CHAPTER 4

PACLOBUTRAZOL SUPPRESSED VEGETATIVE GROWTH AND IMPROVED YIELD AS WELL AS FRUIT QUALITY OF 'TOMMY ATKINS' MANGO (*MANGIFERA INDICA*) IN ETHIOPIA.

4.1 ABSTRACT

The effects of paclobutrazol (1- (4-chlorophenyl) –4,4-dimethyl-2- (1,2,4- triazol-1-yl) pentan-3-ol) on the vegetative growth, reproductive development, total non-structural carbohydrate of the shoots and nutrient mobilisation to the leaves of 'Tommy Atkins' mango trees grown in the rift valley of Ethiopia were evaluated during the 2002/2003 season. The trees used were characterised by excessive vegetative growth, erratic flowering and fruiting with declining productivity that validated the evaluation of paclobutrazol. Uniform trees were selected for a randomised complete block design experiment with two application methods (soil drench and spraying) at four rates of paclobutrazol (0, 2.75, 5.50, 8.25 g a.i. per tree) in factorial combinations. There were three blocks and three trees per plot for each treatment. The results indicated that application of paclobutrazol at rates of 5.50 and 8.25 g a.i. per tree both as a soil drench and spray application, were effective in suppressing vegetative growth as compared to the control. Trees from these treatments also had a higher level of total non-structural carbohydrates in their shoots before flowering. Compared to the control, paclobutrazol treated trees had a higher percentage of shoots flowering, number of inflorescences produced, percentage of

hermaphrodite flowers, yield as well as fruit quality. Applications of paclobutrazol did not affect the leaf macronutrient content levels analysed (N, P, K and Ca), but except for manganese, the micronutrient (Cu, Zn and Fe) levels in the leaves of the treated trees leaves were significantly higher than the control.

Key words: paclobutrazol; mango; leaf mineral content; total non-structural carbohydrate

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4.2 INTRODUCTION

Dwarfing rootstocks can reduce scion vigour, make the tree manageable and stimulate fruiting. The disadvantages of dwarfing rootstocks, such as high establishment and management costs and poor anchorage, associated with scions, led to the introduction of effective chemical retardants (Quinlan, 1980).

The improvements in crop productivity in modern agricultural systems are increasingly dependent on manipulation of the physiological activities of the crop by chemical means (Subhadrabandhu *et al*., 1999). The first report about the use of paclobutrazol (PBZ) on mango came from India where Kulkarni (1988) tested concentrations of 1.25 to 10 g a.i. per tree on the cultivars Dashehari and Banganepalli.

PBZ is a synthetic plant growth regulator, which has been used in fruit tree crops to control vegetative growth and to induce flowering (Swietlik & Miller, 1985). Rademacher (1991); Sterrett (1985) also confirmed that PBZ is one of the known effective retardants in tree crops. PBZ can be applied to mango trees as a foliar spray or as a soil drench (Tongumpai *et al*., 1991). Reports on the use of PBZ in temperate tree fruits show differences between species and locations in response to methods of application. Davenport & Nunez-Elisea (1997) elaborated that unlike the other classes of growth retardants that are normally applied as foliar spray, PBZ is usually applied to the soil due to its low solubility and long residual activity. PBZ is taken up through the root system and is transported primarily in the xylem through stem and accumulates in the leaves and fruit if applied to the soil (Wang *et al.,* 1986; Lever *et*

al., 1982). Hence some of PBZ residues may remain in the fruit. Voon *et al.* (1991) explained that PBZ is systemic and can be taken up by plant roots or through lenticels and bark perforations while foliar spray uptake occurs through shoot tips, young stems and leaves.

In commercial mango plantations, it is desirable to control the vegetative growth and the canopy size to prevent or reduce alternate bearing and to facilitate cultural practices. Flowering in mango is also associated with reduced vegetative growth often induced by lower activity of gibberellins (Voon *et al.,* 1991). Exogenous application of GA as well as endogenous high levels of gibberellins has proven to be a major hindrance in the way of flower bud differentiation in a number of temperate as well as tropical fruits including mango (Tomer, 1984).

Considering the above inhibitory role of GA for flower development in mango, PBZ, owing to its anti-gibberellin activity, (Dalziel & Lawrence, 1984; Quinlan & Richardson, 1984; Webester & Quinlan, 1984; Voon *et al.,* 1991) could induce or intensify flowering by blocking the conversion of Kaurene to Kaurenoic acid. Such alterations could be important in restricting vegetative growth and enhancing flowering by altering assimilate partitioning and patterns of nutrient supply for new growth. The cropping manipulations possible with PBZ ranged from off-season or early season harvests to simply increased yields (Voon *et al*., 1991).

Ethiopia being situated very close to the equator is characterized by two erratic and unreliable flowering periods due to bimodal rainy periods and low temperature (the main raining season is June-August and a shorter one in February-March). This

situation exhausts the tree and usually the yield obtained is below expected. Excessive vegetative growth is a common characteristic of most mango cultivars resulting in unmanageable and large trees. The above situations validated the evaluation of PBZ (Cultar) for growth suppression and consequent advantages in increasing flowering, yield and fruit quality. However, because of negative connotations towards the use of PBZ, regulations for export of fruit from PBZ treated trees to certain countries must be cleared. The maximum residue limit of PBZ accepted by FAO in stone fruit is 0.05 mg/kg (Singh & Ram, 2000).

This report discusses the results of an experiment done to determine the effect of PBZ on vegetative growth, shoot total non-structural carbohydrate contents, leaf mineral content, flowering, yield and fruit qualitative aspects of 'Tommy Atkins' mango trees grown at Upper Awash Agro-industry farm in Ethiopia. This is the first study in Ethiopia on the effect of growth retardants on fruit trees and other crops.

4.3 MATERIALS AND METHODS

4.3.1 Area description

The trial was conducted during the 2002/2003 season at Upper Awash Agro-industry Enterprise in the rift valley of Ethiopia (latitude: 8^0 27'N; longitude: 39^0 43'E; elevation: 1000 m.a.s.l.; mean annual temperature: max. 32.6 ^{0}C, min. 15.3 ^{0}C; mean annual rain fall: 500 mm; soil type: calcic xerosol and 50% loam soil). The area is situated at 180 km South East of Addis Ababa.

4.3.2 Plant material

Ten-year old 'Tommy Atkins' mango trees, uniform in vigour and size were selected for this study based on their volume. The trees were characterized by excessive vegetative growth (average tree height and canopy diameter more than 5.5m and 5.8m respectively), erratic flowering and poor yield. All treatment trees received the standard orchard management practices as applied by the company.

4.3.3 Design, method, rate and time of PBZ application

The experiment was designed in a randomised block with three replications. Three trees were included per plot. Treatments were factorial combinations of two application methods (soil drenching Vs spraying) each at four PBZ levels (0, 2.75, 5.50 and 8.25 g a.i. per tree). The PBZ application rates were determined based on the average volume of the selected uniform trees. A suspension concentrate of Cultar (250 g a.i. paclobutrazol per litre, Zene Co. Agrochemicals SA PTY LTD, South Africa) was used.

