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EFFECT OF POLYMERIC NANOPARTICLE "PNIPAM" (POLY-N- ISOPROPYL ACRYAMIDE) ON THE MICROBIAL INFESTATIONS OF TASAR SILKWORM

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ABSTRACT
The present paper deals with the use of Polymeric compound such as "PNIPAM" for minimizing the fungal growth on various developmental stages of the wild variety of the Daba ecorace of tropical tasar silkworm, *Antheraea mylitta* Drury (Lepidoptera: Saturniidae). "PNIPAM" is a nanoparticle and has ability to form a microfilm which is acting as a barrier both externally and internally around the infected silkworms, thus, preventing the microbial infection therein. These nanoparticles have microfilm forming ability and can produce an additional barrier against the microbial infestations. The microbial diseases cause severe loss both in the productivity as well as silk performances parameters. In the present study an attempt has been made to know the efficacy of "PNIPAM", a polymeric compound for restricting the fungal growth on larval stages of tasar silkworms. It was observed that the formation of microfilms interfered with the growth of fungal infection and finally inhibits the microbial population. It was due to the restricted growth of the fungal hyphae in the microfilm that results in the suppression of mycilial growth both externally and internally body epithelia of the different larval instars. The result indicating that the application of "PNIPAM" at a concentration of 0.4 and 0.6 mg/mL shows significant decrease in microbial infestations in these silkworms. The work will add some valuable breakthrough in minimizing the loss of tasar silk production due to microbial infection, which is a common factor for this wild variety of tasar silkworm.

INTRODUCTION

Tasar silkworm *Antheraea mylitta* Drury is a tropical silkworm of India (Thangavelu, 1991, 1992). India is the home of all the four varieties of silkworm species. The various uses of silkworm byproducts such as silk fibre, faecal matters and pupa have been used since long time by the mankind. All aspects of silkworm depend upon the state of health of the worms. The microbial infestations create stress condition in these silkworms. The stressed silkworm produces relatively low quality products which may be quantitatively affected.

The perusal of available pertinent literature on tasar silkworm indicate that several types of microbes ranging Protozoa, Bacteria, Fungi, Viruses, endo-larval parasitoids such as Uzi Fly have potent role in minimizing the silk production due to infection and pathogenesis (Singh *et al.,* 1991 and Goel, R. K. 2000). The various microbes adopt different strategies to complete their life cycle using silkworm as a host as endo or ecto-pathogen.

Considerable work has been done from the pathogen and the pathogenicity of the tasar silkworm in India and abroad (Sen and Jolly, 1967; Singh and Thangavelu, 1971; Srivastava *et al.,* 1994, Hay *et al.,* 2004 and Leger and Wang, 2009). PNIPAM is polymeric nanomaterial possess all the same physical and chemical properties as its normal dimension (Imtiyaz *et al.*, 2013). Practically no information is available on the use of polymeric compounds in restricting the growth of microbes in the developmental stages of the tropical silkworms. However, the literature suggests the fungal control by other method using medicinal plant extract as well as antifungal agents (Chandrasekaran *et al.*, Nagarajan *et al.,* 1999.). The present work may be helpful as an alternate way of controlling fungal infestations in different larval stages of tasar silkworm and increases its productivity. The objective of the present study is to investigate the role of nanoparticles against fungal diseases of this ecorace cultivated abundantly in Bihar and Jharkhand states.

MATERIALS AND METHODS

Antheraea mylitta was obtained from Tasar Silk Seed Multiplication Centre, Bhagalpur and rearing of the developmental stages was done in P. G. Department of Biotechnology, T. M. Bhagalpur University and Bhagalpur. The fungal strain was isolated after the culture on PDA medium after following the technique of Subramanayam (1971) after some modifications.

The nanoparticle "PNIPAM" was purchased from Sigma Aldrich. The different larval instars were taken along with the control for the present study. The five test batches each containing 10 larval instars was assessed along with 2 control larvae. The freshly grown spores of *A. niger* on PDA were used for inoculation. Each test batch was inoculated with *Aspergillus* spore solution prepared in saline water. The concentration of the spores was determined through

Table 1: Showing different exogenous fungal spreads of control and different test groups

Average spread of fungal colony (cm)	PNIPAM Conc.(mg/mL)	1st Instars	2nd Instars	3rd Instars	4th instars	5th Instars
Control	0.0	1.45	3.56	4.57	6.72	7.93
Test group 1	0.1	1.25	2.83	4.22	5.97	7.13
Test group 2	0.2	1.13	2.36	3.78	5.02	6.45
Test group3	0.4	0.76	1.74	2.93	4.47	5.46
Test group4	0.6	0.52	1.25	2.14	3.89	4.72
Test group5	0.8	0.52	1.12	2.08	3.76	4.69

