Effect of Paclobutrazol (PP 333) and Human Hair-derived Aminoacids Mixture on Growth, Yield and Quality of Tomato cv. PKM-1 (*Lycopersicon esculentum* Mill.)

By

Kalpana .S

A Thesis Submitted to the Avinashilingam Institute for Home Science and Higher Education for Women (Deemed University), Coimbatore - 641 043 in partial fulfilment of the requirements for the Degree of

Master of Science in Biochemistry

May, 1999

EFFECT OF PACLOBUTRAZOL (PP333) AND HUMAN HAIR-DERIVED AMINOACIDS MIXTURE ON GROWTH, YIELD AND QUALITY OF TOMATO cv. PKM-1 (*Lycopersicon esculentum* Mill.)

By

KALPANA. S

A thesis submitted to the Avinashilingam Institute for Home Science and Higher Education for Women (Deemed University), Coimbatore – 641 043

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE IN BIOCHEMISTRY

MAY, 1999

CERTIFIED AS BONAFIDE RESEARCH WORK

6/5/99

Signature of the Head of the Department

6/5/99

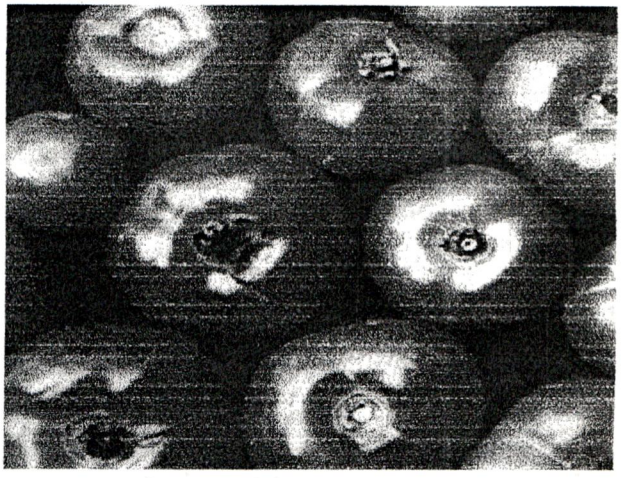
Signature of the Guide

Dedicated to

My

Parents

ACKNOWLEDGEMENT



ACKNOWLEDGEMENT

Many are the persons
many the reasons
and few the words
to give thanks;
here are the some
whom to name,
to whom to show
how much I owe!

I find great pleasure in expressing my reverential gratitude to ***Padmashri Hon. Colonel (Tmt.) Rajammal P. Devadas****, M.A., M.Sc., Ph.D., (Ohio State), Hon.D.Sc. (Madras), Hon.O.H.L. (Oregon State), Hon.D.H.L. (Ohio State), Hon.D.Sc. (C.Azad Agri University, Kanpur), Hon.D.Sc (Ulster, Northern Ireland), Chancellor, Avinashilingam Institute for Home Science and Higher Education for Women (Deemed University), Coimbatore, for providing the opportunity for conducting the study.*

My profound sense of thanks are due to ***Dr.(Tmt.) Lakshmi Santa Rajagopal****, M.S., (Tennessee), Ph.D., (Madras), Vice Chancellor, Avinashilingam Institute for Home Science and Higher Education for Women (Deemed University), Coimbatore, for the kind help rendered.*

I extend my sincere thanks to ***Dr.(Tmt.) Saroja Prabhakaran****, M.A., Dip. in Ed., Ph.D., Registrar, Avinashilingam Institute for Home Science and Higher Education for Women (Deemed University), Coimbatore, for her warm and willing help.*

I express my thanks to to ***Dr. (Tmt.) Sivakama Sundari****, Dean, Faculty of Science, Avinashilingam University, Coimbatore for providing the opportunity to conduct the study.*

I find great pleasure in expressing my deep sense of gratitude for the guidance and help rendered by my Guide ***Dr. S. Saroja****, M.Sc., M.Phil, Ph.D. (Madras), Professor and Head, Department of Biochemistry, Avinashilingam University, Coimbatore. It is no exaggeration to say that my thesis completion would be impossible with out the unceasing inspiration, constant encouragement and well-timed suggestions made available by her. I express my truest and soul-felt gratitude.*

I wish to express my special thanks to ***Mrs. A. Pushpa****, Lecturer, Department of Biochemistry, Avinashilingam University, Coimbatore, for her bountiful help during my course of investigation.*

I am very much thankful to all the ***staff members*** *of Biochemistry Department for their timely help.*

On a personal note, I owe a great deal to ***my father*** *for his help at every stage of shaping this thesis.*

I am also thankful to ***Mr. S. Senthil Murugan & Sree Kumaran Computers*** *for their promptness and neat execution of this work.*

Last but not the least, I thank the Almighty.

(S. KALPANA)

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

INTRODUCTION

1.0. INTRODUCTION

Tomato (*Lycopersicon esculentum*. Mill), though of Mexican origin, has become a popular annual vegetable crop throughout the globe, extending from the tropics to within a few degrees of the Artic circle (Rick, 1976), indicating its high degree of adaptability to a wide range of soils and climates. In India and many other countries, it ranks second only to potatoes in terms of annual production value. The reason for the tomato's popularity are various. It supplies vitamins (particularly vitamin C) and a variety of colour and flavour to the diet.

In India tomatoes are grown for being used as salad as well as vegetable in cooking preparations and value added processed products like ketchup, chutney, juice, etc. Accordingly there are many tomato varieties to suit different needs. Particularly in Tamil Nadu sour type of tomatoes are preferred to salad types, though both are grown commercially. Among the sour type of tomatoes, the variety PKM-1 is most popular among the growers of Tamil Nadu. PKM-1 tomato released by the Tamil Nadu Agricultural University, is known for its high yield and good keeping quality (shelf-life) with an optimal blend of acidity and sweetness that suits the cooking needs of south Indian preparations like rasam, chutney, etc. The nutritive value of tomato, in general, has been well documented. National Institute of Nutrition has published that tomato is rich in minerals such as iron, calcium, manganese, boron, zinc, potassium, copper, chromium, sulphur, phosphorus and chloride and also vitamins such as carotene, thiamine, riboflavin, niacin, folic acid and ascorbic acid besides protein and fat (Narasinga Rao *et al.*, 1996).

It is evident from research reports that many chemical substances can regulate the growth and development of tomato. Use of fertilizers and synthetic growth regulants not only increases the yield but also alters the qualities of the tomato fruit (Baruah *et al.*, 1993).

Particularly several growth retardants have been reported to have enhanced the yield and quality of tomato fruit by causing changes in endogenous biochemical environment of the plant. Among various growth retardants like antiauxin and anti gibberellin, the latter is known to be very effective. Paclobutrazol, a synthetic antigibberellin, is a new class of growth retardant having profound influence on flowering, fruit set, biochemical constituents and nutritive make up of many fruits and vegetables.

Among the nutrients required for the growth of tomato plant, nitrogen is considered to be very important as it forms the basis of aminoacids formation and ultimately the proteins and specific tissues. Aminoacids are now being extracted from human hair and poultry feathers and a new organic product (SRIDIAMIN) developed recently out of such aminoacids makes it possible to use them as a cheaper source of substitute for or supplement to nitrogenous fertilizers in agriculture and horticulture. The research work on fruit and vegetable crops using a mixture of 17 aminoacids and the antigibberellin paclobutrazol separately and in different combinations and concentrations is meagre but emerging fastly in crop research in India and abroad.

The present investigation, therefore, aims at assessing the effect of application of human hair-derived mixture of 17 amino acids (SRIDIAMIN) and paclobutrazol (PP333) on tomato cv. PKM-1 in terms of

1. Vegetative parameters such as plant height, number of laterals per plant and root-shoot ratio.
2. Flowering - Time taken for first flowering, number of flowering clusters and number of flowers per cluster
3. Yield - total number of fruits and fruit weight
4. Biochemical constituents- changes in leaf nutrient concentration and fruit quality characteristics.

REVIEW OF LITERATURE

2.0. REVIEW OF LITERATURE

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important solanaceous fruit vegetables grown throughout the world. The fruits are not only important from yield point of view but are also rich source of minerals and vitamins. The quality of fruit is therefore one of the important considerations for improving the health standard of our masses. Recently PP333, a new inhibitor of biosynthesis of gibberellins has been reported to be most effective in improving the yield potential of certain vegetable crops. There is no precise information on the effect of PP333 in combination with human hair derived aminoacids (SRIDIAMIN), a substitute for nitrogenous fertilizers. Literature pertaining to the role of paclobutrazol and aminoacids have been reviewed.

2.1. ROLE OF AMINOACIDS IN PLANT GROWTH AND DEVELOPMENT

2.2. EFFECT OF PACLOBATRAZOL ON PLANT BIOMETRIC CHARACTERS

2.2.1. Plant height

2.2.2. Stem girth, number of leaves, leaf area and internodal length

2.2.3. Effect of PP333 on flowering, yield and fruit characters

2.3. EFFECT OF PP333 ON BIOCHEMICAL PARAMETERS

2.3.1. Leaf chlorophyll content

2.3.2. Fruit quality

2.3.3. Effect of PP333 on leaf nutrient concentration

2.1. ROLE OF AMINOACIDS IN PLANT GROWTH AND DEVELOPMENT

Aminoacids are the essential building blocks of proteins which inturn are the energy rich compounds abundantly required for crop growth. It is also well known that aminoacids are precursors of growth regulators. Studies on the effect of exogenous aminoacids on plants under *in vivo* conditions are practically lacking. Thus, literatures pertaining to the role of aminoacids in plant growth and development, in general, have been reviewed.

Aminoacids may act as growth regulating substances (Riker and Gutsche, 1948). Asparagine is found to be a fair source of nitrogen for tobacco (Beaumont *et al.*, 1931). Many aminoacids can be used by clover and tomato plants. Alanine, asparagine, glutamine, glycine, histidine, isoleucine, leucine, lysine and phenylalanine prove better as nitrogen sources than nitrates or ammonia. Of all the aminoacids, arginine is known to promote growth in the presence of sugars. Glycine, nicotinic acid and pyridoxine are helpful for continuous growth of the roots. Guanine and adenine are effective for appreciable cell enlargement in beans (Ghosh and Burris, 1950). Free proline is reported to induce stress tolerance in a variety of plants through rehydration of protoplasm (Chauhan *et al.*, 1980). Hence, the proline accumulation has been suggested as a criterion in breeding programmes for the development of drought resistance in the crop plants (Bates *et al.*, 1973).

Andhra Pradesh Agricultural University (APAU) and the ICAR Directorate of Oil seeds Research (DOR) have conducted trials using Sridiamin (a mixture of 17 aminoacids derived from human hair) during 1992-93 and the results revealed that the application of the Sridiamin gave an additional yield of 321 kg groundnut, 106 kg sunflower, 320 kg chillies (dry pod) and 2000 kg tomato per acre (Anon, 1995).

Studies on the effect of Sridiamin at 0.5 per cent concentrations and a total of 2-3 sprays on various crops resulted in increased yield under European conditions; the increase was 10 per cent in maize, 13 per cent in potato, 15 per cent in strawberry, 14 per cent in lettuce, 16 per cent in tomato, 9 per cent in little radish and 6 per cent in sugarbeet. Experiments in Germany showed that nitrogen content of various crops significantly increased by 13 per cent with foliar spray of 0.5 per cent Sridiamin. An increase in protein content of maize has been found after 2-3 spray treatments with 0.7 per cent solution of Sridiamin. Dosages of 0.5 to 0.75 per cent Sridiamin resulted in statistically significant increase in wet matter, dry matter and nitrogen content of the crops. Dosage above 0.75 per cent did not achieve additional increase in crop yield (Anon, 1995).

Increased number of flowers, squares, bolls and branches were recorded in cotton treated with 0.5 per cent Sridiamin both under rainfed and acute water stress conditions. Similarly in chillies good flowering, reduced flower drop, increased pod size and quality were obtained with Sridiamin spray both under irrigated and rainfed conditions (Anon, 1997).

Experiments conducted on tea by United Planters Association of South India using Sridiamin as foliar spray revealed that the aminoacids spray effectively improved the productivity in both normal and moisture stress conditions. The overall quality was also consistently good. In groundnut, there was significant increase in the number of pods and shelling per cent due to Sridiamin spray. Sunflower sprayed with 0.5 per cent Sridiamin showed better seed setting and yield than the unsprayed control. Sridiamin spray at 0.5 per cent improved the crop growth as well as productivity, besides advancing crop maturity in tomato, capsicum, brinjal and bhendi. The quality and size of the output were much higher in treated plots than the controls. Sridiamin spray recorded higher panicle emergence and better grain filling in paddy and significantly increased number of cobs and protein content in maize (Anon, 1997).

2.2. EFFECT OF PP333 ON PLANT BIOMETRIC CHARACTERS

2.2.1. Plant height

PP333 sprays to `Delicious' apple at 85 or 100 mg per litre drastically reduced total shoot growth (55% and 37% respectively). This reduction was primarily due to reduction in the number of laterals and terminal shoots rather than the average length (Greene, 1991). Soil drenching of 25 mg PP333 per litre inhibited shoot elongation on Blueberry cultivars (Ehlenfeldt, 1998). Foliar application of PP333 (250 mg per litre) to grapes led to a reduction in shoot length (Kumar *et al.*, 1998).

In pear cv. Gola, trunk-soil-line-pour (TSLP) application of PP333 at 125 mg per tree effectively reduced terminal shoot growth accompanied by an increase in fruit yield and quality (Bist, 1994). Costa *et al.* (1995) reported that application of PP333 as foliar spray (1000 or 2000 mg/L) at full bloom or when shoots were 25 cm long to trees carrying no crop, inhibited shoot growth in Blanquilla pear and the effect increased linearly with the concentration. PP333 (2 or 4 mg as soil drench) reduced plant height in all species of Acacia (Parletta and Sedgley, 1998).

In apricot, shoot growth reduced as the concentration of PP333 increased. PP333 foliar spray at 1000 ppm reduced the rate of shoot growth to 57.06 per cent and 65 per cent in Canino and Precode de colomer apricot trees respectively (Kuden *et al.*, 1995). Similarly in Sundrop apricot PP333 at 2, 4 or 6 g a.i. per tree applied into a 5 cm deep furrow around the trunk base effectively controlled the vegetative growth (Jacyna and Dodds, 1995).

In mango cv. Alphonso, vegetative flushes were significantly reduced by PP333 as soil drench treatments at 5 and 10 g per tree as compared to foliar sprays of 500, 1000 and 2000 ppm concentrations (Burondkar and Gunjate, 1993).

In papaya var. CO2, PP333 reduced the plant height by 20 per cent, when applied at 1000 ppm foliar spray (Baskaran, 1995).

