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SYNTHESIS AND EVALUATION OF DIAMINO SUBSTITUTED 1,3,4 THIADIAZOLE AS POSSIBLE BOMBYX MORI GROWTH ENHANCER

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ABSTRACT

Sericulture or silk farming is the rearing of silkworms for the production of raw silk. The mulberry silkworm, *Bombyx mori*, is a domesticated and monophagous insect which feeds only on the leaves of mulberry for its nutrition. Thiadiazoles are heterocyclic compounds containing oxygen and nitrogen. 1, 3, 4, thiadiazoles are extensively studied and are known to play diverse biological activities. Diamines or polyamines are found in various biological fluids and are necessary for optimal growth, replication and metabolism of every cell in the body. This report describes the synthesis of diamino substituted thiadiazole and its effect on the growth parameters of *Bombyx mori* silk worm. Results show that the title compound at high concentration resulted in increased worm weight, silk gland weight and cocoon weight and at low concentrations proved to be beneficial for improving the pupal weight. We conclude that the synthesized compound can be fed to *B. mori* silk worms for improving their economic parameters.

KEY WORDS : ***Diamines, Thiadiazoles, Bombyx mori, sericulture, Economic parameters***

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INTRODUCTION

Polyamines are synthesized in cells via highly regulated pathways. They are poly-cationic compounds with a wide range of physiological effects in microorganisms and animal cells. Polyaminesalso play an important role in chromosome stabilization and in DNA repair pathway[1]. They bind to DNA, affect the DNA synthesis by increasing the movement rate of the DNA replication fork [2,3]. Polyamines influence the transcriptional and translational stages of protein synthesis in eukaryotic cells[4]. They stabilize the membranes[5] and alter levels of free calcium[6,7]. Polyamine transporter system is highly active in rapidly proliferating cells[8].

A recent rational approach of drug design involves linking two molecules with individual intrinsic activity into a single hybrid molecule and the molecules thus produced were shown to have improved efficacy and minimum toxicity. Substituted 1,3,4 thiadiazoles posses a wide spectrum of biological activities including antimicrobial[9] anti-inflammatory[10] antitubercular[11] anticonvulsant[12] and antidiabetic[13] activities. The study of growth enhancing properties of thiadiazoles on silkworm, *Bombyx mori* is a novel approachto improve economic and commercial aspects of Sericulture.

Silkworm proteinshave a wide variety of applications like-cardiac tissue engineering[14], skin tissue engineering[15] biomedical applications[16], biotechnological applications[17] and tissue engineering applications[18,19]. Silk gland proteins, sericin and fibroin were useful in many biological processes[20] and biomaterials [21,22].

Enriching mulberry leaves by nutrient supplementation is one of the ways to improve growth rate in *Bombyx mori L*. Effect of mulberry leaves enriched with amino acids on growth of silkworms was studied[23,24,25]. The protein content of silk gland, fat body and muscles was found to increase significantly when fed with ascorbic acid[26]. Mulberry leaves enriched with nickel chloride, potassium iodide and their combinations has increased the cocoon weight at low concentrations[27]. The growth and development of larva, and subsequent cocoon production are greatly influenced by nutritional quality of mulberry leaves[28] .

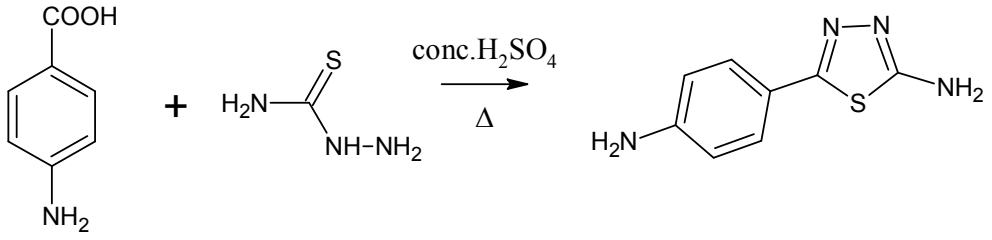
The proposed work based on the effect of amino substituted thiadizoles on the growth of silkworm, *Bombyx mori* was not exploited so far , and we considered it as a novel approach in the field of Sericulture. Results have proventhat the title compound has positive effect on the growth of the *B. mori* silkworms