The required quantity of PBZ was dissolved in 5 liters of water and sprayed uniformly on a single tree. During spray application, the soil underneath the canopy was covered with plastic sheeting to prevent contamination of the soil. PBZ drift to the neighbouring trees was avoided by using a mobile canvas shield. Soil drenching treatments were applied according to Burondkar & Gunjate (1993), in which 10 small holes (10-15 cm depth) were made in the soil around the collar region of the trees, just inside the fertilizer ring. A solution was prepared by mixing the required quantity of

PBZ for each concentration into five litres of water and drenched uniformly (500 ml per hole) into the holes. The control trees were sprayed or soil drenched with pure water. All treatments were applied once only on 15th of August 2002, 90 days before the expected date of flower development.

4.3.4 Data recorded

Flower and fruit related developments

Prior to treatment applications, one hundred uniform terminal shoots per tree were tagged randomly, for recording the percentage of flowering shoots. The beginning of flowering was registered for all treatments, as the number of days passed after treatment application to a stage where at least 25 inflorescences per tree had reached bud break. Twenty inflorescences per tree were also tagged randomly for recording the percentage of hermaphrodite flowers per panicle. Another twenty inflorescences per tree were tagged to observe average fruit set. Fruit set was quantified at pea size stage. Data on fruit number and weight per tree were also recorded during harvesting to estimate yield per tree.

Fruit quality

Fruit quality was determined nine days after harvesting using 30 fruit per tree. The fruit used for the quality test were ripened at room temperature. Fruit Total Soluble Solids (TSS) was measured with a bench top 60/70 ABBE refractometer (No. A90067, Bellingham & Stanley Ltd, England) with a reading range of 0 to 32 ^{0}Brix. After each reading, the prism of the refractometer was cleaned with tissue paper and methanol, rinsed with distilled water and dried before re-use. The refractometer was

standardised against distilled water (0% TSS). Reducing and total sugars were estimated from the fruit mesocarp by using the technique of Somogyi (1945). Titratable acid was determined by applying an acid base titration method using a 5 g sample and 0.1 N NaOH with phenolphthalein colour indicator.

Leaf nutrient content

Thirty matured and completely developed leaves per tree from the central position of branches were collected and analysed both before (1st of August 2002) and six months after PBZ application (15th of February 2003). Nutrient contents of leaves were determined on composite samples where leaves were composited from each tree in each plot per treatment.

The samples were analysed for selected macro (N, P, K, Ca) and micronutrients (Fe, Mn, Zn, Cu). Samples were oven-dried, ground and analysed for Nitrogen using Kjeldahl method (Chapman & Pratt 1973), phosphorous by spectrophotometer and potassium with flame photometer. Calcium and all the minor nutrients were analysed using atomic absorption.

Total non-structural carbohydrates

Samples for determining total non-structural carbohydrates were collected from the leaf flush that occurred on the current year shoots of each tree per plot, two weeks before the expected period of flowering (October 30/2002). Samples were oven-dried, ground and analysed for total non-structural carbohydrates (TNC) using the methods of Hodge & Hofreiter (1962); Smith *et al*. (1964).

Vegetative growth

Vegetative growth parameters (different parameters on new vegetative flushes development) were determined from the 100 shoots tagged before the onset of the experiment. The parameters were studied four times, at three month intervals, during the course of the experiment (data collection dates were Nov. 15 2002, Feb. 15 2003, May 15 2003 and Aug. 15 2003 for the 1^{st}, 2^{nd}, 3^{rd} and 4^{th} rounds respectively). The following growth patterns were observed: tree height (m), canopy diameter (average of N-S and E-W) (m), trunk perimeter (cm), tree volume (m^3), percent tagged shoots with new vegetative flushes, average length of the new shoots (cm), average internode length of the new shoots (cm), average number of leaves developed per tagged shoots and leaf area (cm^2) were observed and data recorded. Leaf area of forty latest matured leaves per tree from the tagged branches was calculated using the formula:

$$Y= -0.146+0.706X \ (r^2=0.995)$$

where Y= leaf area (cm^2) and X = leaf length (cm) × leaf width (cm) (Nii *et al.*, 1995). Tree volume was calculated considering the tree canopy as a cylinder (Westwood, 1988). According to him, the volume of a cylinder equals its cross sectional area times its length; thus tree volume was determined using the formula:

$$V= 1/4\pi D^2 \alpha H$$

where V is the volume (m^3), D= canopy diameter (average of N-S and E-W canopy diameters) (m), α=0.667 (constant), H= tree height (m).

4.3.5 Statistical analysis

Differences between treatments were determined with Analysis of Variance (ANOVA) using MSTATC statistical package (MSTATC, 1989). Whenever significant differences were detected, means were separated using Least Significant Difference (LSD) test at the 5% level of significance. Co-variance analysis was done in analysing data on vegetative growth as well as leaf nutrient status. The means presented in the table for nutrient analysis results are the output of the adjusted means from the co-variance table of means.

4.4 RESULTS

4.4.1 Effect of PBZ on flowering

There was a significant difference for the interaction effects between methods and rate of PBZ application (except for foliar application of 2.75 g a.i. per tree) with respect to percentage tagged branches flowered and days needed for floral bud break after treatment application (Table 4.1). Trees treated with soil drenching at a rate of 8.25 g a.i. per tree produced a significantly higher percentage of tagged branches flowered and the lowest number of days required for attaining bud break stage (Table 4.1). In the foliar spraying treatments, 8.25 g a.i. per tree also produced a significantly higher percentage of tagged branches flowered and the lowest number of days required for attaining bud break stage as compared to the control (Table 4.1). The main treatment effects of application methods and rate, but not the interaction effects, significantly affected the number of inflorescences produced (Table 4.2). Trees treated with soil

applications of PBZ had higher number of inflorescences as compared to spray applications (Table 4.2). Applying PBZ at a rate of 8.25 g and 5.50 g a.i. per tree resulted in the highest number of inflorescences per tree (Table 4.2). Application of 8.25 g a.i. per tree PBZ increased number of inflorescences by 80.95% as compared to the control.

Significant differences between the interaction effects of the method and rate of PBZ application were observed for the percentage of hermaphrodite flowers within the inflorescences (Table 4.1). Trees that received 8.25 and 5.50 g a.i. per tree PBZ as a soil drench or foliar spray and soil drench at 2.75 g a.i. per tree had significantly higher percentages of hermaphrodite flowers per panicle as compared to the control.

