

Development and Validation Trials of User-friendly Disinfectant for Containment of Tasar Silkworm Diseases

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ABSTRACT

As there are no curative measures for the control of silkworm diseases, different preventive methods using formaldehyde or chlorine based chemical disinfectants are being practiced in tasar culture. But these disinfectants are hazardous to the users and environment. Moreover, these chemicals have limitations to be effective in open field. Hence, the project was taken up to develop user friendly disinfectant suitable to the open field rearing conditions of the tasar culture. Based on the *in vitro* inactivation studies of the previous experiment the best performed user friendly chemicals namely Tri-sodium orthophosphate (TSP), Slaked lime (SL), Urea (UR) and Desnet (DN) were selected, prepared certain combinations and conducted *in vivo* studies. The best performed user friendly disinfectant was subjected to on farm trials in CTR&TI field and validation trials in different states of RTRSs and results were statistically evaluated. *In vivo* studies of user friendly disinfectant (TSP+ SL+UR) have shown less mortality due to the various diseases than any other treatments and mortality percentage recorded was below 6% in case of all tested pathogens. Another combination, DN + SL+ UR occupied last position with disappointed results which even did not cross the values of the control. In inoculated control mortality percentage was ranged from 53% to 98.83% which directly indicated effect of the pathogens on the cocoon yield. On farm trials of user friendly disinfectant results revealed that, Cocoon yield/Dfl ranged from 40.00 to 123 cocoons/Dfl with the average of 74.40 cocoons/Dfl. and values recorded for S.D and C.V% were 34.81 cocoons and 46.79% respectively in treated lots where as in control, Cocoon yield/Dfl ranged from 30.50 to 101 cocoons/Dfl with the average of 59.40 cocoons/Dfl and values recorded for S.D and C.V% are 29.77 cocoons and 50.12%, respectively. In treated lots, Single cocoon weight, Single shell weight and S.R% slightly improved over the control. RTRSs of Jagadapur, Bhandara and Warangal conducted validation trials of user friendly disinfectant and results revealed that, Cocoon yield/ Dfl, Single cocoon weight, Single shell weight and S.R% were increased in user-friendly disinfectant lots when compared with control. Data of on farm trials and validation trials were pooled and the improvement was calculated, 13.99 cocoons/Dfl in the yield, 0.30g in Single cocoon weight, 0.07g in Single shell weight and 0.26% in Shell ratio improvement in treated lots, over the control was observed. T-test analysis of pooled data indicated that the mean value of treated lot Cocoon Yield/Dfl is more than control which is significantly different at 5% level, at the same time mean values of treated lots Single cocoon weight, Single shell weight and S.R% are more than control but not significantly different. Newly developed disinfectant is user friendly and acts against all kinds of disease causing pathogens of tasar silkworm. The developed user friendly disinfectant can be useful to the tasar farmers to obtain stable and improved cocoon yield by suppressing diseases.

Key words: *Antheraea mylitta* D., Diseases, Mortality, User-friendly disinfectant

INTRODUCTION

Tasar silkworm, *Antheraea mylitta* D., an economically important insect is mainly reared on *Terminalia arjuna* (Arjuna), *T. tomentosa* (Asan) and *Shorea robusta* (Sal) food plants in out door conditions. Several diseases affect tasar silkworm larvae during rearing. Virosis, pebrine, muscardine and bacteriosis are the commonly prevalent diseases caused respectively by pathogens cytoplasmic polyhedral virus (A rheovirus), *Nosema* species (Microsporidia), *Penicillium citrinum* (Fungus) and different types of bacteria.

