**Effect of feeding diafenthiuron 50 % WP sprayed mulberry leaves on growth and performance of silkworm, *Bombyx mori*** **L.**

**ABSTRACT**

Mulberry is the sole food for silkworm, due to evergreen lush foliage which attracts several pests majorly thrips and mites. Management of these pests demands usage of chemicals. Exposure to the residue of pesticides in the mulberry could affect the growth and quality of economic traits, it is therefore important to observe the suitability of the chemicals used for managing these pests. An experiment was conducted to evaluate the effect of diafenthiuron, being a chemical with novel mode of action and an acaricide as well as insecticide was sprayed on mulberry at 25 and 30th day after pruning and fed to the silkworms at 15 and 20 days after spray on 3rd instar onwards. There was no mortality was observed in the treatment during two different rearing seasons. The larval duration was found to be shortest (24.15 days) after 20 days of spray, the larval weight (31.10 g/10 larvae), effective rate of rearing (97.66 %) was significantly maximum when silkworms were fed with diafenthiuron 50 % WP @ 1g/l sprayed mulberry leaves at 20 days of post spray.

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

1. **INTRODUCTION**

Silkworm, *B. mori* is a monophagous insect which relies exclusively on mulberry foliage for its growth and productivity. Around 70 per cent of total silk proteins are directly derived from mulberry leaves (Awquib et al., 2016). The cocoon productivity and profitability is mainly determined by the quality and quantity of mulberry leaves. Hence, obtaining quality leaf is imminent to obtain high silk productivity. Though mulberry is hardy plant, due to the availability of evergreen luxuriant foliage mulberry attracts several pests and pathogens that significantly lead to poor leaf quality because of reduced photosynthetic activity and consequently lower silk yield and quality. More than 300 insect and non-insect pests infesting mulberry have been well documented, each inflicting a diverse range of damages.

The management option to address several pests in mulberry is limited with regard to chemical control using two chemicals *viz*., dichlorvos and dimethoate. Dichlorvos is the common insecticide used for controlling sucking pests in mulberry (Mahadeva, 2011). But, the Government of India notification, dated 28th December, 2016 has imposed a complete ban on usage of dichlorvos 76 % EC from 31st December, 2020 onwards in the country (Anon., 2016). Dimethoate is an old insecticide with limited efficacy on thrips and does not possess acaricidal properties for management of mites. Most of the times, both mites and thrips occur simultaneously in the mulberry ecosystem. Hence it would be economical to use a chemical having both insecticidal and acaricidal properties for management of both the pests with single application. Diafenthiuron is one such molecule found to be effective against polyphagous mites and several insect pests on a variety of agricultural and horticultural crops worldwide (Lasota, 1990), which can be tested for its safety to silkworm, *Bombyx mori* L. and moth for its productivity traits.

In light of this, a comprehensive study has been formulated with the primary goal, to evaluate the impact of dual active molecule diafenthiuron 50 % WP @ 1g/l with respect to growth and productivity of the parental breeds 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 performance of 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 by 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 while the third instar onwards the larvae were fed with mulberry leaves of treatment plots harvested at 15 and 20 DAS of the 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 larval growth and productivity such as larval duration (days), larval weight (g/10 larvae), larval mortality (%), ERR (%) 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:**

**Larval mortality (%)**

The larval mortality due to feeding with mulberry leaves harvested at different intervals of pesticidal spray was recorded separately in each replication for every treatment and computed as below:

Larval mortality (%) = $\frac{Number of dead larvae}{Total number of larvae treated}$ × 100

**Instar wise larval duration (Days)**

The time taken by larvae to complete each instar was recorded by counting the total duration between two consecutive moults.

**Total larval duration (Days)**

The total time taken by larva from hatching till cocoon spinning was recorded and computed as total larval duration.