MATERIAL AND METHODS

(i) Synthesis of 5-(4- aminophenyl)-1,3,4-thiadiazole-2-amine (scheme1)

A mixture of thiosemicarbazide (0.01mole, 3.6grms), 4-amino benzoic acid (0.01mole) and concentrated sulphuric acid (10ml) were taken in a round bottomed flask and refluxed for 3 hours the mixture was poured on to crushed ice, washed with water and recrystallized from ethanol to give 5-(4-amino phenyl)-1,3,4-thiadiazol-2-amine (79% yield) (scheme 1). Paleyellow compound, melting point 180-182ºC. Mass spectra-m/e is 192.05 (100.0% 193.05 9.8%). The H^1NMR: aromatic protons appeared at δ 7. 33 - 7. 90, NH2 proton at δ 9.14. The IR shows bands at 3314-3336 (vNH2) 3010 -1542 (vC=N), 1520 (vC=C) and at 648 (vCS).

Scheme1



Dietary supplementation of the silk worm with

the synthesised compound *Bombyx mori* (CB Csr2×Csr4 type) larvae were grown on normal mulberry leaves till the end of the 4th instar larval stage. feeding was given from day one of the 5th instar stage as silk glands develop from 5th instar stage. Larvae were divided into four experimental groups, each group consisting of 45 worms. 1mg/ml of the compound is dissolved in 1ml of ethanol and diluted with distilled water to get appropriate of 1mM, 0.1mM and 0.01mM strengths. Fresh mulberry leaves were treated with the synthesized compound by swab method. These treated leaves are fed to larvae three times per day. This treatment was carried out on all the days of fifth instar. The larvae fed with plain mulberry leaves were maintained as a control group.

(ii) Estimation of growth parameters

To study the effect of the compound on silkworm growth, the weight and lengths of silkworm larvae were measured every day during the fifth instar (until the spinning) stage, ten larvae from every replication were randomly selected and their weights and lengths were recorded. Average larval weights and lengths were derived from the above values. Silk glands were dissected out as per the standard procedures on all the days of the 5th instar and the weights were measured.

(iii) Estimation of Economic parameters

The Cocoon and post – cocoon parameters like cocoon weight, shell weight, shell ratio and pupal weight, were measured according to the formulae from standard procedures [29,,30,31] .

(iv) ***2Measurement of cell viability by 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay***

silkworms (both treated and controls) were dissected in the insect ringer solution (7.5gm of Nacl, 0.35gm of Kcl, 0.21gm of $Cacl_2$ in 1000ml of distilled water). Isolated silk glands were weighed and kept in microfuge tubes with 900 µl of insect ringer solution, 100µl of MTT (5mg/ml-MTT) was added and then incubated for one hour in dark. After 1 hr, the solution in the vial was discarded, and then the gland was washed thoroughly with insect ringer solution. Finally 1 ml of 50% (v/v) tween 80 solution was added and incubated for 24 hr in a shaker for extraction. The formed formazan product was collected diluted to 3ml with insect ringer solution, vortexed and optical density was measured at 570 nm using calorimeter.

RESULTS

(i) Estimation of growth parameters:

The result analyses of the effect of title compound on larval weights showed positive effect (Fig 1). Treatment with 0.01mM resulted in the maximum larval weight of 2.6gm on 6th day of 5th instar larval stage. Maximum larval weight of 2.5gm was observed with 1mM of the compound on the 5th day of 5th instar larval stage. 1mM treated larvae entered spinning stage prior to control larvae and larvae fed with lower concentrations of the compound.