Table 4.1 Effect of methods and rates of PBZ application on flower related parameters of 'Tommy Atkins' mango

Treatments		Tagged branches flowered (%)	Number of days for inflorescence development	Hermaphrodite flowers (%)
Soil drench	0 (control)	41.67e	116.0a	43.08ef
	2.75 g a.i. per tree	60.00c	105.0b	56.30c
	5.50 g a.i. per tree	69.00b	87.78d	69.35a
	8.25 g a.i. per tree	76.89a	82.22e	73.09a
Foliar spray	0 (control)	40.78e	116.8a	41.84f
	2.75 g a.i. per tree	48.78d	115.7a	46.21e
	5.50 g a.i. per tree	57.33c	106.3b	50.36d
	8.25 g a.i. per tree	66.44b	99.44c	60.82b

Means followed by different letters in the same column are significantly different by LSD test at $P<0.05$

4.4.2 Effect of PBZ on Total Non-structural Carbohydrates (TNC)

Both rates and methods of paclobutrazol applications affected the shoot's TNC but there was no significant effect for the interaction. Trees treated with soil application of paclobutrazol had higher TNC than sprayed trees (Table 4.2), while irrespective of the rates applied, all PBZ treated trees had a significantly higher TNC than the control (Table 4.2).

4.4.3 Effect of PBZ on fruit development

PBZ treatments enhanced fruit set and total fruit number per tree as compared to the control (Table 4.2). Averaged across the application methods, the highest average fruit set per 20 inflorescences (7.95) was observed with the application of PBZ at a rate of 5.50 g a.i. per tree (Table 4.2) as compared to the control trees (4.29). The main treatment effects of method and rate of PBZ application significantly affected the total fruit number at harvest. The results illustrated that higher numbers of fruit were obtained from soil drenching than from spray applications (Table 4.2). A significantly higher number of fruit per tree at harvest was obtained from trees that received PBZ at a rate of 8.25 g a.i. per tree (299.3) as compared to the control (131.80) (Table 4.2). With the same trend like fruit number per tree, total fruit weight at harvest was significantly increased by soil drenching compared to foliar spray treatments (Table 4.2). Trees treated with PBZ at 8.25 and 5.50 g a.i. per tree had the highest weight of harvested fruit (Table 4.2). The increase in fruit weight per tree was caused by the increased fruit number per tree but not as a result of fruit size (Table 4.2). Applications of 8.25 g a.i. per tree PBZ increased the weight of fruit harvested

by 152.87% when compared to the control. Average weight of fruit was not significantly affected by PBZ application (Table 4.2).

Table 4.2 Effects of PBZ application methods (averaged across PBZ rates) and PBZ rates (averaged across PBZ application methods) on flowering, fruit growth and shoot total non-structural carbohydrate of 'Tommy Atkins' mango.

Treatments	Number of inflorescences developed	Av. fruit set per 20 panicles (no.)	Total fruit no. per tree	Total fruit weight per tree (kg)	Average fruit weight (kg)	Total non-structural carbohydrate (mg glucose/g dw)
Methods						
Soil	164.25a	6.53a	253.17a	95.77a	0.371a	176.25a
Spray	128.00b	5.95a	177.75b	68.35b	0.378a	165.0b
Rates						
0	104.17c	4.29c	131.80d	47.85c	0.368a	149.3c
2.75	131.80bc	6.28b	183.7c	66.12bc	0.362a	168.2b
5.500	160.00ab	7.95a	247.0b	93.28ab	0.368a	176.0b
8.25	188.50a	6.44b	299.3a	121.00a	0.398a	189.0a

Means followed by different letters in the same column are significantly different by LSD test at $P<0.05$

4.4.4 Effect of PBZ on fruit qualitative parameters

All fruit qualitative parameters were significantly affected by PBZ applications as compared to the control (Table 4.3). The main treatment effects, viz., method and concentration of PBZ application affected the TSS of the fruit. Soil drenched trees

with PBZ had a significantly higher TSS (14.77 ^{o}Brix) in their fruit than foliar sprayed trees (14.26 ^{o}Brix). Irrespective of the different rates, all PBZ treatments increased the TSS of the fruit as compared to the control and the highest TSS was recorded at PBZ concentration of 8.25 g a.i. per tree (Table 4.3). The other fruit quality parameters observed in this study (titratable acids, TSS per acid ratio, reducing and total sugars) were significantly affected only by PBZ rates (Table 4.3). Averaged across application methods, PBZ treated trees produced fruit with significantly lower titratable acids than the control (Table 4.3). Regardless of the concentrations used, PBZ treatments significantly increased TSS per acid ratio, reducing and total sugars (Table 4.3).

Table 4.3 Effect of different rates of soil/foliar applied PBZ on fruit qualitative parameters of 'Tommy Atkins' mango

Rates of PBZ (g a.i. per tree)	TSS (^{o}Brix)	Titratable acids (mg/100g)	TSS: Acid	Reducing Sugar (%)	Total Sugar (%)
0 (control)	13.33c	0.51a	26.17b	4.215b	11.22b
2.75	14.42b	0.44b	32.94a	5.212a	12.72a
5.50	14.67b	0.45b	33.43a	5.913a	12.77a
8.25	15.63a	0.45b	35.47a	5.057a	12.93a

Means followed by different letters in the same column are significantly different by LSD test at $P<0.05$

4.4.5 Influence of PBZ application on leaf mineral composition

The result from the current experiment revealed that PBZ had no significant effect with respect to the macronutrient (N, P, K and Ca) content of the leaves analysed (Table 4.4).

Conversely, there was a statistically significant difference (both interaction and main effects) among the treatments (for some elements lower and for others higher than the control) with regard to the analysed leaf micronutrient contents in this study even if no clear trend was observed (Tables 4.4 & 4.5).

Methods and rates of PBZ application affected copper content of the leaves. Soil application had significantly increased the leaf copper content (6.33 ppm) as compared to foliar spray applications (5.89 ppm). PBZ rate that significantly increased the copper content in the leaves was 5.50 g a.i. per tree (Table 4.4). Regardless of the methods and rates used, PBZ treatments increased leaf Zinc contents as compared to the control (Table 4.4).

Significant differences were observed for the interaction results between methods and rates of PBZ applications with respect to leaf iron and manganese content (Table 4.5). Regardless of the concentrations, soil as well as foliar applied PBZ increased leaf iron content and reduced leaf manganese content as compared to the control (Table 4.5).