In India, the extent of cocoon crop loss due to the silkworm diseases is nearly 40% (Sahay *et al.*, 2000). As there are no curative measures in silkworm rearing for the control of silkworm diseases, different preventive methods using chemical disinfectants are practiced (Singh *et al.*, 2002, 2005; Sahay *et al.*, 2005, 2008). Asiphor and Sodium hypochlorite have been used for the control of bacteriosis and virosis in tasar silkworm (Bansal *et al.*, 1996). Hydrochloric acid has been used for the control of microsporidiosis in tasar silkworm (Sukla *et al.*, 1994). Thangavelu *et al.* (1995) used various chemicals as disinfectants for the control of different diseases in tasar silkworm. Bhattacharaya *et al.* (1995), Datta *et al.* (1998), Samson *et al.* (1998) and Patil and Shardamma (1999) used chemicals based body and rearing seat disinfectants like Labex, Sanjeevani, Vijetha, Resham Jyoti for management of diseases in mulberry silkworm *Bombyx mori*. Most of the body disinfectants are para formaldehyde based, which are banned chemicals due to hazardous to the users and environment. Moreover, these chemicals have limitations to be effective in open field. Hence, a broad spectrum formulation by using user's friendly chemicals was developed for protection of tasar silkworm from diseases in rearing and conducted validation trials.

MATERIALS AND METHODS

Based on the in vitro inactivation studies of the previous experiment the best performed user friendly chemicals namely Tri-sodium orthophosphate (TSP), Slaked lime (SL), Urea (UR) and Desnet (DN) were selected, prepared certain combinations and conducted in vivo studies. The best performed chemical combination was subjected for validation trials in different states of RTRSs and results were statistically evaluated.

***In vivo* studies: Challenge with sterilized disease causative pathogens**

Two chemical combinations TSP+SL+UR (1.5+0.5+0.5) and DN+SL+UR (1.5+0.5+0.5) were prepared and sterilized the disease causing pathogens separately for 10 minutes. Tasar silkworms were challenged with sterilized disease causing pathogens, reared up the cocoon formation in indoor conditions and mortality was recorded. Inoculated, healthy and normal (Bleaching powder) controls were also maintained separately. In case of inoculated control the disease causing pathogens were feed to the silkworms without sterilization with the disinfectants, where as in the case of healthy control distilled water was sprayed on the leaf without pathogen load and silkworms were reared up to the cocoon formation. Bleaching powder is existing disinfectant and this treatment was taken as normal control for the comparison.

On form trials: Based on the results of the *in vivo* studies the chemical combination TSP+SL+UR (1.5+0.5+0.5%) was selected for the on form trials. SRT, SST, Entomology, Pathology, Physiology rearing plots of CTR&TI form were selected for the trials. The rearing

fields and plants were disinfected with the chemical combination TSP+SL+UR (1.5+0.5+0.5%). A separate plot was maintained control by spraying 3% bleaching powder solution. Rearing of the tasar silkworms were conducted during first and second crops of 2011 in all the selected plots. The data was collected on different cocoon parameters and results were statistically analyzed.

Validation trials of selected combination: Based on the results of on form trials the chemical combination TSP+SL+UR (1.5+0.5+0.5%) was selected for the Validation trials in different test centers namely, RTRS Warangal, RTRS, Jagadapur and RTRS, Bhandara. The rearing field and rearing plants were disinfected with the chemical combination TSP+SL+UR (1.5+0.5+0.5%). A separate plot was maintained control by spraying 3% bleaching powder solution. Rearing of the tasar silkworms were conducted in all the test centers during first and second crops of 2012. The data was collected on different cocoon parameters and results were statistically analyzed.

RESULTS

In vivo studies:

Results revealed that, TSP+ SL+UR (1.5+0.5+0.5%) chemical combination have shown less mortality due to the various diseases than the any other treatments. Mortality percentage recorded was 5.20%, 4.00%, 4.40% and 2.66% in the case of AmCPV, Pebrine spores, Bacteria and *P. Citrinum*, respectively. DN + SL+ UR treatment occupied least passion among the treated batches in the suppression of various diseases causing pathogens while Bleaching powder treatment has occupied second place. In the case of inoculated control mortality percentage was ranged from 53% to 98.83% where as in the case of health control no mortality due to the any diseases was found (Table 1).

