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Effect of Neem seed kernel extract on the incidence of major pest (tukra) in mulberry leaves on excretory products in Silkworm, *Bombyx mori* L.

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ABSTRACT
The continuous use of pesticides over a period of time cannot sustain the crop yield and also harmful effects on soil and environment. Mulberry leaves are the predominant food source for silkworm, *Bombyx mori* rearing. The incidence of Pink mealy bug occurring in mulberry plantation can cause tukra disease that leads to qualitative loss of mulberry plantation. The present study was undertaken to study the effect of neem seed kernel extract having potential against the pests and insects as natural botanicals origin by foliar spray. The seed kernel extract of ***Azadirachta indica*** sprayed to occurring mealy bugs at the early cause of infection to V1 mulberry variety and reared to Silkworm. The total ammonia, urea and uric acid parameters were studied in tissue like haemolymph. The ammonia and uric acid activity gradually increased this increase however was significant at (P>0.05). There were a gradual decrease of urea level from day 3 to day 6, this decrease was however non-significant. Foliar spray of seed kernel extract hold greater promise for control of tukra infested mulberry leaves and did not affect the excretory system in silkworms.

Keywords: Tukra, Neem seed, excretory products.

1. Introduction

The silkworm, *Bombyx Mori* L. is an important economic insect and also a tool to convert leaf protein into silk. The industrial and commercial use of silk, the historical and economic importance of production and its application in all over the world finely contributed to the silkworm promotion as a powerful laboratory model for the basic research in biology [15].

Mulberry is an important commercial crop grown extensively as a food plant for silkworm *Bombyx mori* L [4]. The mulberry silkworm, *Bombyx mori* is a monophagous insect feeding only on mulberry leaves due to the presence of morin [20]. Mulberry, *Morus* spp is infested by a number of insect pests among these *Macconellicoccus hirstus* a pest which is responsible for the highest damage to mulberry plants. Mulberry sericulture is one among the income assuring occupations to rural folks. It needs low capital and ensures round the year employment opportunities [8]. Recently discussed on early diagnosis method for the tukra incidences in mulberry [18]. Various studies on bio-control of the mealy bug by beetle, *Cryptolaemus montrouzieri* of an exotic enemy as a part of pest management program [2, 9, 13].

Different concentrations of botanicals were reported effective in suppression of Tukra i.e. mealy bugs in mulberry [13]. Several natural enemies were recorded from mulberry agro-ecosystem [1, 17].

In the recent years serious damage to mulberry by tukra has been reported in rain fed sericulture tract of Karnataka and Andhrapradesh. The commonly employed chemicals used for control of tukra are dimethoate, dichlorvos hardly control the disease. Moreover, chemical control of disease leads to environmental pollution as well as bio degradation in soil leads to toxicity.

Neem products having good evaluation characteristics against suppression of pests from different plants and have been reported to possess the inhibiting of mulberry diseases [6]. Use of neem derivatives is being advocated for the protection of different species of silkworm [17].

Neem compound significantly altered the growth and developments of insect dose dependent manner, similar observation were recorded by meny scientists [23, 7, 19]. The present study explores to assess the plant extract sprayed to tukra infested mulberry leaves fed to silkworm and to analyze the role of enzyme activity in tissues of silkworm of cross breed PMxNB4D2 (Bivoltine hybrid) silkworm.

2. Materials and Methods

2.1 Maintenance of Silkworms

For the present investigation, the popular south Indian cross breeds (CB) silkworms PMxNB4D2 of Bivoltine breeds of Mulberry silkworms variety, *Bombyx mori* (L) was used as test materials. The disease free laying (DFLS,) of this cross breed PMxNB4D2 (Bivoltine hybrid) were produced under field conditions and brought to the laboratory.

