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Efficacy of certain medicinal plant extracts for the management of late larval flacherie disease on cocoon and post cocoon parameters of Silkworm, *Bombyx mori* L.

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Abstract

The silkworm cocoon crop is majorly affected by diseases like Flacherie, Grasserie, Muscardine and Pebrine. Among these four diseases, flacherie disease is most sever resulting in cocoon crop loss and poor quality raw silk production. The study on efficacy of nine different medicinal plant extracts for the management of late larval flacherie disease of silkworm *B. mori* L., on cocoon and post cocoon character showed significant results. Among the nine medicinal plant extracts, *Asparagus officinalis* administration to CSR₂ and PM x CSR₂ silkworms breeds was found effective by enhancing the cocoon quality parameters viz., cocoon weight (17.92 and 13.63 g/10 cocoons), shell weight (3.743 and 2.480 g/10 cocoon shells), pupal weight (14.32 and 11.15 g/10 pupae), shell ratio (20.92 and 18.15 %/10 cocoon shells), silk filament length (1066.33 and 752.00 m) and denier (2.61 and 2.64) in both the breeds compared to control.

Keywords: Flacherie, medicinal plant extracts, silkworms and cocoons

1. Introduction

The success of sericulture depends upon proper management and protection of silkworms from diseases. The major diseases affecting silkworm are flacherie (viral and bacterial), grasserie (viral), muscardine (fungal) and pebrine (protozoan). Among the four diseases, flacherie is the major disease in India with an estimated annual cocoon crop loss to the tune of 33.88 per cent (Tayal and Chauhan, 2017) [17].

Silkworm diseases management is one of the important components of successful silkworm rearing for obtaining higher cocoon yield and quality. A wide variety of chemical bed disinfectants and antibiotics are used for the management of flacherie, but the ability of microbes to acquire resistance to drugs makes it ineffective within a short duration and hence attempts are being made for the use of plant compounds especially crude aqueous extracts of plants against silkworm bacterial pathogens (Priyadarshini *et al.*, 2008) [12].

India is rich with a variety of medicinal and aromatic plants. These plants constitute a major source of natural organic compounds widely used in human health care. These plants produce many compounds as secondary metabolites that have no apparent metabolic, physiologic and structural role in the producer. But, often have effects on other organisms. In many cases they are believed to function as biochemical defense (Jain *et al.*, 2004) [6].

Plant extracts have been proven to be a good alternative to synthetic chemical as the antibiotic properties of these plant extracts are of extreme interest in light of the ongoing threat of bacterial strains developing resistance to conventional antibiotics is inferred (Priyadarshini *et al.*, 2009) [13]. Hence, the present investigation has been undertaken to study the efficacy of acetone extract of medicinal plants viz., *Curcuma longa* (Turmeric), *Tinospora cordifolia* (Amruthaballi), *Tridax procumbens* (Coat buttons), *Phyllanthus niruri* (Kirunelli), *Phyllanthus emblica* (Amla), *Punica granatum* (Pomegranate), *Aloe vera* (Aloe vera), *Ocimum tenuiflorum* (Tulasi) and *Asparagus officinalis* (Asparagus) against the late larval flacherie disease on cocoon and post cocoon parameters of Silkworm, *B. mori* L.

Materials and Methods

Isolation of pathogens

Mulberry silkworms exhibiting specific symptoms of late larval flacherie were collected and

surface sterilized. The midgut was dissected to collect midgut juice and alimentary canal from the larvae. After maceration and filtration of alimentary canal through double layered muslin cloth, stock suspension was prepared. Serial dilutions (10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} and 10^{-6}) were prepared using 9 ml sterile water blanks. In the same way haemolymph was also collected by cutting the front pair of prolegs and filtered through filter paper to obtain the stock suspension from which serial dilutions (10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} and 10^{-6}) were prepared using 9 ml sterile water blanks (Nataraju *et al.*, 1999; Siromani *et al.*, 1994; Patil, 1990 and Chitra *et al.*, 1973) [10, 14, 11, 2].

Midgut juice and haemolymph each of 0.5 ml dilution was prepared and each of which were transferred to separate petridishes containing nutrient agar medium and spread thoroughly. Later the culture plates were incubated at 37 °C for three days. The colonies developed on the culture plates were picked, purified by using streak plate method. Pathogenicity of the individual bacterial isolates conforming to the principle of Koch's postulates in causing the disease was identified.

