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Influence of Acetone Extractive of *Oroxylumindicum*on Cocoon characters; Silk Filament Characters and the Electrophoretic patterns of esterase activity of silk worm *Bombyxmori* (L.)(Race: PM x CSR2)

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Abstract

The present study was carried out for the purpose to know the effect of acetone extractives of stem of Oroxylumindicumon the cocoon characters; silk filament characters and esterase enzyme activity of silk worm Bombyxmori(L.) belong to the Race of PM x CSR2. The soxhletation was followed for the obtaining acetone extractive of bark of Oroxylumindicum (L). Three concentrations (5ppm; 10ppm and 20ppm) of extractive were prepared. The fifth instar larvae were utilized for the experimentation. At zero after last but the first ecdysis, the larvae fifth instar were dividedinto five groups (each with hundred individuals) (Untreated control; Acetone treated control; 5ppm extractive; 10ppm extractive and 20ppm extractive). Ten microliters of each concentration of extractives were topically applied to respective group to the individual larva at forty eight hours of age. The larvae were maintained through standard method of rearings. Ten larvae from each group were utilized for analysis of electrophoretic esterase pattern on fifth day. The silk worms were sacrificed,haemolymphand silk gland samples were isolated and analyzed by using 7.5% of native gel electrophoresis. Acetone extractives of O.indicum at 5 ppm, 10 ppm and 20 ppm concentrations recorded maximum cocoon weight (1.95,1.84,1.76 gm), shell weight (0.40, 0.35, 0.33gm), pupal weight (1.55, 1.49,1.43). All concentrations of the bark extract of O.indicum recorded higher cocoon, shell, and pupal weight than the control (untreated and acetone treated). Increase in the concentration of Oroxylum extract used for topical application was found reflected into improvement in the weight of cocoon shell followed by the shell ratio and denier scale of silk filament. Efficient use of acetone herbal extractives, likeOroxylumindicum (L) may open a new avenue in the silk yield.

Keywords: *Bombyxmori*, Esterases, Native gel electrophoresis, *Oroxylum indicum*and Silk yield.

Introduction

The Indian subcontinent is considered to be a rich emporium of medicinal plants in the world. Nature has provided an impressive number of drugs which have been isolated from the medicinal plants. Many of the medicinal plants are known to possess a wide range of medicinal properties such as antibacterial, antimicrobial, analgesic, antioxidant, anticancerous, anti arthritic, antipyretic etc., due to the presence of biologically active compounds otherwise called secondary metabolites. One among such plants possessing a number of medicinal properties is *Oroxylumindicum* (Bignoniaceae). Isolation of secondary metabolitesfrom the bark extract of *O. indicum*showed that itpossessed antimicrobial, analgesic, antifungal[1,2], antibacterial[3] activities and it is being widely employed in the famous tonic formulation*Chyawanaprasha*. *Oroxylumindicum*is one among widely used ayurvedic preparations and is one of the ingredients of *Dasamoolam*, *SidharthaKadhiagadam*, *Misrakasneha*, *Amrotharishta*and *Mashataila*considered to be antiarthritic, anti fungaland antibacterial[4]. The mulberry silkworm *Bombyxmori*L. is a lepidopteron belonging to family Bombycidae. It is a domesticated phytophagous insect feeding on the leaves of mulberry plant and reared indoors. Enriching mulberry leaves by nutrient supplementation is one of the ways to improve growth rate in *Bombyxmori*L[5,6]. It is of great economic importance as a foreign exchange earner for many silkproducing countries of the world[7]. The forms of enzyme, Esterase are varying, especially, based on the type of tissue source, the life stage of insect life cycle and the post embryonic role. Each of them seems to be specific with reference to the substrate. The enzyme Esterases, thus consists of a diverse group of enzymes catalyzing the hydrolysis of organic esters. Esterases (EST, 3.1.1.1) are ubiquitous in living organisms. Several esterases have been isolated from various tissues of microbes, plants and animals and investigated for their biochemical properties[8]. There is a report on reported the heterogeneity of the enzyme esterase corresponding to many more insect species[9]. Esterase polymorphism in haemolymph, silk gland, fat bodies, mid gut, eggs and integuments with differential relative mobility through gel electrophoresis has been evidenced in *B. mori*[10,11]. Hammock and Quistard[12] opined the co-relation between the titer of juvenile hormone and involvement of esterases for it's regulation. Recent investigations on biologically active secondary metabolites from the stem bark of *O. indicum*showed that the stem has a more antimicrobial effect than the root[13]. So

far no work has been done on the effect of the bark extract of *O. indicum*on the esterase activity of silk gland and haemolymph and silk yield of silkworm *Bombyxmori*. On this much background, the study has been planned. The aim of study was to analyze the titer of acetone extractive to be utilized for topical application to the fifth instar larvae of polyvoltine crossbreed race (PM x CSR2) of silkworm, *Bombyxmori* (L).