2.2 Maintenance of Seed Kernel Extract Sprayed Tukra Infested Mulberry Leaves:

Mulberry crop was maintained by following standard agronomic practices. Treatments were imposed on 15th day of pruning in each plot, five plants were randomly selected and the population of pink mealy bug was counted. In each plant, population was counted on three leaves (top, middle and bottom). The total number leaves per plant were also counted and the population was expressed as number per leaf. Observations were made just before spraying (pre-treatment count), 3, 5 and 7 days after spraying. The following seed kernel extract with naturally existing insecticidal properties were selected for preparation of seed kernel extract *Azadirachta indica.*

2.3 Preparation of Neem Seed Kernel Extract

50 gr of neem kernel is required for use in 1 litre of water. The neem kernel is pounded gently. It should be pounded in such a way that no oil comes out. The outer coat is removed before pounding this is used as manure. If pounded with seed coat 1 ½ times (75 gr) seeds is required. The seeds that are used for preparation of neem kernel extract should be at least 3 months old and should not be used after 8-10 months. Before 3 months or after 8 months, the azadirachtin quantity is quite low in the seed and hence it cannot efficiently be used for pest control. The pounded neem kernel powder is gathered in a muslin pouch and this is soaked overnight in the water. The pouch is squeezed and the extract is filtered. To the filtrate, an emulsifier like soap oil or soap cake powder is added. One ml of emulsifier is added to one litre of water. The emulsifier helps the extract to stick well to the leaf surface.

2.4 Studies of Excretory Products in Silkworm Fed with Seed Kernel-Sprayed Mulberry Leaves

A bioassay was conducted to find out the effect of feeding healthy and botanical-Sprayed leaves on silkworm hybrid, PMxNB4D2. Leaves were collected from plots from 0, 2, 5, 7, 10, 15 and 20 days after spray and were fed to fifth instar silkworm. The haemolymph was drawn out from the larvae by puncturing the proleg. The haemolymph was collected in small ice cooled test tubes rinsed with phenylthiourea solution (1% w/v).

All the results obtained in this investigation were subjected to statistical analysis. The standard deviation was calculated and‘t’ values were derived between the control and experimental. The levels of significance were noted from the standard‘t’ values and represented in the respective histogram.

3. Results

The percentage change over of the excretory products in the haemolymph fed on leaves treated with neem seed kernel extract sprayed mulberry leaves. Ammonia levels gradually increased at all the days relative to respective haemolymph controls 6.140, 6.280, 6.350, 6.410 (Table 1) and uric acid levels increased gradually relative to respective controls haemolymph 3.240, 3.620, 4.860, 4.990 (Table 2). The urea levels gradually decreased at day 5 to day 6 relative to controls haemolymph 0.108, 0.120, 0.194, 0.224 (Table 3).

Table 1: Effect of neem seed kernel extract on the incidence of tukra mealy bug (M*aconelli coccus hirsutus)* on ammonia in haemolymph of silkworm.

	Name of the tissue		Days of Vth instar			
			3	4	5	6
RACE BREED	Haemolymph	Control S.D. ±	6.140 0.2400	6.280 0.2300	6.350 0.2900	6.410 0.3200
PMxNB4D2		Sprayedbatch S.D. ± % ‘t’ test	6.360 0.3500 3.580 N.S	6.430 0.2500 2.380 N.S	6.500 0.2800 2.300 N.S	6.600 0.3000 2.960 N.S

S.D.±: Standard deviation P: level of significance. N.S: Non Significant

Table 2: Effect of neem seed kernel extract on the incidence of tukra mealy bug (M*aconellicoccus hirsutus*) on uric acid in haemolymph of silkworm.

	Name of the tissue		Days of Vth instar			
			3	4	5	6
RACE BREED	Haemolymph	Control S.D. ±	3.240 0.1200	3.620 0.1500	4.860 0.2000	4.990 0.2100
PMxNB4D2		Sprayedbatch S.D. ± % ‘t’ test	3.260 0.1400 0.650 N.S	3.650 0.1800 0.820 N.S	4.880 0.1700 1.320 N.S	5.110 0.2200 2.300 N.S

S.D.±: Standard deviation P: level of significance. N.S: Non Significant

Table 3: Effect of neem seed kernel extract on the incidence of tukra mealy bug (M*aconellicoccus hirsutus)* on urea in haemolymph of silkworm.