Preparation of plant extracts

The extracts from nine different plants were prepared as per the procedure adopted by Karthikairaj *et al.*, (2014) [7]. The above mentioned plant samples were collected from 'Sanjeevini vatika' (Herbal garden), Department of Horticulture, UAS, GKVK, Bengaluru and Botanical garden UAS, GKVK, Bengaluru. The collected plant samples made to fine powder using electric blender after shade drying. Ten grams of fine powder was soaked with 100 ml of acetone solution for 6 hours under air tight condition. The content is then stirred for an hour using magnetic stirrer and filtered through a filter paper. The residual extract was collected in a flask and the solvent was allowed to evaporate at room temperature. The extracts was then stored at 4° C till further use. The resultant residue was then made up to required volume (2, 4 and 6 %) using double distilled water and used for the study.

Treatment Details

- T₁ – Turmeric (*Curcuma longa*)
- T₂ – Amruthaballi (*Tinospora cardifolia*)
- T₃ – Coat buttons (*Tridax procumbens*)
- T₄ – Kirunelli (*Phyllanthus niruri*)
- T₅ – Amla (*Phyllanthus emblica*)
- T₆ – Pomegranate (*Punica granatum*)
- T₇ – Aloe vera (*Aloe vera*)
- T₈ – Tulasi (*Ocimum tenuiflorum*)
- T₉ – Asparagus (*Asparagus officinalis*)
- T₁₀ – Distilled water control.

In-vivo efficacy of plant extracts

Depending up on inhibition zone of the bacterial growth by the above plant extracts against the mixed infection by inoculation of *Bacillus* sp. + *Staphylococcus* sp. + *Streptococcus* sp., was evaluated on silkworm larvae of CSR₂ (Pure bivoltine) and PM x CSR₂ (Kolar gold).

Inoculation of silkworms

Inoculation of pathogens to silkworms was done on the third instar first day immediately after second moult. The dilution 10^{-6} involving of *Bacillus* sp. + *Staphylococcus* sp. + *Streptococcus* sp., were mixed and smeared on the mulberry

leaves and fed to the silkworms.

Administration of plant extracts

Administration of plant extracts was done twice on the second day of third instar and the first day of fourth instar. Fresh mulberry leaves were smeared with the plant extracts at the rate of 3 ml/treatment having 6 per cent concentration and allowed to dry for 30 minutes before feeding it to the silkworms. The control lot was maintained with distilled water treatment. In each treatment three replications were maintained (50 larvae / replication).

Economic parameters of cocoons:

Cocoon weight (g)

The ten cocoons were randomly selected from each replication of the treatments and weight was recorded on the fifth day of mounting and average weight was expressed.

Shell weight (g)

After taking cocoon weight, ten cocoons were cut open and the cocoon shell weight was recorded and the average was calculated to get the mean shell weight.

Pupal weight (g)

The ten pupae which were obtained from cutting the weighed cocoons and average weight were calculated expressed as mean pupal weight.

Shell ratio (%)

Shell ratio denotes the total amount of silk available in a single cocoon and is expressed in percentage. Shell ratio was calculated by using the formula

$$\text{Cocoon shell ratio} = \frac{\text{Shell weight (g)}}{\text{Cocoon weight (g)}} \times 100$$

Silk filament length (m)

Five cocoons per replication were selected and each cocoon was reeled using euppovette and silk filament length was recorded. Filament length was calculated by the formula,

$$L = R \times 1.125 \text{ m}$$

Where, in

L = Length of the silk filament (m)

R = Number of revolutions

1.125 m = Circumference of the Euppovette

Denier

Denier represents the size of the silk filament, it was found out by using the formula,

$$\text{Denier} = \frac{\text{Weight of silk filament (g)}}{\text{Length of silk filament (m)}} \times 9000$$

Results and Discussion

Influence of plant extracts on cocoon weight (g)

Supplementation of acetone plant extract at six per cent concentration increased the cocoon weight against flacherie induced silkworms of CSR₂ and PM x CSR₂ breeds. However, the maximum cocoon weight (17.92 and 13.63 g/10 cocoons) was registered in the silkworm breeds CSR₂ and PM x CSR₂, respectively fed on T₉ (*Asparagus officinalis*) treated mulberry leaves, followed by T₈ (*Ocimum tenuiflorum*) (17.58 and 12.62 g/10 cocoons) and T₅ (*Phyllanthus emblica*) (17.47 and 11.52 g/10 cocoons) treated silkworm lots which are found significantly on par with each other. However, lowest

cocoon weight (14.37 and 9.47 g/10 cocoons) was noticed in the T₁₀ (control) batch (Table 1; Plate 1 & 2).