Table 4.4 Effect of different rates of soil/foliar applied PBZ on leaf nutrient contents of 'Tommy Atkins' mango

Rates of PBZ (g a.i. per tree)	Nitrogen (%)	Phosphorous (%)	Potassium (%)	Calcium (%)	Copper (ppm)	Zinc (ppm)
0 (control)	0.962a	0.10a	0.66a	2.01a	8.60bc	12.81b
2.75	1.053a	0.10a	0.65a	2.45a	9.01ab	16.61a
5.50	1.000a	0.08a	0.66a	1.67a	9.38a	16.72a
8.25	1.010a	0.07a	0.64a	2.06a	8.24c	16.65a

Means followed by different letters in the same column are significantly different by LSD test at P<0.05

Table 4.5 Effect of methods and rates of paclobutrazol applications on leaf iron and manganese contents of 'Tommy Atkins' mango

		Methods of PBZ application	
	Rates of PBZ applied	Soil	Spray
Iron (ppm)	0 (Control)	282.1b	283.8b
	2.75	323.6a	315.3a
	5.50	328.4a	336.5a
	8.25	326.8a	318.5a
Manganese (ppm)	0 (Control)	244.1a	246.5a
	2.75	226.5b	226.4b
	5.50	226.5b	226.5b
	8.25	226.4b	226.4b

Means followed by different letters in the same column are significantly different by LSD test at P<0.05

4.4.6 Effect of PBZ on Vegetative growth

During the first round of observations, three months after treatment application, no statistically significant differences were found in trunk perimeter, percent tagged shoots with new vegetative flushes or leaf number between the control and treated trees (data not shown). For canopy diameter and leaf area parameters, only the rate of applied PBZ had an effect, irrespective of the methods of applications. Regardless of the different rates used, PBZ treatment significantly reduced canopy diameter and total leaf area as compared to the control (Fig. 4.1). On the other hand, there were significant differences for the interaction results between method and rate of PBZ application with respect to tree height, tree volume and shoot length (Table 4.6). With respect to tree height, both soil and spray applications of PBZ treatments at a rate of 8.25 g a.i. per tree and soil application of PBZ at 2.75 g a.i. per tree had significantly lower values as compared to the control (Table 4.6). Regardless of the concentrations applied, PBZ treated trees had lower values for tree volume and length of new shoots compared to the control. In all the figures below for the different rounds of observations, treatments 1, 2, 3 and 4 represent application of 0 (control), 2.75, 5.5 and 8.25 g a.i. PBZ per tree respectively.

During the second round of observations, six months after treatment application, there were significantly lower results for PBZ treated trees than the control trees for all the vegetative parameters considered. (Table 4.7). In all of the cases, rates of PBZ applied had an impact on the parameters and the methods of application did not affect the results. PBZ at a rate of 8.25 g a.i. per tree significantly reduced leaf number and leaf area as compared to the control trees (Table 4.7). Irrespective of the rates used, tree height & canopy diameter (Fig. 4.2); trunk perimeter & tree volume (Fig. 4.3),

internode length, percent tagged shoots with new vegetative flushes and shoot length (Table 4.7) were significantly reduced by PBZ application as compared to the control.

During the third (Table 4.7, Fig. 4.4 and 4.5) and fourth (Table 4.7, Fig. 4.6 and 4.7) round of observations, nine and twelve months after treatment application respectively, similar trends like those of the second round were recorded.

Table 4.6 Effect of methods and rates of PBZ application on tree height, volume and length of new shoots of 'Tommy Atkins' mango three months after treatment application

Treatments		Height of trees (m)	Tree volume (m^3)	Length of new shoots (cm)
Soil drench	0 (control)	5.64a	98.55a	26.50a
	2.75 g a.i. per tree	5.24b	90.06b	23.09b
	5.50 g a.i. per tree	5.31ab	90.07b	23.24b
	8.25 g a.i. per tree	5.22b	86.53bc	22.99b
Foliar spray	0 (control)	5.62a	95.99a	26.02a
	2.75 g a.i. per tree	5.30ab	89.96b	23.16b
	5.50 g a.i. per tree	5.30ab	87.85bc	23.13b
	8.25 g a.i. per tree	5.19b	85.78c	22.96b

Means followed by different letters in the same column are significantly different by LSD test at $P<0.05$

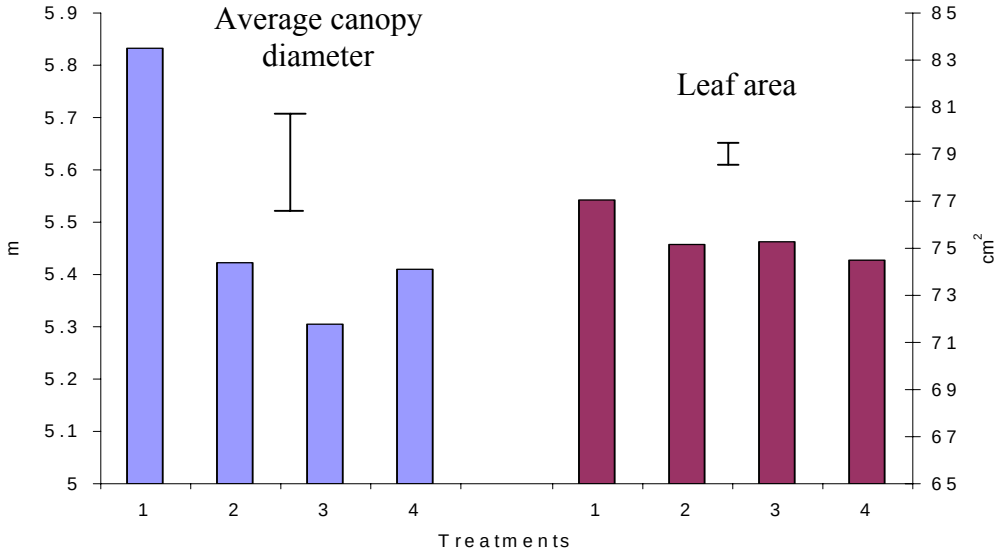

Figure 4.1 **Effect of different rates of soil/foliar applied PBZ on canopy diameter and leaf area three months after treatment application. The vertical line bars indicate LSD between means at P<0.05 level.**

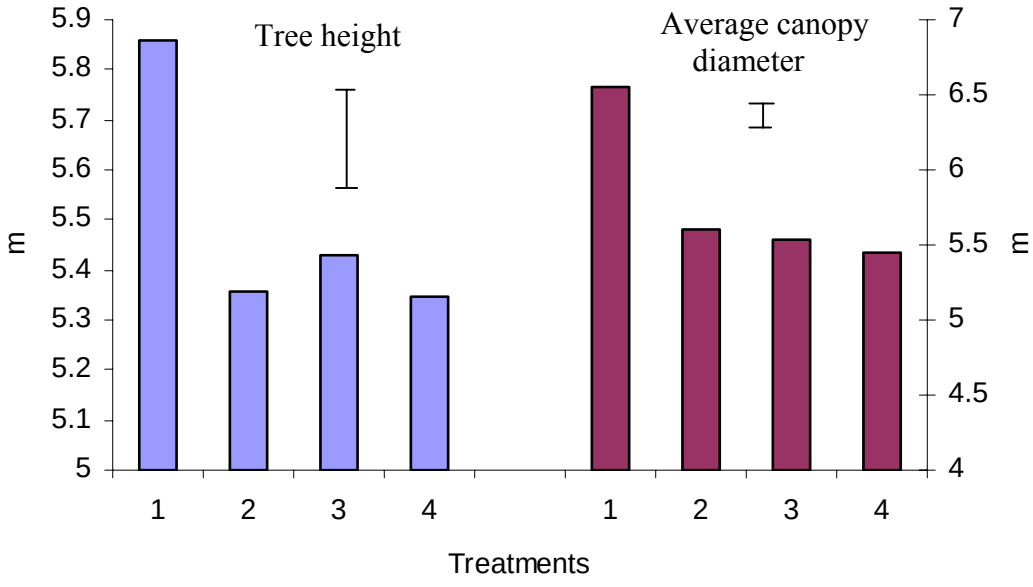

Figure 4.2 **Effect of different rates of soil/foliar applied PBZ on tree height and average canopy diameter six months after treatment application. The vertical line bars indicate LSD between means at P<0.05 level.**

Table 4.7. The effects of different rates of soil/foliar applied PBZ on some vegetative growth parameters of 'Tommy Atkins' mango six, nine and twelve months after treatment application.

