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RESEARCH ARTICLE :

Solvent extracts of medicinal plants against *Bm*NPV

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SUMMARY : Grasserie, a viral disease caused by nuclear polyhedrosis virus inflicts great loss to mulberry silkworm, *Bombyxmori*L. This disease causes extensive larval mortality thereby reducing cocoon yield and ultimately silk yield. Five medicinal plants *viz,. Eucalyptus citriodora, Cymbopogon citrates, Thymus vulgaris, Rosmarinusofficinalis* and *Annonasquamosa* were evaluated against grasserie on bivoltine double hybrid {(CSR 6 × CSR 26) × (CSR 2 × CSR 27)} revealed that *R. officinalis* (1000ppm) was not toxic to the silkworm and also enhanced the economic parameters of silkworm. *A. squamosa* (1000ppm) was highly toxic and caused 100 per cent larval mortality. Two different solvents, *viz.*, chloroform and hexane were used for extracting active principles from four medicinal plants. The results revealed that chloroform extracts of *R. officinalis* (1000ppm) was found to be more effective against grasserie compared to other botanical extracts with the larval mortality of 12.66 per cent, whereas the larval mortality was 100 per cent in virus alone treatment. *R. officinalis* (1000ppm) enhanced the economic parameters of silkworm such as larval weight, cocoon weight, shell weight, filament weight. In the present work, GCMS studies with *R. officinalis* spots eluted through TLC revealed the presence of 22 compounds *viz.*, Eucalyptol, Caryophyllene, Camphor, Borneol, *etc.*

KEY WORDS :
*Bm*NPV, Medicinal plants, Mortality and economic parameters, GCMS

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BACKGROUND AND OBJECTIVES

India is the second largest producer of silk in the world next to China. The mulberry raw silk production in the country was 13,991 MT in 2012-13 compared to 14,153 MT in 2011-12 with a decrease of 1.1%. Silkworms are susceptible to a number of diseases caused by different infectious agents (Doreswamy *et al.*, 2004). It is the main factor seriously affecting the cocoon production (Watanabe, 1994). The infectious silkworm diseases are caused by pathogenic microorganisms. The mulberry silkworm, *Bombyxmori L.* is well known to suffer from bacterial, viral, fungal and protozoan diseases. Due to continuous rearing of mulberry silkworms, they become highly susceptible to various diseases which accounts for 30-40 per cent loss in the cocoon yield (Chandrasekharan *et al.*, 2006). Generally, 70 % of damage caused by diseases is due to viruses. Among viruses, nuclear polyhedrosis viruses (NPV) have caused the highest damage to silkworm, *B. mori*in tropical regions (Sivaprasad *et al.*, 2003; Biabani *et al.*, 2005). It infects all larval instars but more commonly in the 4th and 5th instars, during all seasons and cause 20-50% cocoon

crop losses in India (Sivaprakasam and Rabindra, 1995; Biabani, 1998; Sivaprasad *et al*., 2003; Khurad *et al*., 2004). It is also known as *"Haul- hula"*in Karnataka, *"Rasa"*in West Bengal, *"Polapurugu"*in Andhra Pradesh and *"PalPoochhi"* in Tamil Nadu (Ramesh Babu, 2009)

Various methods are available for disease management *viz.*, chemicals and botanicals. Increased use of chemical disinfectants is not only hazardous to silkworms but also to human beings employed for the purpose. The use of plant molecules for management of diseases inside the rearing house is appropriate for the current scenario because of their cost effectiveness and eco friendly nature.

RESOURCES AND METHODS

Silkworm rearing :

Experiments were conducted using the bivoltine double hybrid, DH1 ((CSR 6 × CSR 26) × (CSR 2 × CSR 27)) as this is mostly reared by Tamil Nadu farmers. Chawki worms for the experiments were purchased from the private Chawki Rearing Centre, Amman Chawki Centre, Udumalpet, Thiruppur district. The rearing rooms along with its appliances were thoroughly disinfected prior to the commencement of experiments. A day before disinfection, the rooms and appliances were washed with 5% bleaching powder solution and sun dried for 3-4 hours. A day after, the rooms and appliances were properly disinfected with chlorine dioxide + slaked disinfectant. Room was disinfected at the rate of 1 litre per square metre floor area.The doors and windows were kept open for 24 hours before the commencement of rearing (Chandrashekar, 2003)