Material and Methods

The experimentation was divided into the parts like: Preparation of plant extractive; Rearing of silkworm larvae; Topical application of plant extractive to the fifth instar larvae; Bioassay of esterase activity and Analysis of economic parameters.

Preparation of Plant Extractive: The stem bark of *Oroxylumindicum* (Bignoniaceae) was collected from trees growing in Malegaon Sheti farm of Agriculture Development Trust, Baramati (India). The collected plant material was was hed thoroughly with distilled water to remove the surfacecontaminants. The stem bark was the shade dried. It was finely powdered usingan electric blender and stored in airtight containers untilrequired. 25gm of the dried stem bark powder was subjected for soxhletation using acetone as solvent, for twenty four hours. The soxhelet extract was filtered through a muslin cloth and filtrates were centrifuged at 3000 rpm for 15 min. The supernatants were maintained as a stock solution (100%). Three concentrations (5ppm; 10ppm and 20ppm) of extractive were prepared.

Rearing of Silkworm Larvae: The race of silkworm, *Bombyxmori* (L) selected for the study was PM x CSR2 (Polyvoltine crossbreed). The egg mass in the form standard form of layings (DFLs) were brought from the Malegaon Sheti Farm through the "Dr. APIS" Laboratories and processed for black boxing, rearing of early instars, rearing of late age instars, provision of mountage for spinning the cocoon and cocoon harvesting through the standard methods[14].

Topical Application of Plant Extractiveto The Fifth Instar Larvae: The fifth instar larvae were utilized for the experimentation. Just after the completion of fourth ecdysis, the larval instars were divided into the control groups (one control group and one acetone treated group) and three groups (5 ppm; 10 ppm and 20 ppm) for treatment of acetone extractives of stem of *Oroxylumindicum* (L). Each of the group in the study was with hundred larval instars. Forty eighth hour of age of the fifth instar larvae seems to be sensitive with reference to topical application of juvenoid compounds and the herbal extractives[15]. Therefore, at this sensitive period, ten microliters of each concentration of Oroxylumextractives were topically applied to respective group to the individual larva. The standard schedule of feeding larvae with appropriate amount and quality mulberry leaves was followed. Rearing was conducted in wooden trays withfour feedings per day.

Analysis of Electrophoreti Esterase Pattern: Ten larvae(each for haemolymph and silk glands) from each group were utilized for analysis of electrophoretic esterase pattern on fifth day. The silk worms were sacrificed, haemolymph and silk gland samples were isolated and analyzed by using 7.5% of native gel electrophoresis. silkworm larvae were dissected, haemolymph and thesilk gland were isolated and collected into prechilledeppendorftubes containing 0.025% Phenyl thiourea. The silk gland were homogenizedin (10%) 0.01 M Tris-HCl buffer (pH 7.4) having sodium chloride (0.9%). The centrifugation of the homogenate was carried. The supernatant resulted was diluted. Likewise, haemolumph was diluted.The ratio of dilution of both was 1:1 with 20% sucrosecontaining bromophenol blue as tracking dye. Aliquot of 0.1ml of these solutions was loaded directlyonto the separating gel. Esterase pattern was separatedon thin layer (1.5mm thick). Native Poly acryl amidegels (7.5%). The standard method[16] was followed for the preparation of gel mixture. Gelling was allowed for 45 min. After loading on to gel, the samples were overlaid with electrode buffer and gel plates were connected to the Electrophoretic tank. Tris (0.05M),Glycine (0.38M) buffer (PH 8.3) was used as electrodebuffer. A constant current of 50 volts for the first 15minutes followed by 150 volts for the rest of the runwas supplied during electrophoresis esterases were visualized on the gels by adopting the staining procedures[17, 18]. Electrophoretic bands ofesterases resulted from stained gel with α-naphthylacetate. The relative mobility of the individual subunits was calculated using the following formula. The reading of Distance travelled by the esterase was divided by the Distance travelled by tracking dye from the origin. The quotient thus obtained was considered as relative mobility of individual subunits.

Analysis of Economic Parameters: The cocoons from the mountage were harvested on fifth day after mounting for spinning. Twenty cocoons from each group were selected randomly, deflossed and used for recording the weight of entire cocoon. Each cocoon was cut vertically using the blade. Weight of shell of cocoon and pupa were noted. Through the use of readings of weight of entire cocoon and weight of pupa, the shell ratio was calculated. The experimentation was repeated for three times for consistency in the results. The collected data was subjected for statistical analysis.