Table 2: Different endogenous fungal spreads of control and different test groups

Average spread of fungal colony (cm)	PNIPAM Conc.(mg/mL)	1st Instars	2nd Instars	3rd Instars	4th instars	5th Instars
Control	0	1.03	1.72	2.81	3.44	4.93
Test group 1	0.1	0.94	1.44	1.96	2.58	3.62
Test group 2	0.2	0.87	1.26	1.44	2.15	2.95
Test group3	0.4	0.63	1.15	1.29	1.77	2.36
Test group4	0.6	0.57	1	1.11	1.69	2.11
Test group5	0.8	0.54	0.94	1.09	1.65	2.08

Haemocytometry. The spore solution contained One million spores per mL. Then each test larva was washed thrice for the infection. After 72h of incubation at 27 ± 2ºC and RH 80% each larva was tested for the exogenous infection through Swab test in PDA media. The sacrificed instars were tested on the PDA media after surface sterilization through 0.2% $HgCl_2$ to remove the surface pathogen. The gut was isolated, opened and inoculated upon PDA media.

The different concentration of "PNIPAM" solution was prepared using double distilled water. The "PNIPAM" treated test batches at concentration ranges from 0.1, 0.2, 0.4, 0.6 and 0.8 mg/mL were tested for its antifungal activity against exogenous and endogenous fungal pathogens. Exogenous treatment of "PNIPAM" to different test group of larval instars was given in the manner of surface washing under aseptic condition. The different test group instars were maintained at 27 ± 2ºC and RH 80% .The exogenous treatment of "PNIPAM" was given twice for 2 days at the interval of 12h. The sacrificed instars were aseptically transferred to the sterilized PDA media. The inoculated plates were incubated for 72h at 27 ± 2ºC and RH 80% for the assessment of fungal growth.

For the endogenous treatment the different larval instars were first made fungus free with the antifungal agent "Blue Copper M-45". The sterilized leaves of *Terminalia arjuna* were inoculated with fungal spore solution and then treated with different concentration of "PNIPAM" and fed to the different test group instars. The 24h fed different instars larvae were sacrificed to isolate the gut. The gut was chopped and cut opened aseptically to expose the internal lining and transferred to the PDA media aseptically.

The regression analysis and correlation coefficient between microbial strains and "PNIPAM" concentration has been worked out according to (Weil, 1952).

RESULTS

The results obtained are depicted in Table 1 for exogenous fungal spreads of control and different test groups and Table 2

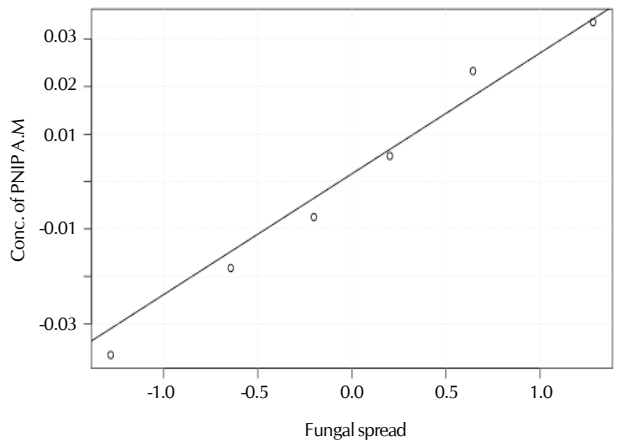

Figure 3.0: Regression graph of Endogenous fungal Spread for Instars 1st, 2nd and 3rd

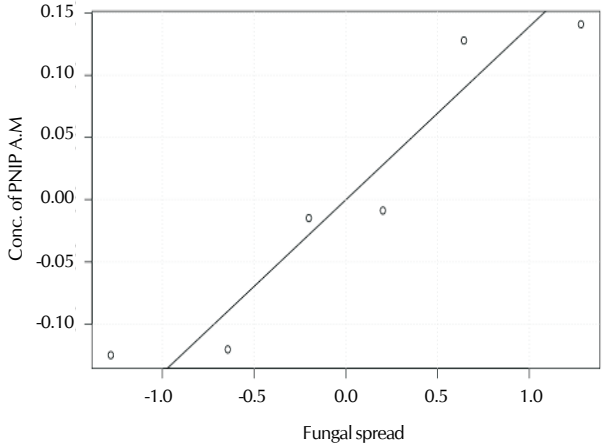

Figure 4.0: Regression graph of Endogenous fungal Spread for Instars 3rd, 4thand 5th

