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Evaluation of certain medicinal plant extracts for the management of late larval Flacherie disease of silkworm, *Bombyx mori* L.

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Abstract
The study on evaluation of nine different medicinal plant extracts against late larval flacherie disease of silkworm *B. mori* L. showed significant results. Among the nine medicinal plants extracts, *Asparagus officinalis* administration in silkworms (CSR_2 and PM x CSR_2) was found effective by enhancing the larval growth parameters *viz.*, fifth instar larval duration (197.48 h and 189.10 h), mature larval weight (33.66 and 29.28 g / 10 larvae), ET_{50} value for larval mortality (184.66 and 205.84 h), reduced larval mortality (18.00 and 12.66 %) and ERR (79.33 and 86.67 %) in both the silkworm breeds respectively compared to control.

Keywords: Flacherie, medicinal plant extracts and pathogen inoculation.

Introduction
Sericulture is an art of rearing of silkworms for the production of raw silk, which is used in various agro based industries. During silkworm rearing the worms are affected by number of diseases *viz.*, Flacherie, Grasserie, Muscardine and Pebrine due to various biological, chemical, physical, nutritional and environmental factors, which leads to cocoon crop loss and poor quality raw silk production. Among these diseases Flacherie disease is most severe and accounting for the cocoon crop loss to the tune of 71 per cent as reported by Sidhu and Singh (1968) [13] and 30 to 40 per cent by Chitra *et al.* (1975) [2], also 47.9 per cent reported by Savanurmath *et al.* (1992) [12], whereas 33.88 per cent was reported by Tayal and Chauhan (2017) [17]. Flacherie is the condition caused by both viruses and bacteria. The bacterial agents that are induces flacherie are, *Bacillus* sp., *Streptococcus* sp., *Staphylococcus* sp., *Bacillus thuringiensis*, *Serratia marcescens etc*., (Chitra *et al.*, 1975) [2]. Prolonged use of chemical antibiotics may lead to development of resistant in microbes to the drug. The present investigation has been undertaken to study the antibacterial 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 of silkworm *B. mori* L.

Material and methods
Preparation of plant extracts
The extracts from nine different medicinal plants were prepared as per the procedure adopted by Karthikairaj *et al*., (2014) [5]. The above mentioned plants were collected from 'Sanjeevini vatika' (Herbal garden) and Botanical garden UAS, GKVK, Bengaluru. The collected plant samples after shade drying made to fine powder using electric blender. 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 magnet 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 (Plate 1).

Treatment details

T_1 – Turmeric (*Curcuma longa*)
T_2 – Amruthaballi (*Tinospora cardifolia)*
T_3 – Coat buttons (*Tridax* procumbens)
T_4 – Kirunelli (*Phyllanthus niruri*)
T_5 – Amla (*Phyllanthus emblica*)
T_6 – Pomegranate (*Punica granatum*)
T_7 – Aloe vera (*Aloe vera*)
T_8 – Tulasi (*Ocimum tenuiflorum*)
T_9 – Asparagus (*Asparagus officinalis*)
T_{10} – Distilled water control.

Isolation of pathogens

Mulberry silkworms exhibiting specific symptoms of late larval flacherie were collected and surface sterilized. The midgut juice was collected from the larvae. Further, midgut was dissected to collect the alimentary canal. 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) [9, 14, 10, 1].

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 ^{0}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 koches' postulates in causing the disease was identified.

***In-vivo* efficacy of plant extracts**

Depending upon 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 CSR_2 (Pure bivoltine) and PM x CSR_2 (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).

Results and Discussion

Influence of plant extracts on fifth instar larval duration (h)

Silkworm breeds CSR_2 and PM x CSR_2 fed on mulberry leaves fortified with nine different medicinal plant extracts at six per cent concentration against mixed infection by bacterial isolates (*Bacillus* sp., *Staphylococcus* sp. and *Streptococcu* sp.) causing late larval flacherie showed significant effect with respect to extending the fifth instar larval duration of both the breeds. The maximum fifth instar larval duration of 197.48 and 189.10 hours was recorded in T_9 (*Asparagus officinalis*) treated silkworm batch of CSR_2 and PM x CSR_2 respectively, followed by T_8 (*Ocimum tenuiflorum*) recorded 196.27 and 185.27 hours of fifth instar larval duration. However, in silkworm breed CSR_2, among the treatments shorter fifth instar larval duration 190.33 hours was recorded in control. Whereas in PM x CSR_2 silkworm breed the sorter fifth instar larval duration 171.24 hours was registered in T_7 (*Aloe vera*) treated silkworm batch compared to control (173.90 h) (Table 1).