The improvement in cocoon weight in both the silkworm breeds (CSR₂ and PM x CSR₂) was noticed due to supplementation of plant extracts of *A. officinalis* and *O. tenuiflorum* compared to control. It could be due to plants containing many biochemical factors that may contributed for improvement of cocoon weight. The present results are comparable with findings of Manjunath *et al.* (2009) [9] who reported that silkworm larvae of PM x CSR₂ infected with 10⁻¹, 10⁻² and 10⁻³ dilution of *Bacillus* sp., fed on *Aegle marmelos* extract recorded maximum cocoon weight (1.34 and 1.34 g) at 1:1 and 1:3 proportions, respectively. Harish Babu *et al.* (2011) [5] who also reported that, silkworm larvae (PM x CSR₂) fed on mulberry leaves smeared with *Aloe vera gel* extract at 100 per cent concentration against inoculation by 10⁻² *Bacillus* sp. spore dilution had effective enhancement of cocoon weight (1.93 g) compared to other treatments and control. According to Divya and Patil (2016) [3] oral supplementation of vitamin C (ascorbic acid) rich botanical extract amla juice at 1.5 per cent and lime juice at 3 per cent to fifth instar silkworm *Bombyx mori* L. had showed positive impact on cocoon weight (16.72 and 16.32 g/10 cocoons).

Influence of plant extracts on cocoon shell weight (g)

Cocoon shell weight of CSR₂ and PM x CSR₂ breeds were differed significantly among the treatments. The maximum Shell weight (3.743 and 2.480 g/10 cocoon shells) was recorded in T₉ (*Asparagus officinalis*) followed by T₈ (*Ocimum tenuiflorum*) (3.370 and 2.263 g/10 cocoon shells), T₅ (*Phyllanthus emblica*) (3.293 and 1.897 g/10 cocoon shells) and T₂ (*Tinospora cordifolia*) (3.153 and 1.827 g/10 cocoon shells) treated silkworms and are found statistically on par with each other. Whereas, the lowest cocoon shell weight (2.033 and 1.227 g/10 cocoon shells) was recorded in T₁₀ (control) (Table 1).

The cocoon shell weight was greatly influenced by application of different plant extracts in both silkworm breeds (CSR₂ and PM x CSR₂). The improvement in shell weight with the application of different plant extracts may be due to increased nutritional efficiency of food which is utilized for the maximum protein content of the cocoon shell. The results are in agreement with the findings of Priyadarshini *et al.* (2009) [13] who reported that shell weight was significantly higher in treatments with amla (0.30 g) and boerhavia (0.29 g) against *Bacillus* sp., whereas, significantly lesser shell weight (0.15 g) was recorded in control batch. Harish Babu *et al.* (2011) [5] who also reported that, silkworm larvae (PM x CSR₂) fed on mulberry leaves smeared with *Aloe vera gel* extract at 100 per cent concentration against inoculation by 10⁻² *Bacillus* sp. spore dilution had effective enhancement of shell weight (0.340 g) compared to other treatments and control. Further, Waktole Sori and Bhaskar (2015) [17] also reported fortification of M₅ mulberry leaves with botanicals *Psoralea coryleifolia* and *Phyllanthus niruri* significantly recorded maximum shell weight (3.68 and 3.72 g/10 cocoons) in PM x CSR₂ silkworm hybrid.

Influence of plant extracts on pupal weight (g)

Supplementation of different plant extracts at six per cent concentration against the late larval flacherie pathogens infected silkworms showed significant difference with respect to pupal weight. Among the treatments in silkworm breeds CSR₂ and PM x CSR₂, maximum pupal weight (14.32 and

11.15 g/10 pupae) was recorded in T₉ (*Asparagus officinalis*) administered silkworm breeds of CSR₂ and PM x CSR₂ followed by T₈ (*Ocimum tenuiflorum*) (14.21 and 10.36 g/10 pupae) and T₅ (*Phyllanthus emblica*) (14.17 and 9.62 g/10 pupae) which were found significantly on par with each other. The, least pupal weight (12.30 and 8.22 g/10 pupae) was recorded in T₁₀ (control) (Table 1; Plate 3 & 4).

