



Research Paper

**EXPLORING POSSIBILITIES OF INDUCTION OF WATER STRESS
TOLERANCE IN MULBERRY IN RAINFED CONDITION BY APPLICATION
OF PACLOBUTRAZOL**

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Abstract

Mulberry is the sole food plant of silkworm *Bombyx mori*. Mulberry is grown in North Western states of India in rain-fed conditions to utilize its leaves for silkworm rearing where it is subjected to low and irregular precipitation causing mild to severe water deficit/ stress. Mulberry is susceptible to water stress damage during both nursery and early plantation stage in field. After transplantation saplings show low survival. The tree in field produces bad quality leaf due to water deficit/water stress. Good quality leaf is the prerequisite for production of quality cocoons by silkworm rearing. Hence there is need to find out a technology which can lead to higher survival and production of quality leaves under rain-fed water stress condition as irrigation is a limiting factor at farmer's level. Soil water availability represents major environmental constraint under rain-fed condition and predictions suggest that the decline in total rainfall in the north western India will be drastic. Under such conditions, it is likely that plants will experience increasing water deficit/ stress in their natural communities. Water stress tolerance is seen in almost all plant species but its extent varies from species to species. The numerous physiological responses of plant to water deficit generally vary with the severity as well as the duration of water stress. Paclobutrazol (PBZ) is a triazole-based plant growth regulator. The retardant activity is not accompanied by phytotoxicity or scorch, even when applied at higher rates. The principal mode of action of PBZ is through inhibition of gibberellin biosynthesis. PBZ is readily taken up through the roots, stems, and leaves but is transported almost exclusively in the xylem to its site of action, the sub apical meristem, where it has a persistent effect. The present work was taken up to explore the possibilities of inducing water stress in mulberry saplings raised through cuttings in rain-fed condition in July 2014. Two treatments were given at an interval of 15 days after planting cuttings. 0 (water -control), 5, 10, 25, 50, 100, 500 mg/litre of Paclobutrazol (pure compound 95%) was applied in the nursery in RBD as soil drench. Survival of saplings after 180 days was found higher in treated series of cuttings and was in

the range of 2.00 % to 7.00 % in treated series in comparison to controls. Since the Paclobutrazol as a growth regulator has the ability to improve the leaf quality in terms of moisture %, and yield of leaf, an experiment was conducted on existing trees of mulberry (S-146 Genotype:ten year old growing) to find out the impact of the paclobutrazol on these aspects in rain fed condition in the proposed third crop in April 2014 which is the hottest season with regard to sericulture crop.(water -control),5,10,25,50,100,500 mg/litre of paclobutrazol (pure compound 95%) was applied to trees in RBD as soil drench on 15th of March and on first of April .The data on leaf yield and moisture retention were found to be higher in some of the treatments which indicates that Paclobutrazol treatment has induced tolerance to high temperature which was substantiated by higher photosynthesis and lower rate of transpiration in most of the treated series.

Key words: Sericulture, Water Stress, transpiration, Triazole, Paclobutrazol, Growth regulator, Sapling, Survival and leaf quality.

INTRODUCTION

Sericulture provides gainful employment, economic development and improvement in the quality of life to the people in rural area and therefore it plays an important role in poverty alleviation programme and prevents migration of rural people to urban area in search of employment.

Mulberry which is the sole food plant of silkworm *Bombyx mori* belonging to Family Moraceae and Genus *Morus*. About 150 species of the genus *Morus* have been described and among these most of them have been considered as varieties of the same species. In India, there are many species of *Morus* found naturally, of which *Morus alba*, *M indica*, *M serrata* and *M. laevigata* grow wildly in the Himalayas. The major area of mulberry cultivation is the tropical zone covering Karnataka, Andhra Pradesh and Tamil Nadu states in India. In the sub tropical zone, West Bengal, Himachal Pradesh, Jammu and Kashmir and the North- Eastern states have major area under mulberry cultivation. Various varieties of mulberry have been developed through traditional breeding methods.