Period of observations	Rates of PBZ (g a.i. per tree)	Leaf number	Leaf area (cm^2)	Shoot length (cm)	Internode length (cm)	Tagged shoots with vegetative flushes (%)
6 months	0 (control)	13.59a	77.77a	25.41a	3.87a	50.37a
	2.75	12.31b	74.77b	22.93b	3.71b	47.78b
	5.50	11.97b	74.89b	22.98b	3.67bc	46.92bc
	8.25	10.62c	73.64c	22.83b	3.51c	46.46c
9 months	0 (control)	14.27a	78.60a	26.56a	4.04a	52.55a
	2.75	12.81b	74.49b	22.96b	3.70b	47.78b
	5.50	11.97b	74.96b	22.98b	3.66b	46.38bc
	8.25	10.45c	71.46c	22.79b	3.47c	46.19c
12 months	0 (control)	15.26a	79.94a	27.55a	4.18a	55.09a
	2.75	13.31b	74.36b	22.98b	3.71b	47.85b
	5.50	12.14bc	74.75b	22.98b	3.65b	46.35bc
	8.25	10.62c	69.40c	22.75c	3.45c	45.94c

Means followed by different letters in the same column are significantly different by LSD test at $P<0.05$

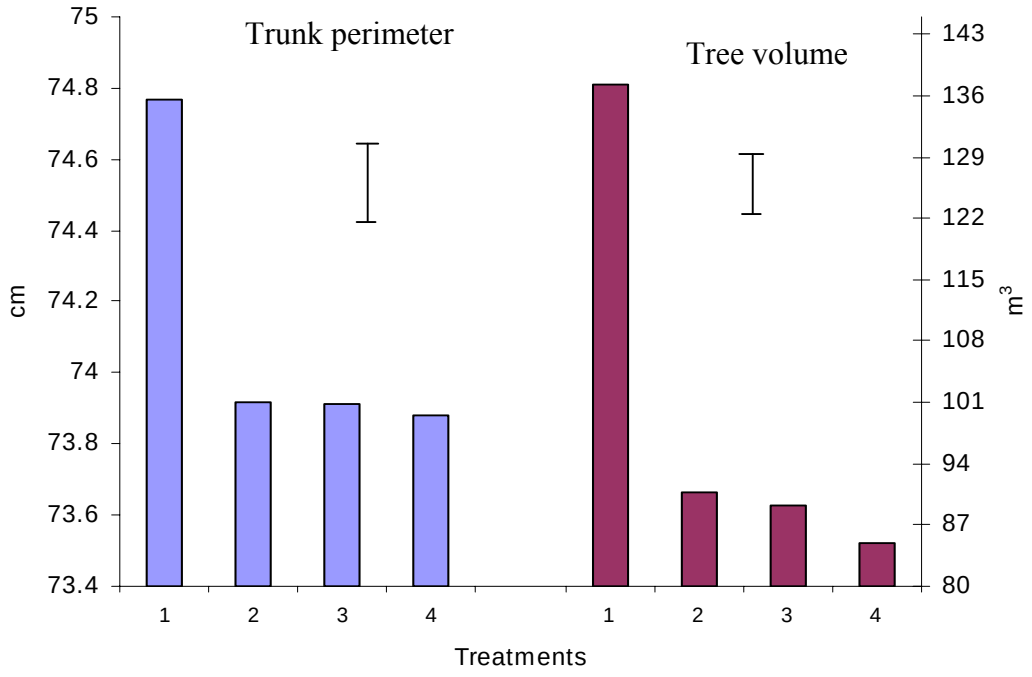

Figure 4.3 **Effect of different rates of soil/foliar applied PBZ on trunk perimeter and tree volume six months after treatment application. The vertical line bars indicate LSD between means at P<0.05 level.**

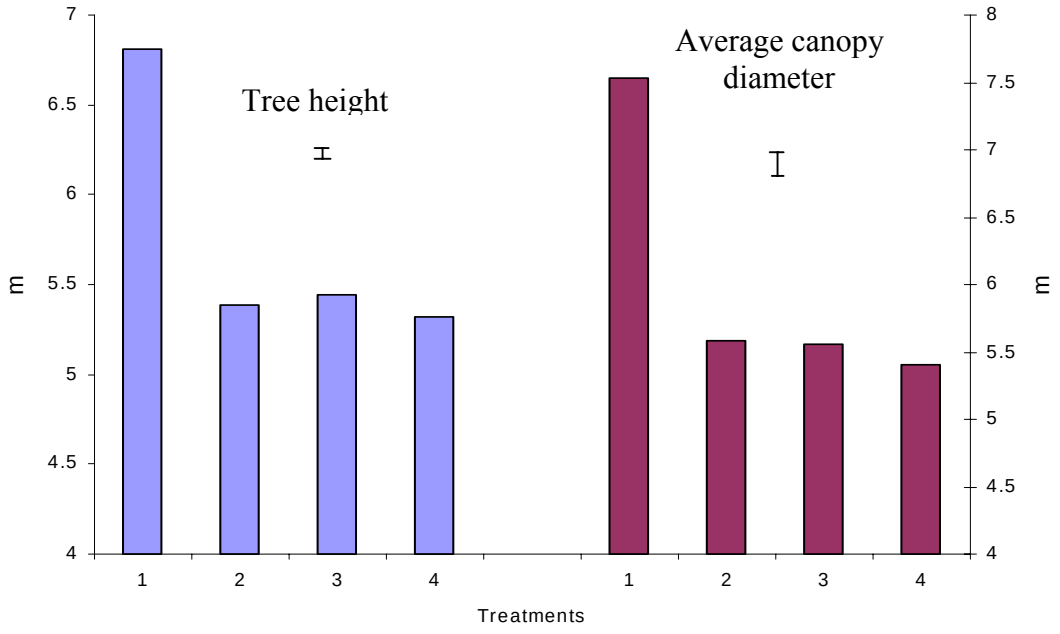

Figure 4.4 **Effect of different rates of soil/foliar applied PBZ on tree height and average canopy diameter nine months after treatment application. The vertical line bars indicate LSD between means at P<0.05 level.**