PP333 modified canopy structure in the interspecific grapes cv. Seyral Blane by inhibiting the growth of the main shoot apex and axillary bud and shoots (Hunter and Procter, 1992). PP333 applied at 100, 250 or 500 mg per plant to the soil of container grown sweet orange cv. Valencia suppressed plant growth (Vu and Yelenosky, 1992). According to Okuda *et al.* (1996), PP333 1000 mg as soil drench reduced new shoot sprouting by 48 per cent and shoot growth by 37 per cent in Satsuma mandarin.

When tomato cv. Pusa Ruby plants were sprayed with 0, 100 and 150 ppm, the plant height and leaf area decreased as PP333 rate increased (Baruah *et al.*, 1995).

2.2.2. Stem girth, number of leaves, leaf area and internodal length

PP333 spray at 50 or 500 ppm, increased leaf thickness and decreased leaf area, but the number of leaves and stem girth did not get altered (Abod and Webster, 1991). There was a reduction in pedicel length when PP333 was sprayed at 250 mg per litre to `Delicious' apple (Greene, 1991).

PP333 at 250 mg per tree effectively reduced trunk diameter, terminal shoot length and its internodal length in Pear cv. Gola, when applied as trunk-soil-line-Pour (Bist, 1994). On the contrary, Costa *et al.* (1995) observed a decrease in trunk cross sectional area due to PP333 at 1000 and 2000 ppm.

According to Snowball *et al.* (1994), PP333 as soil drench (100 and 500 mg a.i. per plant) reduced number of branches and branch internodal length in Marsh grape fruit.

Mehonachi *et al.* (1996) reported that PP333 foliar spray at 100 µg per ml daily for one week inhibited shoot growth, reduced length of the stem (21%) and also the dry weight (19%).

2.2.3. Effect of PP333 on flowering, yield and fruit characters

PP333 plus N resulted in increased yield by 69 per cent and fruit weight by 49 per cent and reduced fruit number in peach cv. Florida Prince (George *et al.*, 1995).

In pear cv. Doyenna-du-comice, PP333 stimulated flower initiation throughout the tree (Browning *et al.*, 1992). In Nashi pear trees soil application of 1000 or 2000 mg PP333 at full bloom resulted in greater reduction of fruit set, fruit size and almost total loss of the crop, in Blanquilla (Klinac *et al.*, 1991) and pear (Costa *et al.*, 1995). PP333, 250 ppm foliar spray significantly increased fruit yield in apricot cv. Canino (Kuden *et al.*, 1995).

In papaya var. CO2, PP333 foliar spray at 250 ppm induced flowering earlier by 40 days and at 500 ppm the percentage of fruit set increased by 92 per cent. Number of fruits, pedicel length, seed content were increased by PP333, whereas fruit length, circumference, weight and volume were reduced (Baskaran, 1995).

In citrus also (lime, mandarin hybrids, grape fruit) PP333 increased the levels of flowering (Snow ball *et al.*, 1994). Soil application of PP333 at 1000 mg per tree increased total number of inflorescence by 66 per cent but reduced the new shoot sprouting by 48 per cent and shoot growth by 37 per cent (Okuda *et al.*, 1996).

PP333 as spray at 0, 25, 50, 75 and 100 ppm, has increasingly delayed flowering as the concentration increased (Yewale *et al.*, 1997).

Asao *et al.* (1996) reported that PP333 100ppm spray increased the yield of hydroponically grown tomato. Baruah *et al.* (1995) reported that in tomato cv. Pusa Ruby, the fruit set and yield increased with the highest dose of N (150 kg/ha) coupled with 150 ppm spray of PP333.

2.3. EFFECT OF PP333 ON BIOCHEMICAL PARAMETERS

2.3.1. Leaf chlorophyll content

PP333 has been found to increase the chlorophyll content of the leaf of many fruit crops. An increase in chlorophyll content was recorded in rice leaves (Yim *et al.*, 1997).

In banana, both chlorophyll `a' and `b' increased linearly with the concentration of PP333, but the ratio of chlorophyll `a' and `b' decreased linearly (Costa *et al.*, 1995).

Foliar spraying of PP333 treated *Brassica carinata* plants exhibited higher chlorophyll content (Setia *et al.*, 1995).

On the contrary, PP333 did not alter the leaf chlorophyll `a' and `b' content in the sweet orange cv. Valencia (Vu and Yelenosky, 1992).

2.3.2. Fruit quality

PP333 at higher concentrations increased the total soluble solid content in apricot (Kuden *et al.*, 1995). The seeds of PP333 treated *Brassica carinata* plants had higher levels of total soluble solids (Setia *et al.*, 1995).

PP333 application sometimes caused reduction in soluble solid content, as observed in `Mc Intosh' apple (Elfving *et al.*, 1990) and in `Delicious' apple (Greene, 1991) .

Titrable acidity content decreased in tomato cv. Pusa Ruby (Baruah *et al.*, 1993) due to PP333.

Paclobutrazol (PP333) enhanced the levels of total free aminoacids in mature seeds of *Brassica juncea* (cv.) Czern and Coss (Setia *et al.*, 1996).

On the contrary, in apricot, Kuden *et al.* (1995) reported that PP333 treatment had no effect on fruit quality characters like total soluble solids and titrable acidity. Such contradictory effects were also observed in mango (Burondkar and Gunjate, 1993).

2.3.3. Effect of PP333 on leaf nutrient concentration

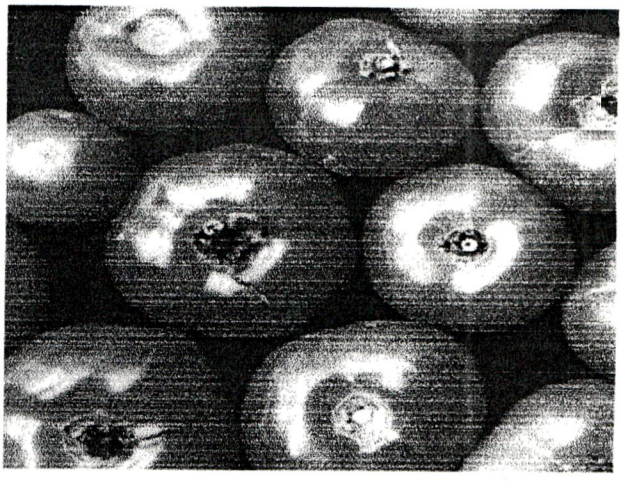
Limited attempts were made to study the effect of PP333 on leaf nutrient concentration. PP333 either as foliar spray or soil drench has been found to increase the leaf N, Fe and Mn content in citrus root stock seedlings (Yelenosky *et al.*, 1995).

PP333 was found to increase the leaf calcium content in apple (Greene, 1991). Increase in leaf Mn and Fe contents were observed in many fruit crops due to PP333 treatments.

Contradictory results were reported in respect of Zn and Cu contents. In mango cv. Blanco, soil application of 1500 ppm PP333 increased the Zn content in leaves and decreased the copper content (Werner, 1993).

With the above findings in view, the present study was undertaken to assess the effect of paclobutrazol (PP333) and human hair derived aminoacids (SRIDIAMIN) on growth, yield and quality of tomato cv. PKM-1 which is a sour type popular in Southern states in general and Tamil Nadu in particular.

EXPERIMENTAL PROCEDURE



3.0. EXPERIMENTAL PROCEDURE

The present investigations on the effect of paclobutrazol (PP333) and a mixture of human hair derived aminoacids (SRIDIAMIN) on tomato cv. PKM-1 were carried out under field conditions at the Avinashilingam University, Coimbatore, during 1998-99.

3.1. EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

The experiment was laid out in Randomized Block Design with four replications.

3.2. CROP HUSBANDRY

3.2.1. Nursery management

The breeders quality seeds of tomato cv. PKM-1 was sown in raised beds adopting the procedure recommended by the Tamil Nadu Agricultural University.

3.2.2. Transplanting

Thirty days old seedlings were transplanted on the sides of the ridges spaced at 60 cm in the row and 60 cm between rows.

3.3. TREATMENT SCHEDULE

Paclobutrazol (PP333) was applied as soil drench one month after transplanting.

Human hair derived aminoacids mixture (SRIDIAMIN) was foliar sprayed four times at fifteen days interval, commencing from 15th day of transplanting.

Paclobutrazol : Chemical name PP333=1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4,-triazol-1-yl) -pentan-3-ol).

Human hair derived aminoacids (SRIDIAMIN)

A product derived from human hair containing 32.52 per cent of 17 L-aminoacids fortified with vitamins (folic acid). It is available as proprietary product in the market as SRI DIAMIN. The technical details of SRI DIAMIN are furnished below :

SRI DIAMIN - Product specifications

(Liquid aminoacids for foliar applications)

Aminoacids (total)	-	32.52%
Folic acid	-	100 ppm
Density	-	1.10-1.30 per ml
pH (undiluted)	-	4.0-5.0
Colour	-	Brown
Odour	-	Specific and spicy

Amino acid composition

Alanine	2.09%	Lysine	1.84%
Arginine	3.67%	Methionine	0.49%
Aspartic acid	2.70%	Phenylalanine	0.60%
Cystine	0.17%	Proline	3.25%
Glutamic acid	5.38%	Serine	4.54%
Glycine	1.74%	Threonine	2.97%\
Histidine	0.54%	Tyrosine	0.20%
Isoleucine	0.37%	Valine	1.41%
Leucine	0.56%		

TREATMENTS

T_1	-	Control
T_2	-	0.5% Sridiamin
T_3	-	1.0% Sridiamin
T_4	-	25 mg paclobutrazol (cultar 1 ml / plant)
T_5	-	50 mg paclobutrazol (cultar 2 ml / plant)
T_6	-	0.5% Sridiamin + 25 mg paclobutrazol
T_7	-	1% Sridiamin + 25 mg paclobutrazol
T_8	-	0.5% Sridiamin + 50 mg paclobutrazol
T_9	-	1% Sridiamin + 50 mg paclobutrazol

3.4. PLANT BIOMETRIC CHARACTERS

Data on plant height, time taken for flowering, number of laterals per plant, number of flowering clusters, number of flowers per cluster were recorded.

a. Plant height

The height of the plant from the cotyldenary node to the tip of the plant was measured and expressed as centimeters.

b. Number of laterals per plant

The branches arising from the main stem were counted at the time of final harvest and expressed as number per plant.

c. Number of days to first flowering and flower clusters per plant

From the day of transplanting, till the appearance of the first flower days were counted and expressed as number of days to first flowering. The individual flower clusters were tagged as and when they appeared and counted.

d. Number of flowers per cluster

The number of flowers in the first five clusters were counted in each plant and the mean number of flowers per cluster was arrived at.

e. Root-shoot ratio

At peak harvest, the plants were uprooted carefully with root system intact and washed thoroughly. The root and shoot were separated, chopped into pieces and dried separately in hot air oven at 60°C.

$$\text{The ratio was worked out using the formula} = \frac{\text{Shoot weight (g per cent)}}{\text{Root weight (g per cent)}}$$

3.5. FRUIT CHARACTERS

a. Mean fruit weight

Mean fruit weight was obtained by dividing the total yield per plant by the total number of fruits per plant and was expressed as grams.

b. Number of fruits per plant

The number of ripe fruits plucked at every harvest was counted, the total obtained and the mean worked out. The value was expressed as number of fruits per plant.

c. Yield per plant

All the ripe fruits harvested were weighed and such weights were added to get the yield per plant which was expressed as kilograms.

3.6. BIOCHEMICAL ANALYSIS

a. Estimation of total leaf chlorophyll

Total leaf chlorophyll was estimated in fresh mature leaves adopting the procedure of Arnon (1949) and expressed as milligram per 100 g (Appendix - I).

b. Starch estimation in leaves

The starch content of leaves was estimated and expressed as grams per 100 g as per the method adopted by Hedge and Hofreiter (1962) (Appendix-II).

c. Determination of total sugars in fruits

Total sugars in fruits was determined by the method described by Hedge and Hofreiter (1962) and expressed as grams per 100 g (Appendix - III).

d. Estimation of reducing sugars in fruits

Reducing sugars in fruits were estimated and expressed as grams per 100 g (Miller, 1972) (Appendix - IV).

e. Non-reducing sugars

The difference between the total sugars and reducing sugars was recorded as non-reducing sugars and expressed as grams per 100 g.

f. Total soluble solids (TSS)

The TSS of fruit pulp was determined using ERMA Hand Refractometer and expressed as degree brix.

g. Titrable acidity in fruits

Titrable acidity in fruits was determined by the method A.O.A.C. (1960) and expressed in percentage using the principle that the acidity in the fruit juice is estimated by titration with a standard alkali with phenolpthalein as indicator (Appendix-V).

h. Ascorbic acid in fruits

The amount of ascorbic acid present in fruits was estimated adopting the method of Harris and Ray (1935) and expressed as grams per 100 g (Appendix-VI).

i. Lycopene estimation in fruits

The lycopene content of the fruits was determined by the method of Ranganna (1976) and expressed as mg per 100 g (Appendix-VII).

j. Total protein in fruits

Total protein content of fruits was determined by the method of Lowry (1951) and expressed as grams per 100 g (Appendix-VIII).

k. Total free aminoacids in leaves and fruits

The total free aminoacids present in leaves and fruits was estimated as per the method of Moore and Stein (1948) and expressed as grams per 100 g (Appendix-IX).

3.7. NUTRIENTS UPTAKE BY PLANTS

3.7.1. Macronutrients

a. Nitrogen

Total nitrogen content of the leaf was estimated by microkjeldahl method (APHA, 1976) and expressed as percentage (Appendix-X).

b. Phosphorus

Total leaf phosphorus was estimated adopting the procedure of APHA (1976) and expressed as percentage (Appendix-XI).

c. Potassium

Potassium content of leaf was estimated using Flame Photometer (Dean, 1960) and expressed as percentage (Appendix-XII).

3.7.2. Micronutrients

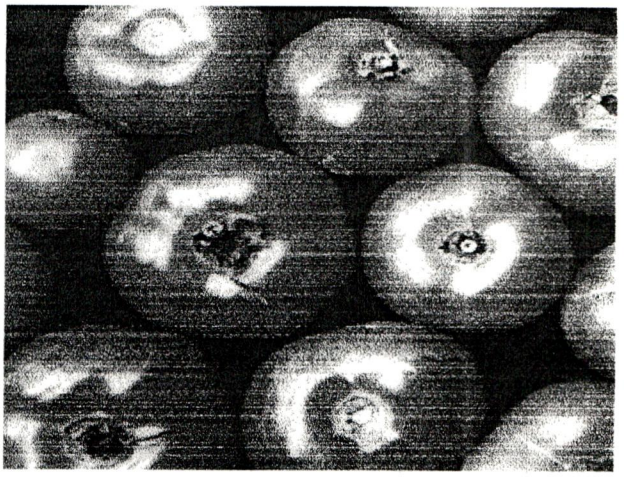
Manganese, zinc and iron

Total manganese, zinc and iron contents were estimated from double acid extract (Toth *et al.*, 1948) (Appendix-XIII) using Atomic Absorption Spectrophotometer and expressed in parts per million (ppm).

