 | 523.70 | 4.69 | 57.56 |
| B3: FC1 | 529.05 | 4.05 | 57.82 |
| B4: FC2 | 547.77 | 4.32 | 58.24 |
| F-test | \* | \* | \* |
| S.Em± | 2.889 | 0.088 | 0.448 |
| CD 0.05 | 8.483 | 0.259 | 1.316 |
|  **Safety period (S)** |
| S1: 15 DAS | 502.75 | 4.67 | 55.10 |
| S2: 20 DAS | 530.13 | 4.11 | 57.73 |
| S3: Control | 537.59 | 3.83 | 58.41 |
| F-test | \* | \* | \* |
| S.Em± | 2.502 | 0.076 | 0.388 |
| CD 0.05 | 7.346 | 0.224 | 1.140 |
| **Interaction (B×S)** |
| B1S1 | 481.16 | 4.22 | 54.02 |
| B1S2 | 496.13 | 3.65 | 54.98 |
| B1S3 | 503.05 | 3.40 | 55.10 |
| B2S1 | 503.48 | 5.21 | 55.02 |
| B2S2 | 529.11 | 4.61 | 58.54 |
| B2S3 | 538.50 | 4.24 | 59.13 |
| B3S1 | 504.93 | 4.53 | 55.26 |
| B3S2 | 537.10 | 3.93 | 58.65 |
| B3S3 | 545.13 | 3.69 | 59.54 |
| B4S1 | 521.45 | 4.71 | 56.12 |
| B4S2 | 558.18 | 4.23 | 58.75 |
| B4S3 | 563.70 | 4.01 | 59.87 |
| F-test | NS | NS | NS |
| S.Em± | - | - | - |
| CD 0.05 | - | - | - |

 \*Significant at 0.05; NS: Non-significant; DAS- Days after spray; The mentioned values

 represent the average of two rearing.

residual toxicity of the chemical on fecundity in different parental breed of both bivoltine and multivoltine which is also a breed characteristic.

**3.2 Egg retention in ovary (%)**

The gravid female moths were dissected after 24 hours of egg laying and the number of eggs retained in the ovary are counted that showed a significant variation among different treatments

The retention of eggs in the ovary varied significantly among different silkworm breeds when they fed with diafenthiuron 50 % WP treated mulberry leaves. The bivoltine pure breed, CSR2 retained a higher number of eggs in the ovary (4.69 %). On the other hand, the multivoltine breed, PM retained fewer eggs (3.76 %) in the ovary. The residual toxicity of the chemical exhibited a notable impact on egg retention in ovary when the silkworms were fed with diafenthiuron 50 % WP sprayed mulberry leaves harvested at different durations after spray. The highest egg retention percentage was recorded in 15 DAS (4.67 %) and the least was in control (3.83 %) and 20 DAS (4.11 %), respectively. The interaction between breeds and duration after spray did not show any significant difference, suggesting that the impact of spraying on egg retention was consistent across all breeds (Table 2)

**3.3 Weight of 100 eggs (mg)**

The egg weights reflect health of developing embryo and decides the cocoon productivity in sericulture and is expressed in terms of weight 100 eggs that showed significant difference among the parental breeds and different safety period post spay of the pesticide.

The egg weight recorded significantly minimum in multivoltine breed, PM (54.70 mg) and the bivoltine hybrid, FC2 recorded maximum egg weight of 58.24 mg. The residual toxicity of pesticide showed significant influence on egg weight. The lowest egg weight of 55.10 mg was recorded in 15 days after spray. However, the increased egg weight of 57.73 mg was observed in 20 days after spray and control. The interaction between parental breeds and duration after spray was non-significant for weight of 100 eggs (Table 2).

The toxic effects of chemical residues can hinder nutrient distribution or interfere with normal egg development, resulting in smaller and less viable eggs laid by moths when silkworms consume leaves from pesticide treated mulberry gardens. Cheng *et al*. (2019) found that egg weight decreased in the acetamiprid treated group (0.524 mg/egg) compared to the control group (0.580 mg/egg).

**3.4 Egg recovery (g/kg of cocoons)**

The egg recovery represents the quantity of eggs that can be recovered from total moth pairs obtained per kg of cocoons and is an important parameter in the grainage for maximizing the profit.