**Fifth instar larval weight (g/10 larvae)**

The weight of ten randomly picked silkworms was recorded on 5th day of 5th instar. The observations were recorded separately for each replication and the average of the same was computed to observe the effect of each treatment on the larval weight (g/10 larvae)

**Effective rate of rearing (ERR) (%)**

The number of cocoons harvested at the end of rearing were counted and the ERR was calculated by using formula:

ERR (%) = $\frac{Number of cocoons harvested }{Total number of larvae brushed }$ × 100

**3. RESULTS**

Feeding silkworms with the chemical sprayed leaves was initiated from second feed of 3rd instar onwards and the observation recorded on larval growth parameters are as follows

**3.1 Instar wise larval duration (Days)**

A significant difference in larval duration was observed when silkworms were fed with mulberry leaves treated with diafenthiuron 50 % WP @ at 15 and 20 days after spray during the third, fourth and fifth instar of *B. mori*. Among the parental breeds, the bivoltine pure breed, CSR2 exhibited shortest larval duration of 3.21, 4.49 and 7.76 days while, it was longest in multivoltine, Pure Mysore (PM) (4.18, 5.40- and 8.38-days during 3rd, 4th and 5th instar, respectively). Feeding on leaves harvested from the control plot resulted in the shortest larval duration during the 3rd, 4th and 5th instar with duration of 3.27, 4.42 and 7.66 days. However, feeding on the leaves harvested from the plot treated with diafenthiuron at 15 days after spray led to prolonged larval duration. There was no significant difference was observed in response of parental breeds to the toxicity of diafenthiuron 50 % WP with respect to the larval duration of silkworms, indicating that the pesticide impact is uniform across different silkworm breeds (Table 2).

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

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Treatments** | **3rd instar (Days)** | **4th instar (Days)** | **5th instar (Days)** | **Total larval duration (Days)** |
| **Breeds (B)** |
| B1: PM | 4.18 | 5.40 | 8.38 | 25.95 |
| B2: CSR2 | 3.21 | 4.49 | 7.76 | 23.46 |
| B3: FC1 | 3.34 | 4.59 | 7.93 | 23.85 |
| B4: FC2 | 3.23 | 4.50 | 7.76 | 23.49 |
| F-test | \* | \* | \* | \* |
| S.Em± | 0.045 | 0.027 | 0.040 | 0.072 |
| CD 0.05 | 0.131 | 0.079 | 0.120 | 0.212 |
| **Safety period (S)** |
| S1: 15 DAS | 3.67 | 5.08 | 8.31 | 25.07 |
| S2: 20 DAS | 3.52 | 4.72 | 7.90 | 24.15 |
| S3: Control | 3.27 | 4.42 | 7.66 | 23.36 |
| F-test | \* | \* | \* | \* |
| S.Em± | 0.039 | 0.023 | 0.035 | 0.062 |
| CD 0.05 | 0.114 | 0.069 | 0.104 | 0.183 |
| **Interaction (B×S)** |
| B1S1 | 4.30 | 5.72 | 8.73 | 26.76 |
| B1S2 | 4.24 | 5.39 | 8.32 | 25.96 |
| B1S3 | 3.99 | 5.08 | 8.07 | 25.15 |
| B2S1 | 3.38 | 4.81 | 8.12 | 24.31 |
| B2S2 | 3.22 | 4.48 | 7.70 | 23.40 |
| B2S3 | 3.04 | 4.19 | 7.45 | 22.68 |
| B3S1 | 3.59 | 5.00 | 8.27 | 24.86 |
| B3S2 | 3.38 | 4.50 | 7.88 | 23.80 |
| B3S3 | 3.03 | 4.22 | 7.63 | 22.89 |
| B4S1 | 3.43 | 4.82 | 8.09 | 24.34 |
| B4S2 | 3.22 | 4.48 | 7.71 | 23.42 |
| B4S3 | 3.04 | 4.20 | 7.47 | 22.71 |
| F-test | NS | 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.

**3.2 Total larval duration (Days)**

The larval duration showed significant difference among the breeds when silkworms were fed with mulberry leaves harvested at different duration after spray of diafenthiuron 50 % WP @ 1g/l. Among the different parental breeds of *B. mori* used in the experiment, the shorter total larval duration was observed in bivoltine pure breed, CSR2 (23.46 days) and the total larval duration was longer in multivoltine, PM (25.95 days). Irrespective of the parental breeds, the total larval duration extended significantly when the silkworms were fed with diafenthiuron 50 % WP sprayed mulberry leaves harvested at 15 DAS (25.07 days). There was no significant difference was found among the interaction between parental breeds and toxicity of diafenthiuron 50 % WP with respect to total larval duration (Table 2).

