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Effect of Bio-Inoculants to M_5 Mulberry and *Bm*CPV Development in Silkworm, *B. mori* L. (PM × CSR_2)

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Abstract

Administration of *Bm*CPV to IV and V instar larvae of PM × CSR_2 on LC_{50} for larval mortality revealed significant effect. The LC_{50} for mortality was ranged from 35.22 to 51.00% and 33.22 to 44.77% for T_1 and T_5 respectively. Application of bio-inoculants to mulberry and the resultant leaf fed to both the instars recorded low per cent of mortality as was reflected in the experimental data (T_2 39.55 and 41.22, T_3- 47.44 and 42.44, than T_4-37.44 and 35.88, T_5-51.00 and 44.77%) for IV and V instars respectively. However, the local check recorded highest LC_{50} value of 55.88 and 47.22% for IV and V instar (PM × CSR_2). The lowest lethality was noticed in the treatment T_1, where mulberry raised only through recommended NPK + recommended FYM (33.22%) and the higher lethality was recorded in local control T_8 (47.22%) followed by T_5 (44.77), T_6 (44.55%), T_7 (42.66%), T_3 (42.44%) T_2 (41.22%) and T_4 (35.88%). Significantly lower LC_{50} mortality was recorded from *Bm*CPV dilutions 10^{-1} (43.66%) to 10^{-3} (37.00%), and non-significant results were observed between the interaction effect of treatments and the concentration.

Key words : Bio-inoculants, *Bm*CPV, Larval weight, Mulberry, Silkworm.

To achieve maximum leaf yield potential of mulberry, the native soil fertility alone cannot be relied upon and hence, the soil should be continuously replenished through periodical application of organic manures and fertilizers. Hence, nutrient management plays an important role in improving the yield and quality of mulberry leaves which is directly responsible for quality and quantity and cocoon production. The quality of mulberry plays an important role in silkworm nutrition. However, feeding of nutrient deficient leaves not only cause malnutrition but also act as pre-disposing factor for many other diseases of silkworm. Therefore, supply of nutrient rich mulberry leaves to silkworm is the only answer for minimizing the crop loss due to diseases. Cytoplasmic polyhedrosis virus of silkworm *Bombyx mori* L (*Bm* CPV) is known to cause flacherie. Its incidence has been reported to be 27.76% in Karnataka, out of the total loss of cocoon crop in India, 71% was due to flacherie (2). Flacherie may be due to the viruses, bacterial and physiological abnormalities. The CPV disease is caused by an occluded double stranded RNA virus which multiplies in cytoplasm of columnar cells of midgut epithelium. The disease is characterized by the manifestation of symptoms like, body flaccidity, paleness of skin, vomiting and semisolid whitish-green feces. The severity of the disease mainly dependent upon the quality of mulberry leaf, silkworm breed and rearing conditions. The quality of the leaf further, enhanced due to application of chemical fertilizers and bio-inoculants in right time is essential to bring down the flacherie disease (3). The application bio-inoculants to mulberry and their role on nature and spread is studied based on LC_{50} for mortality in PM × CSR_2.

Methods

Isolation and Purification of BmCPV Polyhedra

The worms (PM × CSR_2) showing typical symptoms of *Bm*CPV are collected from farmer's field and inclusion bodies were isolated from whitened midgut. The whitish mid-gut were homogenized in distilled water (1:4 wt/vol) in a beaker and churned using double layered muslin cloth. Water sent on the mus-

Table 1. Combined effect of bio-inoculants and *Bm*CPV infection on LC_{50} for mortality (%) of silkworm, *B. mori* L. (PM × CSR_2).

Treat-ments	IV Instar inoculated batch *Bm*CPV viral dilutions 10^{-1}	10^{-2}	10^{-3}	Mean	V Instar inoculated batch *Bm*CPV viral dilutions 10^{-1}	10^{-2}	10^{-3}	Mean
T_1	41.33	36.33	28.00	35.22	37.33	32.00	30.33	33.22
T_2	46.00	38.66	34.00	39.55	47.33	38.33	38.00	41.22
T_3	50.00	44.66	47.66	47.44	41.66	46.66	39.00	42.44
T_4	44.00	37.33	31.00	37.44	37.66	36.00	34.00	35.88
T_5	59.33	47.00	46.66	51.00	49.00	45.66	39.66	44.77
T_6	58.33	46.00	47.33	50.55	46.33	47.00	40.33	44.55
T_7	58.66	45.00	39.00	47.55	39.00	34.00	31.00	42.66
T_8	63.00	54.66	50.00	55.88	51.00	47.00	43.66	47.22
Mean	52.58	43.70	40.45		43.66	4083	37.00	
Test of significance	*F* test	SE ±	CD 0.05%		*F* test	SE ±	CD 0.05%	
Concentration	*	0.353	1.005		*	0.395	1.124	
Treatment	*	0.577	1.641		*	0.645	1.835	
Interaction	*	1.000	2.843		*	1.118	3.179	

lin cloth to force the polyhedra to percolate through the cloth. The filtrate was stored in refrigerator for 24 hours. Later these inclusion bodies are inoculated to V instar silkworm. The worms showing the same symptom were crushed in sterile distilled water and pass through double layer muslin cloth. Further, the filtrate was filtered through Watman No. 1. The same will be subjected for repeated centrifugation at 5,000 rpm for ten minutes (4) till recovery of whitish pellet. The resultant pellet was suspended in distilled water to prepare stock suspension. The polyhedral body/ml was enumerated by using hemocytometer and confirmed by methyl green pyronin-G stain.