The above observations are in conformity with results of Sridevi (2003) [15] who reported that spraying the extracts of *Tagetes erecta* and *Adathoda vasica* increased the fifth instar larval duration (146.34 and 146.00 h) significantly at 0.50 per cent concentration in silkworm PM x CSR_2. Further, Mahesha *et al.* (1999) [6] also confirmed that foliar supplementation of Cassia serica, Lantana camara, Amaranthus spinorus and Eupatorium odoratum extended the larva duration silkworm *B. mori* L. Mala *et al.* (2017) [7] also reported that, silkworm PM x CSR_2 reared on mulberry leaves fortified with aqueous extracts of *Aloe vera* at 100 per cent concentration had showed the reduced larval duration (7.76 days) when compared to other treatments and control.

Influence of plant extracts on fifth instar larval weight (g)

Silkworm larvae of CSR_2 and PM x CSR_2 fed on mulberry leaves treated with medicinal plant extracts at six per cent concentration showed significant difference among the treatments with respect to fifth instar larval weight. In silkworm breeds CSR_2 and PM x CSR_2, the maximum fifth instar larval weight of 33.66 and 29.28 g/10 larvae was recorded in T_9 (*Asparagus officinalis*) administered silkworm batch, followed by 31.33 and 28.02 g/10 larvae was recorded in T_8 (*Ocimum tenuiflorum*) treated batch, respectively. However, the lowest fifth instar larval weight (24.74 and 22.41 g/10 larvae) was recorded in control for both the breeds of silkworms compared to other treatments (Table 1; Plate 3 & 4).

The results are comparable with the findings of Sujatha *et al.* (2015) [16] who reported that when silkworms were fed on mulberry leaves administered with aqueous leaf extract of *Ocimum sanctum* at 3 per cent concentration recorded highest fifth instar larval weight (31.704 g/10 worms). Divya and Patil (2016) [3] also reported that supplementation of amla juice at 1.5 per cent and lime juice at 3 per cent had showed positive impact on larval growth resulted in increased larval weight of 40.41 and 39.05 g, respectively. Gobena and Bhaskar (2015) [5] also reported that silkworm PM × CSR_2 fed on M_5 mulberry leaves fortified with *Psoralea coryleifolia* and *Phyllanthus niruri* plant extracts recorded maximum mature larval weight (28.63 and 30.13 g/10 worms).

Influence of plant extracts on ET_{50} for larval mortality (h)

The ET_{50} value pertaining to larval mortality of CSR_2 and PM x CSR_2 silkworm breeds differed significantly among the treatments., the longer ET_{50} value for mortality (184.66 and 205.84 h) was recorded in silkworms administered with T9 (*Asparagus officinalisi*), followed by T_8 (*Ocimum*

tenuiflorum) (180.00 and 201.19 h), T_5 (*Phyllanthus emblica*) (179.17 and 197.66 h) and T_2 (*Tinospora cardifolia*) (179.00 and 192.44 h) which were significantly on par with each other in silkworm breeds CSR_2 and PM x CSR_2, respectively. Whereas, the shorter ET50 value for larval mortality (147.84 and160.38 h) was recorded in T_{10} (control) (Table 1).

The current study showed that the ET_{50} value for mortality is longer in plant extract administered silkworms whereas it is found shorter in control, which clearly shows that plant extracts possess certain secondary metabolites which may suppressed the pathogenicity of flacherie organisms in inoculated silkworms.

Influence of plant extracts on larval mortality (%)

The larval mortality of CSR_2 and PM x CSR_2 silkworm breeds was greatly decreased by administration of medicinal plant extracts compared to control and found significant. In CSR_2 and PM x CSR_2 silkworm breeds, the minimum larval mortality (18.00 and 12.66 %) was recorded in T_9 (*Asparagus officinalis*) followed by T_8 (*Ocimum tenuiflorum*) (19.33 and 12.67 %) treated silkworm batch. However, the maximum larval mortality (31.33 and 22.67 %) was recorded in control (Table 1; Plate 2).