**3.3 Larval weight (g/10 larvae)**

Significantly the maximum larval weight was observed in the bivoltine parental breed FC2 (34.73 g/10 larvae) followed by the FC1 (32.49 g/10 larvae). In contrast, the multivoltine parental breed, PM recorded the lowest larval weight of 20.63 g/10 larvae. Regardless of the parental breeds, the maximum larval weight was recorded at 20 days after spray (31.10 g/10 larvae) followed by 15 DAS (29.81 g/10 larvae) and minimum larval weight was observed in control plot (28.79 g/ 10 larvae). There was no significant difference was found with respect to the interaction between parental breeds and duration of spray concerning the larval weight (Fig. 1).

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

**Table 3: Effect of feeding mulberry leaves treated with diafenthiuron 50 % WP at different days after spray on larval mortality and effective rate of rearing (ERR) of parental breeds of silkworm, *B. mori***

|  |  |  |
| --- | --- | --- |
| **Treatments** | **Larval mortality (%)** | **ERR (%)** |
| **Breeds (B)** |
| B1: PM | 0.00 | 99.00 |
| B2: CSR2 | 0.00 | 95.55 |
| B3: FC1 | 0.00 | 97.44 |
| B4: FC2 | 0.00 | 97.11 |
| F-test | NS | \* |
| S.Em± | - | 0.608 |
| CD 0.05 | - | 1.780 |
| **Safety period (S)** |
| S1: 15 DAS | 0.00 | 95.75 |
| S2: 20 DAS | 0.00 | 97.66 |
| S3: Control | 0.00 | 98.41 |
| F-test | NS | \* |
| S.Em± | - | 0.527 |
| CD 0.05 | - | 1.483 |
| **Interaction (B×S)** |
| B1S1 | 0.00 | 98.33 |
| B1S2 | 0.00 | 99.00 |
| B1S3 | 0.00 | 99.66 |
| B2S1 | 0.00 | 93.66 |
| B2S2 | 0.00 | 96.00 |
| B2S3 | 0.00 | 97.00 |
| B3S1 | 0.00 | 95.66 |
| B3S2 | 0.00 | 97.66 |
| B3S3 | 0.00 | 99.00 |
| B4S1 | 0.00 | 95.33 |
| B4S2 | 0.00 | 98.00 |
| B4S3 | 0.00 | 98.00 |
| F-test | 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.

**3.4 Larval mortality (%)**

The molecule, diafenthiuron 50 % WP was sprayed on the mulberry plants @ 1g/l and the leaves were fed to the worms at 15 and 20 days after spray. The treatment-wise larval mortality was recorded across the four parental breeds (PM, CSR2, FC1 and FC2). There was no report of larval mortality across the breeds in both the safety durations after spray of the chemical similar to the control treatment and also the interaction between breeds and the safety period, which is documented in the (Table 3).

**3.5 Effective rate of rearing (ERR) (%)**

The effective rate of rearing showed significant differences among the parental breeds of silkworm, *B. mori*. The highest ERR was recorded in multivoltine pure breed, PM (99.00 %) followed by bivoltine hybrid, FC1 which had ERR of 97.44 per cent. The lowest ERR was found in bivoltine pure breed, CSR2 (95.55 %). The duration of spray also significantly affected the ERR. The highest ERR was recorded in the absolute control (98.41%) followed by the treatment applied at 20 days after spray (97.66 %). The lowest ERR was recorded at 15 days after spray (95.75 %). The interaction between parental breeds and duration of spray was found to be non- significant regarding ERR (Table 3).

**4. DISCUSSION:**

The variation in the larval duration of different parental breeds is attributed to the breed character (Ashoka *et al*., 2016). However, the chemicals used for management of pests in mulberry may alter the length of the larval duration. The high residual content of insecticide molecules present in the initial days after spray can disrupt the normal physiological processes of silkworms. This residual toxicity may diminish over time with an extended safety period (Kumutha *et* *al*., 2013). Insecticides interfere with the release of hormones necessary for metamorphosis, leading to a delay in instar duration (Tiwari *et* *al*., 2006). This disruption in physiology may result in an extended larval duration (Singh *et al.,* 2008). Kalpana *et al*. (2022)recorded a significant reduction in the total larval duration when leaves harvested from abamectin 1.9 % EC sprayed plot (23.72 and 22.50 days, respectively at 15 and 20 DAS) (PM ×CSR2). The earlier studies conducted by Kenchappa *et a*l. (2024) also confirmed the present investigation with respect to total larval duration that recorded (22.30 and 23.22 days) when the silkworms were fed with the leaves harvested from abamectin 1.9 % EC sprayed treatment plot of mulberry at 15 and 20 DAS, respectively in the bivoltine double hybrid.