Immediately after third moult the silkworms were per-orally administered with the polyhedral inclusion bodies and smeared on to the leaves of respected bio-inoculated plots and the respective batches of worms reared.

Infectivity Technique

The IV and V instar larvae of PM × CSR_2 were used. Mulberry leaves are harvested from bio-inoculated plots, washed with tap water and surface sterilized with 70% ethyl alcohol by using sterile cotton. The leaves are cut into 10 cm^2 and 2 ml of viral suspension will be administered (uniform spraying) to 50 larvae. Standard control batches were maintained during experimentation. The LC_{50} value was determined for the IV and V instar worms separately. The LC_{50} value was determined by the Reed and Meunch method (5). The arithmetic estimate of the LC_{50} value was obtained and expressed in percentage.

Results and Discussion

The combined effect of bio-inoculants and *Bm*CPV infection on LC_{50} for mortality revealed significant effect on IV and V instar inoculated lots of PM × CSR_2. The LC_{50} for mortality ranged from 35.22 to 51.00% and 33.22 to 44.77% for T_1 and T_5 respectively. The per cent mortality of other treatments were also found significant with bio-inoculants and chemical fertilizers.

Whereever the bio-inoculants are added to mulberry and the resultant leaf fed to both the instars recorded lesser per cent of mortality as it is reflected in the experimental data (T_2 39.55 and 41.22 and T_3 47.44 and 42.44 than T_4 37.44 and 35.88, T_5 51.00 and 44.77% recorded for IV and V instar respectively. However, the local check recorded highest LC_{50} value of 55.88 and 47.22% mortality for IV and V instar larvae of PM × CSR_2, respectively. Worms fed with the leaves harvested from bio-inoculated mulberry plots of V instar worms showed 50% lethality at 50% concentration of *BM*CPV dilution administered (Table 1).

The lowest lethality was noticed in the treatment T_1 imposed only through recommended NPK + recommended FYM (33.22%) and the higher lethality was recorded in local control T_8 (47.22%) followed by T_5

(44.77), T_6 (44.55%), T_7 (42.66%), T_3 (42.44%) T_2 (41.22%) and T_4 (35.88%).

Significantly lower LC_{50} mortality was recorded from the dilutions 10^{-1} (43.66%) to 10^{-3} (37.00%), and non-significant results was observed between the interaction of treatments and the concentration.

The experimental data on LC_{50} for mortality registered positive effect due to combined effect of bio-inoculants and *Bm*CPV infection. The treatments with bio-inoculants and inorganic fertilizers revealed significant results in both the instars (IV and V) of PM × CSR_2. However, significantly lower 35.22 to 51.00 and 33.22 to 44.77% mortality was encountered in T_1, T_5 respectively which were comparatively superior over other treatments.

The additive effect of both was vivid with respect to treatment T_2 (39.55 and 41.22) and T_3 (47.44 and 42.44) than T_4 (37.44 and 35.88) and T_5 (51.00 and 44.77). These results are parity with the findings of Bhaskar et al. (6) who opined that LC_{50} for mortality was the lowest for second instars (0.7510×10^7), followed by third instars (0.87848×10^7), fourth instars (1.0870×10^7) and fifth instars (1.014×10^7) of *B. mori*. The early instars were more sensitive to *Bm*CPV as compared to later instars. Even following Aruga and Watanabe (7), the susceptibility to per oral infection of *Bm*CPV in *B. mori* decreased with larval age from first to fourth instars.

These results are in line with findings of Bhaskar et al. (6) who reported breed susceptibility of silkworm, *Bombyx mori* L. to *Bm*CPV. Among biovoltine breeds, NB_4D_2 and KA showed LC_{50} values of 1.603×10^7 and 1.158×10^7 PIBs/ml for mortality, respectively showing their high sensitiveness to CPV than multivoltines (0.034 to 5.689×10^7).

LC_{50} for mortality was lowest for II instar (0.7510×10^7), followed by III instar (0.8748×10^7), IV instar (1.0870×10^7) and for V instar (1.014×10^7) without much differences IV and V instars. This indicates that early instars of *B. mori* were more sensitive to *Bm*CPV as compared to later instars. Even Aruga and Watanabe (7) reported susceptibility between IV and V instar larva resistance to *Bm*NPV infection increased ten fold with each moult except that the susceptibility of just moulted; V instar larvae was same as that of IV instar.

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