Foliar treatment with optimum doses of agrochemicals can improve the quality of oak-tasar cocoons

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A study has been carried out on the effect of foliar treatment with mineral nutrients (N, P, K, Mg, Zn, Mo), plant growth regulators (IAA, GA_3, kinetin), an insecticide (Dimecron) and a fungicide (Bavistin) on carbon metabolites (chlorophyll, total sugar, crude fat and crude fibre), nitrogen metabolites (total free amino acids, soluble protein and crude protein) and nitrate reductase activity in the leaves of *Quercus serrata* seedlings, and optimum concentrations were determined for each. The optimum concentrations of mineral nutrients determined for seedlings were applied to the foliage of 5-year-old plants in various combinations and carbon and nitrogen metabolites were also studied. The foliage from 5-year-old plants was also used for rearing of *Antheraea proylei* which showed better performance with one of its optimum combinations.

THE quality of foliage is an important factor which determines the quality of the silk[1,2]. Hence, improvement of the quality of foliage in respect of chemical constituents of leaves seems to be essential for the present day need.

In India about 58 species of oak flora are distributed from Jammu & Kashmir in the west to Manipur in the north-east all along the temperate and sub-Himalayan belt. Some of these species have been introduced for rearing of oak-tasar silkworm (*Antheraea proylei*) and *Quercus serrata* is one of the most suitable hosts. Improvement of shoot and root growth of oak seedlings has been achieved by foliar applications of fertilizers[3,4]. However, no work has been done with respect to application of agrochemicals for the improvement of the foliage quality of *Q. serrata*. The agrochemicals constitute an important ingredient in the cultivation of oak trees. Their input, like all other energy ingredients, must thus be considered from the viewpoint of optimal utilization. In the present study, three major groups of agrochemicals, viz. mineral nutrients, plant growth regulators and pesticides, have been used for the improvement of chemical constituents of the foliage as well as quality of the oak-tasar cocoons.

For the determination of optimum concentration of various agrochemicals, foliar spray was given once for each of the agrochemicals to 2-month-old seedlings. The seedlings were grown in polythene bags filled with soil medium (farm yard manure : soil : sand :: 1 : 1 : 1). The soil contained 0.27% N, 0.43% P and 0.92% K, and 2.27% organic carbon with soil pH nearly 6.0. During the course of the 2-month experiment, no additional treatment of any fertilizer was given through basal dose. In the beginning the experiments were conducted using a number of concentrations of the various agrochemicals. The concentrations for $(NH_4)_2SO_4$, KH_2PO_4, H_2NCONH_2, KCl, $MgSO_4 \cdot 7H_2O$ and $ZnSO_4 \cdot 7H_2O$ were 1.0, 2.5, 5.0 and 10.0 mM and for ammonium molybdate the concentrations were 0.005, 0.01, 0.05 and 0.1 mM. IAA, GA_3 and kinetin were sprayed at the concentrations of 0.1, 1.0, 5.0, 10 0 and 50.0 ppm while for pesticides the concentrations were 0.01, 0.02, 0.04, 0.1 and 0 2% prepared on the basis of their formulation strength (v/v for Dimecron and w/v for Bavistin).

For determination of carbon metabolites – Arnon's method for chlorophyll[5], Plumer's[6] method for total sugars, AOAC[7] method for crude fat and crude fibre were used. For determination of nitrogen metabolites –

Figure 1. *Quercus serrata* plantation with 4ft × 4ft spacings.