Preparation of medicinal plant extract :

Leaf materials from plants *viz., E. citriodora, C. citrates, T. vulgaris, R. officinalis*and *A. squamosa* was thoroughly washed with running tap water and shade dried at room temperature for two weeks. The dried plant leaves were ground into fine powder and packed into air tight polythene bags and stored in normal condition. Soxhlet apparatus was used for extraction purpose.25 g of the powdered leaves of medicinal plants were weighed separately into 200 ml hexane/chloroform and percolated for 24 hours. The sample tube of the unit was fitted with a filter disc at the bottom and filled with ground samples, sealed with another filter disc and compressed. This was fitted to electric heating mantle with soxhlet unit, filled with 240 ml of hexane/ chloroform, and temperature of 40 ºC and 60 ºC was maintained for hexane/chloroform, respectively. The unit was regulated with water to give a slow controlled flow of the solvent through the compressed sample. The filtrate was collected in a rained bottom flask. The residual extract was collected in a flask and transferred to a rotary flask vacuum evaporator for evaporation of the solvent. The residue thus obtained was stored at 4^0C in airtight bottles for future use (Khatune, 2000)

Isolation and processing of nuclear polyhedrosis virus :

The grasserie infected larvae were collected and the abdominal legs of larvae were cut open, and the haemolymph containing the polyhedral bodies (POBs) were collected in a sterile container. The haemolymph was passed through a double layer of muslin cloth to remove the crude tissue debris and the pellet was collected. The POBs were then semi-purified by differential centrifugation. First, the suspension was centrifuged at 500 rpm for about a minute to sediment the crude tissue debris, if any. Then the supernatant was centrifuged at 3000 rpm for three minutes. The pellet was washed three times in distilled water and finally suspended in distilled water (Khurad, 2004)

Method of application :

For experimental purposes, fourth instar silkworms were used. Before the treatment, 25 larvae were transferred to small bamboo trays of 30 cm diameter and 3 replications were maintained per treatment. The larvae were fed four times a day. Bed cleaning was carried out daily.

The viral suspension with the concentration of 10^6 POBs/ml was prepared in distilled water. The mulberry leaves were dipped in the viral suspension, shade dried and fed to the larvae. After inoculation, the solvent extracts (chloroform / hexane) of medicinal plants (at 500 and 1000ppm) were prepared in distilled water. Mulberry leaves were dipped in these suspensions, shade dried and fed to the experimental worms. Observations on larval mortality (%), larval duration (h), cocoon weight (g), shell weight (g), shell ratio (%), filament length (m), filament weight (g) and denier were recorded.

OBSERVATIONS AND ANALYSIS

The results obtained from the present study as well as discussions have been summarized under following heads :

Larval mortality :

Among the five medicinal plants tested, chloroform leaf extracts of *R. officinalis* (1000ppm) was found to be most effective against *Bm*NPV and recorded lesser larval mortality of 12.66 per cent, whereas untreated control recorded nil larval mortality. Larval mortality in *Bm*NPV alone treatment was 100 per cent (Table 1).

Padma and Manimegalai (2007) reported that the aqueous extract of *Plectranthusamboinicus* and *P. corylifolia*were effective in suppressing grasserie with mortality of 24.00 and 25.33 per cent in the cross breed, PM X CSR 2, respectively. The per cent mortality was found to be higher in bivoltine single hybrid than in bivoltine double hybrid.

Mahalingam *et al.* (2010) reported that least mortality of 1.35 per cent was recorded in the treatment with TNAU seridust +*Psoralia* extract *per os* application followed by 1.50 % in vijetha+ *Psorali*extract *per os* application. Highest mortality of 3.28 per cent was recorded in untreated control.

Economic parameters :

The bioassay study on silkworms treated with *Bm*NPV and chloroform extracts of medicinal plants yielded significant results than hexane extracts on larval weight, cocoon weight, shell weight and filament weight. Chloroform extracts of *R. officinalis* (1000ppm) treatment apart from reducing *Bm*NPV infection recorded higher larval weight (4.56g), cocoon weight (2.00g); shell weight (0.40g) and filament weight (0.32) which was on par with untreated control (4.05; 2.06; 0.42; 0.33) (Table 2).