The present findings are supported by the observations of Manjunath *et al.* (2009) [9] who reported that administration of medicinal botanical extracts on third and fourth instar larvae of PM x CSR₂ infected with 10⁻¹, 10⁻² and 10⁻³ dilution of *Bacillus* sp. (hemolymph) resulted in reducing bacterial flacherie disease. However, *Aegle marmelos* extract sprayed lots recorded maximum pupal weight (1.12 and 1.12 g) at 1:1 and 1:3 proportions, respectively. According Divya and Patil (2016) [3] the oral supplementation of vitamin C (ascorbic acid) rich botanical extract to fifth instar silkworm *B. mori* L. amla juice at 1.5 per cent and lime juice at 3 per cent significantly increased the pupal weight (14.78 and 14.55 g) compared to other treatments and control.

Influence of plant extracts on ten cocoon shell ratio (%)

Supplementation of different plant extracts to silkworm breeds CSR₂ and PM x CSR₂ showed significant difference with respect to cocoon shell ratio. In silkworm breeds CSR₂ and PM x CSR₂ silkworms fed on mulberry leaves fortified with T₉ (*Asparagus officinalis*) recorded higher shell percentage (20.92 and 18.15 %) when compared to other treatments, followed by T₈ (*Ocimum tenuiflorum*) (19.09 17.91 %), T₅ (*Phyllanthus emblica*) (18.83 and 16.46 %), T₂ (*Tinospora cordifolia*) (18.08 and 16.04 %) and T₁ (*Curcuma longa*) (17.29 and 15.32 %). The Lowest cocoon shell ratio (14.13 and 13.14 %) was recorded in control silkworm batch (Table 1).

The present observations are comparable with findings of Divya and Patil (2016) [3] who reported that oral supplementation of amla juice at 1.5 per cent and lime juice at 3 per cent to fifth instar silkworm, *Bombyx mori* L had the positive impact on ten cocoon shell ratio (21.61 and 20.86 %). Sujatha *et al.* (2015) [15] also reported that when silkworms were fed on mulberry leaves fortified with aqueous leaf extract of *Ocimum sanctum* in the second instar increased average silk ratio (15.975 %).

Influence of plant extracts on silk filament length (m)

The supplementation of different plant extracts to the silkworm breeds CSR₂ and PM x CSR₂ through mulberry leaves showed positive effect with respect to single cocoon filament length. Significantly longer filament length (1066.33 and 725.00 m) was recorded in T₉ (*Asparagus officinalis*) treated silkworm breed CSR₂ and PM x CSR₂, followed by T₈ (*Ocimum tenuiflorum*) (1034.67 and 69733 m) treated silkworm batches, respectively. However, shorter silk filament length (830.00 and 476.67 m) was recorded in T₁₀ (control) (Table 1; Plate 5 & 6).

The increase in the single filament length in the treatments of *A. officinalis*, and *O. tenuiflorum* of silkworm breeds (CSR₂ and PM x CSR₂) might be due to more shell weight obtained in the respective treatments. The present results are supported by the findings of Harish Babu *et al.* (2011) [5] who observed that, silkworm larvae (PM x CSR₂) fed on mulberry leaves smeared with *Aloe vera gel* extract at 100 per cent concentration against inoculation by 10⁻² *Bacillus* sp. spore dilution had effective enhancement of filament length (903.94

m). According to Kumari *et al.* (2011) [7] administration of mulberry leaves treated with aqueous leaf extract of *Phyllanthus niruri* increased filament length (912.50 m) in PM x CSR₂ silkworm hybrid. Further, Sujatha *et al.* (2015) [15] also observed that fortification of mulberry leaves with leaf extract of *Ocimum sanctum* at 3 per cent concentration increased the silk filament length (838.01 m).

Influence of plant extracts on cocoon filament denier

Administration of plant extracts to flacherie induced silkworms exhibited significant results with respect to silk

filament denier for both CSR₂ and PM x CSR₂ breeds. However, in CSR₂ and PM x CSR₂ silkworm breeds, the thicker denier (2.61 and 2.64) was recorded in T₉ (*Asparagus officinalis*) treated silkworms silkworm batches. In case silkworm breed CSR₂, the thinner denier was recorded in T₃ (*Tridax procumbens*) (2.20) followed by T₄ (*Phyllanthus niruri*) (2.21) treated silkworms and also recorded thinner denier than T₁₀ (control) silkworm batch (2.27). Whereas, in silkworm breed PM x CSR₂, the thinner denier 1.99 was registered in T₁₀ (control) compared to other treatments (Table 1).