Weight analysis of control and drug treated worms in 5th instar larval stage

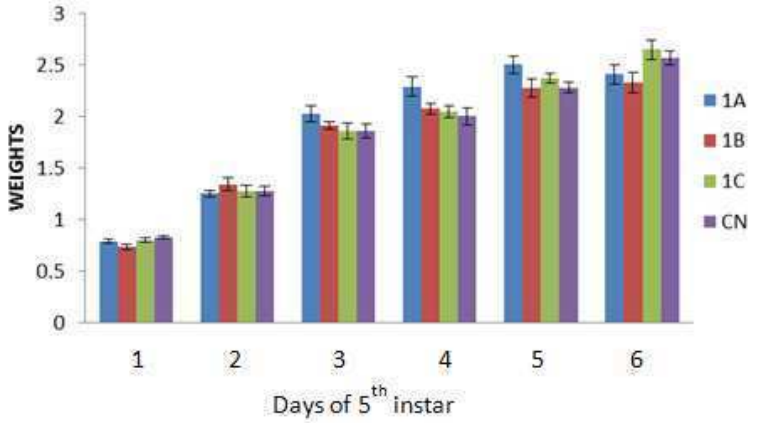


Figure 1

Graph representing the weights of the silkworms during different days of 5th instar larval stage. X-axis represents the days and Y-axis represents the weights of the worms in grams. 1A, 1B and 1C are 1mM, 0.1mM and 0.01mM concentrationsof the compoundrespectively

Maximum larval length of 6.03cm was observed when fed with 1mM of the compound (Fig 2). The lower concentrations of the title compound, 0.1mM and 0.01mM also showed increased larval lengths in comparision with the control group. Overall, an increase in larval length with increase in concentration of the compound ( ) was observed by the end of 5th instar stage.

Length analysis of control and treated worms in 5th instar larval stage

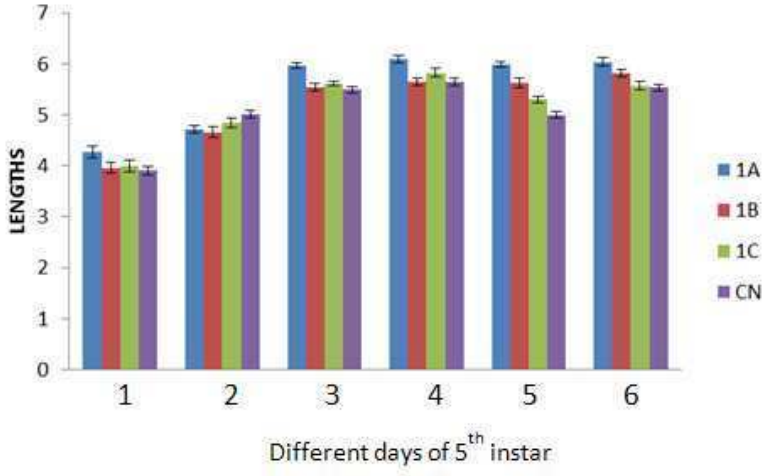


Figure 2

Graph representing the lengths of the silkworms during different days of 5th instar larval stage at different concentrations of the title compound. X-axis represents the days and Y-axis represents the lengths of the worms in centimeters. 1A, 1B and 1C are 1mM, 0.1mM and 0.01mM concentrationsrespectively.

As silk is secreted by the silk glands, we checked the effect of the title compound on the silk gland weights. Larvae treated with the compound showed better gland weights (Fig 3). All the

concentrations showed similar gland weights till day 4 of 5th instar. Maximum weight gain of the silk glands was observed on the 8th day with 0.01mM compound.

Analysis of silk gland weights in control and drug 5th instar silkworms

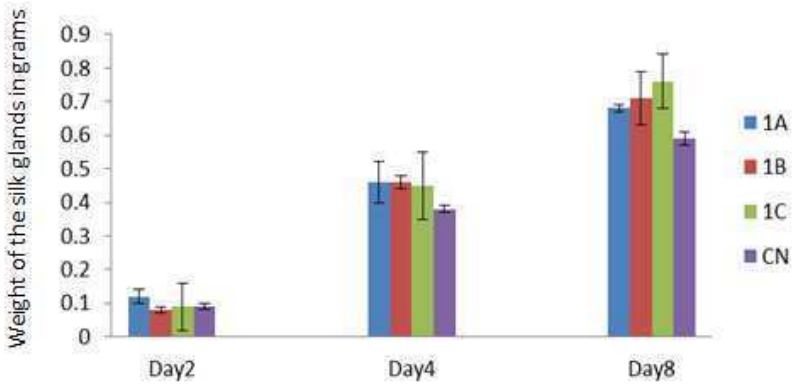


Figure 3

Graph representing the weights of the silk glands during different days of 5th instar larvae fed with different concentrations of the test compound. (Glands were dissected out and weighed on day 2, 4 and 8 of 5th instar larval stage.) X-axis represents the days and Y-axis represents the weights of the silk glands in grams. 1A, 1B and 1C are 1mM, 0.1mM and 0.01mM concentrations of the compoundrespectively.