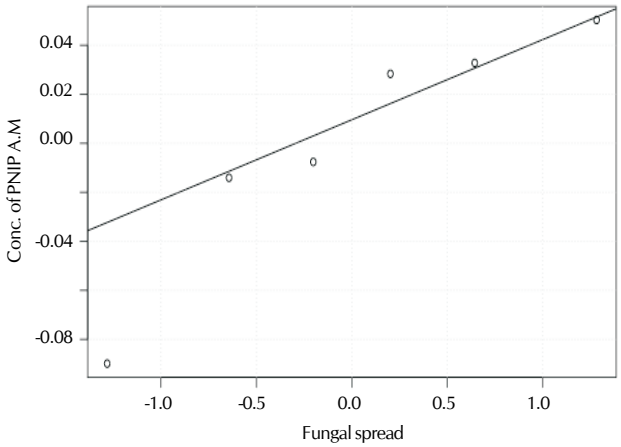

Figure 3.0: Regression graph of Endogenous fungal Spread for Instars 1st, 2nd and 3rd

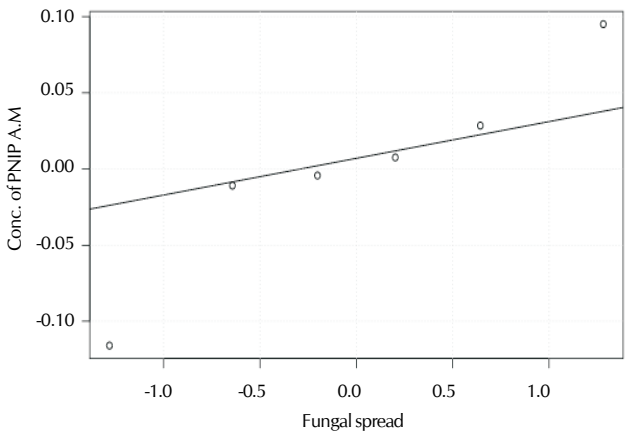

Figure 4.0: Regression graph of Endogenous fungal Spread for Instars 3rd, 4thand 5th

for endogenous fungal spreads respectively. Histograms-1.0 and 2.0 showing the exogenous and endogenous spreads of control and test groups of fungal strains. The Fig. 1 and 2 showing the regression analysis and correlation coefficients of the fungal strains with polymeric compound "PNIPAM".

The "PNIPAM" treated test batches at concentration 0.4 and 0.6 mg/mL shows considerable decrease in both exogenous and endogenous fungal infections. The 0.1 and 0.2mg/mL concentration of "PNIPAM" shows no obvious decrease in the fungal infection. The exogenous growth of fungus shows considerable decrease with the increase of "PNIPAM" concentration after the threshold concentration of 0.1 and 0.2mg/mL. The exogenous and endogenous fungal spread shows difference within the same incubation period under same condition of temperature and humidity.

DISCUSSION

The "PNIPAM", Poly (N-isopropylacrylamide) is a thermoresponsive polymer. It forms a three-dimensional hydrogel when crosslinked with N, N'- methylene- bis-acrylamide (MBAm (Imtiyaz *et al.,* 2013). The "PNIPAM" Nanoparticles have polymerizing effect and it can seal the outer coating of the larvae and inner gut from the microbial infestations (Leger and Wang, 2009). Sen and Jolly, 1967 and Singh and Thangavelu, 1991 have emphasized on the destruction of different larval instars and cocoon by microbial infections. Several workers stressed on the role of *Aspergillus niger* as a common fungal weed with wide range of hosts. *Aspergillus niger* is associated both exogenously and endogenously on the different larval stages of silkworms. *Aspergillus* changes the normal metabolism, natural flora and fauna of the silkworm (Srivastava *et al.*, 1994). In the present study it was observed that there was a gradual decrease in the fungal spread in correlation with the length of different treated larval instars test groups (Table I and II). "PNIPAM" has antifungal activity which may be due to the formation of Nanofilms inside the gut and at the outer surface of different instars. The Nanofilms may suppress the interaction of fungus with the body epithelium of the different instars. The concentration of 0.4 and 0.6mg/mL of aqueous solution of "PNIPAM" in correlation with the body length of different larval instars was found effective in the present study which is in conformity with the work of Leger and Wang, 2009. The 0.1 and 0.2 mg/mL concentration of "PNIPAM" shows slight reduction of fungal spread than control. This may be due to the formation of very thin Nano-film inside and outside the body surface of the different instars under experimentation. The concentration of 0.8 mg/mL shows saturation with the inhibitory effect of fungal growth. Thus, 0.4 and 0.6 mg/mL concentration of the "PNIPAM" solution seems to be more potent and have maximum inhibitory effect of fungal growth. The further study at ultramicroscopic level may reveals the better conclusion about the method of inhibition. Histological studies may provide the clue about the level of "PNIPAM" concentration invasion within the tissues and the physiological changes occurred after the application of "PNIPAM". The results (Table 1 and 2) along with their regression data shows that the compound "PNIPAM" may be employed against the fungal diseases of silkworms.

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