3.9. Statistical analysis

The data collected were subjected to statistical analysis as per the methods of Panse and Sukhatme (1967).

RESULTS & DISCUSSION



4.0. RESULTS AND DISCUSSION

Tomato finds a place in many processed products and diet. Particularly in Tamil Nadu, sour (acid) type of tomatoes are preferred for culinary usage, rather than salad types. Among the sour type tomatoes, the variety PKM-1 developed by the Tamil Nadu Agricultural University, is being grown extensively in southern states of India. Several research has been done to enhance yield and quality of sour type of tomatoes in India and elsewhere. Use of human hair derived aminoacids mixture (Sridiamin) is a new product which has the potential as substitute for chemical nitrogenous fertilizers. Investigations to assess the effect of such aminoacid mixture is very meagre. Similarly the application of paclobutrazol (PP333), an antigibberellin individually and in combination with aminoacids and its effect on growth and yield is a new concept of research in tomato. Present study bridge such a research gap. The results of the research on various aspects of growth, yield and quality of tomato cv.PKM-1 are discussed below.

4.1 PLANT BIOMETRIC CHARACTERS

4.1.1. Plant height

4.1.2. Number of laterals per plant

4.1.3. Number of days to first flowering

4.1.4. Number of flower clusters and flowers per clusters per plant

4.1.5. Root-shoot ratio

4.2. FRUIT CHARACTERS

4.2.1. Mean fruit weight

4.2.2. Number of fruits per plant

4.2.3. Fruit yield

4.3. BIOCHEMICAL ANALYSIS

4.3.1. Leaf total chlorophyll and starch

4.3.2. Total, reducing and non-reducing sugars in the fruit

4.3.3. TSS, titrable acidity, ascorbic acid and lycopene in the fruit

4.3.4. Total protein in fruit and total free aminoacids in leaves and fruits

4.4. NUTRIENTS UPTAKE BY PLANTS

4.4.1. Macronutrients - N, P and K

4.4.2. Micronutrients - Zn, Mn and Fe

4.1. PLANT BIOMETRIC CHARACTERS

Plant biometric characters viz., plant height, numbers of laterals as per plant, number of days to first flowering, flower clusters per plant, number of flowers per cluster and root-shoot ratio in tomato cv.PKM-1 were found to be influenced by different concentrations of Sridiamin (human hair-dried aminoacids) and PP333 (the antigibberellin paclobutrazol) when applied individually and in combinations of both (PLATES 1-9).

4.1.1. Plant height

Biometric parameters of the tomato plant were influenced by the exogenous application of Sridiamin and PP333. This is depicted in Table I and Figure Ia. The combination treatments had caused further reduction of height as compared to control. The spraying of one per cent Sridiamin had resulted in a maximal mean height of 83.25 cm which was 18 per cent more than that of the control plants. However, spraying of 0.5 per cent Sridiamin also enhanced the plant height and the effect was on par with that of one per cent Sridiamin. Aminoacids have been reported to enhance growth of many plants by way of enhanced cell division and cell enlargement, thereby causing increase in plant height and girth (Ghosh and Burris, 1950). More recently, Auxcilia (1998) has observed increase in height of papaya cv.CO2 due to application of Sridiamin at a concentration of 0.4 per cent. This lends supports to the observations of the present study.

Soil drenching of PP333 has significantly reduced the plant height. The reduction was 33.7 per cent at 50 mg per plant (T5) and it was 26.0 per cent at 25 mg level (T4). The reduction was profound when PP333 was applied in combination with Sridiamin. Plant height was 44.50 cm in T8 (Aminoacid 0.5% + PP333 50 mg) which was 37.7 per cent

less than the control. All the combination treatments were on par with each other except T6. General reduction in plant height, according to Dalziel and Lawrence, (1984) could be a consequence of inhibition of gibberellin biosynthesis. Major biochemical effect of PP333 is suppression of gibberellin production by inhibiting the oxidation of kaurene to kaurenoic acid in the biosynthetic pathway, ultimately resulting in reduced rates of cell division and cell enlargement. The direct morphological consequence, therefore, is reduction in plant height. Similar retardation of height and rate of shoot elongation was also reported in papaya var.CO2 (Baskaran, 1995), grapes (Reynolds *et al.*, 1992) and blueberry (Ehlenfeldt, 1998). Thus it is evident that human hair derived mixture of 17 aminoacids can exert a positive effect on plant height, while PP333 suppressed the plant height individually and more effectively in combination with aminoacid mixture in PKM-1 tomato.

4.1.2. Number of laterals per plant

The data set out in Table I and Figure Ib revealed, that the differences in the number of laterals per plant differed significantly among the treatments. Foliar spraying of Sridiamin in both the concentrations (0.5 and 1%) did not cause any significant change in the number of laterals, which was the highest in T5 (PP333 50 mg). All the combination treatments however, did not differ among themselves. The increase in the number of laterals due to soil drenching of PP333 at the rate of 50 mg per plant (T5) was 111 per cent efficient as compared to control. However, T5 was on par with T4, T9, T8 and T7. The increase in number of laterals could probably due to the antigibberellin effect of PP333 which might have suppressed the apical dominance ultimately inducing the quiescent lateral buds to sprout (Dalziel and Lawrence, 1984). In tomato cv. Pusa Ruby, however, Baruah *et al.*, (1995) has observed decrease in plant height, leaf area and general canopy size, by spraying PP333 at 150 ppm. On the contrary, the number of laterals have been reduced due to PP333 in tree fruits like, apple (Greene, 1991); peaches (Wang *et al.*, 1993); mango (Khader, 1991 and Kurian and Iyer, 1993) and citrus (Okuda *et al.*, 1994).

4.1.3. Number of days to first flowering

Different treatments of Sridiamin and PP333 significantly influenced the days taken for first flowering (Table I and Figure Ic). Sridiamin in both the concentrations reduced the days for flowering as compared to control from 2.5-4.5 days. However, the effects both the concentrations of Sridiamin were on par with each other. On the other hand, PP333 conspicuously delayed flowering in tomato. PP333 at the lower dose (T4-25mg) took 47.5 days for first flowering while the higher concentration of 50 mg took 53.5 days. Both the concentrations however, induced early flowering as compared to control (71 days). The combination of Sridiamin and PP333 significantly reduced the days for flowering. The combination treatment of T6 (Sridiamin 0.5% + PP333 25mg) took only 46 days as compared to 71.0 days in control. Aminoacids as a source of nitrogen was only less effective than the growth retardant PP333. Auxcilia (1998) has observed delayed flowering in papaya var. CO2, when PP333 was applied as soil drench at 25 mg per plant. The induction of flowering in plants owing to PP333 has been well documented in several crop plants; in apple (Kim *et al.*, 1990 and Hao *et al.*, 1991), peach (Szewazuka, 1994) and mango (Medina-Urrutia, 1994). The induction of flowering was attributed to appropriate Carbon : Nitrogen ratio brought about by PP333 and aminoacids (Zorasingh and Dhillon, 1992).

4.1.4. Number of flower clusters and flowers per clusters per plant

All the treatments differed significantly compared to the control (Table I and Figure Id and c). Foliar spraying of Sridiamin at 1.0 per cent level increased the number of flower clusters (47%) and flowers per cluster (100%) as compared to that of the control. However, the effect of both the concentrations was almost the same and did not differ significantly. The increase in flower clusters and flowers per cluster was observed in both the concentrations of PP333 as compared to control. The combination of Sridiamin and PP333 also increased the flower clusters and flowers per cluster per plant. The combination treatment T6 (Sridiamin 0.5% + PP333 25 mg) showed 22 clusters per plant as compared to

16 in control and in T8 (Sridiamin 0.5 + PP333 50 mg) flowers per cluster was 8.5, as compared to control which was 4.5.

Increased number of flowers was recorded with 0.5 per cent Sridiamin spray in cotton and in chillies (Anon, 1997). In mango, PP333 stimulated flower initiation throughout the tree (Khader, 1991), also in pear (Browning, *et al.*, 1992), papaya (Baskaran, 1995) and citrus (Snowball *et al.*, 1994).

4.1.5. Root-shoot ratio

Root shoot ratio is an important parameter that decides the growth vigour in plant system (Leopald, 1964). In the present study the root-shoot ratio has been found to significantly differ among the treatments (Table I and Figure If). The ratio was the highest (0.13) in T5 (PP333 50mg) followed by T4 (PP333 25 mg) though both were on par. The lowest ratio of 0.031 was in T2 (Sridiamin 0.5%) while the highest concentration of Sridiamin (1.0%) had a ratio of 0.058 which was on par with control as well as many combination treatments. The higher ratio recorded in PP333 treatments was due to considerable reduction in root growth rates. Observations in respect of root-shoot ratio is quite meagre. Yelenosky *et al.*, (1995) have reported reduced root growth in many citrus species due to soil application of PP333 which also caused shorter and thicker roots. Antigibberellin effect of PP333 could have also caused metabolic imbalance resulting in root growth. Sridiamin, on the other hand, has not only enhanced the shoot growth but also proportionate root growth which was evident from the results obtained for control and 1 per cent Sridiamin (T3) which are on par with each other.

Overall appraisal of the data on biometric parameters indicates that PP333 and Sridiamin work on opposite direction in proportion to their concentrations and one per cent Sridiamin (T3) has increased the number of flowering clusters per plant and flowers per cluster.

Table I

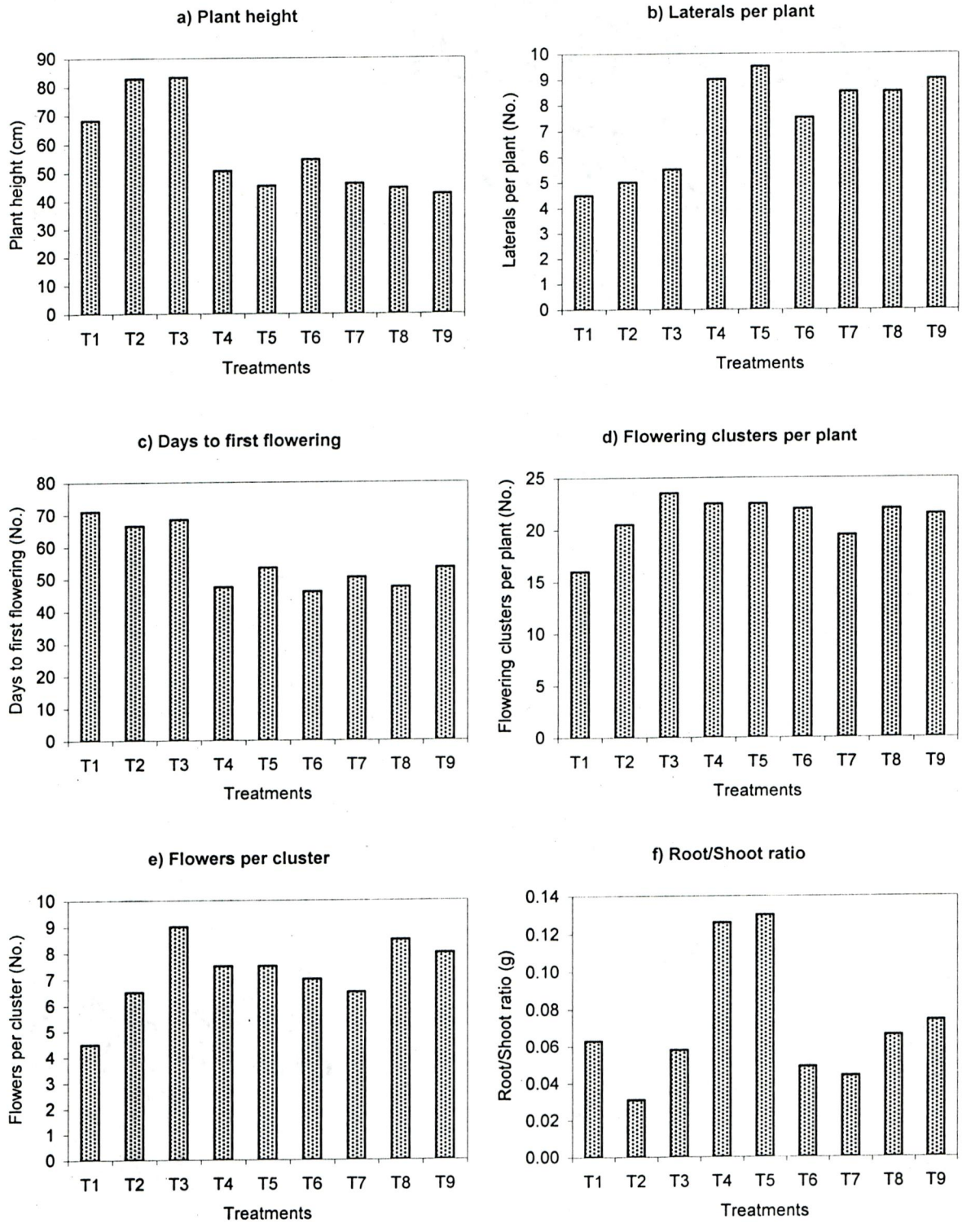
BIOMETRIC PARAMETERS

Treatments	Plant height at flowering (in cms)	Laterals per plant (in number)	Days to first flowering (in number)	Flowering clusters per plant (in number)	Flowers per cluster (in number)	Root-shoot ratio (in grams)
T1	68.25	4.5	71.0	4.5	16.0	0.063
T2	82.75	5.0	66.5	6.5	20.5	0.031
T3	83.25	5.5	68.5	9.0	23.5	0.058
T4	50.50	9.0	47.5	7.5	22.5	0.126
T5	45.25	9.5	53.5	7.5	22.5	0.130
T6	54.50	7.5	46.0	7.0	22.0	0.049
T7	46.00	8.5	50.5	6.5	19.5	0.044
T8	44.50	8.5	47.5	8.5	22.0	0.066
T9	42.50	9.0	53.5	8.0	21.5	0.074
SED	2.1794	0.7169	1.8295	0.5893	1.2748	0.0133
CD (0.05%)	5.03	1.65	4.22	1.36	2.94	0.031

Values mean of four replications

T1 - Control
T2 - Sridiamin 0.5 per cent
T3 - Sridiamin 1.0 per cent
T4 - PP333 25 mg
T5 - PP333 50 mg
T6 - Sridiamin 0.5 per cent + PP333 25 mg
T7 - Sridiamin 1.0 per cent + PP333 25 mg
T8 - Sridiamin 0.5 per cent + PP333 50 mg
T9 - Sridiamin 1.0 per cent + PP333 50 mg

Figure - I
Biometric parameters



4.2. FRUIT CHARACTERS

The changes in fruit characters like mean fruit weight, number of fruits per plant and total yield per plant are the important parameters that decide the practical utility of the present study. Various treatments differed significantly in respect of fruit characters and yield (PLATES 10 - 12).