Table 1. Mortality of tasar silkworm (indoor) challenged with sterilized disease causative pathogens

Disinfectant	Conc. (%)	Mortality %			
		AmCPV (1x10 ⁴ PIB/ml)	Pebrine spore (1 x 10 ⁴ Sp/ml)	Bacteria (1x10 ⁻⁴ cells/ml)	<i>P. Citrinum</i> (1x 10 ⁴ Sp/ml)
DN + SL+UR	1.5+0.5+0.5	23.00	25.33	11.00	10.00
TSP+ SL+UR	1.5+0.5+0.5	5.20	4.00	4.40	2.66
Bleaching. P	3.0	13.83	9.80	4.80	6.33
Control (I)	--	98.83	80.60	58.8	53.00
Control (H)	--	0.00	0.00	0.00	0.00

On form trials of user friendly disinfectant

Results revealed that, Cocoon yield/ Dfl, Single cocoon weight, Single shell weight and S.R% were increased in user-friendly disinfectant (TSP+ SL+UR) lots when compared with control (Table 2). In treated lots, Cocoon yield/Dfl ranged from 40.00 to 123 cocoons/Dfl with

the average of 74.40 cocoons/Dfl. and values recorded for S.D and C.V% were 34.81 cocoons and 46.79% respectively where as in the case of control, Cocoon yield/Dfl ranged from 30.50 to 101 cocoons/Dfl with the average of 59.40 cocoons/Dfl. and values recorded for S.D and C.V% are 29.77 cocoons and 50.12% respectively. In treated lots, Single cocoon weight ranged from 10.09 to 11.89g with the average of 10.90g and values recorded for S.D and C.V% are 0.78g and 7.17%, respectively where as in the case of control, Single cocoon weight ranged from 10.00 to 11.50g with the average of 10.67g and values recorded for S.D and C.V% are 0.75g and 7.02% respectively. In treated lots, Single shell weight ranged from 1.04 to 1.39g with the average of 1.20g and values recorded for S.D and C.V% are 0.15g and 12.32%, respectively where as in the case of control, Single shell weight ranged from 1.03 to 1.30g with the average of 1.15g and values recorded for S.D and C.V% are 0.12g and 10.46% respectively. S.R% ranged from 10.35 to 11.70% with the average of 11.00% and values recorded for S.D and C.V% are 0.58% and 5.29%, respectively in treated lots, where as in the case of control, S.R% ranged from 10.32 to 11.36% with the average of 10.75% and values recorded for S.D and C.V% are 0.46% and 4.25% respectively.

Test plot	Cocoon Yield/Dfl		Single cocoon wt.		Single shell wt.		S.R%	
	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control
SST	40.00	30.50	10.09	10.00	1.04	1.03	10.35	10.32
SRT	41.00	30.50	10.47	10.00	1.10	1.03	10.49	10.32
Physiology	83.00	70.00	10.47	10.42	1.16	1.15	11.05	11.04
Pathology	123.00	101.00	11.89	11.50	1.39	1.23	11.70	10.70
Entomology	85.00	65.00	11.56	11.44	1.32	1.30	11.42	11.36
Average	74.40	59.40	10.90	10.67	1.20	1.15	11.00	10.75
S.D	34.81	29.77	0.78	0.75	0.15	0.12	0.58	0.46
C.V%	46.79	50.12	7.17	7.02	12.32	10.46	5.29	4.25

Validation trials of user friendly disinfectant:

RTRSs of Jagadapur, Bhandara and Warangal conducted validation trials of user friendly disinfectant. Results revealed that, Cocoon yield/ Dfl, Single cocoon weight, Single shell weight and S.R% were increased in user-friendly disinfectant lots when compared with control lots (Table 3). In treated lots, Cocoon yield/Dfl ranged from 37.90 to 68.20 cocoons/Dfl with the average of 55.37 cocoons/Dfl and values recorded for S.D and C.V% are 15.67 cocoons and 28.31% respectively where as in the case of control, Cocoon yield/Dfl ranged from 29.20 to 59.00 cocoons/Dfl with the average of 43.07 cocoons/Dfl. and values recorded for S.D and C.V% are 15.01 cocoons and 34.85% respectively. In treated lots, Single cocoon weight ranged from 11.10 to 13.00g with the average of 12.01g and values recorded for S.D and C.V% are 0.95g and 7.93%, respectively where as in the case of control, Single cocoon weight ranged from 11.02 to 12.00g with the average of 11.59g and values recorded for S.D and C.V% are 0.51g and