Table 1. Parameters on carbon metabolism in *Quercus serrata* leaves of control plants and in those sprayed with optimum doses of seven minerals, three plant hormones, one insecticide and one fungicide. Two-month-old plants were used for this experiment

Treatment	Chlorophyll (mg/g dry wt)	Total sugar (% dry wt)	Crude fat (% dry wt)	Crude fibre (% dry wt)
0 (control)	0.65 ± 0.013*	10.14 ± 0.01	3.56 ± 0.05	16.43 ± 0.09
Minerals				
$(NH_4)_2SO_4$ (5.0 mM)	0.81 ± 0.019	11.55 ± 0 02	3 98 ± 0.02	13.39 ± 0.12
H_2NCONH_2 (2.5 mM)	0.73 ± 0.015	10.55 ± 0.04	3 62 ± 0.10	14.33 ± 0.07
KH_2PO_4 (5.0 mM)	0.78 ± 0.024	11.57 ± 0.004	4.12 ± 0.09	15.25 ± 0.08
KCl (5.0 mM)	0.82 ± 0 024	11.95 ± 0.03	4 05 ± 0 09	14.43 ± 0.20
Mg-sulphate (2.5 mM)	1.30 ± 0.008	13.19 ± 0 01	3.61 ± 0 02	16.35 ± 0.05
Zn-sulphate (2 5 mM)	0.76 ± 0 012	11.32 ± 0.007	3.72 ± 0.02	16.77 ± 0.10
Ammonium molybdate (0.01 mM)	0.76 ± 0.014	11.24 ± 0.008	4.21 ± 0.01	16.00 ± 0.13
CD at 5%	0.051	1.219	0.237	1 682
Growth hormones				
IAA (10 ppm)	0.70 ± 0.018	10.21 ± 0.04	3.73 ± 0.03	14.52 ± 0.07
GA_3 (5 ppm)	0.74 ± 0 019	11.40 ± 0.02	3.70 ± 0.05	15.02 ± 0.11
Kinetin (10 ppm)	0.74 ± 0 014	10.39 ± 0 05	3.75 ± 0.05	14 18 ± 0.06
CD at 5%	0.038	1 025	0.209	1.328
Insecticide				
Dimecron (0.04%)	0.70 ± 0.012	10.44 ± 0.004	3.70 ± 0 09	15 26 ± 0.06
Fungicide				
Bavistin (0 02%)	0.72 ± 0 010	10.30 ± 0.005	3.68 ± 0.01	15.92 ± 0.11
CD at 5%	0.036	1 042	0 214	1 028

* S E.m. ($n = 3$)

Hageman and Hucklesby's[8] method for nitrate reductase activity (NRA), Yemm and Cocking's[9] method for total free amino acids, Lowry *et al.*'s[10] method for phosphate–buffer-soluble protein and AOAC[7] method for crude protein were used. All these parameters were studied at different ages with fifteen days' interval and continued up to 60 days and the results of only one day, when the levels were optimum/maximum, are reported here. The experiments with plant hormones and pesticides were restricted to 2-month-old seedlings for determination of optimum doses only.

After determining the optimum doses of all the minerals in relation to their carbon and nitrogen metabolites, the next step was to conduct the experiments for field trials on 5-year-old plants with 4' × 4' spacings (Figure 1). The nutrient levels (N, P and K) of the soil were the same as mentioned for the seedlings. In this experiment, only the effect of mineral nutrients was studied by spraying their respective optimum concentrations and in combinations followed by determination of chemical constituents of leaves and finally quality of leaves was assessed by rearing of oak tasar silkworm in the *in situ* condition. The rearing parameters included larval length, width and body weight, average cocoon and shell weight and silk ratio.

Table 2. Some parameters on nitrogen metabolism in *Quercus serrata* leaves of control plants and of those sprayed with optimum doses of seven minerals, three plant hormones, one insecticide and one fungicide. Two-month-old seedlings were used for this experiment