(ii) Estimation of Economic parameters

Finally, the effect of the compound on economic trait of silk worm, cocoon weight, was checked. Cocoon weight is the most important character as it indicates the approximate quantity of the raw silk that can be reeled. Cocoon weights increased with increase in the concentration of the compound (Fig 4). The compound at higher concentration has no considerable effect on the cocoon weight in comparison with the control. Lower concentrations of the compound i.e. 0.1mM and 0.01mM showed increased cocoon weight. This data was found to be in correlation withthe weight of the worms and silk glands during late 5th instar larval stage.

Evaluation of economic parameters in control and drug treated worms

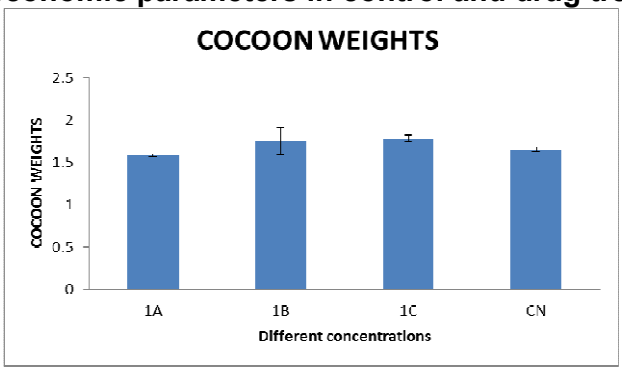


Figure 4

Graph representing the cocoon weights of the silkworms B. mori. 1A, 1B, 1C are the different concentrations of the compound. 'CN' is control. Y-axis represents the weights of the cocoons in grams.

(iii) Measurement of cell viability-MTT assay

The result analyses of MTT assay on treated and untreated (control) silk gland showed maximum cell viability on 4th day (Fig 5). Decreased cell viability was observed on 8th day. The silk glands of

larvae treated with 0.01mM concentration showed maximum cell viability on 8th day. Increase in cell viability leads to more metabolic rate.

MTT assay of silk glands during different days of 5th instar larval development in control and treated silkworms with the compound

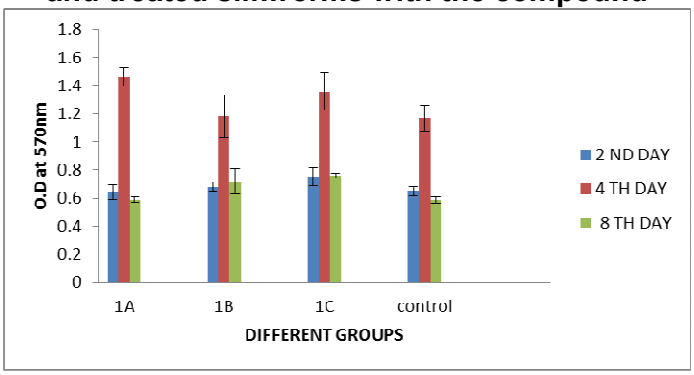


Figure 5

Graph representing the MTT assay absorbance measurements of the silk glands during different days of 5th instar larval stage. Y-axis represents the absorbance values at 570nm.

DISCUSSION

This is the first study of the diamino substituted thiadiazole on the growth of *Bombyx mori* silkworms.. We observed an increase in the growth of B.mori silkworm when their diet is supplemented with aminosubstituted thiadiazole Though wide literature is available for substituted thiadiazolesas possible anticancer agents[32,33,34,35,36,37], there are no reports on their effect on normal cells and tissues[38]. Diamine incorporated thiadiazoles proved as growth enhancers of *B. mori* larval weights, silk gland weights, cocoon weights and viability with 0.01mM compound in comparision with the control worms.

CONCLUSION

This is the first report showing growth enhancing effect of the diamino substituted thiadiazole compound on *B. mori* silkworms. Further investigations are also necessary to find the mechanism for this activity. It can be concluded from the present study that the compound has positive effect on sericulture.

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