Table 3. Validation trials of user friendly disinfectant at different RTRSs

Name of RTRS	Cocoon Yield/Dfl		Single cocoon wt.		Single shell wt.		S.R%	
	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control
Jagadapur	68.20	59.00	11.93	11.75	1.92	1.87	16.10	15.91
Warangal	60.00	41.00	11.10	11.02	1.26	1.22	11.35	11.07
Bhandara	37.90	29.20	13.00	12.00	2.00	1.80	15.38	15.00
Average	55.37	43.07	12.01	11.59	1.73	1.63	14.28	13.99
S.D	15.67	15.01	0.95	0.51	0.41	0.36	2.56	2.57
C.V%	28.31	34.85	7.93	4.39	23.52	21.89	17.92	18.38

4.39% respectively. In treated lots, Single shell weight ranged from 1.26 to 2.00g with the average of 1.73g and values recorded for S.D and C.V% are 0.41g and 23.52%, respectively where as in the case of control, Single shell weight ranged from 1.22 to 1.87g with the average of 1.63g and values recorded for S.D and C.V% are 0.36g and 21.89% respectively. S.R% ranged from 11.35 to 16.10% with the average of 14.28% and values recorded for S.D and C.V% are 2.56% and 17.92%, respectively in treated lots, where as in the case of control, S.R% ranged from 11.07 to 15.91% with the average of 13.99% and values recorded for S.D and C.V% are 2.57% and 18.38%, respectively (Table 3).

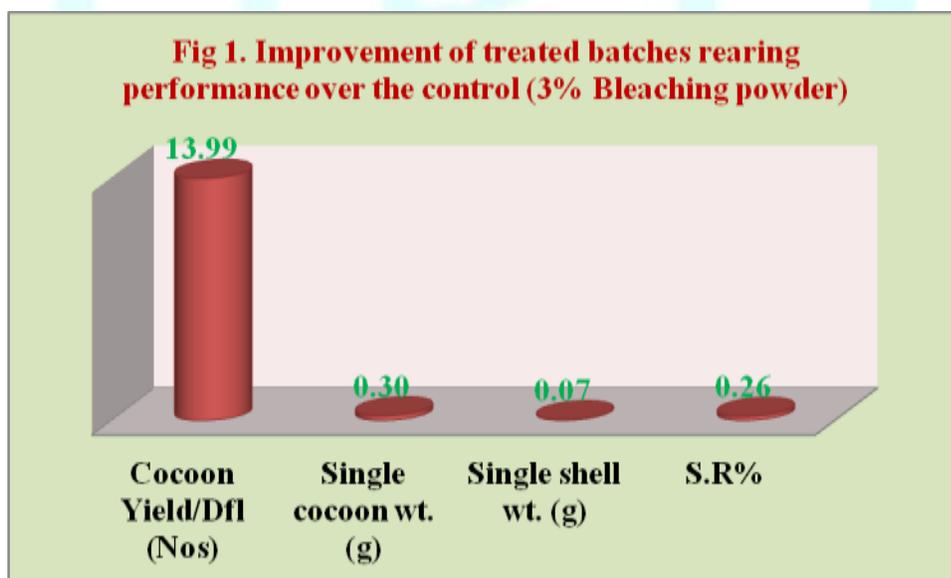
Statistical analysis for the performance values of user friendly disinfectant:

T-test analysis of pooled data of on form trials and validation trials revealed that, mean value of treated lot Cocoon Yield/Dfl is more than control which is significantly different at 5% level, at the same time mean values of treated lots Single cocoon weight, Single shell weight and S.R% are more than control but not significantly different (Table 4).