Treatment	NRA (μ mol $NO_2^- h^{-1} g^{-1}$ leaf fresh wt)	Total free amino acids (mg/g leaf dry wt)	Soluble protein (mg/g fresh wt)	Crude protein (% dry wt)
0 (control)	2.55 ± 0 05*	28 2 ± 0 06	53.71 ± 0.21	22.37 ± 0.28
Minerals				
$(NH_4)_2SO_4$ (5 0 mM)	5 11 ± 0.16	37.4 ± 0 16	238.72 ± 0 19	27 39 ± 0 18
H_2NCONH_2 (2.5 mM)	3.90 ± 0.07	34 6 ± 0.05	187.99 ± 0.11	26.03 ± 0 08
KH_2PO_4 (5.0 mM)	4.68 ± 0 08	38.3 ± 0.06	226.78 ± 0 26	25 42 ± 0.22
KCl (5 0 mM)	5.11 ± 0.01	31.5 ± 0.02	128.31 ± 0.22	26.19 ± 0 21
Mg-sulphate (2.5 mM)	4.26 ± 0.02	34.5 ± 0.02	170.08 ± 0.21	23 19 ± 0.15
Zn-sulphate (2.5 mM)	4.75 ± 0.06	32.5 ± 0.05	122.34 ± 0.19	23 00 ± 0.17
Ammonium molybdate (0.01 mM)	5.32 ± 0.04	40.5 ± 0.06	259 60 ± 0.32	28 23 ± 0.18
CD at 5%*	0.314	1.289	2.794	1 832
Growth hormones				
IAA (10 ppm)	5.32 ± 0.04	34.9 ± 0 02	116.37 ± 0 21	25.34 ± 0.25
GA_3 (5 ppm)	4 87 ± 0.08	30 4 ± 0.03	101.45 ± 0 21	24 02 ± 0.31
Kinetin (10 ppm)	4.54 ± 0.21	32.6 ± 0.06	114 28 ± 0.17	24 29 ± 0 27
CD at 5%	0 283	1.003	2 893	1 627
Insecticide				
Dimecron (0 04%)	5 32 ± 0 06	35.9 ± 0 02	149 20 ± 0.15	24 91 ± 0.22
Fungicide				
Bavistin (0.02%)	3 76 ± 0.02	36.2 ± 0 03	179.04 ± 0.14	24 87 ± 0 23
CD at 5%	0.297	1 012	3.482	1.596

* S.E.m. (n = 3)
NRA = Nitrate reductase activity

The levels of total chlorophyll and total sugar were higher with magnesium sulphate followed by KCl (Table 1). The levels of crude fat were maximum with the optimum concentration (0.01 mM) of ammonium molybdate. The crude fibre was found significantly less than control with the optimum (5.0 mM) dose of $(NH_4)_2SO_4$. The optimum doses recorded for plant hormones were – 10, 5 and 10 ppm for IAA, GA_3 and kinetin respectively. Dimecron was found effective at 0.04% and Bavistin at 0.02% concentrations[11]. All the parameters under nitrogen metabolism were maximum with the optimum concentration of ammonium molybdate (Table 2). $(NH_4)_2SO_4$ and KCl were also effective in increasing the level of NRA[12].

In the case of 5-year-old plants, out of 17 combination treatments (T_0 and T_1 to T_{17}) the results of only three effective treatments (T_8, T_{14} and T_{16}) are given in Table 3. (T_8 = combination of all the minerals at their optimum doses except urea; T_{14} = combination of all except urea and KCl; T_{16} = combination of all except urea and KH_2PO_4.) Of the six parameters studied, the levels of chlorophyll, total sugars, total free amino

Table 3. Carbon and nitrogen metabolites in *Quercus serrata* leaves of control plants and in those sprayed with different combinations of the optimum doses of seven minerals on 5-year-old plants. Of the 17 combination treatments, the three that were most effective are given below