The reduced larval mortality in silkworm batches administered with medicinal plant extracts compared to control might be due to the fact that plants contain secondary metabolites, which may helped in reduction in mortality of silkworms against mixed infection by pathogens (*Bacillus* sp. + *Staphylococcus* sp. and *Streptococcus* sp.) of late larval flacherie. These results are comparable with the findings of Manjunath (2007) [8] who reported that larval mortality of PM x CSR_2 was greatly influenced by application of different medicinal plant extracts. The minimum larval mortality (2.66 and 4.22 %) was found in *Adathoda vesica* as compared to control (9.77 and 12.44 %) against *Bacillus* sp. at 1:1 and 1:3 proportions. Sridevi (2003) [15] also reported that the mortality due to grasserie and flacherie was lower in medicinal plant extracts supplemented silkworm hybrids CSR_2 x CSR_4 and PM x CSR_2 compared to control. The lowest grasserie (5.50 and 4.00 %) and Flacherie (3.00 and 2.16 %), incidence was noticed in *Withania somnifera*.

Influence of plant extracts on Effective Rate of Rearing (%)

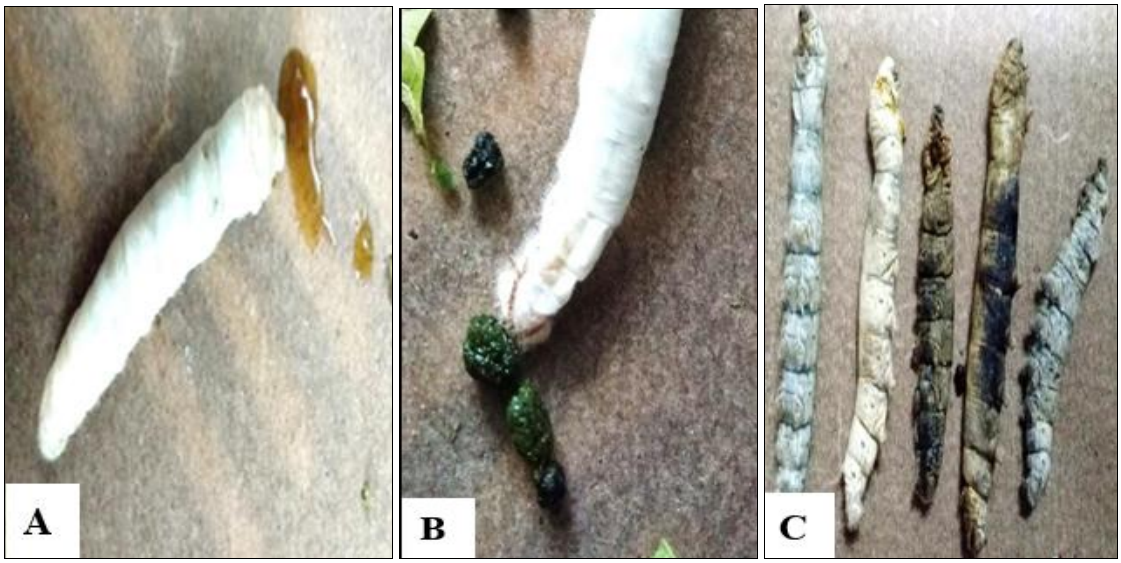
Feeding of silkworms (CSR_2 and PM x CSR_2) with mulberry leaves applied with acetone extract of different medicinal plant extracts increased the effective rate of rearing (ERR) significantly. However, in the silkworm breed CSR_2 and PM x CSR_2, the maximum ERR (79.33 and 86.67 %) was recorded in T_9 (*Asparagus officinalis*) treated silkworm batch followed T_8 (*Ocimum tenuiflorum*) (76.00 and 83.33 %) and T_5 (*Phyllanthus emblica*) (74.67 and 83.14 %) treated silkworm batches.