The egg recovery was found to have been significantly influenced by the parental breeds, pesticide toxicity and their interactions. Among the parental breeds, the egg recovery was highest in bivoltine hybrid, FC2 (54.67 g/kg of cocoons) and it was least in bivoltine pure breed, CSR2 (50.61 g/kg of cocoons). The duration of spray significantly affected egg recovery when the silkworms were fed with diafenthiuron 50 % WP sprayed mulberry leaves harvested at 15 and 20 DAS. Shorter safety period of 15 DAS recovered significantly least number of eggs (48.50 g/kg of cocoons) compared to 20 DAS (54.66 g/kg of cocoons) that was on par with control (55.45 g/kg of cocoons). There was a significant difference among interaction between parental breeds and duration of the spray regarding egg recovery. For instance, FC2 at 20 days after spray recorded the highest egg recovery of 57.98 g/kg of cocoons (Fig. 1), which is probably the breed characteristics. Interestingly, the bivoltine hybrids recorded relatively higher egg recovery compared to the pure breed where the heterosis might have played role.

**3.5 Hatching eggs (%)**

A good seed in sericulture is the one which has higher percentage of hatched eggs in the laying. Hatching percentage showed significant difference among the silkworm breeds. The highest hatching percentage was recorded in multivoltine pure breed, PM (97.72 %). The lowest was found in bivoltine pure breed, CSR2 (95.95 %). Regardless of parental breeds the highest per cent hatching was recorded in the treatment with the safety duration of 20 DAS (97.03 %) and least in 15 DAS (95.40 %). Interaction between breeds and duration of spray also varied significantly for hatching percentage. The highest per cent of hatching was observed in the multivoltine breed, PM at 20 DAS (98.18 %) followed by PM in control (Table 3).

**Fig. 1: Effect of feeding mulberry leaves treated with diafenthiuron 50 % WP at different days after spray on egg recovery 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

 The mentioned values represent the average of two rearing.

The hatching percentage of eggs improved as the safety period was extended while spraying chemicals during pest management in mulberry, the sole food plant of silkworm, *B. mori*. The current observations are in line with findings of Patnaik *et* *al*. (2011) that reported a better hatching percentage in the eggs laid by moths that emerged from thiamethoxam @ 0.015% (96.11 and 98.12 %, respectively at 14 and 21 DAS) and clothianidin @ 0.0047% (96.64 and 97.46 %, respectively at 14 and 21 DAS) treated groups when the safety period was extended to 21 DAS.

**Table 3: Effect of feeding mulberry leaves treated with diafenthiuron 50 % WP at different days after spray on hatching percentage, dead and unfertilized eggs of parental breeds of *B. mori***

|  |  |  |  |
| --- | --- | --- | --- |
| **Treatments** | **Hatching (%)** | **Dead eggs (%)** | **Unfertilized eggs (%)** |
| **Breeds (B)** |
| B1: PM | 97.72 | 1.61 | 0.65 |
| B2: CSR2 | 95.95 | 3.20 | 0.84 |
| B3: FC1 | 95.76 | 3.39 | 0.83 |
| B4: FC2 | 96.42 | 2.73 | 0.84 |
| F-test | \* | \* | \* |
| S.Em± | 0.044 | 0.036 | 0.028 |
| CD 0.05 | 0.130 | 0.106 | 0.082 |
| **Safety period (S)** |
| S1: 15 DAS | 95.40 | 3.50 | 1.08 |
| S2: 20 DAS | 97.03 | 2.32 | 0.64 |
| S3: Control | 96.95 | 2.38 | 0.65 |
| F-test | \* | \* | \* |
| S.Em± | 0.038 | 0.031 | 0.024 |
| CD 0.05 | 0.112 | 0.092 | 0.071 |
| **Interaction (B×S)** |
| B1S1 | 96.94 | 2.20 | 0.84 |
| B1S2 | 98.18 | 1.25 | 0.55 |
| B1S3 | 98.03 | 1.38 | 0.57 |
| B2S1 | 95.04 | 3.87 | 1.07 |
| B2S2 | 96.50 | 2.77 | 0.71 |
| B2S3 | 96.29 | 2.95 | 0.74 |
| B3S1 | 94.56 | 4.17 | 1.26 |
| B3S2 | 96.37 | 3.01 | 0.61 |
| B3S3 | 96.37 | 2.99 | 0.63 |
| B4S1 | 95.06 | 3.75 | 1.17 |
| B4S2 | 97.08 | 2.24 | 0.67 |
| B4S3 | 97.11 | 2.21 | 0.67 |
| F-test | \* | \* | \* |
| S.Em± | 0.077 | 0.063 | 0.048 |
| CD 0.05 | 0.225 | 0.184 | 0.142 |

 \*Significant at 0.05; NS: Non-significant; DAS- Days after spray; The mentioned values

 represent the average of two rearing.