RACE BREED	Name of the tissue		Days of Vth instar			
			3	4	5	6
PMxNB4D2	Haemolymph	Control S.D. ±	0.108 0.0043	0.120 0.0045	0.194 0.0055	0.224 0.0060
		Sprayedbatch S.D. ± % 't' test	0.123 0.0048 13.600 P<0.001	0.118 0.0049 -1.660 N.S	0.180 0.0053 -6.200 P<0.01	0.220 0.0055 -8.800 N.S

S.D.±: Standard deviation P: level of significance. N.S: Non Significant

4. Discussion

The tukra infected mulberry leaves with symptoms of minute mealy bugs, infected mulberry garden, curling of apical leaves. The mulberry infested with *M. hirsutus* (green) is a major pest of mulberry in southern parts of India and has become regular pest of mulberry in Andhra Pradesh and Tamil Nadu and other southern states especially during warmer. It has been reported that most of the mulberry varieties were susceptible for the mealy bug, *M. hirsutus* (green) attack [13]. Leaf curling with mealy bug is the symptoms of tukra infested mulberry and to find out whether spray of the aqueous seed kernel extracts of *Azadirachta indica* extract was sprayed to tukra incindence at earlier of V1 mulberry variety and fed to Silkworm (PMxNB4D2 Bivoltine hybrid). As the control of mealy bug, application of chemical pesticides are not advised since they harm the silkworms and recently non-chemicals avenues like botanicals acted as an efficient alternative for the pesticides in mulberry garden. Mukhopadhyay [13] reported that when silkworms fed with botanical sprayed of infested mulberry leaves after observing the waiting period and feeding to silkworms there was no impact on the economic parameters of cocoons. Neem leaves extract demonstrated a strong ability against the development of many disease causing fungi through its addition to the soil or by its direct application. Some extracts from neem plant have been shown to be toxic to fungal pathogens such as *Poria monticolad* infecting wood [3]. The impacts of mealy bug infestation in mulberry (malformed, curled leaves) were assessed for their nutritive value and relative ability to support the growth and development of *Bombyx mori* of this cross breed PMxNB4D2 [21]. Neem extracts do not normally kill pests right away rather they repel them or affect their growth and its repellent and pesticidproperties are broad spectrum in nature [5]. Neem seed kernel extract maybe can induce plant defens reactions and useful in management of leaf stipe disease. Extracts of neem are being extensively used to control pests [12].

Ammonia is a toxic nitrogenous end product, and it is released endogenously intotissues mainly through the catabolism of the amino acids, pyrimidines, purines, amino sugars and gangliosides [10, 11]. The mealy bugs, M.hirsutus (green) infected mulberry leaves when fed to silkworm the metabolites of phenylalamine ammonia lyses, showed marked increased in infected leaves when compared with healthy leaves fed to larvae [5]. Many amino acids and their derivates yield sufficient quantities of ammonia by transamination and deamination reactions. Further, hydrolysis of urea by ureas results in ammonia formation. The steady elevation in the ammonia level in the haemolymph when fed with tukra effected leaves from 3 to day 6 due to elevation of transaminases and GDH activity is concerned for transdeamination the increase in the ammonia level in the haemolymph could be due to other aminogenic system such as the operation of purine nucleotide cycle under pest stress or starvation [22].

The urea ornithine cycle in insect is less significant. Hence the urea level is low in the haemolymph of silkworm due the lack of nutrition (or) pathophysiological condition created in tissues of silkworm indicates the little conversion of ammonia to urea might be suppressed gradually (or) on active tranamination in to uric acid by a rise in ammonia level is observed [16]. The progressive reduction of urea levels suggest the physiological stability up to some extent created is the tissues of silkworm when fed with tukra affected leaves, hence instead of discarding tukra leaves the farmers can make use tukra leaves.

5. Conclusion

Based on the results of the study mulberry growers may use botanical extracts instead of chemicals which is used for the suppression of mealy bugs in mulberry fields. Feeding of silkworm with the mulberry leaves treated with botanical extracts showed marked improvement in the silkworms instead of feeding with tukra infected or contaminated mulberry.

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