Mahalingam *et al.* (2010) reported that larval weight, cocoon weight, shell weight, shell ratio and cocoon yield was 3.68 g, 1.74 g, 0.323 g, 18.56 per cent and 78.20 kg/ 100 dfls, respectively in TNAU seri dust +

Table 1 : Effect of solvent extracts of medicinal plants on larval mortality of silkworm.

	Larval mortality (%)			
Solvents	Chloroform		Hexane	
Treatments (ppm)	1000	500	1000	500
Rosmarinusofficinalis	12.66^{b} (20.84)	19.66^{c} (26.32)	18.00^{b} (25.10)	21.33^{c} (27.50)
Thymus vulgaris	21.00^{cd} (27.27)	23.66^{d} (29.10)	22.33^{c} (28.20)	23.33^{c} (28.88)
Eucalyptus citriodora	47.33^{e} (43.47)	48.33^{e} (44.04)	46.66^{d} (43.08)	48.33^{d} (44.04)
Cymbopogon citrates	55.00^{f} (47.87)	56.66^{f} (48.82)	48.33^{d} (44.04)	50.00^{d} (45.00)
*Bm*NPV alone	100.00^{g} (89.42)		100^{e} (89.42)	
Untreated Control	0.0^{a} (0.57)		0.0^{a} (0.57)	
C.D.	2.08		2.1	
S.E. ±	0.1		1.01	

Table 2 : Effect of solvent extracts of medicinal plants on economic parameters of silkworm

Solvents	Chloroform								Hexane							
Characters	Larval weight (%)		Cocoon weight (%)		Shell weight (%)		Filament weight (%)		Larval weight (%)		Cocoon weight (%)		Shell weight (%)		Filament weight (%)	
Treatments (ppm)	1000	500	1000	500	1000	500	1000	500	1000	500	1000	500	1000	500	1000	500
R.officinalis	4.56^{a}	3.96^{bc}	2.00^{a}	1.93^{a}	0.40^{a}	0.37^{ab}	0.32^{ab}	0.30^{bc}	4.16^{ab}	3.96^{ab}	2.00^{a}	1.83^{ab}	0.37^{a}	0.33^{ab}	0.30^{a}	0.28^{b}
T.vulgaris	3.76^{c}	3.40^{cd}	1.76^{ab}	1.08^{ab}	0.33^{bc}	0.30^{bc}	0.29^{cd}	0.28^{cd}	3.83^{bc}	3.43^{bc}	1.63^{b}	1.53^{bc}	0.29^{b}	0.28^{b}	0.26^{c}	0.26^{c}
E.citriodora	3.26^{cde}	3.09^{def}	1.16^{bc}	1.05^{cd}	0.27^{cd}	0.21^{de}	0.27^{d}	0.27^{d}	3.16^{cd}	3.08^{d}	1.50^{bc}	1.23^{cd}	0.22^{c}	0.19^{cd}	0.25^{c}	0.25^{c}
C. citrates	2.83^{ef}	2.73^{f}	1.16^{cd}	1.00^{d}	0.20^{de}	0.16^{e}	0.22^{e}	0.20^{e}	2.43^{e}	2.02^{e}	1.00^{cd}	1.02^{d}	0.15^{d}	0.17^{d}	0.20^{d}	0.20^{d}
*Bm*NPV	-		-		-		-		-		-		-		-	
Control	4.05^{ab}		2.06^{a}		0.42^{a}		0.33^{a}		4.46^{a}		2.06^{a}		0.35^{a}		0.31^{a}	
C.D.	0.58		0.39		0.07		0.02		0.52		0.33		0.05		0.05	
S.E. ±	0.28		0.19		0.04		0.01		0.25		0.16		0.02		0.02	

*Psoralea*extract treated batch whereas untreated control recorded 3.54g, 1.53g, 0.277g, 18.10% and 66.57 kg/ 100 dfls, respectively.

Identification of biochemical constituents from *R. officinalis* through GC-MS :

In the present work, GCMS revealed the presence of 22 active compounds. Among these most of the compounds are used against human diseases, may be those compounds have the capacity to reduce grasserie disease in silkworm also (Table 3). And the Total ion chromatogram (TIC) of *R.officinalis* is shown in the Fig. 1.