At higher concentration of dichlorvos at 0.0005 % significantly reduced egg hatchability to 79.67 per cent, whereas, at a lower concentration of 0.0001 %, egg hatchability was 91.86 per cent, which was comparable to the control group (93.39 %) (Kumutha *et al.,* 2013). The hatching rate of control group was 98.47 per cent and in the 0.001 g/l pyriproxyfen treated group were 94.48 and 89.43 per cent, respectively (Qian *et* *al*., 2020). The decrease in hatching rate with higher pyriproxyfen concentrations suggests that increased doses have a more detrimental effect on embryonic development.

**3.6 Dead eggs (%)**

The quality of a laying is considered as poor if the percentage of dead eggs is more in a laying. There was a significant influence of breeds, safety period and their interaction with respect to dead eggs percentage observed in the present study using the chemical, diafenthiuron 50 % WP sprayed @ 1g/l for management of thrips and mites in mulberry while cultivating the food plant *B. mori*.

Among the four different parental breeds of *B.* mori, significantly lowest percentage of dead eggs was observed in multivoltine breed, PM (1.61 %) that was highest in bivoltine hybrid, FC1 (3.39 %). The duration of the spray significantly influenced the percentage of dead eggs. When the silkworms were reared on diafenthiuron 50 % WP sprayed mulberry leaves harvested at different safety periods, the lowest percentage of dead eggs was recorded in the batch reared with 20 DAS (0.69 %) treatment while, it was highest in the treatment with 15 DAS (3.50 %). The interaction effect of parental breeds and duration after spray varied significantly concerning to dead eggs percentage with higher dead egg percentage of 4.17 % in FC1 followed by CSR2 with the dead egg percent of 3.87 %, respectively at 15 DAS. In contrast, the PM exhibited the lower percentage of dead eggs (1.25 %) at 20 DAS (Table 3), indicating the contribution of the breed and the chemical toxicity on the per cent dead eggs in a layings.

**3.7 Unfertilized eggs (%)**

The highest percentage of unfertilized eggs was found in bivoltine hybrid, FC2 (0.84 %) and bivoltine pure breed, CSR2 (0.84 %) and least number of unfertilized eggs were found in in the multivoltine breed, PM (0.65 %).The duration of spray significantly affected the percentage of unfertilized eggs that was lowest at 20 DAS (0.64 %) while, the highest was recorded at 15 DAS (1.08 %).There was a significant difference among the interaction between breeds and duration of spray for percentage of unfertilized eggs. PM at 20 days after spray recorded the lowest percentage of unfertilized eggs (0.55 %). In contrast, FC1 at 15 days after spray recorded the highest percentage of unfertilized eggs (1.26 %) (Table 3).

Kumutha *et* *al*. (2013) reported maximum rate of unfertilized eggs in the treatment with higher concentration @ 0.005% neem pesticide (12.85 %) and @ 0.0005 % dichlorvos compared to (8.94 %) and control (2.77 %) and lower concertation of dichlorvos @ 0.0001 % and Vijay neem @ 0.001 % (3.20 and 5.70 %, respectively). Cheng *et* *al.* (2019) observed low rate of unfertilized eggs in the acetamiprid-treated group (8 %) that was comparable with control group (5 %) suggesting that low concentration of chemical (acetamiprid) may not significantly impact the fertilization process and probably does not interfere directly with the reproductive mechanisms involved in fertilization process. Similar observations were reported in the present study where the extended safety period showed improved hatchability and reduced number of unfertilized and dead eggs.

**4. Conclusion**

The study on the impact of diafenthiuron 50 % WP on the reproductive performance of silkworm, revealed its dual role in effectively managing thrips and mites in mulberry cultivation while safeguarding the grainage parameters of *B. mori*. The selected molecule diafenthiuron 50 % WP @ 1g/l was found safer to parental breeds of silkworm when the leaves are used at 20 DAS, which was reflected through the maximum fecundity, hatching percentage, egg weight, egg recovery and minimum number of eggs retained in the ovary, per cent dead and unfertilized eggs. This suggests that spraying diafenthiuron 50 % WP, judiciously can be a viable option for pest management in mulberry cultivation without compromising the reproductive performance of silkworms.

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