Mulberry is grown in North Western states of India in rain-fed conditions where due to low and irregular precipitation, it is subjected to mild to severe water deficit/ stress. S-146(*Morus alba*:origin India)) variety developed after years of breeding by CSR & TI ,Behrampur is extensively grown in rainfed areas of north western states like UP, Uttarakhand, HP, Haryana, Punjab and tropical belts of J&K.It shows leaf yield of 12-15 MT/ha/year in two harvest of spring and autumn. Hence the variety was chosen for the present experimental work.

Mulberry is susceptible to water stress damage during both nursery and early plantation stage in field. After raising cuttings in nursery, saplings show low survival. The tree in field produces bad quality leaf due to water deficit/water stress. Good quality leaf is the pre-requisite for production of quality cocoons by silkworm rearing. Hence there is need to find out a technology which can lead to higher survival and production of quality leaves under rain-fed water stress condition as irrigation is a limiting factor at farmer's level.

Soil water availability represents a major environmental constraint under rain-fed conditions and predictions suggest that the decline in total rainfall in the north western India will be drastic. Under such conditions, it is likely that plants will experience increasing water deficit stress in their natural communities Jaleel *et al*, (2009). Water stress tolerance is seen in almost all plant species but its extent varies from species to species Jaleel *et al*, (2007). A better understanding of the physiological strategy adopted by a variety to cope with water deficit requires thorough study of the relationship between water use efficiency and transpirations. In Plants and trees the detrimental effects of water deficit on the harvest index also minimizes the impact of the water limitation on crop productivity and increase the efficiency of water use Jaleel *et al*, (2008). The numerous physiological responses of plant to water deficit generally vary with the severity as well as the duration of water stress Jaleel *et al*, (2008 a & b)

Water stress is considered to be a moderate loss of water, which leads to stomatal closure and limitation of gas exchange. Desiccation is a much more extensive loss of water which can potentially lead to gross disruption of metabolism and cell structure and eventually to the cessation of enzyme catalyzed reaction. Water stress characterized by reduction of water content, turgor, total water potential, wilting closure of stomata and decrease in cell enlargement and growth. Severe water stress may result in arrest of photosynthesis, disturbance of metabolism and finally death of plant Sankar, et al ,(2006).

There are two ways to meet this challenge , one either the plantation at farmers level be uprooted and planted with drought resistant varieties which is a long and time taking activity, may take at least a decade or even more. Hence we need to find-out technology which can induce drought tolerance in the existing huge plantation. Hence use of triazole Paclobutrazol and screening of suitable doses will be a breakthrough in this direction. This chemical has been successfully used in various plants and trees but their use on mulberry has been negligible. Abu *et al* and Sreethar *et al* (1991 & 2014)

Therefore the present work was taken up to provide economic, eco-friendly and easy to operate technology for mulberry foliage improvement under rain fed water stress condition.

The establishment of trees after planting is difficult because of the changing environmental conditions and continuous reduction in rainfall leading to depletion of water availability in rain-fed areas and thus creating water stress. Such stresses contribute to tree decline through reduced translocation of photosynthates to roots, impaired stomatal function, premature leaf senescence, clogged lenticels, branch dieback, stem/trunk lesions, leaf scorch and eventual tree death Percival *et al*(2001&2006) and Pinhero and Fletcher (1994).

During the first few years of establishment, water is vital to trees for survival. Plant cells are killed or injured by desiccation resulting from moisture lost by transpiration through leaves as the stomata that facilitate photosynthetic gaseous exchange also allow water vapor to escape. "Maintaining favourable water status is crucial for successful establishment of newly planted trees. Because trees are grown in perfect conditions in nurseries, when they are planted outside into harsher environments, it is difficult for a tree to adapt. Consequently, the percentage of survival after out planting has often been low Watson (2001). Improving hardiness against drought before out planting (in nursery) may ensure greater post planting survival of newly planted trees. This in turn will reduce labour and replacement cost. Hence, finding exogenous chemicals that improve a plant's stress tolerance may save trees during severe drought conditions Still and Pill (2004).