4.2.1. Mean fruit weight

The mean fruit weight (Table II and Figure IIa) ranged between 67.7 g in T3 (Sridiamin 1.0%) to 15.7 g in T5 (PP333-50 mg) both of which differed significantly from the control (30.3 g). Sridiamin improved the fruit weight in proportion to their concentration. Treatments with Sridiamin 0.5 per cent (T2) and 1.0 per cent (T3) differed significantly. On the other hand, PP333 has reduced the mean fruit weight in proportion to their concentrations as is evident from the lowest fruit weight of 15.7 g in PP333 at 50 mg and 21.5 g at 25 mg of PP333. The increase in fruit weight due to 1.0 per cent Sridiamin (T3) was 124 per cent while the reduction due to PP333 at 50 mg was 49 per cent over the control. Among the four combination treatments the mean fruit weight was greater than the control. However, was far less than the fruit weight observed in T2 (0.5% Sridiamin) and T3 (1.0% Sridiamin). Auxcilia (1998), however, has reported that human hair-derived aminoacid mixture (Sridiamin) at 0.4 per cent increased the fruit weight of papaya. There are many reports on nitrogenous fertilizers enhancing the fruit weight to the tune of 49 per cent in peach (George *et al.*, 1995 and Manago *et al.*, 1994). Similar effects have been recorded in plum (Chandel and Jindal, 1991), apricot (Kuden *et al.*, 1995) and in grapes (Basiouny, 1994). The present finding that the tomato fruit weight can be manipulated using human hair-derived aminoacid mixture therefore, assumes importance. On the contrary PP333 reduced not only the plant height but also the fruit weight. Fruit weight was reduced in papaya var. CO2 at 500 ppm of PP333 (Baskaran, 1995). Hao *et al.*, (1991a) and Rizzolo *et al.*, (1993) reported that PP333 333 did not have any effect on fruit weight. In the present investigation also PP333 at 25 mg and 50 mg has been found to reduce fruit weight enormously.

4.2.2. Number of fruits per plant

Significant differences between the treatments was observed with regard to the number of fruits per plant (Table II and Figure IIb).

Among the treatments, number of fruits ranged from 59 (T7) to 48 (T9). The treatment with one per cent Sridiamin + PP333 25 mg (T7) significantly increased the number of fruits which was on par with T3, T4 and T6.

The combination treatment T8 (Sridiamin 0.5 per cent + PP333 50 mg) significantly increased the number of fruits per plant (50.0) as compared to control (34.0). The treatments T2 and T9 were on par with T8.

Application of PP333 either alone or in combination with Sridiamin significantly increased the number of fruits per plant corroborating the results obtained in many fruit crops. Increased yields were observed due to PP333 in apple (Kim *et al.*, 1990; Huang *et al.*, 1995), pear (Salem *et al.*, 1991), peach (Monge *et al.*, 1994 and George *et al.*, 1995) and cherry (Lauri, 1993)

4.2.3. Fruit yield

Significant differences were obtained for fruit yield in terms of total weight among treatments and interaction effects.

The Sridiamin spray at one per cent level (T3) significantly increased the weight of fruits per plant (3.9 kg) as compared to 1.0 kg in control. Increased dosage of PP333 from 25 mg (T4) to 50 mg (T5) significantly reduced the total weight of fruits. It was 1.2 kg in T4 and 0.8 kg in T5. Lower dose of 0.5 per cent Sridiamin (T2), PP333 (25 mg) combined with 0.5 per cent Sridiamin (T6) and PP333 50 mg with one per cent Sridiamin (T7) however, increased the yield of fruits per plants. It was 2.7 kg, 2.3 kg and 2.2 kg respectively (Table II and Figure IIc).

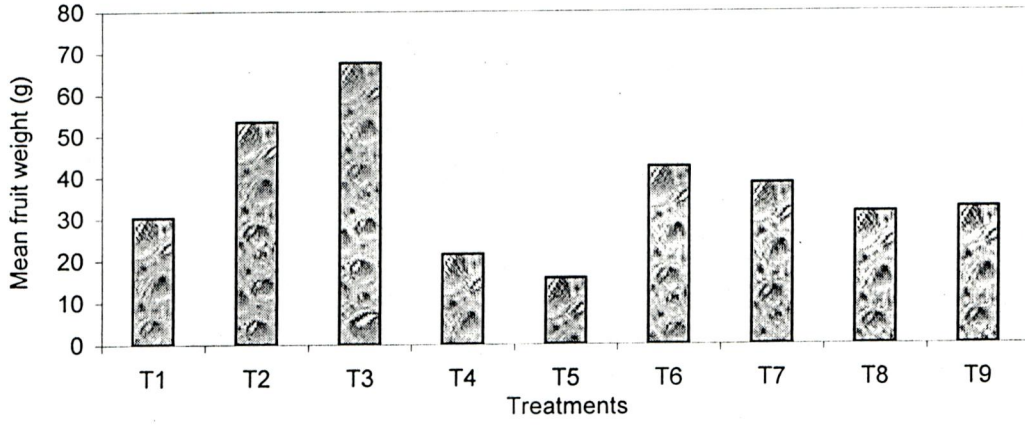
Table II
FRUIT CHARACTERISTICS

Treatments	Mean fruit weight (g)	Number of fruits per plant	Yield per plant (kg)
T1	30.3	34.0	1.0
T2	53.4	50.0	2.7
T3	67.7	58.0	3.9
T4	21.5	56.0	1.2
T5	15.7	48.0	0.8
T6	42.5	55.0	2.3
T7	38.5	59.0	2.2
T8	31.5	50.0	1.6
T9	32.6	48.0	1.5
SED	3.052	1.696	0.194
CD (0.05%)	7.04	3.91	0.45

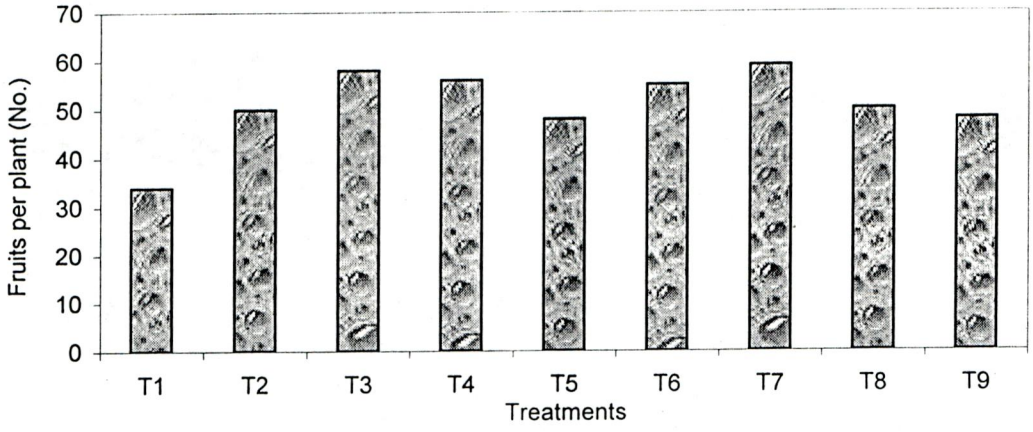
Values mean of four replications

Figure - II
Fruit characteristics

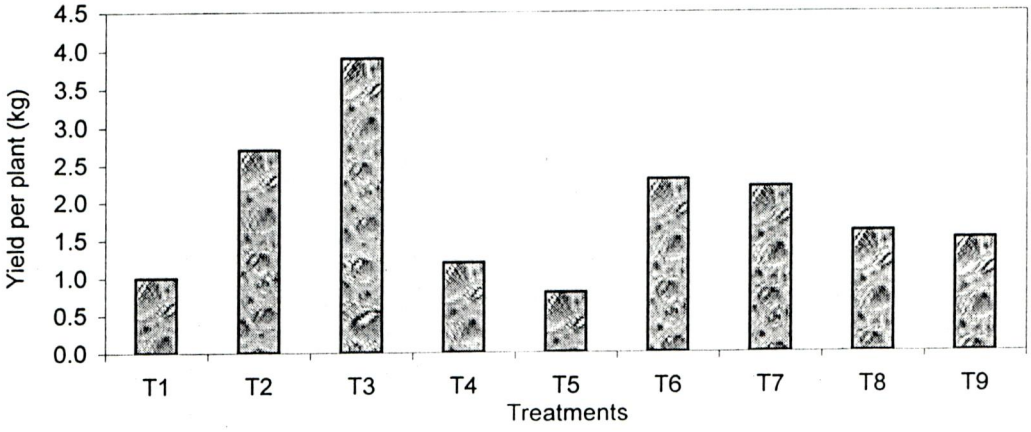
a) Mean fruit weight



b) Number of fruits per plant



c) Yield per plant



Amino acids at both the concentrations increased the fruit yield significantly in terms of number and weight, the response being in tune with the increase in concentrations.

Asao *et al.* (1996) reported that PP333 100 ppm spray increased the yield of hydroponically grown tomato. In tomato cv. Pusa ruby, Baruah *et al.* (1995) reported that the fruit set and yield increased with the highest dose of N (150 kg/ha) coupled with 150 ppm spray of PP333.

This study, however, with PP333 and Sridiamin on growth, yield and quality of tomato proved that application of Sridiamin alone increased the yield of tomato. In combination with PP333, although the number of fruits set increased, the yield did not increase.

With regard to the fruit characteristics, T3 (1% Sridiamin) proved to be the best. This was followed by T2 (0.5% Sridiamin).

4.3. BIOCHEMICAL ANALYSIS

The edible quality of tomato fruit is judged by the biochemical constituents like TSS, titrable acidity, sugars, proteins, ascorbic acid etc. The leaf nutrient concentration of major elements like N, P, K and micro nutrients like Zn, Mn and Fe have a direct bearing on yield and quality of tomato. Leaf analysis is often being used as a guide for application of fertilizers to the crop plants. In the present study leaf N, P, K, Zn, Mn and Fe contents were determined at the harvest stage and the biochemical constituents of the fruit was analysed at the fully-ripened stage (all-red fruit) and the results are discussed below.

4.3.1. Leaf total chlorophyll and starch

a) Total chlorophyll

Total leaf chlorophyll content varied significantly among the treatments. Chlorophyll serves as an useful index of the leaf N concentrations (Takebe *et al.*, 1990). The highest chlorophyll content was observed in T5 (PP333 50 mg) followed by T4 (PP333 25 mg) (Table III and Figure IIIa).

Among the combination treatment T7 (Sridiamin 1% + PP333 25 mg) has the highest value of 2.05 mg per 100 g followed by T8. The lowest chlorophyll content of 1.44 mg per 100 g was recorded in control. Sridiamin has increased the chlorophyll content by 13 per cent and 18 per cent over control at concentrations 0.5 and 1.0 per cent respectively. The chlorophyll content increased by 86 per cent over control due to application of PP333 50 mg.

Treatments with PP333 individually and in combination with Sridiamin exhibited a better positive response to leaf total chlorophyll content than did the treatments with Sridiamin alone. It is possible that the retardants enhanced chlorophyll content by inhibiting chlorophyllase enzyme as reported by Paricha *et al.*, (1977). The results are in agreement with the reports of Hao *et al.*, (1991b) and Xiachunsen *et al.*, (1994) in apple, Yelenosky *et al.*, (1994) in orange and El-Otmani *et al.*, (1992) in banana. Contradictory results were also obtained in orange (Mauk *et al.*, 1987) and apple (Weiland and Wample, 1984) under cold hardening conditions. On the other hand, Vu and Yelenosky (1992) in sweet orange and Monge *et al.*, (1994) in peach did not find any alterations in leaf chlorophyll content due to PP333 application. This study reveals that by altering the ratio of Sridiamin and PP333 in the combination, a still greater chlorophyll content of the leaves could be obtained.

b) Starch estimation in leaves

The starch content of the leaf varied from 3.40 g to 0.63 g per 100 g, the lowest being obtained with 0.5 per cent Sridiamin (T2), which is on par with T3 (1% Sridiamin) and T8 (0.5% + PP333 50 mg).

The treatment T5 (PP333 50 mg) recorded the highest content of 3.4g per 100 g of leaves followed by T4 (PP333 25 mg) registering 2.8 g per 100 g.

Table III

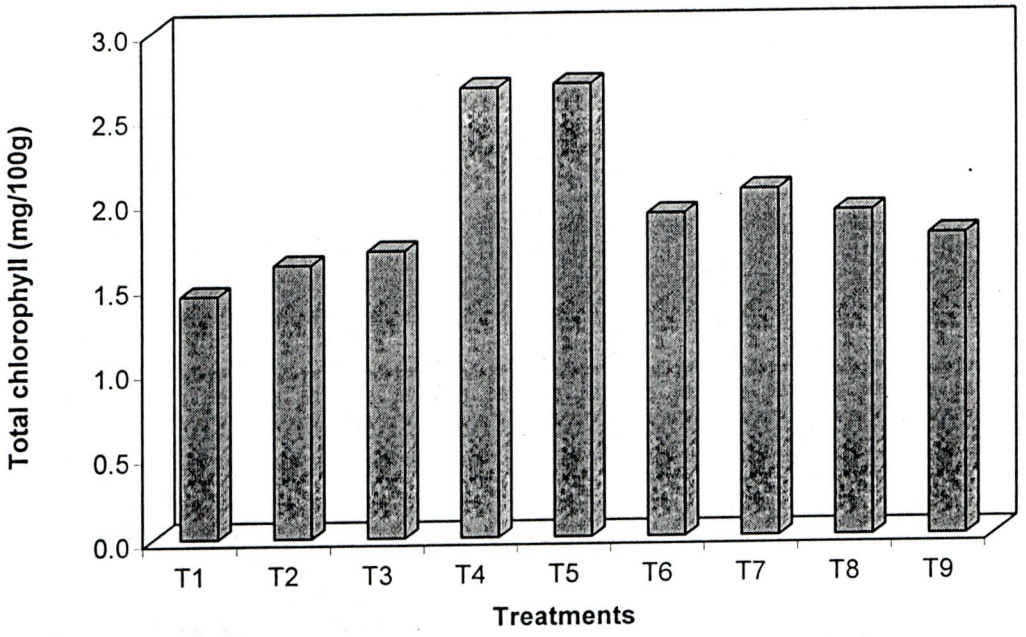
TOTAL CHLOROPHYLL AND STARCH CONTENT IN LEAVES

Treatments	Total chlorophyll (mg/100 g)	Starch content (g/100g)
T1	1.44	0.80
T2	1.62	0.63
T3	1.70	0.75
T4	2.66	2.80
T5	2.68	3.40
T6	1.91	0.85
T7	2.05	0.80
T8	1.92	0.70
T9	1.78	0.93
SED	0.069	0.062
CD (0.05%)	0.160	0.142

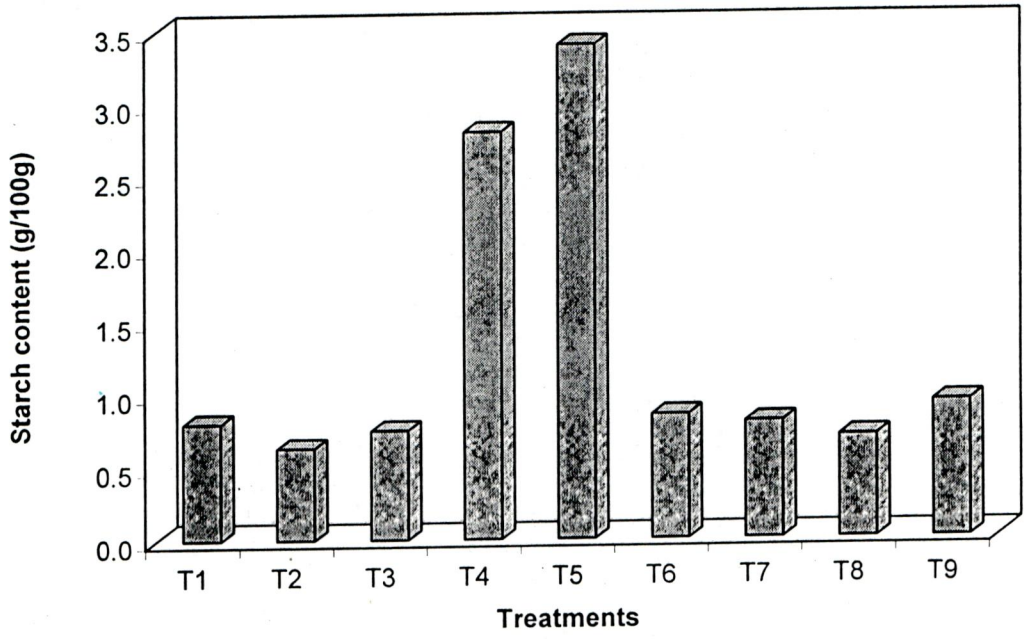
Values mean of four replications

Figure - III
Total chlorophyll and starch content in leaves

a) Total chlorophyll



b) Starch content



Among the combination treatments, T9 (1% Sridiamin + PP333 50 mg) recorded the highest starch content, which was on par with T6, T7 and T1 (Table III and Figure IIIb).