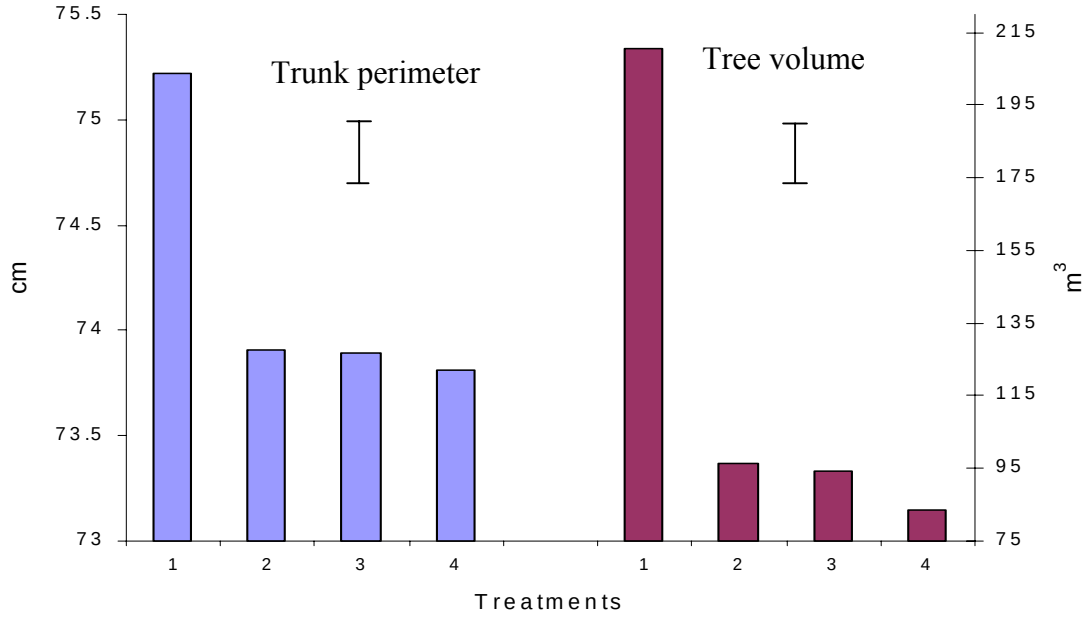

Figure 4.5 **Effect of different rates of soil/foliar applied PBZ on trunk perimeter and tree volume nine months after treatment application. The vertical line bars indicate LSD between means at P<0.05 level.**

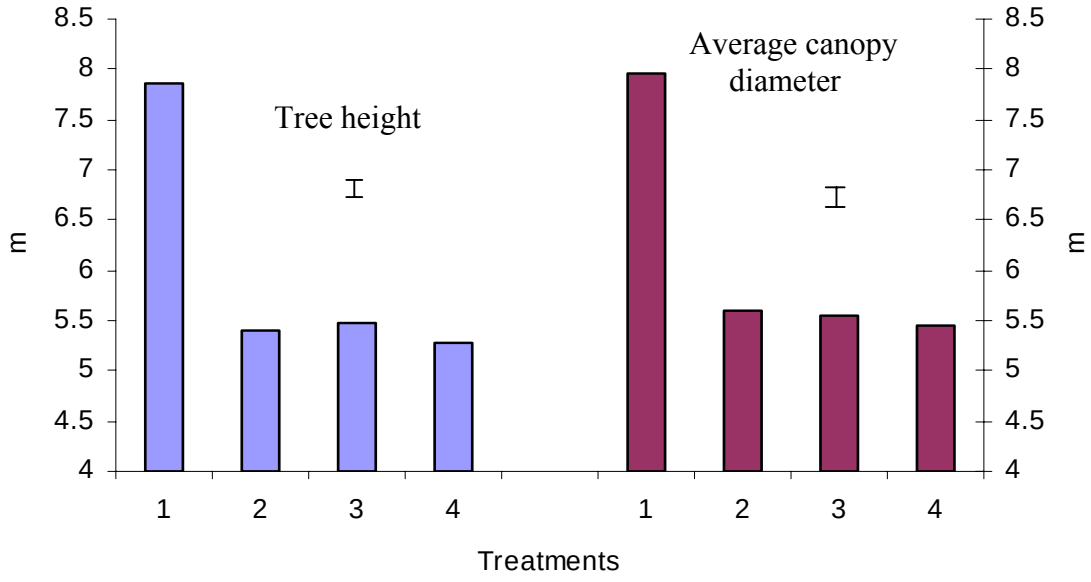

Figure 4.6 **Effect of different rates of soil/foliar applied PBZ on tree height and average canopy diameter one year after treatment application. The vertical line bars indicate LSD between means at P<0.05 level.**

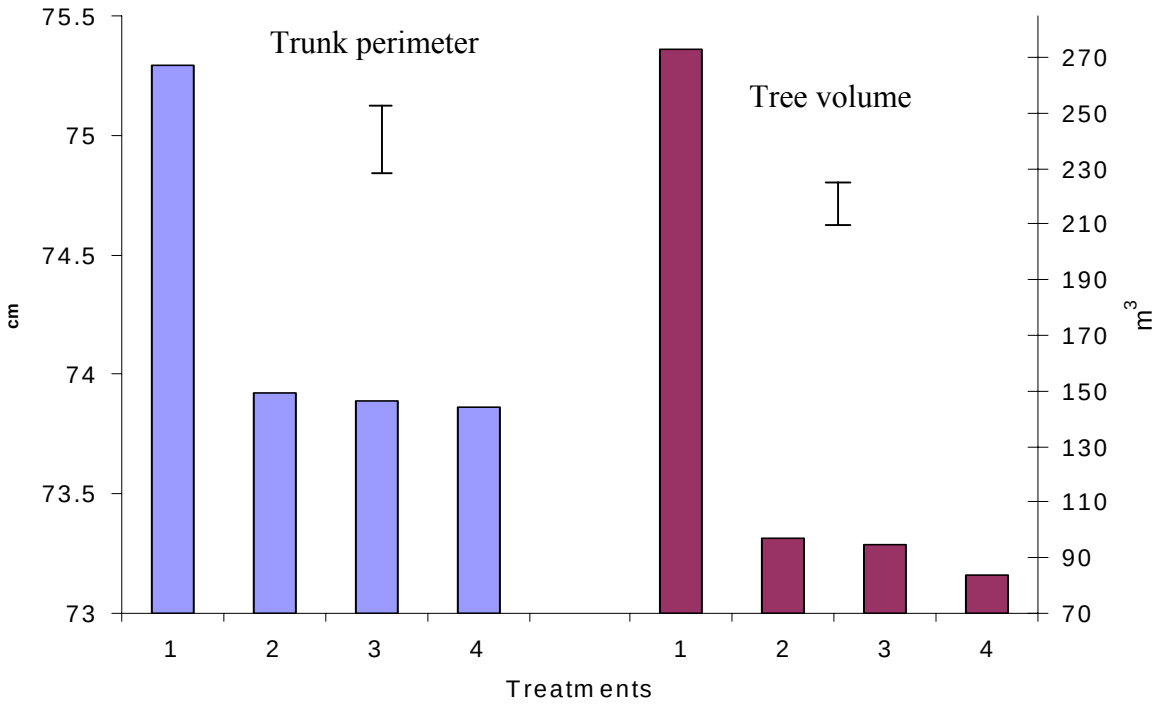

Figure 4.7 **Effect of different rates of soil/foliar applied PBZ on trunk perimeter and tree volume one year after treatment application. The vertical line bars indicate LSD between means at P<0.05 level.**

4.5 DISCUSSION

Most of the results obtained in the current experiment (with respect to vegetative and reproductive growth) are in line with a previously conducted controlled experiment, where PBZ (1- (4-chlorophenyl) –4,4-dimethyl-2- (1,2,4- triazol-1-yl) pentan-3-ol) was applied to potted plants grown in a temperature regulated growth chambers (Chapter 6 in this Thesis).