Paclobutrazol (PBZ) is a triazole-based plant growth regulator The retardant activity is not accompanied by phytotoxicity or scorch, even when applied at higher rates. The principal mode of action of PBZ is through inhibition of gibberellins biosynthesis Dalziel and Lawrence(1984). PBZ is readily taken up through the roots, stems, and leaves but is transported almost exclusively in the xylem to its site of action, the subapical meristem, where it has a persistent effect Anon(1984).

Exogenous application of Paclobutrazol makes plant adaptive to various environmental conditions. It was reported that their application shows contrasting results depending upon the concentration of the chemical, genus and even sometimes species specific response. Therefore experiments were conducted to determine suitable doses of paclobutrazol to alleviate water stress.

More than 80% of area under sericulture crop in North Western India is rain fed. Both quality and quantity of leaf yield of mulberry are reduced in rainfed areas. This is due to water stress created by low and irregular rain fall The undesirable effects of plant water stress caused by the poor soil water supply can be met advantageously by the use of Triazole compounds by Somsundaram *et al* (2009) in *Sesamum indicum*. Non-enzymatic antioxidative defence in drought-stressed mulberry (*Morus indica* L.) genotypes which can be very useful while using Triazoles on mulberry. Paclobutrazol, an Inhibitor of GA biosynthesis, has shown a good promise both as a soil drench and foliar sprays In cultvars like Satsuma and Nagpur mandarin Srivastav *et al*(2010) and they also recommended ,Soil drench: One of the most effective ways to achieve maximum tree response is to apply paclobutrazol to the soil. The only report on uses

of Triazoles on mulberry was in the form of report *Sreethar (1991)* and Babu *et al* (2014) on Proline accumulation and reduced transpiration in the leaves of triazole treated mulberry. It was reported that their application shows contrasting results depending upon the concentration of the chemical, genus and even sometimes species specific response. Hence the work was taken up.

Name of the Chemical used in the work

- Common Chemical Name: Paclobutrazol (**PBZ** acronym may be used)
- IUPAC name: 2RS,3RS-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl) pentan-3-ol.

MATERIALS & METHODS

FIELD TREATMENTS

S-146 genotype of mulberry was selected for observing the effects of Paclobutrazol on photosynthesis and growth characteristics in mulberry because S-146 genotype is extensively growing at field and farmers level in the whole north western India. Uniform mulberry trees of S-146 variety, growing at the experimental station of Regional Sericultural Research Station, Jammu (altitude 300 m AMSL 32°38.294'N 74°48.534'E), were selected for the experiment. A total of sixty three trees were taken for application of paclobutrazol. A total of 7 treatments ranging from 0, 05, 10, 50, 25, 100 and 500 mg/litre of paclobutrazol PBZ (95% purity; procured from Ms/- Vinit Fertilizers and Chemicals, Rajkot (CAS No. 76738-62-0) were given as soil drench at 15 day interval (15-03-2014 and 01-4-2014) as per standard procedure given in the literature. The treatment was given in randomised block design. Separate suspensions of the requisite concentration of Paclobutrazol (95% purity) were prepared in water. The chemical was applied into a shallow trench around the tree as close to the tree trunk as possible, as described by Chaney (2005). Controls were treated with only water in the same amount. Similarly 100 cuttings for each treatment in triplicate were planted in nursery in First week of January 2014 in same randomized block design and similarly two soil drench treatments were given at an interval of 10 days in February 2014. The cuttings were planted at an spacing of 9"X6".

GROWTH PARAMETERS

Data on growth parameters in respect of all treated and control trees were collected. Randomly 3 tree from each replication were selected and data recorded after 40 days of first treatment. The following growth parameters were recorded:

No. of new shoots per tree; number of new shoots / tree were counted and recorded.

Leaf yield: Leaf yield per tree was recorded in Kg by plucking all the leaves through standard plucking method.