The results are in agreement with the findings of Setia *et al.*, (1995), who reported that the seeds from paclobutrazol treated plants had, higher levels of starch content.

4.3.2. Total Reducing and Non-reducing sugars in the fruit

Among the treatments, total and reducing sugars were the highest in T4 (PP333 25 mg) and T5 (PP333 50 mg), whereas the non-reducing sugars were the highest (1.73%) in T6 (Sridiamin 0.5% + PP333 25 mg). The lowest total and reducing sugars were recorded in T1 (control), and non-reducing sugars in T5 (PP333 50 mg). The application of Sridiamin in both the concentrations were on par with PP333 25 and 50 mg, although application of PP333 (both 25 and 50 mg) caused a higher amount of total, reducing and also non-reducing sugar contents of the fruit (Table IV and Figure IV). PP333 increased the total sugars in mango (Khader, 1990), citrus (Shrestha, 1988) and pear (Salem *et al.*, 1991).

4.3.3. TSS, TITRABLE ACIDITY, ASCORBIC ACID AND LYCOPENE IN THE FRUIT

a) Total soluble solids (TSS)

The various treatments do not seem to have any significant effect on the TSS of the fruit (Table V).

b) Titrable acidity

The titrable acidity of the fruit was significantly higher in treatments compared to control. Application of Sridiamin one percent (T3) and 0.5 per cent (T2) showed a significant increase in titrable acidity over the control. It was however less than that caused by T5 (PP333 50 mg) and T4 (PP333 25 mg). The combination treatments, however, showed the highest per cent (62%) of increase in titrable acidity in T8 (Sridiamin 0.5% + PP333 50 mg) which was on par with T7, T9, T5 and T6 (Table V).

Table IV

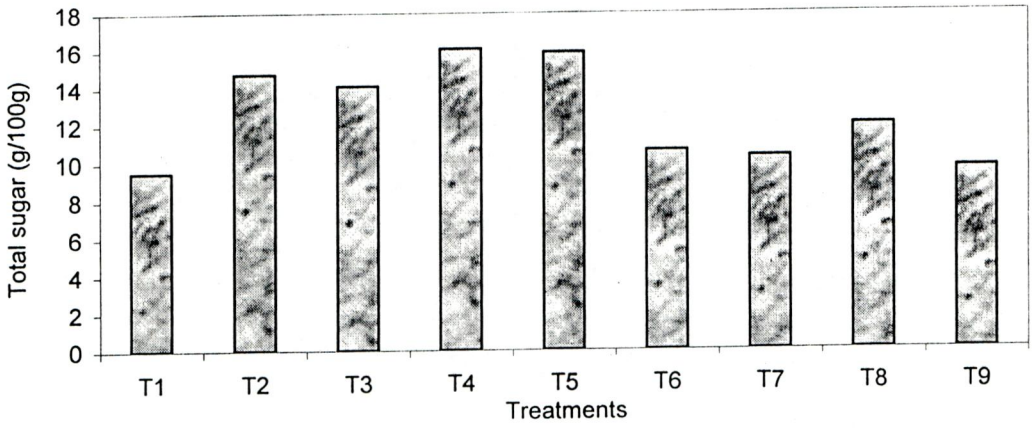
TOTAL, REDUCING AND NON-REDUCING SUGAR CONTENTS OF THE FRUIT

Treatments	Total sugar (g/100g)	Reducing sugar (g/100)	Non-reducing sugar (g/100g)
T1	9.50	8.15	1.35
T2	14.75	13.50	1.25
T3	14.10	12.40	1.70
T4	16.10	15.00	1.10
T5	15.90	15.55	0.35
T6	10.63	8.90	1.73
T7	10.32	9.00	1.32
T8	12.00	11.05	0.95
T9	9.69	8.55	1.14
SED	1.4249	1.3710	0.1942
CD (0.05%)	3.286	3.162	0.448

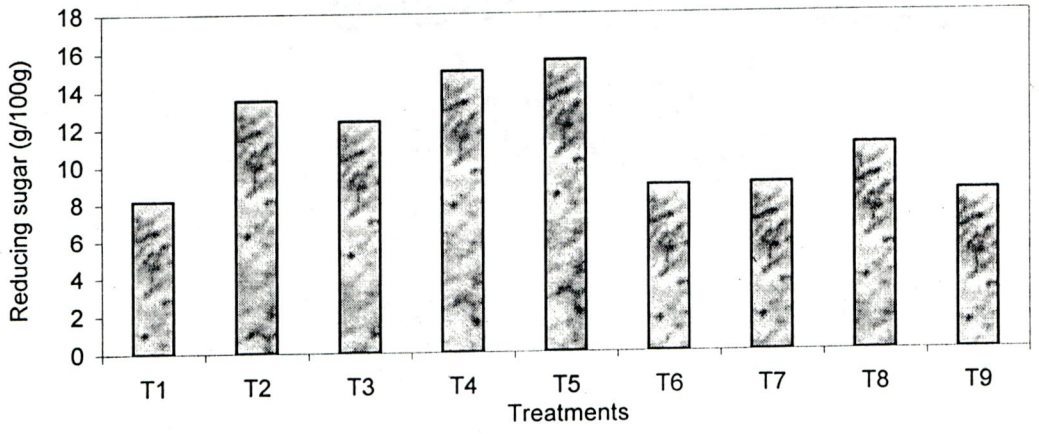
Values mean of four replications

Figure - IV
Total, Reducing and Non-reducing sugar contents of the fruit

a) Total sugar



b) Reducing sugar



c) Non-reducing sugar

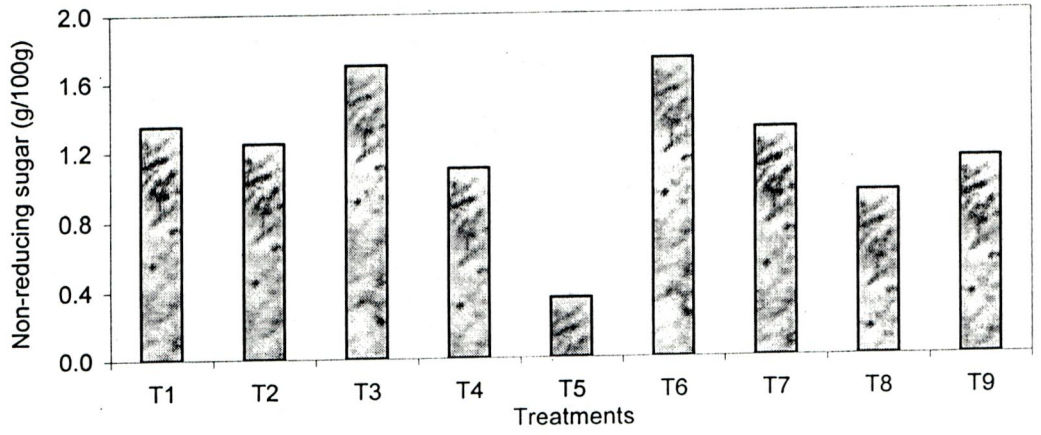


Table V

TSS, TITRABLE ACIDITY, ASCORBIC ACID AND LYCOPENE CONTENT IN FRUITS

Treatments	TSS (Degree Brix)	Titrable acidity (g/100g)	Ascorbic acid (g/100 g)	Lycopene (mg/100g)
T1	4.30	0.55	12.50	1.36
T2	5.00	0.62	15.15	1.49
T3	5.15	0.75	16.25	1.60
T4	6.20	0.78	16.90	2.83
T5	6.60	0.81	13.75	2.89
T6	4.60	0.80	15.00	2.44
T7	5.25	0.84	16.25	1.74
T8	4.70	0.88	20.00	2.76
T9	5.55	0.83	18.75	2.48
SED	0.358	0.036	1.627	0.027
CD (0.05%)	0.827	0.082	3.751	0.062

Values mean of four replications

Titrable acidity content decreased in plum (Chandel and Jindal, 1991), grapes (Zoecklein *et al.*, 1991) and tomato (Baruah *et al.*, 1993) due to PP333.

On the contrary, in apricot, Subhadrabandhu *et al.*, (1990) reported that PP333 treatment had no effect on fruit quality characters like titrable acid and TSS. Such contradictory effects were also observed in apple (Rizzolo *et al.*, 1993), peach (Wang *et al.*, 1993), plum (Harangozo *et al.*, 1996) and loquat (Pilone and Scaglione, 1996).

c) Ascorbic acid

The individual and combination treatments of Sridiamin and PP333 did not cause any significant effect on ascorbic acid content of the fruit (Table V).

d) Lycopene

Lycopene is responsible for the red colour of tomato. It has no nutritional value but its contribution to the colour of tomato has a great role in consumer acceptability (Sadasivam and Manickam, 1992). Lycopene is also considered to be an antioxidant.

Significant difference in the lycopene contents of the fruits was observed with the T5 (PP333 50 mg) and T4 (PP333 25 mg) registering maximum amount ie., 2.89 and 2.83 mg per 100g of the fruit respectively. Application of Sridiamin alone did not cause as much lycopene formation as the rest of the treatments (Table V).

4.3.4. Total protein in fruits and total free aninoacids in leaves and fruits

a) Total protein in the fruit

The Table VI and Figure Va depicts that the protein content of fruits due to various treatments differed significantly from that of the control. The treatment T3 (Sridiamin 1%) recorded the highest protein content (34.4 g) which was on par with T2 (Sridiamin 0.5%) and T6 (Sridiamin 0.5 + PP333 25mg). PP333 at 50 mg increased the protein content by 11 per cent compared to the control.

Table VI

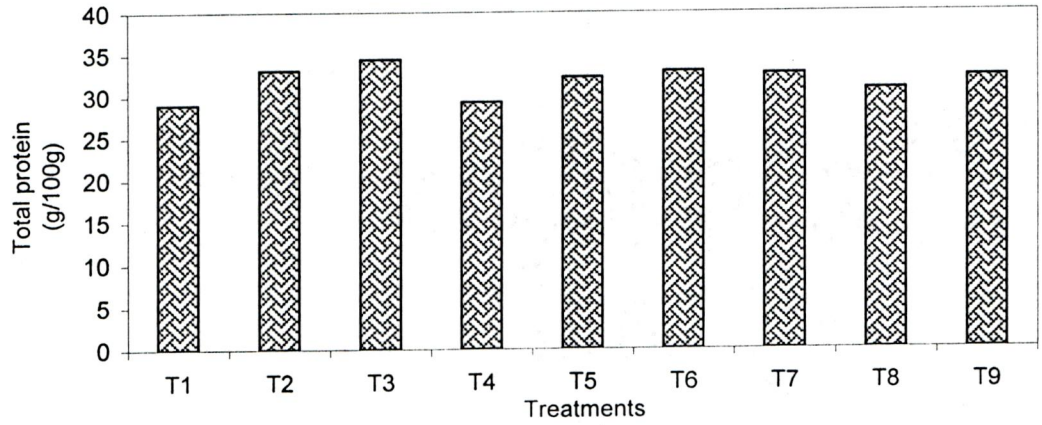
TOTAL PROTEIN AND FREE AMINOACIDS

Treatments	Total protein in fruits (g/100g)	Total free amino acids (g/100g)	
		Fruits	Leaves
T1	29.00	11.55	5.5
T2	33.10	14.00	9.1
T3	34.40	16.30	8.8
T4	29.3	9.30	9.6
T5	32.2	8.25	11.7
T6	32.9	3.25	8.7
T7	32.6	16.20	12.0
T8	30.7	16.65	13.9
T9	32.2	4.45	14.0
SED	0.743	0.266	0.902
CD (0.05%)	0.714	0.613	2.081

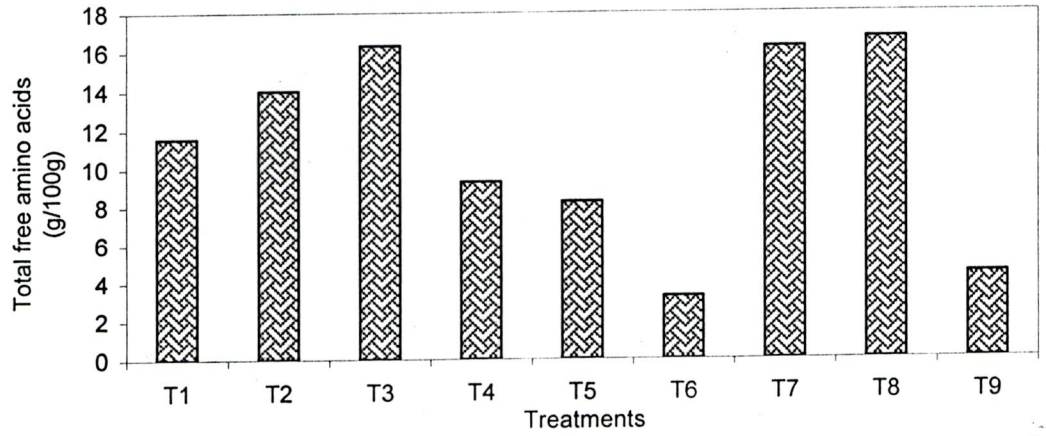
Values mean of four replications

Figure - V
Total protein and free amino acids

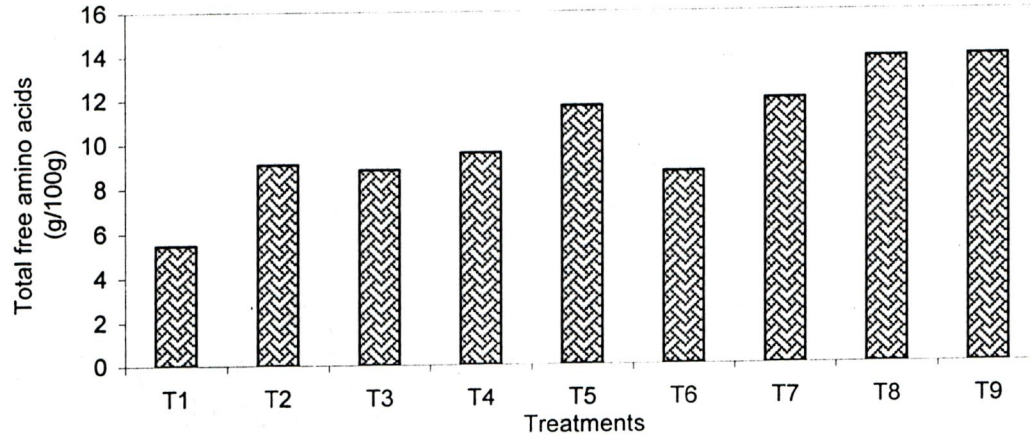
a) Total protein - Leaves



b) Total free amino acids - Fruits



c) Total free amino acids - Leaves



A similar result was observed in *Brassica juncea* plants (Setia *et al.*, 1996) and *Brassica carianta* (Setia, *et al.*, 1995) which added support to the present study.

b) Total free aminoacids in leaves and fruits

Total free aminoacids content of the leaves and fruits varied significantly among the treatments (Tables VI and Figure Vb and C).