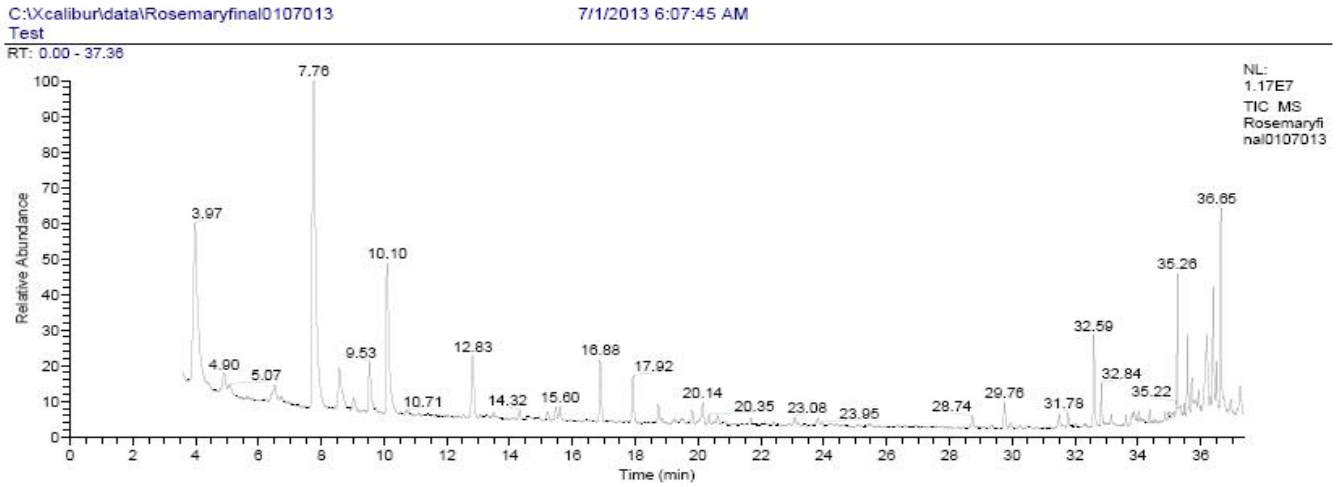

Fig. 1 : Total ion chromatogram (TIC) of *R. officinalis*

Table 3 : Lists of active compounds found in *R. officinalis* extract analyzed by GC-MS

Sr. No.	Compound	RT (min)	Molecular formula	Molecular weight (g/mol)	Probability
1.	Eucalyptol	3.97	C10H18O	154	75.34
2.	Camphor	7.76	C10H16O	152	9.22
3.	Borneol	8.57	C10H18O	154	15.79
4.	trans-α-Terpinylpentanoate	9.55	C15H26O2	238	1.21
5.	D-Verbenone	10.1	C10H14O	150	1.16
6.	Bornyl acetate	12.83	C12H20O2	196	17.55
7.	Caryophyllene	16.88	C15H24	204	21.26
8.	α -Caryophyllene	17.92	C15H24	204	73.28
9.	Naphthalene	18.74	C15H24	204	10.28
10.	Cedrene	19.82	C15H24	204,	8.05
11.	Copaene	20.14	C15H24	204	2.39
12.	trans-Z- α -Bisabolene epoxide	23.81	C15H24O	220	35.87
13.	1-Heptatriacotanol	31.49	C37H76O	536	17.6
14.	n-Hexadecanoic acid	31.79	C16H32O2	256	61.21
15.	Heptamethyl-3-phenyl-1,4-cyclohexadiene	32.61	C19H26	254	48.92
16.	Trenbolone Acetate	32.84	C20H24O3	312	8.13
17.	9,12-Octadecadienoyl chloride, (Z,Z)-	33.85	C18H31ClO	298	5.46
18.	2-Phenanthrenol, 4b,5,6,7,8,8a,9,10-octahydro-4b,8,8-trimethyl-1-(1-methylethyl)-, (4bS-trans)-	35.26	C20H30O	286	81.83
19.	Spirost-8-en-11-one, 3-hydroxy(3α,5α,14α,20α,22α,25R)-	35.89	C27H40O4	428	6.14
20.	Squalene	36.2	C30H50	410	1.43
21.	Benzo[b]furan, 3-(4-methoxyphenyl)-2,6-dimethyl-	36.4	C17H16O2	252	35.41
22.	1,2-Benzenedicarboxylic acid, diisooctyl ester	36.65	C24H38O4	390	61.2

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