Moisture retention (%) after 6 hrs of harvest: The leaves harvested for fresh weight were stored in open at room temperature in the rearing house. Every hour they were turned up down. After 6 hours of harvest, weight of 100 leaves was taken by using electronic top pan balance (Sartorius-MA 40) up to two decimal place in grams. The moisture retention after 6 hours of harvest in % was calculated by the following formula:

$$\text{Moisture Retention (\%)} = \frac{(\text{Wt of leaf after 6 hrs} - \text{Dry wt of leaf}) \times 100}{\text{Leaf weight after 6 hrs}}$$

Moisture content in leaves: Moisture content in leaf was calculated by plucking 100 leaves from all replications randomly at 10 am and weighed immediately by using electronic top pan balance (Sartorius-MA 40) up to two decimal place in gram. Thereafter the leaves were dried perfectly at 80°C for 48 hours into hot air oven to determine the total moisture content. Moisture % was calculated by the following formula:

$$\text{Moisture \%} = \frac{(\text{Wt of Fresh leaf} - \text{Wt of dry leaf}) \times 100}{\text{Wt of fresh leaf}}$$

PHOTOSYNTHETIC PARAMETERS

Net CO_2 assimilation (P_N) rates of photosynthesis were measured using a LICOR-6400 XT infrared gas analyzer (Licor, Lincoln, NE, USA) fitted with red-blue LED light source essentially as described by the manufacturer's protocol. Fourth leaf from top that was well exposed to sunlight was used for photosynthetic measurements. Leaf was kept under conditions of ambient CO_2 concentration of $380 \mu\text{mol mol}^{-1}$ and $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$ light was used for measurements after 30 days of first treatment.

BIOASSAY

To observe the effect of the PBZ on survival of silkworm and cocoon quality, rearing was done during autumn season 2014 with PBZ treated leaves. Treated leaves were fed to silkworm from 3rd instar till cocoon formation.

After complete harvesting, ten cocoons from each treatment and replication were randomly picked and cocoon weight including pupa was recorded. Later, these cocoons were cut open and pupae were removed. The empty shell was weighed again to get the shell weight. Shell percentage was calculated as: $\text{Shell \%} = (\text{Shell wt} / \text{Cocoon wt}) \times 100$

STATISTICAL ANALYSIS

Two way analysis of variance was done with the package available in windows 07 version of excel.

RESULTS

The survival of saplings raised through cuttings after 180 days showed highest survival in 50mg/litre PBZ(51.33%) followed by , 25 mg/litre PBZ(49%), 10 mg/litre PBZ(46.33%) and 10 mg/litre of PBZ(46.33%) against control(44.33%).05mg/litre and 100 mg/litre PBZ showed survival at par with control while as 500mg/litre showed reduction in survival. The results were not proportional to the concentration of the chemical used.(Table:1)

The data presented in Table:1 shows that leaf yield/tree (Kg) was highest in 25 mg/litre (26.47% higher than control) Paclobutrazol treatment followed by 50 mg/litre (24.47% higher than control) ,05 mg/litre (4.65 % higher than control),100 mg/litre (3.67% higher than control),10 mg/litre(3.43% higher than control) of Paclobutrazol treatment while as the leaf yield was reduced with 500 mg/litre PBZ (19.31 % lesser than control). The values were significantly higher in comparison to controls. This increase is supported by the increase in number of shoots/tree and the higher weight of 100 fresh leaves. The results were not proportional to the concentration of the chemical used. (Table-1).

The moisture retention capacity was highest in treatment of 05 mg/litre followed by 10 mg/litre,500 mg/litre and 50 mg/litre of paclobutrazol.This is supported by the fact that the transpiration rate was lower in all the treatments except 25 mg/litre. The Paclobutrazol treatment appears to have supported the trees to with stand the water stress which is evident by the fact that the photosynthesis was higher in all the treatments in comparison to controls. The carbon di oxide concentration and stomatal conductance also supported the results.(Table 1 & 2)

When bioassay was performed to check the effect of PBZ treatment on the production of silkworm cocoons, it was found that there was no damaging effect on silk production. The number and weight of single cocoon weight, single shell weight and SR % were either found similar or better in silkworms that were fed treated leaves (Table 3).

DISCUSSION:

The principal mode of action of PBZ is through inhibition of gibberellins biosynthesis Dalziel et al(1984). PBZ is readily taken up through the roots, stems, and leaves but is transported almost exclusively in the xylem to its site of action, the sub-apical meristem, where it has a persistent effect Anon (1984).