The highest total free aminoacids content in leaf (13.9g) was obtained with T9 (Sridiamin 1% + PP333 50mg) which was on par with T8 and T7. The effect of application of Sridiamin and PP333 in both the concentrations individually differed significantly from that of the control.

The total free aminoacids in fruit was highest in T8 (Sridiamin 0.5% + PP333 50 mg) which was on par with T3 (Sridiamin 1%) and T7 (Sridiamin 1% + PP333 25 mg). The treatment T2 (Sridiamin 0.5%) resulted in 21 per cent more free aminoa acids when compared with the control.

PP333 enhanced the levels of total free aminoacids in mature seeds of *Brassica juncea* cv. Czern and Coss (Setia *et al.*, 1996).

Combinations of Sridiamin and PP333 have enhanced the free aminoacids levels in both the leaf and fruit while Sridiamin alone or PP333 alone did not enhance that much.

4.4. NUTRIENTS UPTAKE BY PLANTS

Leaf nutrients viz., N, P, K, Mn, Zn and Fe were determined in the leaves at of harvest stage.

4.4.1. Macronutrients - N, P, K

Application of Sridiamin at one per cent level (T3) recorded 88 percent increase in nitrogen content over the control followed by 77 per cent increase in T2 (0.5% Sridiamin). The highest level of nitrogen, however, was observed in T9 (1% Sridiamin + PP333 50mg)

Table VII

MACRONUTRIENTS N, P AND K

Treatments	Nitrogen (%)	Phosphorus (%)	Potassium (%)
T1	2.84	0.455	1.98
T2	5.03	0.511	2.001
T3	5.18	0.637	4.13
T4	6.93	0.644	4.23
T5	7.01	0.678	4.26
T6	6.54	0.719	4.43
T7	6.76	0.561	4.57
T8	7.11	0.629	5.12
T9	7.64	0.701	4.88
SED	0.8250	0.0858	0.1708
CD (0.05%)	1.903	0.198	0.394

Values mean of four replications

169 per cent higher than the control. But the treatments T8, T5, T4, T7 and T6, were on par with T9 implying that at this stage no significant difference in the nitrogen content of leaf was observed between various treatments (Table VII).

A similar observation was made with regard to phosphorus also (Table VII).

The increase in potassium content of the leaf varied from 5.12 per cent to 2.01 per cent among the treatments over the control. The highest being 5.12 per cent recorded in T8 (Sridiamin 0.5% + PP333 50mg) followed by T9 (Sridiamin 1.0% + PP333 50mg) which was on par with T7, T6, T5, T4 and T3. The treatment T2 (0.5% Sridiamin) however was on par with control (Table VII).

Paclobutrazol influenced leaf N, P and K content in the present study. This is in concurrence with the reports in mango (Werner, 1993). Spraying Sridiamin at all the concentrations, though increased the leaf N content, it was less than the N content obtained with the application of PP333. This lends support to the earlier findings with 0.5 to 0.75 per cent Sridiamin spray in many crops (Anon, 1995).

The leaf N, P and K contents increased with the application of increasing concentrations of aminoacids. This indicates that the aminoacids could be used as an organic N source.

4.4.2. Micronutrients - Zn, Mn and Fe

The highest level of zinc was observed to be 1.694 ppm in T9 (Sridiamin 1% + PP333 50 mg). However, the treatments did not differ significantly from that of the control (Table VIII).

The highest level of manganese was observed in T9 (Sridiamin 1% + PP333 50 mg), followed by T4 (PP333 25 mg) which was on par with T5 (PP333 50 mg). The other treatments however, did not differ significantly from that of the control (Table VIII).

Table VIII

MICRONUTRIENTS – Zn, Mn AND Fe

Treatments	Zinc (ppm)	Manganese (ppm)	Iron (ppm)
T1	1.164	47.3	1.300
T2	1.650	59.3	1.416
T3	1.216	109.2	1.626
T4	1.458	112.7	2.538
T5	1.446	74.3	2.010
T6	1.406	119.5	1.686
T7	1.355	62.5	1.750
T8	1.277	80.8	1.712
T9	1.694	100.5	2.782
SED	0.3050	4.2800	0.3029
CD (0.05%)	0.703	9.870	0.699

Values mean of four replications

The significant difference was noticed among treatment in the level of iron. The highest being recorded in T6 (0.5% Sridiamin + PP333 25 mg) which was on par with T4, T3 and T9. The lowest level of iron was observed in the treatment T2 (0.5% Sridiamin), which was 25 per cent higher when compared to that of the control (Table VIII).

A general appraisal of the data on leaf micronutrients showed that the application of 50 mg of PP333 increased the leaf Mn and Fe content and had no significant effect on zinc.

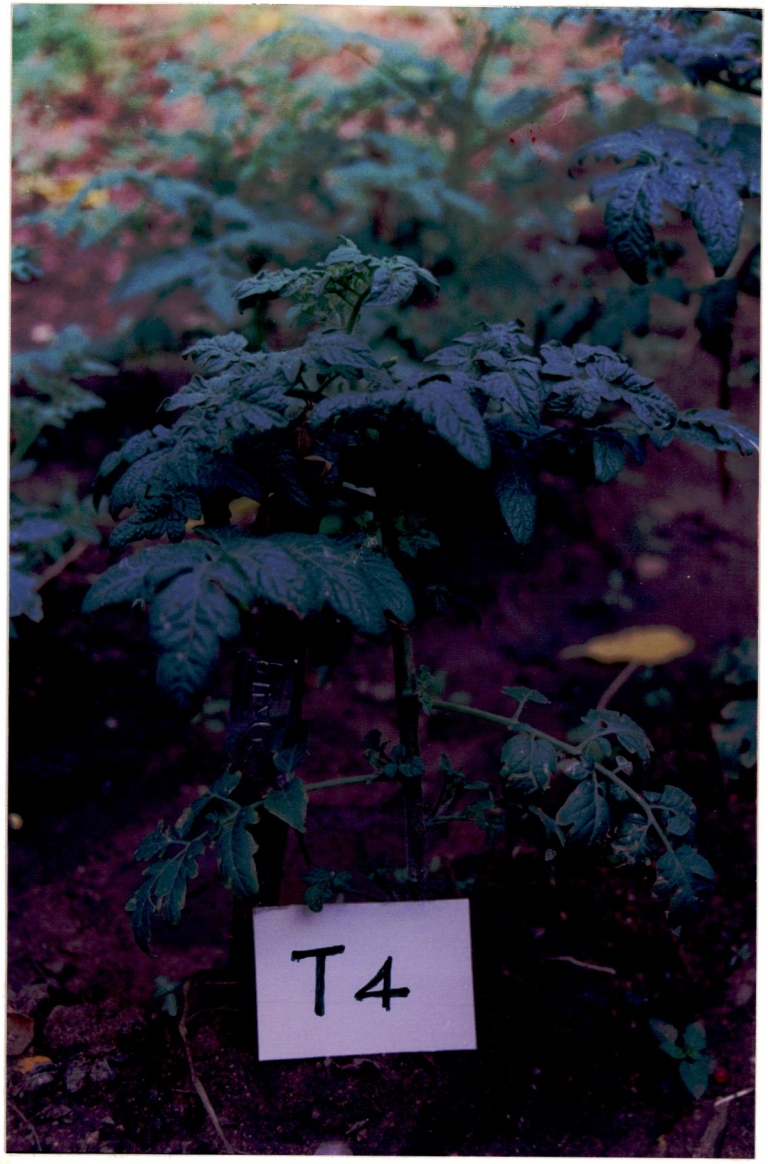
PP333 increased leaf Mn and Fe and decreased the zinc content in peach (Monge *et al.*, 1993 and 1994).

T1 - Control

PLATE 1 & 2

T2 - Sridiamin 0.5 per cent

T3 - Sridiamin 1.0 per cent



T4 - PP333 25 mg

PLATE 3 & 4

T5 - PP333 50 mg

PLATE 5 & 6

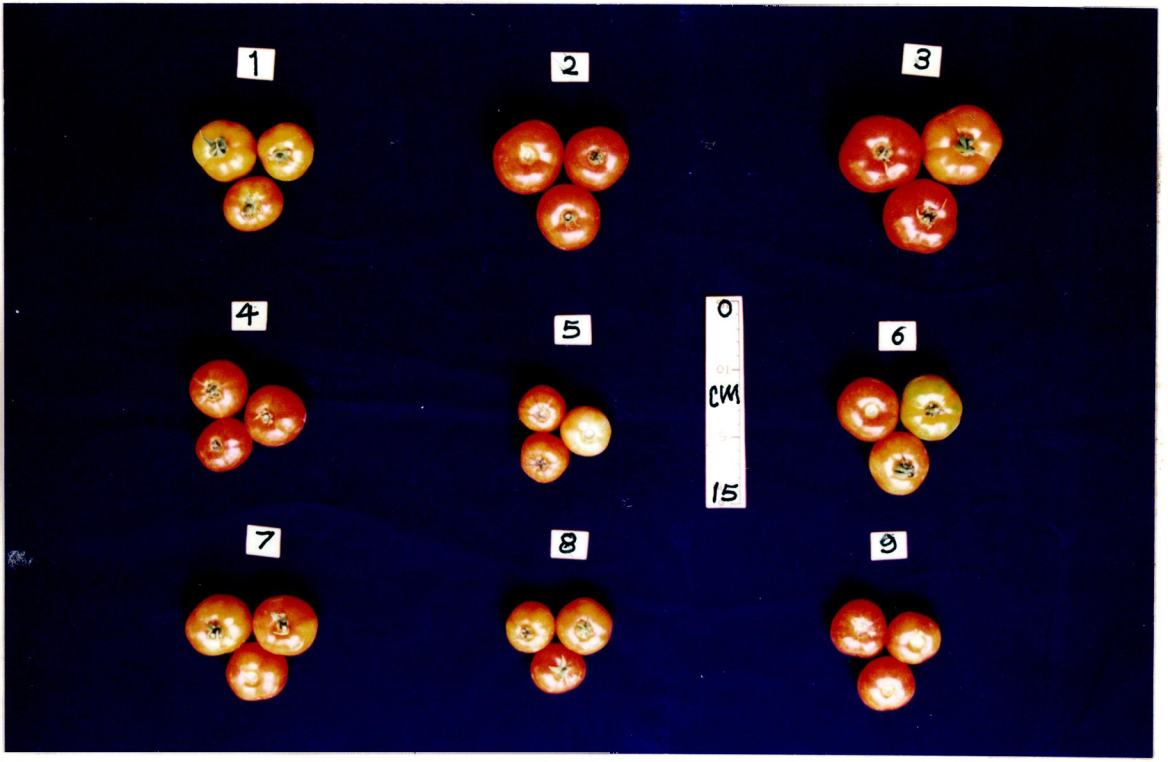
T6 - Sridiamin 0.5 per cent + PP333 25 mg

T7 - Sridiamin 1.0 per cent + PP333 25 mg

PLATE 7 & 8

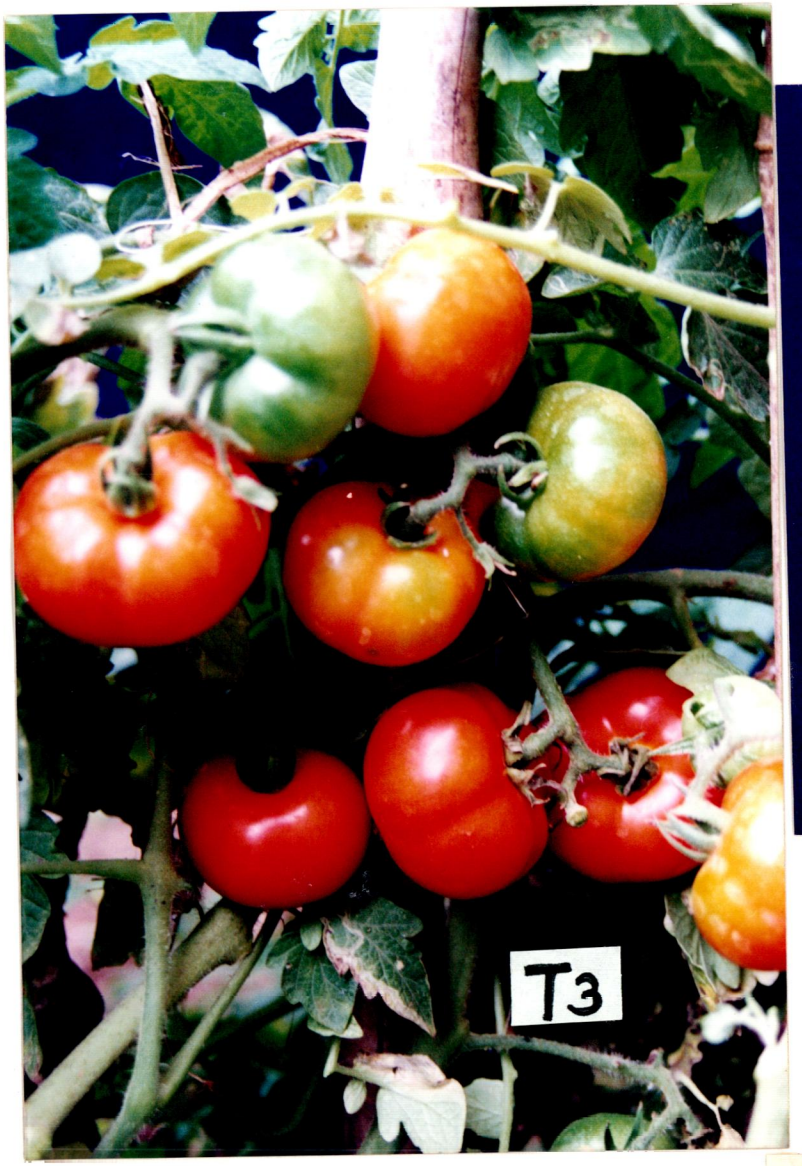
T8 - Sridiamin 0.5 per cent + PP333 50 mg

T9 - Sridiamin 1.0 per cent + PP333 50 mg



FRUIT SIZE IN DIFFERENT TREATMENTS

PLATE 9 & 10



FRUIT IN BEST TREATMENT

T3 - Sridiamin 1.0 per cent

T1 - Control

PLATE 11 & 12

SUMMARY & CONCLUSION

5.0. SUMMARY AND CONCLUSIONS

The results obtained in the study on the '**Effect of paclobutrazol (PP333) and human hair-derived mixture of aminoacids (Sridiamin) on growth, yield and quality of tomato cv. PKM-1**', carried out under field conditions at the Avinashilingam University, Coimbatore, are as follows:

1. Plant biometric characters viz., plant height, days to first flowering, flowering clusters and flowers per cluster per plant significantly increased with the application of Sridiamin alone when compared to control, whereas the number of laterals and root-shoot ratio were found to be higher in T4 (PP333-25 mg) and T5 (PP333-50 mg) treatments.