Parameters	Treatments				CD at 5%
	T_0	T_8	T_{14}	T_{16}	
Chlorophyll (mg /g dry wt)	0.73 ± 0 014*	0 86 ± 0 004	0 84 ± 0 028	0.94 ± 0 045	0.0452
Total sugars (% dry wt)	10.45 ± 0 01	12 69 ± 0.02	12 66 ± 0.01	12.73 ± 0 011	1.171
Crude fat (% dry wt)	4.20 ± 0 03	4 64 ± 0.03	4.76 ± 0.02	4.74 ± 0.04	0.243
Crude fibre (% dry wt)	17 85 ± 0 11	16 07 ± 0 24	16.21 ± 0.09	15.82 ± 0.13	1.402
Total free amino acids (mg /g dry wt)	28 42 ± 0 09	38.79 ± 0.02	37.92 ± 0 02	38.92 ± 0.04	1.823
Crude protein (% dry wt)	24 31 ± 0 08	27.62 ± 0.02	27.13 ± 0.12	27.75 ± 0.05	1.129

* S.E. m. ($n = 3$)

T_0 = Control, T_8 = Combination of 5 0 mM of $(NH_4)_2SO_4$, KH_2PO_4, KCl + 2.5 mM of Mg-sulphate and Zn-sulphate + 0.01 mM ammonium molybdate in equal volume of each.

T_{14} = Combination of 5 0 mM of $(NH_4)_2SO_4$, KH_2PO_4 + 2.5 mM of Mg-sulphate and Zn-sulphate + 0 01 mM ammonium molybdate in equal volume of each.

T_{16} = Combination of 5.0 mM of $(NH_4)_2SO_4$, KCl + 2 5 mM of Mg-sulphate and Zn-sulphate + 0.01 mM ammonium molybdate in equal volume of each.

Table 4. Rearing parameters during spring crop by using foliage of control plants of *Antheraea proylei* and of those treated with different combinations of the optimum doses of seven minerals on 5-year-old plants. Out of 17 combination treatments, the three that were most effective are given below

Parameters	Treatments				CD at 5%
	T_0	T_8	T_{14}	T_{16}	
Larval length (mm) at final instar	74 2 ± 0.015*	96 1 ± 0.014	110 0 ± 0.017	112.0 ± 0.014	1.873
Larval width (mm) at final instar	12.1 ± 0.01	13 2 ± 0.012	15.5 ± 0.007	16.2 ± 0.01	0 692
Body wt of larva (mg dry wt) at last moult	469.17 ± 0 21**	531.32 ± 0.33	640 19 ± 0.25	671.32 ± 0.17	27.105
Larval period (days)	36.7	35.0	32.0	31 0	3.37
Effective rate of rearing (%)	68 4	75.1	92.1	98.3	5.739
Av. cocoon wt (g)					
Male	3.59	4 72	5.62	5 88	0.206
Female	4 45	5.62	6.78	6.92	0.231
Av. shell wt (g)					
Male	0.33	0.52	0 69	0.75	0 042
Female	0 40	0.68	0.77	0.82	0.048
Silk ratio (%)	9 08	9.70	11.82	12.29	–

* S E.m ($n = 5$); ** S E.m. ($n = 3$)

acids and crude protein were found maximum with T_{16}. The level of crude fat was found high with T_{14} but the level of crude fibre was less with T_{16}.

Table 4 summarizes the rearing performance where T_{16} treatment was found most effective and the larval period was less compared to control and other treatments.

The elements N, P and K are important in more than one way. Being macro-nutrients, they are not only essential but also have a specific role to play in general cell physiology[13–17]. Some of the micro-nutrients (Mo and Zn) are involved in the oxidation–reduction in plant metabolism. Foliar fertilization has been proven a satisfactory method of supplying nutrients to oak[3].

It has been established by Johnson *et al.*[18], that nitrate reductase (NR) activity can be considered as a predictive test for crop yield. Hence, any treatment which affects NR would certainly affect the leaf biomass. In the present study it was noticed that the levels of total free amino acids, phosphate buffer soluble protein and crude protein were higher in those treatments where the NR activity was also higher and *vice versa*. Thus the *in vivo* NR activity can be taken as an index of higher protein level in the leaves of this plant also.