According to Watson (2001), the application of PBZ as soil drench treatment at planting time may be able to stimulate root elongation and reduce water stress of trees after transplanting. A further study by Watson (2001) reported that after 10 weeks of drought stress, stem water

potential of elms treated with PBZ was the same as the well-watered controls. Indeed, a number of workers conclude that PBZ can be used as a pre-treatment to protect plants against drought and increase the percentage of plant survival after transplanting in semiarid climatic conditions Asare *et al* (1986), Fletcher *et al* (2000) and Fletcher and Arnold (1986). In most cases, PBZ-induced drought tolerance has been associated with a decrease in transpiration, and an increase in stomatal resistance. The growth control creates physiological benefits that help trees & woody shrubs withstand stresses, such as poor soil, limited root space, & drought Watson (1996) and Chaney (2005). This is in conformity with the findings of this work in terms of survival of saplings from cuttings and increase in photosynthesis, carbon dioxide concentration, stomatal conductance and decrease in transpiration rate Srivastav *et al* (2010).

Triazole compounds have been used to induce water stress tolerance in some plants such as trees like Peach, Apple, Oak, Bougenvillea etc and *Arachis hypogaea* L. (Leguminosae, Papilioideae) and *Phillyrea angustifolia* L. (Oleaceae) [6]. The induction of water stress tolerance results from some biochemical changes which induce the chlorophyll a and chlorophyll b contents in leaf. The proline content also increases with increasing water stress tolerance Srivastav *et al* (2010). When plants are subjected to water stress, proline is synthesized from glutamic acid to act as osmo-protectant for keeping the water balance in cells and the outer environment. Higher water stress tolerance is also caused from increase of antioxidants, decrease of electrolyte leakage, and lipid peroxidation, which is well known as an indicator to evaluate cell membrane damage Gopi *et al* (2007). Furthermore, PBZ treatment may reduce drought avoidance of non-irrigated trees by decreasing the soil volume occupied by roots Marc and Giancarlo (1990) and Marshall *et al* (2000).

The soil drench method seems to be more effective than the other way William and Edgerton. Triazoles function by inhibiting cytochrome P-450 which mediates oxidative demethylation reactions, including those which are necessary for the synthesis of ergosterol and the conversion of kaurene to kaurenoic acid in the gibberellins biosynthetic pathway, Fletcher *et al* (2000). These have been used to induce water stress tolerance in some plants such as *Arachis hypogaea* L. (Leguminosae, Papilioideae) and *Phillyrea angustifolia* L. (Oleaceae) Sankar *et al* (2007) and Fernandez *et al* (2006). The induction of water stress tolerance results from some biochemical changes which induce the chlorophyll a and chlorophyll b contents in potato leaf Tekalign and Hammes (2004).

Root response to paclobutrazol is an important question because root growth and vigour influence not only water uptake but many other aspects of tree health. Trees treated with paclobutrazol generally have leaves with a rich green color, suggesting high chlorophyll content. There are two possible explanations for this response. One is that the leaves of both treated and untreated trees contain the same number of cells, but the chlorophyll is more concentrated in the reduced cell volume because the cells in leaves of treated trees are smaller. In addition, however, there is evidence that the amount of chlorophyll is actually increased too because phytyl, an essential part of the chlorophyll molecule, is produced via the same terpenoid pathway as gibberellins, Chaney (2005).

It is previously reported that the application of PBZ can increase the xylem water potentials Zhu *et al* (2004) and Thakur *et al* (1998) can increase the cytokinins under drought conditions. Paclobutrazol has been shown to reduce the shoot growth of many tree species Davis *et al* (1985 & 1988). According to Watson (2001), the application of PBZ as soil drench treatment at planting time may be able to stimulate root elongation and reduce water stress of trees after transplanting. A further study by Watson (2001) reported that after 10 weeks of drought stress, stem water potential of elms treated with PBZ was the same as the well-watered controls. Indeed, a number of workers conclude that PBZ can be used as a pre-treatment to protect plants against drought and increase the percentage of plant survival after transplanting in semiarid climatic conditions Asare *et al* (1986), Fletcher *et al* (1986 & 2000). In most cases, PBZ-induced drought tolerance has been associated with a decrease in transpiration, plant height, biomass, and leaf area and an increase in stomatal resistance. The growth control creates physiological benefits that help trees & woody shrubs withstand stresses, such as poor soil, limited root space, & drought Chaney (2005) and (Watson (1996).