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

Treatments	CSR_2					PM x CSR_2				
	5th instar larval duration (h)	Larval weight (g/10 larvae)	ET_{50} (%) larval mortality	Larval mortality (%)	ERR (%)	5th instar larval duration (h)	Larval weight (g/10 larvae)	ET_{50} (%) larval mortality	Larval mortality (%)	ERR (%)
T_1	192.87^{de}	28.30^{bcde}	164.83^{c}	22.00^{bcde}	74.00^{abcd}	181.20^{cd}	26.36^{ab}	190.55^{c}	15.30^{bcd}	81.20^{bc}
T_2	193.44^{cd}	29.11^{bcd}	179.00^{b}	20.67^{cde}	74.66^{abc}	180.78^{cd}	27.59^{ab}	192.44^{c}	15.33^{bcd}	80.00^{bc}
T_3	191.38^{def}	26.96c^{def}	158.81^{d}	25.33^{bc}	70.67^{bcde}	178.82^{d}	26.50^{ab}	183..04de	18.00^{abc}	77.33^{cd}
T_4	191.46^{def}	28.00^{cde}	161.00^{cd}	24.00^{bcd}	72.64^{bcd}	178.33^{cd}	26.72^{ab}	185.00^{d}	17.67^{bc}	79.33^{bc}
T_5	194.97^{bc}	30.20^{abc}	179.17^{b}	22.00^{bcde}	74.67^{abc}	183.62^{bc}	27.96^{ab}	197.66^{b}	14.00^{cd}	83.14^{ab}
T_6	191.60^{def}	26.81^{def}	152.07^{e}	27.33^{ab}	68.62^{de}	178.57^{d}	26.79^{ab}	179.95^{de}	18.00^{abc}	77.33^{cd}
T_7	191.03^{ef}	25.33^{ef}	151.66^{e}	26.67^{ab}	70.00^{cde}	171.24^{e}	25.40^{bc}	178.95^{e}	20.00^{ab}	74.67^{cd}
T_8	196.27^{ab}	31.33^{ab}	180.00^{b}	19.33^{de}	76.00^{ab}	185.27^{ab}	28.02^{ab}	201.19^{ab}	12.67^{d}	83.33^{ab}
T_9	197.48^{a}	33.66^{a}	184.66^{a}	18.00^{e}	79.33^{a}	189.10^{a}	29.28^{a}	205.84^{a}	12.66^{d}	86.67^{a}
T_{10}	190.33^{f}	24.74^{f}	147.84^{e}	31.33^{a}	65.33^{e}	173.90^{e}	22.41^{c}	160.38^{f}	22.67^{d}	72.67^{d}
F - test	*	*	*	*	*	*	*	*	*	*
S. Em ±	0.711	0.674	1.401	1.968	1.932	1.460	0.823	1.880	1.560	1.886
C D at 5 %	2.061	3.127	4.508	5.701	5.422	4.243	3.455	5.222	4.847	5.563

* - Significant a 5 % level.

Plate 1: Stock solutions of acetone extract of medicinal plants materials



A: Vomiting of gut juice **B: Excreta with high moisture content** **C: Dead worms**

Plate 2: Late larval flacherie symptoms exhibited in CSR_2 and PM x CSR_2 silkworm breeds

Plate 3: CSR_2 Silkworms administered with (A) *Asparagus officinalis,* (B) *Ocimum tenuiflorum* and (C) control (distilled water).

Plate 4: PM x CSR_2 Silkworms administered with (a) *Asparagus officinalis,* (b) *Ocimum tenuiflorum* and (c) control (distilled water).

However, the minimum ERR 65.33 and 72.67 per cent were recorded in T_{10} (control) for both the silkworm breeds CSR_2 and PM x CSR_2, respectively (Table 1).

The increased ERR was observed in the present study may be due to less incidence of disease during silkworm rearing and also be due to the phagostimulant compounds present in these antimicrobial plants may have resulted in increased silkworm survival rate. Similar results were observed by Sridevi (2003) [15] that increased ERR of 91.82 and 93.80 per cent (CSR_2 x CSR_4 and PM x CSR_2) was recorded in *Withania somnifera* compared to control (66.06 and 77.84 %). Further, Priyadarshini *et al.* (2009) [11] observed that, under *in-vivo* conditions, amla and boerhavia were found to be effective in managing flacherie with a survivability of 75 and 74 per cent, respectively.

Conclusion

The enhance in larval growth parameters of silkworm breeds CSR_2 and PM x CSR_2 may be due to the presence of possible antimicrobial activity along with certain growth stimulant bio-active compounds present in the plant extracts may activating the velocities of bio chemical reactions catalyzed by the mid gut enzymes and thereby increase the digestibility in the larvae of silkworm *B. mori* L. Improved digestibility reflects in the wealthy performance of the silkworm in terms of qualitative and quantitative characters. I

It can be concluded from this study that, administration of acetone plant extract at 6 per cent concentration found

beneficial in inhibiting the bacterial growth of late larval flacherie organisms and also improving the rearing parameters of CSR_2 and PM x CSR_2 silkworm breeds.

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