Flowering in mango is associated with reduced vegetative growth often induced by lower activity of gibberellins (Voon *et al*. 1991). In the current experiment, higher values for the percentage of tagged branches flowered was obtained by all PBZ treated trees compared to an excessive vegetative growth on the control trees. The buds in the treated trees were forced to be in a quiescent state for some time while some of the buds on the control trees burst into vegetative shoots before the normal flowering period. Forcing the buds to a quiescent state, might be linked to reduction of an expansion growth in the treated trees due to lower activity of GA_3. During this period, the buds had sufficient cold units of the winter and vegetative parameters like canopy diameter, tree volume and shoot length were highly suppressed in the treated trees. PBZ might have also supplemented the insufficient cold units for the buds. Following the reduction of the vegetative growth parameters in response to PBZ treatment, there was a higher TNC in the shoots of the treated trees, compared to the control, as per the analysis made two weeks before flowering. As indicated in Fig. 4.8, there was a significant positive correlation (r=0.98*) between shoot TNC and number of flowers developed. This signifies that a higher TNC in the shoots two weeks prior to flowering likely encouraged higher intensity of flowering in the treated trees (77% of the tagged shoots were flowering as compared to only 41% in the control trees) beside the sufficient cold spell the buds received. The results of an experiment by Burondkar & Gunjate (1993) also indicated that PBZ application increased the number of flowering shoots due to lower vegetative growth and higher reserves in the tree. A higher accumulation of reserves in the current year's shoots prior to flowering was also observed by Stassen & Janse Van Vuuren (1997b); Phavaphutanon *et al*. (2000).

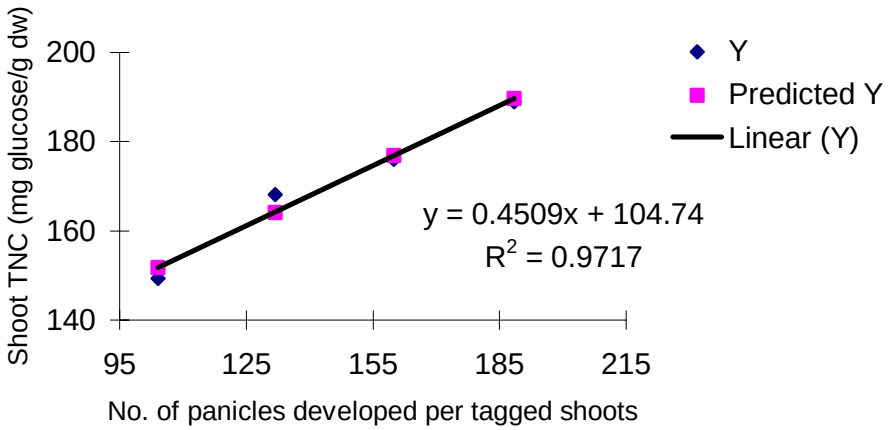

Figure 4.8 **Regression line indicating a positive relationship between shoots total non-structural carbohydrate and number of inflorescences developed.**

The majority of the dormant buds of the treated trees were released from their quiescent state more or less simultaneously soon after the cold period. This situation in addition to the higher TNC in the trees led to earlier and intense flowering in the treated trees. In this experiment, soil drenched trees that received PBZ treatment at a rate of 8.25 g a.i. per tree required 82.22 days for visible inflorescence development as compared to the control trees that needed 116.0 days as can be seen from Table 4.1. Hence, flower initiation in the PBZ soil drenched trees with 8.25 g a.i. per tree occurred about 34 days earlier than those of the control. It is probable that the application of PBZ caused an early reduction of endogenous gibberellin levels within the shoots as also observed by Anon (1984), causing them to reach maturity earlier

than those of untreated trees. This result is similar to that of Van Hau *et al*. (2002) where PBZ induced flowering 85 days after treatment application.

One of the principal effects of GA_3 is to mobilise carbohydrate by stimulating their degradation to glucose (Jacobson & Chandler, 1987). According to them, in an environment where GA levels are high, no starch accumulation can take place and consequently there will be lower tendency of flowering. The hormonal concept of flowering in mango implies that the cyclic synthesis of floral stimulus in the leaves and the difference between two such cycles would determine the flowering behaviour of a cultivar (Kulkarni, 1986). In general, PBZ, owing to its anti-gibberellin activity, could induce or intensify flowering by blocking the conversion of kaurene to kaurenoic acid (Dalziel & Lawrence, 1984; Quinlan & Richardson, 1984; Webester & Quinlan, 1984; Voon *et al.*, 1991).

The most important advantage observed on the flowering behaviour of the trees due to application of higher PBZ application was that, the bimodal flowering nature of the trees was greatly reduced. It could be due to an increased flowering intensity during the main flowering period and greatly reduced vegetative growth of the trees.

The development of complete (hermaphrodite) flowers probably needs more reserves from the tree than unisexual flowers due to the additional structures. Singh (1987) estimated that less than 0.1% of the hermaphrodite flowers develop into mature fruit while the rest falls to the ground. Assuming there are 100,000 flowers and each flower contains 10 micro gram of nitrogen, then each time a tree flowers, it loses 1 kilogram of nitrogen. The tree will, therefore, need to have adequate reserves for flower and

subsequent fruit formation. The higher TNC level (reserve) in the shoots due to PBZ soil drenching especially at rates of 8.25 as well as 5.50 g a.i. per tree increased the percentages of hermaphrodite flowers and consequently fruit set as can be seen from Table 4.1 and 4.2. These results are similar to the observations made by Vijayalakshmi & Srinivasan, (2002); Hoda *et al*. (2001).

Fruit set showed a direct impact on yield depending on number of fruit retained. The impact of higher rates of PBZ in enormously suppressing vegetative growth, especially during peak fruit development stage, contributed to the superior yield observed. In the literature, soil application of PBZ has consistently been found to increase tree yield (Kulkarni, 1988; Burondkar & Gunjate, 1993; Kurian & Iyer, 1993; Singh & Dhillon, 1992; Singh, 2000). Our results confirm the findings of Hoda *et al*. (2001) that soil treatment is more effective than foliar spraying for increasing yield.