Because applications of PBZ increased the stress tolerance of woody plants, it can be suggested that pre-treatment application with PBZ would cause an increase in the stress tolerance of mulberry trees species widely planted for silkworm rearing. Such a response may reduce out planting losses with limited capital investment and only small adjustment to management procedure, the cost of which is negligible compared with the risk of tree death. Besides this existing trees at farm and field level, if treated with PBZ will make the tree to withstand water stress and produce quality leaves for silkworm rearing

Hence, the judicious application of triazole may prove to be a useful tool for decreasing transpiration and in turn inducing drought avoidance mechanisms. It can be concluded that triazole may be useful to trigger drought avoidance mechanisms in plants like *mulberry* also.

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Table1: Growth and Photosynthetic parameters of Mulberry (S-146) affected by Soil drench treatment of Paclobutrazol

Treatments of Paclobutrazol	Nursery Survival	No of new Shoots	Weight of 100 Fresh leaves	Moisture	Moisture retention capacity	Leaf yield
mg/litre	%	Per tree	g	%	%	Kg/tree
O(Water)	44.33	59.11	139.11	71.19	79.77	4.08
05 mg	43.33	63.22	141.33	72.78	83.09	4.27
10 mg	46.33	63.55	169.33	72.84	82.50	4.22
25 mg	49	69.77	134.44	70.87	79.51	5.16
50 mg	51.33	69.55	152.88	72.81	80.92	5.08
100 mg	44.33	67.55	134.88	72.80	78.99	4.23
500 mg	30.66	59.00	124.22	69.65	82.02	3.29
CD at 5 % level	2.66	NS	22.08	NS	NS	1.01

Table 2: Photosynthetic parameters of Mulberry (S-146) affected by Soil drench treatment of Paclobutrazol

Treatments	Transpiration Rate	Photosynthetic rate	Stomatal conductance	Carbon di-oxide availability
Paclobutrazol mg/litre	($\mu\text{mol m}^{-2}\text{s}^{-1}$)	($\mu\text{mol m}^{-2}\text{s}^{-1}$)	($\mu\text{mol m}^{-2}\text{s}^{-1}$)	ppm
O(Water)	2.97±0.87	8.05±1.92	0.12±0.03	183.27±11.95
05	1.53±0.45	10.40±1.46	0.10±0.01	186.41±22.69
10 mg	3.53±0.97	16.25±1.32	0.12±0.03	142.95±40.51
25 mg	1.98±0.30	13.27±0.50	0.08±0.01	179.82±8.3750
50 mg	2.15±0.56	11.59±0.47	0.15±0.03	233.65±26.06
100 mg	1.40±0.24	11.16±0.84	0.11±0.01	214.42±6.653
500 mg	1.53±0.39	17.98±0.39	0.09±0.01	196.53±3.72
CD at 5 % level	2.01	1.14	0.025	19.36

Table3: Bioassay of Mulberry (S-146) affected by Soil drench treatment of Paclobutrazol Combination: SH6XNB4D2 Season: Autumn 2014 worms/replicate:150

Treatments	BIOASSAY						
Paclobutrazol mg/litre	Total Cocoon produced (By No)	Total Cocoon produced By wt (g)	Yield /10,000 larvae		Single Cocoon wt (g)	Single Shell wt (g)	SR %
			By No	By wt (Kg)			
0(Water)	138	216.66	9200	14.44	1.754	0.384	21.90
05 mg	140	228.33	9333	15.22	1.728	0.392	22.54
10 mg	141	218.66	9400	14.57	1.745	0.391	22.42
25 mg	138	227.33	9200	15.15	1.757	0.389	22.18
50 mg	139	226.66	9266	15.11	1.783	0.392	22.01
100mg	137	226.33	9133	15.08	1.775	0.386	21.77
500 mg	138	227.33	9200	15.15	1.741	0.388	22.28
CD at 5 % level	NS	4.06	--	---	NS	NS	NS

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