2. The fruit characteristics i.e. the mean fruit weight, number fruits and yield per plant were significantly enhanced by Sridiamin (both 0.5 and 1% concentrations).

3. The quality in terms of chlorophyll, starch, total and reducing sugars, TSS, titrable acidity, ascorbic acid and lycopene content was found to be significantly higher in T5 and T4 (PP333 50 mg and 25 mg), treatments when compared to other treatments and the control.

4. The highest total protein content of leaf and total free aminoacids in fruit, were recorded in T3 (1% Sridiamin), whereas the total free aminoacids in leaf was found to be higher in combination treatments T8 and T9.

5. The leaf macronutrients viz., N, P and K contents increased with the application of increasing concentrations of Sridiamin. This indicates that aminoacids could be used as an alternate organic N source.

6. The application of PP333 50 mg (T5) increased the leaf Mn and Fe content and had no significant effect on Zn.

Tomato, besides being an universal fruit vegetable known for its nutritive value, has industrial utility for the manufacture of many value – added products.

The present study has clearly revealed that spraying Sridiamin (mixture of 17 aminoacids derived from human hair) at one per cent concentration three times, commencing from 15th day of transplanting with 15 days interval has improved the plant biometric characters and enhanced the yield.

Soil drenching of PP333 at 25 mg per plant applied one month after transplanting individually as well as in combination with aminoacids improved the fruit quality in terms of TSS, titrable acidity and lycopene.

Recommendation for future study

A suitable combination of Sridiamin and paclobutrazol (PP333) can be worked out further by increasing Sridiamin concentration and decreasing PP333 to get a better yield and quality of tomato cv. PKM-1.

BIBLIOGRAPHY

BIBLIOGRAPHY

Abod, S.A. and Webster, A.D. 1991. The influence of foliar sprays of tetcyclasis or paclobutrazol on the growth and water use as transplanted *Malus, Tilia and Betula* stocks. **J. Hort. Sci., 66**(1) : 85-94.

Anon, 1995. 'SRIDIAMIN' product profile.

Anon, 1997. 'SRIDIAMIN' product profile.

APHA, 1976. American Public Health Associaiton, American water Works Association and Water Pollution Control Federation. Standard methods for the examination of water and waste water. Fourteenth Edition, American Public Health Association Inc., New York, 2.35, 2.52, 2.71-2.73.

Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts, polyperoxidase in *Beta-vulgaris*. **Plant Physiology, 24**(1).

Asao, T., Ito, N., Hosoki, T., Ohta, K. and Endo, K. 1996. Effects of plant growth retardants and root pruning on growth and yield of tomato cultured hydroponically at high temperature during summer. **J. Jap. Soc. Hort. Sci., 65**(1) : 89-94.

Auxcilia, J. 1998. Effect of paclobutrazol (PP333) and aminoacids at different levels of nitrogen on growth, yield and quality of papy (*Carica papaya* L.) var. CO2. Ph.D. (Hort.) Thesis submitted to the Tamil Nadu Agrl. Univ., Coimbatore -3.

A.O.A.C. 1960. **Official methods of A.O.A.C.** Washington, D.C.

Baruah, G.K.S., Arora, S.K. and Pandita, M.L. 1993. Effect of paclobutrazol (PP333) in combination with different nitrogen levels on fruit quality of tomato cv. Pusa Ruby. **Haryana Agric. Univ. J. Res., 23** : 100-109.

Baruah, G.K.S., Arora, S.K. and Pandita, M.L. 1995. Effect of paclobutrazol (PP333) and nitrogen levels on growth, flowering and yield of tomato (*Lycopersicon esculentum* Mill.). **Ann. Agric. Res., 16**(4) : 490-492.

Basiouny, F.M. 1994. Effects of paclobutrazol, gibberellic acid and ethephon on yield and quality of muscadine grape. **Phyton** (Buenos Aires), 56:1-6.

Baskaran, A. 1995. Effect of growth retardants on growth, flowering, yield and quality of papaya (*Carica papaya* Linn.) var. CO2. M.Sc.(Hort.). Thesis submitted to the TamilNadu Agrl. Univ., Coimbatore - 3.

Bates, L.S., Waldeer, R.P. and Teare, I.D. 1973. Rapid determination of free proline in water stress studies. **Plant and Soil, 39** : 205.

Beaumont, A.B., Larsinos, G.J., Piekenbrock. P. and Nelson, P.R. 1931. **J. Agr. Res., 43** : 559.

Bist, L.D. 1994. Long term effect of paclobutrazol and promalin on growth, yield and fruit quality of pear (*Pyrus pyrifolia* (Burm) Nakai) cv. Gola. **Prog. Hort., 26**(3-4): 125-132.

Browning, G., Ayzinkuden and Blake, P. 1992. Site of (2RS, 3RS) paclobutrazol promotion of axillary flower initiation in pear cv. Doyenne du comice. **J. Hort. Sci., 67**(1) : 121-128.

Burondkar, M.M. and Gunjate, R.T. 1993. Control of vegetative growth induction of regulator and early cropping in 'Alphonso' mango with paclobutrazol. **Acta Hort., No.341** : 206-215.

Chandel, J.S. and Jindal, K.K. 1991. Effect of triacontanol (TRIA) and paclobutrazol (PP333) on fruit set, yield and quality of Japanese plum (*Prunus salicina* Lind). **Hort. J., 4**(1): 21-25.

Chauhan, R.P.S., Chauhan, C.P.S. and Kumar, D. 1980. Free proline accumulation in cereals in relation to salt tolerance. **Plant Soil, 57** : 167-176.

Costa, J., Bosch, M. and Blanco, A. 1995. Growth and cropping of 'Blanquilla' pear trees treated with pactobutrazol. **J. Hort. Sci., 70**(3) : 433-443.

Dalziel, J. and Lawrence, D.K. 1984. Biochemical and biological effects of Kaurenc oxidase inhibitors such as paclobutrazol. In biochemical aspects of synthetic and naturally occuring plant growth regulators. **in: British Plant Growth Regulators Growth Manograph, 11**: 43-51.

Dean, J.A. 1960. **Flame Photometry**. McGraw Hill Publishing Company, New York. 35-40.

Ehlenfeldt, M.K. 1998. Enhanced bud production in high bush blueberry (*Vaccinium corymbosum* L.) in response to PP333. **Hort. Science, 33**(1) : 75-77.

Elfving, D.C., Lougheed, E.C., Chu, C.L. and Cline, R.A. 1990. Effects of Daminozide, paclobutrazol and Uniconazole treatments on 'Mc Intosh' apples at harvest and following storage. **J. Amerc. Soc. Hort. Sci., 115**(5) : 750-756.

El-Otmani, M., Jabri, K. and Sedki, M. 1992. Paclobutrazol effect on development of green house grown banana: a 2 year assessment. **Acta Hort., 296**: 89-96.

George, A.P., Nissen, R.J., Collins, R.J. and Rasmussen, T.S. 1995. Effects of fruit thinning, pollination and paclobutrazol on fruit set and size of persimmon (*Diospyros kaki* L.) in subtropical Australia. **J. Hort. Sci., 70**(3) : 477-484.

Ghosh, B.P. and Burris, R.H. 1950. Utilization of nitrogenous compounds by plants. **Soil Sci., 70** : 187-203.

Greene, M.D. 1991. Reduced rates and multiple sprays of paclobutrazol control growth and improve fruit quality of 'Delicious apples'. **J. Amer. Soc. Hort. Sci., 116**(5) : 807-812.

Hao, S.Q., Yang, H. and Sun, Z.M. 1991b. Physiological changes induced by paclobutrazol in `Delicious' apple. **Fruit Science Reports, 18**(4): 163-172.

Hao, S.W., Yang, H. and Sun, Z.M. 1991a. Effects of PP333 on the growth and fruiting of young `Delicious' apple trees. **Acta Horticulturae Sinica, 18**(4): 318-322.

Harangozo, T., Szabo, Z. and David, M. 1996. Chemical thinning of plum varieties. **Hort. Sci., 28**(1/2): 35-39.

Harris, L.J. and Ray, S.N. 1935. **Lancer, 1** : 462.

Hedge, J.E. and Hofreiter, B.T. 1962. **in : carbohydrate chemistry 17 (Eds. Whistler, RL and Be Miller, J.N.)**. Academic Press, New York.

Huang, W.D., Shen, T., Han, Z.H. and Liu, S. 1995. Influence of paclobutrazol on photosynthesis rate and dry matter partitioning in the apple tree. **J. Pl. Nutr., 18**(5): 901-910.

Hunter, D.M. and Proctor, J.T.A. 1994. Paclobutrazol affects growth and fruit composition of potted grape vines. **Hort. Sci., 27** : 319-321.

Jacyna, T. and Dodds, K.G. 1995. Paclobutrazol in managing mature cropping apricot trees. **Acta Hort., 240** : 139-142.

Khader, S.E.S.A. 1990. Orchard application of paclobutrazol on ripening, quality and storage of mango fruits. **Scientia Hort., 41**: 329-335.

Khader, S.E.S.A. 1991. Control of tree height, trunk girth, shoot growth and total assimilation in young grafted mango trees by paclobutrazol. **Indian J. Hort., 48**(2): 112-115.

Kim, J.K., Kim, K.Y., Kim, J.B. and Kim, S.B. 1990. The effect of paclobutrazol on shoot growth, photosynthetic activity, leaf and fruit characteristics and flower bud formation in Fuji apples. **Research Reports of the Rural Development Administration Horticulture, 32**(2): 10-15.

Klinac, D.J., Rohitha, H., Pevreal, J.C. 1991. Effect of cultar (Paclobutrazol) on vegetative growth and fruit production by nashi (*Pyrus secrobina* Rehd.). **New Zealand J. Crop and Hort. Sci., 19** : 229-235.

Krishna, G., Ranjan, S.K. 1991. **Special analytical techniques in Nutritional Biochemistry**, First Edition, Kalyani Publishers, New Delhi, 61-81.

Kuden, A., Kuden, A.B. and Kaska, N. 1995. Physiological effects of foliage applied paclobutrazol on canino and Precode decolomer Apricot cultivars. **Acta Hort., 384** : 419-421.

Kumar, A.K., Murti, G.S.R., Shikhamany, S.D. 1998. Effect of cycocel and paclobutrazol on morphological attributes, bunch characteristics and endogenous gibberillin leaves in 'Arkavati' grapes (*Vitis vinifera* L.) trained on two systems. **Gartenbanwissenichaft, 63**(2) : 63-65.

Kurian and Iyer, C.P.A. 1993. Chemical regulation of tree size in mango *(Mangifera indica* L.) cv. Alphonso, Effects of growth retardants on vegetative growth and tree vigour. **J. Hort. Sci., 68**(3): 349-354.

Lauri, P.E. 1993. Long-term effects of paclobutrazol on longivity of sweet cherry spurs. **Acta Hort., 329**: 190-193.

Leopald, A.C. 1964. **Plant growth and development** 206, 274, 275. Tata McGraw Hill Pub. Com.. Ltd., Bombay, New Delhi.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with folin phenol reagent. **J. Biol. Chem.,** (193) : 265-275.

Manago, N., Kimura, N. and Sakakibara, M. 1994. The influence of paclobutrazol on the growth and the fruit quality of peach in green houses. **Research Bulletin of the Aichi-ken Agricultural Research Centre, 26**: 267-273.

Mauk, C.S., Bausher, M.S. and Yelenosky, G. 1987. Physiological effect of temperature and growth regulators on foliar chlorophyll, soluble protein and cold hardiness in citrus. **Plant Growth Regulators, 5**(2): 141-154.

Medina-Urrutia, V.M. 1994. Advancement of flowering in mango 'Tommy Atkins' with PBZ applications. **Proc. Inter. Amer. Soc. Trop. Hort., 38**: 56-61.

Mehouachi, J., Tadeo, F.R., Zaragoza, S., Primo-millo, E., Talon, M. 1996. Effect of gibberellic acid and paclobutrazol on growth and carbohydrate accumulation in shoots and roots of citrus rootstock seedlings. **J. Hort. Sci., 71**(5) : 747-754.

Miller, G.L. 1972. **Anal Chem., 31**: 426.

Monge, E., Aguirre, R. and Blanco, A. 1994. Application of paclobutrazol and GA_3 to adult peach trees: effects on nutritional status and photosynthetic pigments. **J. Plant Growth Reg., 13**(1): 15-19.

Monge, E., Madevo, P. Val, J. and Blanco, A. 1993. Effects of paclobutrazol application and fruit load on micro element concentrations in peach leaves. In optimization of plant nutrition.. **Eigth International Colloquium for the optimization of plant nutrition**, 31 August and September, 319-323.

Moore, S. and Stein, W.H. 1948. **in : Methods in Enzymol**. (Eds. Colowick, S.P. and Kaplan, N.D.). Academic Press New York (3) : 468.