Results and Discussion

The results on the topical application of acetone extractive of Oroxylumindicum (L) to thepolyvoltine crossbred race (PM x CSR2) race of silkworm, Bombyxmori (L) are presented tables (1 and 2). Acetone extractives of *O.indicum* at 5 ppm, 10 ppm and 20 ppm concentrations recorded maximum cocoon weight (1.95; 1.84; 1.76 gm), shell weight (0.40; 0.35; 0.33 gm), pupal weight (1.55; 1.49;1.43gm). All concentrations of the bark extract of *O.indicum* recorded higher cocoon, shell, and pupalweight than the untreated control and acetone treated control groups of larvae. Increase (5 ppm; 10 ppm and 20 ppm)

in the concentration of Oroxylum extractives for topical application was found reflected on gradual increase in the yield of silk.

The serial two fold dilution of the samples followed by the visibility of the zone in electrophoresis was used to score activity intensity of the zone. Relative mobility indicates (table-1) that the Rm valueis 57.142 in the middle region. 1-naphthyl acetate wasused as a substrate to score the activity of esterases ongels. The esterase patterns obtained indicatethat the silk gland in control and in the treated (5 ppm, 10 ppm and 20 ppm) group of larvae showed a single hyper active band. The relative mobility(Rm value) was found calculated 57.142 (table-1) in the middle region. The 20 ppmconcentration of extract has low intensity compared to5 ppm and 10 ppm but high intensity than the control. The haemolymphextract contained a singleesterase band with Rm 57.142 in the middle region. The pattern observed indicates that the haemolymph at5 ppm concentration of the plant extract has low intensity compared to control. Esterase pattern showed highintensity at 10 ppm concentration compared to 20 ppm concentration. The Rm value 57.142 obtained from the bothtissues indicates that there is a homogeneity in theesterase bands in both tissues.Various authors reported different number of esterase fractions in the gut spectrum of differentbreeds of silkworm, *Bombyx mori*[19,20,21,22]. The differences in fractions of esterase may be due to the degree of geneticheterogeneity[23,24]. The stem barkextract *O. indicum*showed the influenceon the enhancement of silk production. The plant secondary metabolite scan be used not only to control diseases of silkworm,but also to increase the commercial characters ofsilkworm[25]. The effectiveness of the plant was not only due to main active constituents but also due to combine action of other chemical compounds such as alkaloids, flavonoids, triterpenoids, and other compounds ofphenolic nature[26, 27,6].The present results clearly indicate that the isolation of bioactive compounds is being done to discover new compounds for pharmaceutical and agricultural applications. Plant extracts have tendency to increase biological characters such as larval, cocoon, pupal and shell weight, shell ratio percentage and length of silk filament[3,25] which isevidenced in the present investigation on the effect of *O. indicum*bark extract which influenced the esterase intensity and hence there is an increase in the silk yield. Hence, it can be used as a nutrient supplement to improve silk yield in *B. mori*. Efficient use of acetone extractives of *Oroxylumindicum* (L) may open a new avenue in the silk yield.

Table–1
Influence of topical application of acetone extractives of bark of *Oroxylumindicum* (L) on the parameters of cocoon and silk filament in silkworm, *Bombyxmori* (L) (Race: PM x CSR2)

Group	Cocoon Weight (gm)	Pupal Weight (gm)	Shell Weight (gm)	Shell Ratio	S. F. L. (m)	S.F. W. (gm)	Denier Scale of S. F.
Control (U.T.)	1.71 (±0.08)	1.392 (±0.04)	0.318	18.596	714.11 (±8.721)	0.164 (±0.009)	2.066
Control (A.T.)	1.71 (0±.08)	1.392 (±0.04)	0.318	18.596	714.11 (±8.018)	0.164 (0. ±009)	2.066
5 ppm	1.95* (±00.11)	1.551** (±0.10)	0.399**	20.461*	989.78** (±41.234)	0.251** (±0.011)	2.282**
10 ppm	1.84* (±0.05)	1.490* (±0.07)	0.350**	19.021**	921.126** (±16.786)	0.249** (±0.018)	2.432**
20 ppm	1.83*** (±0.03)	1.439*** (±0.04)	0.391**	21.366***	1012.23*** (±12.071)	0.291*** (±0.023)	2.587***

Each figure is the mean of the three replications. Figure with ± sign in the bracket is standard deviation. Figure below the standard deviation is the increase for calculated parameter and percent increase for the others over the control.
*- $P < 0.05$, **- $P < 0.005$, ***- $P < 0.01$

Conclusion

Acetone extractives of *O.indicum* at 5 ppm, 10 ppm and 20 ppm concentrations recorded maximum cocoon weight (1.95,1.84,1.76 gm), shell weight (0.40, 0.35, 0.33gm), pupal weight (1.55, 1.49,1.43). All concentrations of the bark extract of *O.indicum* recorded higher cocoon, shell, and pupal weight than the control (untreated and acetone treated). Increase in the concentration of Oroxylum extract used for topical application was found reflected into improvement in the weight of cocoon shell followed by the shell ratio and denier scale of silk filament. Efficient use of acetone herbal extractives, like Oroxylumindicum (L) may open a new avenue in the silk yield.

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