Table 4. T - test for the performance values of user friendly disinfectant

Test centers	Cocoon Yield/Dfl		Single cocoon wt.		Single shell wt.		S.R%	
	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control
SST	40.00	30.50	10.09	10.00	1.04	1.03	10.35	10.32
SRT	41.00	30.50	10.47	10.00	1.10	1.03	10.49	10.32
Physiology	83.00	70.00	10.47	10.42	1.16	1.15	11.05	11.04
Pathology	123.00	101.00	11.89	11.50	1.39	1.23	11.70	10.70
Entomology	85.00	65.00	11.56	11.44	1.32	1.30	11.42	11.36
Jagadapur	68.20	59.00	11.93	11.75	1.92	1.87	16.10	15.91
Warangal	60.00	41.00	11.10	11.02	1.26	1.22	11.35	11.07
Bhandara	37.90	29.20	13.00	12.00	2.00	1.80	15.38	15.00
T-test at 5%	2.15*		0.674		0.404		0.237	

With the perusal of the results, the improvement was calculated which revealed that 13.99 cocoons/Dfl in the yield, 0.30g in Single cocoon weight, 0.07g in Single shell weight and 0.26% in Shell ratio improvement in treated lots, over the control was observed (Fig 1.).



DISCUSSION

In vivo studies of user friendly disinfectant (TSP+ SL+UR) have shown less mortality due to the various diseases than the any other treatments which indicated less disease that in turns leads

to the high yielding. Mortality percentage recorded was below 6% in the case of all tested pathogens. The similar results were observed in the experiment conducted by Balavenkatasubbaiah *et al.* (1994). Another combination, DN + SL+ UR occupied last position with disappointed results which even did not cross the values of the control. In inoculated control mortality percentage was ranged from 53% to 98.83% which directly indicated effect of the pathogens on the cocoon yield.

On form trials of user friendly disinfectant, Qualitative and quantitative improvement in user-friendly disinfectant lots was observed. In treated lots, the average cocoon yield of 74.40 cocoons/Dfl was noticed which is 15.00 cocoons more than the average cocoon yield of the control. Average Single cocoon weight was 10.90g in case of treated batches and values of S.D and C.V% are 0.78g and 7.17% respectively which are in permitted level. Single shell weight and S.R% are directly proportional to each other, in treated lots both the parameters recorded higher values compared with control batches. In Validation trials Cocoon yield/ Dfl, Single cocoon weight, Single shell weight and S.R% were increased in user-friendly disinfectant lots in all test centers similar kind of results were observed in the experiments of Baig *et al.* (1989), Bhattacharya Krishnan *et al.* (1995), Datta *et al.* (1998) and Thangavelu *et al.* (1995). T-test analysis of pooled data of on form trials and validation trials revealed that mean value of treated lot Cocoon Yield/Dfl is more than control which is significantly different at 5% level, at the same time mean values of treated lots Single cocoon weight, Single shell weight and S.R% are more than control but not significantly different 13.99 cocoons/Dfl in the yield, 0.30g in Single cocoon weight, 0.07g in Single shell weight and 0.26% in Shell ratio improvement in treated lots, over the control was observed.

Advantages of newly developed eco-user friendly disinfectant are, it doesn't emit any pungent smell, user friendly, useful for the qualitative and quantitative improvement of the food plant leaf, acts against all kinds of disease causing pathogens of tasar silkworm and the general disinfectant can be sprayed in the field, on the bushes and even on silkworms when the rearing is under progress.

CONCLUSION

- *In vivo* studies of user friendly disinfectant (TSP+ SL+UR) have shown less mortality due to the various diseases than the any other treatments. Mortality percentage recorded was below 6% in the case of all tested pathogens.
- In on form trials and validation trials, Qualitative and quantitative improvement in user-friendly disinfectant lots was observed.
- T-test analysis of pooled data of on form trials and validation trials revealed that mean value of treated lot Cocoon Yield/Dfl is more than control which is significantly different at 5% level, at the same time mean values of treated lots Single cocoon weight, Single shell weight and S.R% are more than control but not significantly different.
- 13.99 cocoons/Dfl in the yield, 0.30g in Single cocoon weight, 0.07g in Single shell weight and 0.26% in Shell ratio improvement in treated lots, over the control was observed.

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