When diafenthiuron 50 % was sprayed at a rate of 1g/l, it effectively controls the pests such as mites and thrips, promoting the growth and quality of mulberry plants. This, in turn, provide essential nutrients to the larvae, potentially leading to increased larval weight when sprayed leaves were fed to the worms at 15 and 20 DAS. These results are in accordance with the findings of Patnaik *et al.* (2011) who reported feeding silkworm with the mulberry leaves treated with thiamethoxam about 20 days after spraying resulted in improvement in larval weight in cross breed. Kalpana *et al*. (2022) recorded maximum larval weight of 23.03 and 25.21g/10 larvae in diafenthiuron 50 % WP @ 1 g/l at 15 and 20 DAS. Kenchappa *et al.* (2024) noticed that the maximum larval weight of 4.17 and 4.06 g in silkworm double hybrid, FC1× FC2 when the worms fed with diafenthiuron 50 % WP sprayed mulberry leaves harvested at 15 and 20 DAS, respectively.

The silkworm, *B. mori* is a beneficial insect cultivated for cocoon production. Therefore, any chemical used to control pests in mulberry plants can affect the growth and productivity of silkworms. Thus, a specific waiting period is recommended to ensure the safe use of the chemicals (Yokoyama, 1962). Pyridine-based compounds such as flonicamid 50 WG at 0.3 g/l (Yeshika *et al*., 2019), ryanoid-class substances like chlorantraniliprole and the new- generation flubendiamide (Sunil Kumar and Naika, 2019) were all observed to cause 93-100 percent mortality in silkworms. This high mortality rate results from the long-lasting toxicity of these substances in mulberry leaves at 10 DAS. However, the pyrrole insecticide chlorfenapyr at 1.5 ml/l resulted in a lower mortality rate of 12.09 % in silkworms at 10 DAS. (Sunil Kumar *et al.,* 2016). The current study is supported by the findings of Kalpana *et al*. (2022) and Kenchappa *et al*. (2024) reported that among the six selected chemical compounds diafenthiuron 50 % WP at 1 g/l were found to be safe for silkworms at 15 and 20 DAS, resulting in zero mortality during the third, fourth, and fifth instar.

Yeshika *et al.,* (2019) reported the highest ERR of 97.77, 97.77 and 98.87 per cent when silkworms (PM×CSR2) were fed with mulberry leaves treated with a neonicotinoid, dinotefuron 20 SG @ 0.25 g/l at 10, 20 and 30 DAS. Kalpana *et al*. (2022)found that using abamectin 1.9 per cent EC at 0.75 ml/l and diafenthiuron 50 per cent WP at 1 g/l as foliar spray resulted in higher ERR percentage at both 15 and 20 days after spraying. Among the different chemicals used to control the thrips and mites in mulberry spraying abamectin 1.9 % EC @ 0.75ml/l and diafenthiuron 50% WP @ 1 g/l recorded significantly maximum ERR of 97.78**,** 97.78 % and 96.65, 96.65 %, respectively at 15 and 21 DAS, in the bivoltine double hybrid (Kenchappa *et al.,* 2024), which is in accordance with the current study.

**5. CONCLUSION**

Mites and thrips commonly infest mulberry simultaneously during dry season. Therefore, management of these pests with a single application of pesticide would be more beneficial. The present investigations indicated the safety of diafenthiuron 50 % WP to the silkworm, *B. mori* when the molecule was employed for management of thrips and mites in mulberry, the sole food of silkworm which was reflected through zero mortality, shortest larval duration, maximum larval weight and ERR when silkworms were fed with diafenthiuron 50 % WP @ 1g/l sprayed mulberry leaves harvested after 20 days of post spray.

**Disclaimer (Artificial intelligence):**

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript

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