Table 1: Influence of medicinal plant extracts on late larval flacherie disease in relation to cocoon parameters of silkworm, *Bombyx mori* L.

Treatments	CSR ₂						PM x CSR ₂					
	Cocoon weight (g/10 cocoons)	Shell weight (g/10 shells)	Pupal weight (g/10 pupae)	Shell Ratio (%/10 shells)	Filament length (m)	Filament Denier	Cocoon weight (g/10 cocoons)	Shell weight (g/10 shells)	Pupal weight (g/10 pupae)	Shell Ratio (%/10 shells)	Filament length (m)	Filament Denier
T ₁	16.59 ^{bc}	2.863 ^{bc}	13.87 ^{ab}	17.29 ^{bcd}	941.33 ^{cd}	2.38 ^{bcd}	11.15 ^b	1.720 ^{cd}	9.43 ^{cd}	15.32 ^{cde}	664.00 ^{de}	2.47 ^{ab}
T ₂	17.41 ^{ab}	3.153 ^b	14.17 ^a	18.08 ^{abc}	975.00 ^{bc}	2.45 ^{abc}	11.37 ^b	1.827 ^c	9.54 ^{bc}	16.04 ^{abcd}	643.31 ^{cd}	2.30 ^{bc}
T ₃	16.00 ^{cd}	2.583 ^{cd}	13.41 ^{abc}	16.09 ^{cde}	923.00 ^{cd}	2.20 ^d	10.47 ^{bcd}	1.543 ^{cde}	8.93 ^{cde}	14.73 ^{cde}	608.33 ^{de}	2.16 ^{bc}
T ₄	15.33 ^{cde}	2.443 ^{cd}	12.89 ^{bcd}	15.99 ^{cde}	967.67 ^{bc}	2.21 ^d	10.51 ^{bcd}	1.610 ^{cd}	8.90 ^{cde}	15.35 ^{bcd}	546.29 ^{de}	2.09 ^c
T ₅	17.47 ^{ab}	3.293 ^{ab}	14.17 ^a	18.83 ^{abc}	977.00 ^{bc}	2.51 ^{ab}	11.52 ^b	1.897 ^{bc}	9.62 ^{bc}	16.46 ^{abc}	687.00 ^{bc}	2.61 ^{ab}
T ₆	15.00 ^{de}	2.263 ^d	12.73 ^{cd}	15.05 ^{de}	903.67 ^{cde}	2.28 ^{cd}	10.61 ^{bc}	1.623 ^{cd}	8.99 ^{cde}	15.25 ^{cde}	519.00 ^{de}	2.14 ^c
T ₇	15.58 ^{cde}	2.237 ^d	13.09 ^{bcd}	14.32 ^e	882.33 ^{de}	2.38 ^{bcd}	10.02 ^{cd}	1.383 ^e	8.64 ^{de}	13.77 ^{de}	482.33 ^e	2.04 ^c
T ₈	17.58 ^{ab}	3.370 ^{ab}	14.21 ^a	19.09 ^{ab}	1034.67 ^{ab}	2.52 ^{ab}	12.62 ^{ab}	2.263 ^{ab}	10.36 ^{ab}	17.91 ^{ab}	697.33 ^b	2.58 ^{ab}
T ₉	17.92 ^a	3.743 ^a	14.32 ^a	20.92 ^a	1066.33 ^a	2.61 ^a	13.63 ^a	2.480 ^a	11.15 ^a	18.15 ^a	725.00 ^a	2.64 ^a
T ₁₀	14.37 ^e	2.033 ^d	12.30 ^d	14.13 ^e	830.00 ^e	2.27 ^{cd}	9.47 ^d	1.227 ^e	8.22 ^e	13.14 ^e	476.67 ^e	1.99 ^C
F - test	*	*	*	*	*	*	*	*	*	*	*	*
S. Em ±	0.462	0.109	0.372	0.833	28.460	0.061	0.384	0.049	0.251	0.301	20.061	0.068
C D at 5 %	1.306	0.561	1.039	2.946	83.958	0.180	1.056	0.376	0.853	2.584	71.437	0.199

* - Significant at 5 % level.



Plate 1: Cocoon of CSR₂ silkworm breed administered with (A) *Asparagus officinalis*, (B) *Ocimum tenuiflorum* and (C) control (distilled water).