It has been noticed that by giving higher doses (supra-optimum) of the agrochemicals either it has no effect or the effect is at par with optimum dose or sometimes counterproductive also. In addition to these, foliar spray is more economical than soil application and this method is ideally suited for hilly areas. Therefore, foliar application of the optimum doses of these agrochemicals may be recommended to achieve high productivity of oak-tasar silk.

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Neuropeptide FMRFamide induces hypoglycemia in rats

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Neuropeptide FMRFamide on intracerebroventricular administration reduced significantly ($P < 0.001$) the blood glucose levels in normal and diabetic rats. FMRFamide also increased the serum insulin levels in both the category of rats, thus eliciting hypoglycemic effect. Intracerebroventricular administration of FMRFamide antiserum enhanced significantly ($P < 0.01$) the blood glucose levels in normal rats, suggesting the involvement of neuropeptide FMRFamide in the regulation of blood glucose levels.

FMRFamide (Phe–Met–Arg–Phe–NH_2) was first isolated from the ganglia of clam *Macrocalista nimbosa*[1]. Subsequent findings have revealed the existence of FMRFamide in mammalian central nervous system (CNS) and gastrointestinal tract (GIT)[2]. The neuropeptide has been shown to be involved in a variety of physiological processes in mammals, including cardiovascular action[3], inhibition of morphine[4] and defeat-induced analgesia[5], selective amnesia[6], excessive grooming on intrathecal administration[7], inhibition of morphine- and amphetamine-stimulated locomotor activity[8] and stimulation of gastric acid secretion on intracerebroventricular (ICV) administration[9].

Relatively large quantity of immunoreactive FMRFamide material has been detected in the exocrine pancreas of the rats[2]. With the existence of FMRFamide in the CNS and pancreas, it was proposed to investigate the role of FMRFamide in the regulation of blood glucose levels.

Male albino rats (Haffkine strain, 180–200 g) maintained under 12 h light/dark cycle and 25 ± 2°C were selected for the study. Food (gold mohur, Bombay) and water was *ad libitum* up to the time of experiment. The following drugs were used: alloxan monohydrate (Loba Chemie, Australia), pentobarbitone sodium (Loba Chemie, Australia), FMRFamide antiserum (anti-FMRFamide, Incstar Inc., USA). FMRFamide was obtained from Prof. M. J. Greenberg, C. V. Whitney Laboratory, St. Augustine, Florida, USA. All the drugs were dissolved in 0.9% NaCl (saline).

In the first set of experiments, rats were fasted overnight and blood glucose levels were determined 15, 30 and 60 min after the administration of FMRFamide (5–10 µg, ICV). For ICV administration, rats were anaesthetized using pentobarbitone sodium (45 mg/kg, intraperitoneally) and polyethylene cannulae (outer diameter 0.75 mm, inner diameter 0.30 mm) were implanted stereotaxically[10]. The cannulae were fixed to the skull with dental cement. The coordinates for ICV administration were: 0.8 mm posterior from bregma, 1.8 mm lateral from midline and 3.3 mm ventral to the surface of skull. Animals were housed individually after the surgery and a recovery period of seven days was allowed before the start of experiments.

Blood glucose levels were measured with reflective photocell glucometer (Reflolux-II, Boehringer Mannheim, GmbH, Germany) using glucose strips. Blood samples were collected by milking of tail and were placed on the glucose strip for 60 s. The strip was then washed with distilled water, blotted and placed in the reflector photocell of the glucometer for direct reading of the blood glucose in mg/dl. The entire procedure took about 2 min for a single estimation.

Further, serum insulin levels were estimated 15 min after the injection of FMRFamide (5–10 µg, ICV). Radioimmunoassay kit (RIAK 1) developed by the Bhabha Atomic Research Centre (BARC), Bombay, was employed for the estimation[11] of serum insulin levels. In another set of experiments, diabetes was induced in rats by injecting alloxan monohydrate[12] (180 mg/kg) subcutaneously. After about two weeks, when the blood