Fruit quality improvements with respect to TSS, TSS to acid ratio, total sugars and reducing sugars in response to PBZ treatments can be related to assimilate partitioning of the plant. As the assimilate demand is unidirectional to the developing fruit, due to the great suppression of vegetative growth, PBZ treated trees had higher fruit quality attributes. With the same justification, the control trees had lower TSS and sugars but higher titratable acidity as can be seen in Table 4.3. In agreement with the current experiment, Vijayalakshmi & Srinivasan (1999); Hoda *et al*. (2001) also reported that PBZ treatments improved fruit quality. The result of Medonca *et al*. (2002) was, however, contradictory to these findings. Caution, however, must be taken to the export regulations of some countries about fruit from PBZ treated trees. Singh & Ram (2000) calculated that an application of PBZ at 2.3 g a.i. per meter tree canopy on

'Dashehari' and 'Langra' mangoes resulted in fruit containing 0.004 mg kg^{-1} PBZ, which was much lower than the international maximum value (0.05 mg/kg fruit weight). Subhadrabandhu *et al.* (1999) also used 8 g a.i. per tree on 'Nam Dok Mai' mango and no chemical residues were detected in the mature fruits. They suggested that the rate of PBZ they applied could be used for mango production in terms of food safety. The rates of PBZ used in the current experiment were lower than the rate used by Subhadrabandhu *et al*. (1999).

It can be understood from the current study that, there is no increased mobilisation of major elements (N, P, K, Ca) to the leaves, either from the soil or from other plant parts, due to PBZ treatment. Leal *et al*. (2000) also reported a non-significant effect of PBZ on the macronutrient levels of leaves. Werner (1993), however, reported increase of N, Ca and Mg due to PBZ application and reduction in P and K. Salazar-Gracia & Vazquez-Valdivia (1997) reported a decrease in P, Mg and Ca due to PBZ rates above 10 g PBZ/tree and no effect on N and K. The significant effect of PBZ in increasing the Cu and Fe concentration and a significant decrease in Mn concentration in the leaves, was contrary to and the increase in Zn was in line with the findings of Werner (1993) who reported an increase of Mn and Zn but reductions of Cu while no effect on Fe. This topic has not yet been researched properly and needs further investigation.

The effect of PBZ on reducing most of the vegetative growth parameters was noticed especially with higher concentrations. A cultivar difference in response to PBZ was previously observed where PBZ was far more effective in retarding extension and expansion growth in 'Tommy Atkins' than in 'Sensation' (Oosthuyse & Jacobs, 1997). In the current experiment, the effect of PBZ on all the vegetative growth

parameters was recorded soon after treatment application but enormously higher values were observed six months after treatment application. It was also observed that, after the first round of observation, there was no significant difference between the two methods of PBZ application and only the rates of PBZ had an effect on the vegetative parameters. High concentrations of both spray and soil application of PBZ treatments produced the most obvious inhibiting effect for almost all the vegetative parameters during the third round (Fig. 4.4 & 4.5). This period coincided with a stage after peak fruit set and development (fruit about to be harvested) that had an additional impact on vegetative growth. During this time, most of the assimilate might have been partitioned to the developed fruit, and therefore restricted new vegetative growth on the trees in addition to the effect of PBZ treatment.

The positive correlation (r=0.95*) observed between internode length and leaf area, indicated that while the internode length tapered (as a result of PBZ treatment), the leaves became crowded. This can possibly be ascribed to a limited cell enlargement in the leaves of the treated trees, which ended up with reduced leaf surfaces. The regression line in Fig. 4.9 indicates the positive relation between internode length and leaf area.

According to Steffens *et al*. (1985) PBZ has the greatest effect on immature tissues, which are still growing and differentiating, through which it affects predominantly the apical growth. Vijayalakshmi & Srinivasan (1999) reported PBZ to increase the leaf area of the treated trees, which is contrary to the observation of this report as well as to that of Kurian & Iyer (1993).

According to Esau (1977), the plate meristem constitutes a major part of the intercalary growth by means of which the leaf reaches its mature size. PBZ treatment might then reduce leaf size as observed in the current study, by diminishing the enlargement of cells derived from the plate meristems. This is due to its obvious effect on reducing levels of gibberellins, since gibberellins encourage cell growth. Generally, triazoles, reduce leaf area, but increase epicuticular wax, width and thickness (Gao *et al*., 1987) and hence leaf dry weight per unit area (Davis & Curry, 1991). According to Gao *et al*. (1987), PBZ increased chloroplast size along both the long and short axes, being 34 and 30% longer than the control, respectively, intensifying the dark green colour compared to the controls (Fletcher *et al*., 2000). This situation perhaps might increase the photosynthetic potential of the treated trees.

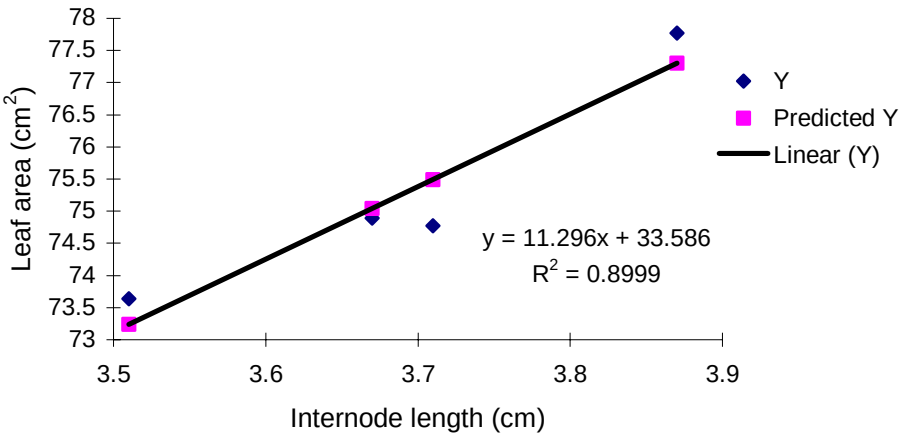

Figure 4.9 Regression line indicating a positive relationship between internode length and leaf area.

4.6 CONCLUSION

Although this chapter was based on the results of one season, the following important outcomes were noted that can have practical values to Ethiopian mango farmers. The productivity of the trees was increased due to higher intensity of flowering, higher percentages of hermaphrodite flowers and higher fruit set. The increase in the flowering parameters and fruit set is linked to reduced vegetative vigour, increased non-structural carbohydrate content of the shoots and increased chlorophyll content of the leaves. These situations ultimately increased the yield obtained. The fruit quality was also improved. Moreover, the bimodal flowering behaviour of the trees was reduced. Generally, soil application of PBZ was recommended and rates of either 5.50 or 8.25 g a.i. per tree can be used.