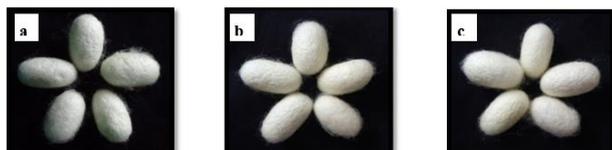


Plate 2: Cocoon of PM x CSR₂ silkworm breed administered with (a) *Asparagus officinalis*, (b) *Ocimum tenuiflorum* and (c) control (distilled water).

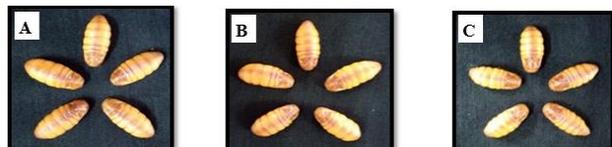


Plate 3: Pupa of CSR₂ Silkworm breed administered with (A) *Asparagus officinalis*, (B) *Ocimum tenuiflorum* and (C) control (distilled water).

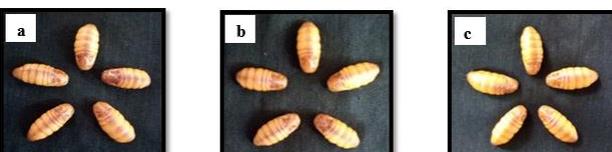


Plate 4: Pupa of PM x CSR₂ Silkworm breed administered with (a) *Asparagus officinalis*, (b) *Ocimum tenuiflorum* and (c) control (distilled water).

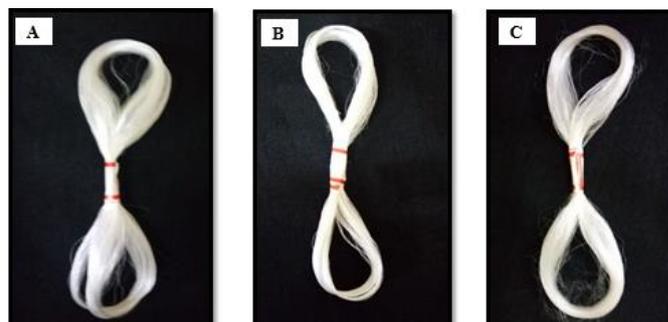


Plate 5: Cocoon filament of CSR₂ silkworm breed administered with (A) *Asparagus officinalis*, (B) *Ocimum tenuiflorum* and (C) control (distilled water).

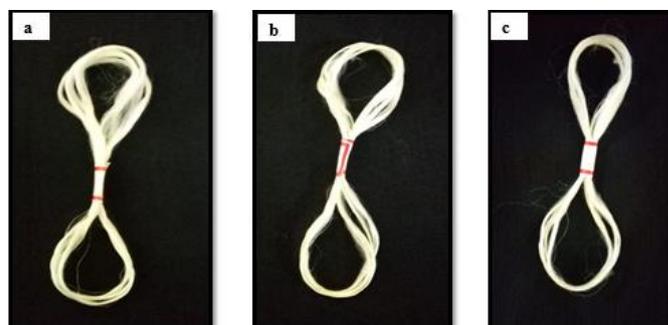


Plate 6: Cocoon filament of PM x CSR₂ silkworm breed administered with (a) *Asparagus officinalis*, (b) *Ocimum tenuiflorum* and (c) control (distilled water).

Thicker denier might be attributed to increased filament weight per unit length of silk filament. The present results are supported by findings of Chavan and Bhavane (2016) [1] who reported that ethanolic plant extract of *Bougainvillea*

spectabilis supplementation in silkworms PM and CSR₂ inoculated with *BmNPV* recorded increased denier (3.90 and 1.78) compared to control (2.40 and 0.96). Sridevi (2003) ^[14] also observed that the application of *Sauropus androgynous* at 0.10 per cent concentration increased the filament denier (2.52) in silkworm hybrids CSR₂ x CSR₄ compared to control (2.45).

Conclusion

The enhancement in economic parameters like cocoon weight, shell weight, pupal weight, shell ratio, filament length and filament denier of silkworm breeds CSR₂ and PM x CSR₂ is may due to presence of possible antimicrobial activity along with certain bio-active compounds in the medicinal plant extracts which reflected in the wealthy performance of the silkworm in terms of qualitative and quantitative characters.

It can be concluded from the study that, administration of acetone plant extract of *Asparagus officinalis* at 6 per cent concentration found beneficial in improving the cocoon and post cocoon parameters of CSR₂ and PM x CSR₂ silkworm breeds.

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