Narasinga Rao, B.S., Deosthale, Y.G. and Pant, K.C. 1996. **Nutritive value of Indian Foods**, National Institute of Nutrition, Hyderabad, 55, 65, 72 and 91.

Okuda, H., Kihara, T., Iwagaki, T. and Kawase, K. 1994. Effects of annual foliar applications of PBZ for 9 years on the growth, yield and fruit quality of Satsuma mandarin trees. **Bulletin of the Fruit Tree Research Station, 26**: 61-69.

Okuda, H., Kihara, T. and Iwagaki, I. 1996. Effects of paclobutrazol application to soil at the beginning of maturation on sprouting, shoot growth, flowering and carbohydrate contents in roots and leaves of satsuma mandarin. **J. Hort. Sci., 71**(5) : 785-789.

Panse, V.G. and Sukhatme, P.V. 1967. **Statistical method for Agricultural Workers**. ICAR Pub., New Delhi.

Paricha, P.C., Ghosh, B.K. and Sahoo, N.C. 1977. Further studies on the significance of cycocel in enhancing drought resistance in rice. **Sci. Cult., 43**: 230-231.

Parletta, M.A. and Sedgley, M. 1998. Acacia as potted plants. **Acta Horticulturae, 454** : 183-190.

Pilone, N., Scaglione, G. 1996. Effects of paclobutrazol on growth of loquats. **Rivista di frutticoltura e di ortofloricoltura, 58**(3): 69-71.

Ranganna, S. 1976. **in : Manual of Analysis of Fruits and Vegetable Products**, Mc Graw Hill, New Delhi. 77.

Reynolds, A.G., Wardle, D.A. Cottrell, A.C. and Gaune, A.P. 1992. Advancement of Riesting fruit maturity by paclobutrazol induced reduction of lateral shoot growth. **J. Amer. Soc. Hort. Sci., 117**(3): 430-435.

Rick, M.C. 1976. **Evolution of Crop Plants**. First Edition, 268-272.

Riker, A.J. and Gutsche, A.E. 1948. **Am. J. Botany, 35** : 227-238.

Rizzolo, A., Visai, C. and Vanoli, M. 1993. Influence of paclobutrazol on the quality of apples during growth. **Acta Hort., 329**: 140-142.

Sadasivam, S. and Manickam, A. 1992. **Biochemical Methods for Agricultural Sciences**, Wiley Eastern Limited, 182.

Salem, A.T., Kilany, A.E. and Shaltout, A.D. 1991. Vegetative growth, yield and fruit quality of heconte pear trees as affected by cultar treatments. **Bulletin of Faculty of Agriculture**, University of Cairo, 42 (4, Suppl. 1): 1353-1368.

Setia, R.C., Bhathal, G., Setia, N. 1995. Influence of paclobutrazol on growth and yield of *Brassica carinata*. A. Br. **Plant Growth Reg., 16**(2) : 121-127.

Setia, R.C., Kaur, P., Setia, N., Anuradha, 1996. Influence of paclobutrazol on growth and development of fruit in *Brassica juncea* (L.). Czern and Coss. **Plant growth regulation, 20**(3) : 307-316.

Shrestha, T.N. 1988. Studies on chemical control of flowering and fruiting in acid lime (*Citrus aurantifolia* swing). **South Indian Hort., 36**(5): 278-279.

Snowball, A.W., Warrington, I.J., Hallingan, E.A. and Mullins, M.G. 1994. Phase change in citrus : the effects on main stem node number, branch habbit and paclobutrazol application on flowering in citrus seedlings. **J. Hort. Sci., 69**(1) : 149-160.

Subhadrabandhu, S., Rakngan, J. and Pipattanawongs, N. 1990. Effect of paclobutrazol on growth, flowering and yield of Japanese agricot. **Acta Hort., 279**: 389-398.

Szewazuk, A. 1994. Effect of irrigation and paclobutrazol on the growth, flowering and fruiting of peach. **Journal of Fruit and Ornamental Plant Research, 2**(2): 37-47.

Takebe, M., Yoneyama, T., Inada, K. and Murakami, T. 1990. Spectral reflectance ratio of rice canopy for estimating N status. **Plant and Soil, 122**: 295-297.

Toth, S.J., Prince, A.L., Wallace, A. and Mikkelsen, D.S. 1948. Rapid quantitative determination of mineral element in plant tissue by a systematic procedure involving use of a flame photometer, **Soil Sci., 66** : 456-466.

Vu, J.C.V. and Yelenosky. 1992. Growth and photosynthesis of sweet orange plants treated with paclobutrazol. **J. Plant Growth Regulation, 11** : 85-89.

Wang, S.P., Jia, H.J. Gao, Z.J. and Wang, S.Z. 1993. Study on PP333 application to the growth and development of young peach tree. **Acta Horticulture Sinica, 20**(2): 139-144.

Werner, H. 1993. Influence of paclobutrazol on growth and leaf nutrient content of mango (cv. Blanco). **Acta Hort.**, 341, 225-231.

Wieland, W.F. and Wample, R.L. 1984. Effect of paclobutrazol on shoot growth, photosynthesis and root growth in apple. **Hort. Sci., 19**(3): 529.

Xiachunsen, Zhon Ping, Shen Meng Ping. 1994. Physiological effects of chenghuabao and gridling on apple trees. Jiangsu. **Journal of Agricultural Sciences, 10**(3): 57-60.

Yelenosky, G., Vu, J.C.V. and Wutscher, H.K. 1995. Influence of paclobutrazol in the soil on growth, nutrient elements in the leaves and flood or freeze tolerance of citrus root stock seedlings. **J. Plant Growth Reg., 14**(3) : 129-134.

Yelenosky, G., Vu, J.C.V. and Wutscher, H.K. 1995. Influence of paclobutrazol induced dwarfing of `Valencia' orange trees. **Proceedings of the Florida State Horticultural Society**, 106: 329-332.

Yewale, A.K. 1997. Effect of growth retardant PP333 on flowering of chrysanthemum. **Journal of Soils and Crops,** 7(2) : 175-177.

Yim, K.O., Kwon, Y.W., Bayer, D.E. 1997. Growth responses and allocation of assimilates of rice seedlings by paclobutrazol and gibberellin treatment. **J. Plant. Growth Reg., 16**(1) : 35-41.

Zoecklein, B.W., Wolf, T.K. and Judge, J.M. 1991. Paclobutrazol effects on fruit composition and fruitset of `Riesling' (*Vitis vinefera*) grapes in Viriginia. **Plant Growth Regulator Society of American Quartely, 19**(2): 101-111.

Zora Singh and Dhillon, B.S. 1992. Effect of paclobutrazol on floral malformation, yield and quality of mango (*Mangifera indica* L.) **Acta Hort., 296**: 51-53.

APPENDICES

APPENDIX - I

ESTIMATION OF TOTAL LEAF CHLOROPHYLL

(Arnon, 1949)

PRINCIPLE

Chlorophyll was extracted in 80 per cent acetone and the absorption was read at 663 nm and 645 nm in a spectrophotometer. Using the absorption coefficients, the amount of chlorophyll was calculated.

PROCEDURE

One gram of leaf sample was homogenised with 20 ml of 80 per cent acetone, centrifuged and the supernatant was transferred to 100 ml volumetric flask. This procedure was repeated until the residue was colourless. The volume was made up to 100 ml with 80 per cent acetone. The absorbance was read at 663 and 645 nm against the blank.

The amount of chlorophyll present in the extract was calculated using the following equations,

$$\text{mg chlorophyll a/g tissue} = 12.7\,(A_{663}) - 2.69\,(A_{645}) \times \frac{V}{1000 \times W}$$

$$\text{mg chlorophyll b/g tissue} = 22.9\,(A_{645}) - 4.68\,(A_{663}) \times \frac{V}{1000 \times W}$$

and

$$\text{mg total chlorophyll/g tissue} = 20.2\,(A_{645}) + 8.02\,(A_{663}) \times \frac{V}{1000 \times W}$$

where A= absorbance at specific wavelengths,

V = final volume of chlorophyll extract in 80 per cent acetone

W = fresh weight of tissue extracted

APPENDIX II

ESTIMATION OF STARCH

(Hodge and Hofreiter, 1962)

PRINCIPLE

The sample is treated with 80 per cent alcohol to remove sugars and then starch is extracted with perchloric acid. In hot acidic medium starch is hydrolysed to glucose and dehydrated to hydroxymethyl furfural. This compound forms a green coloured product with anthrone.

MATERIALS

1. Anthrone reagent : 200 mg anthrone was added to 100 ml of ice-cold 95 per cent sulphuric acid
2. 80 per cent ethanol
3. 52 per cent perchloric acid
4. Standard glucose : Stock - 100 mg was dissolved in 100 ml water. Working standard - 10 ml of stock was diluted to 100 ml with water.

PROCEDURE

0.1-0.5 g of the sample was homogenised in hot 80 per cent ethanol to remove sugars, centrifuged and the residue retained. The residue was dried well over a water bath. To the residue 5.0 ml of water and 6.5 ml of 52 per cent perchloric acid were added, extracted at 0°C for 20 minutes, centrifuged and the supernatant saved. The extraction was repeated using fresh perchloric acid, the supernatants pooled and volume made up to 100 ml. 0.4 ml of the supernatant was pipetted out and the volume made up to 1 ml with water. A series of standards (0.2-1.0 ml) was run using glucose. 4 ml of anthrone reagent was added, heated for eight minutes in a boiling water bath and cooled rapidly. The intensity of green to dark green colour was read at 630 nm. The glucose content in the sample was assessed using the standard graph. The value was multiplied by a factor 0.9 to arrive at the starch content.

APPENDIX - III

DETERMINATION OF TOTAL SUGARS

(Hedge and Hofreiter, 1962)

PRINCIPLE

Carbohydrates were first hydrolyzed into simple sugars using hot dilute hydrochloric acid. In hot acidic medium glucose was dehydrated to hydroxymethyl furfural. This compound forms with anthrone a green coloured product with an absorption maximum at 630 nm.

MATERIALS

1. 2.5 N HCl
2. Anthrone reagent: 200 mg anthrone was dissolved in 100 ml of ice cold 95 per cent H_2SO_4
3. Standard Glucose : 100 mg was dissolved in 100 ml water. Working standard - 10 ml of stock was diluted to 100 ml with distilled water (100 µg/ml)

PROCEDURE

100 mg of the sample was weighed and hydrolysed by keeping it in a boiling water bath for three hours with 5.0 ml of 2.5 N HCl and then cooled to room temperature. It was neutralised with solid sodium carbonate. The volume was made up to 100 ml and centrifuged. The supernatant was collected and 0.5 ml aliquot was taken for analysis. Series of standards was prepared (0.2-1.0 ml). The volume was made up to 1.0 ml with distilled water. Then 4.0 ml of anthrone reagent was added. Heated for eight minutes in a boiling water bath. Cooled and read the green to dark green colour at 630 nm. A standard graph was drawn by plotting concentration of the glucose on x-axis versus absorbance on the y-axis. From the graph the amount of carbohydrate present in the sample was calculated and expressed as grams glucose per 100 g.

APPENDIX IV

ESTIMATION OF REDUCING SUGARS

(Miller, 1972)

MATERIALS

1. Dinitrosalicylic acid (DNS) reagent: One gram DNS, 200 mg crystalline phenol and 50 mg sodium sulphite was dissolved by stirring in 100 ml of 1 per cent NaOH. Stored at 4°C.
2. 40 per cent Rochelle salt solution.
3. Glucose working standard: 100 µg/ml.

PROCEDURE

Hundred mg of the sample was weighed and extracted the sugar with hot 80 per cent ethanol twice. Evaporated the supernatant and dissolved the sugars with 10 ml of water. Pipette out 0.2 ml aliquot. The volume was made up to 3.0 ml with water. Added 3.0 ml of DNS reagent. Heated the contents in a boiling water bath for 5 minutes. When the contents of the tubes was still warm, 1.0 ml of 40 per cent Rochelle salt solution was added, cooled and the intensity of dark red colour read at 510 nm. A series of standards was run using glucose (0.2-1.0 ml). From the standard graph the amount of reducing sugars present in the sample was calculated and expressed in grams per 100 g of the sample.

APPENDIX V

ESTIMATION OF TITRABLE ACIDITY

(A.O.A.C., 1960)

PROCEDURE

Five grams of tomato pulp was mixed with 50 ml of hot distilled water and titrated against 0.1 N NaOH using phenolphthalein as indicator. The end point was the appearance of light pink colour.

The titrable acidity as per cent citric acid was calculated using the following formula,

$$\frac{\text{Titre value (ml) x 0.0064}}{\text{Weight of the sample (g)}} \times 100$$

APPENDIX VI

ESTIMATION OF ASCORBIC ACID

(Harris and Ray, 1935)

PRINCIPLE

Ascorbic acid is dehydrogenated by bromination. The dehydroascorbic acid is then reacted with 2,4, dinitrophenyl hydrazin (DNPH) to form osazone and dissolved in sulphuric acid to give an orange-red colour solution which is measured at 540 nm.

MATERIALS

1. 4 per cent oxalic acid solution
2. 2 per cent DNPH reagent: 2 g DNPH was dissolved in 100 ml 0.5 N H_2SO_4. Filtered and used.
3. 10 per cent thiourea solution
4. 80 per cent sulphuric acid.
5. Bromine water
6. Ascorbic acid standard solution : 100 mg ascorbic acid was dissolved in 100 ml of 4 per cent oxalic acid solution in a standard flask. Working standard 10 ml of stock was converted into dehydroform by bromination and diluted to 100 ml with 4 per cent oxalic acid.

PROCEDURE

0.5-5 g of the sample was ground in 25-50 ml of 4 per cent oxalic acid solution, centrifuged and the supernatant solution was collected. Bromine water was added, when the extract turned orange yellow. A known volume was made up with 4 per cent oxalic acid solution.

10-100 µg standard dehydroascorbic acid was pipetted out into a series of tubes and added 0.2 ml of brominated sample extract. The volume in each tube was made up to 3 ml by adding distilled water and one ml of DNPH reagent was added followed by 1-2 drops of 10 per cent thiourea to each tube. The contents of the tubes were mixed thoroughly and incubated at 37°C for 3 hours. After incubation the orange red osazone crystals formed which were dissolved by adding 7 ml of 80 per cent sulphric acid. The absorbance was read at 540 nm and plotted a graph with ascorbic acid concentration versus absorbance and the ascorbic acid content was calculated and expressed as grams per 100 g of the sample .

APPENDIX VII

LYCOPENE ESTIMATION

(Ranganna, 1976)

PRINCIPLE

The carotenoids in the sample are extracted in acetone and then taken up in petroleum ether. Lycopene has absorption maxima 503 nm. One mole of lycopene when dissolved in one litre of light petroleum (40-60°C) and measured in a spectophotometer at 503 nm in one cm light path gives an absorbance of 17.2×10^4. Therefore, a concentration of 3.120 µg lycopene / ml gives unit absorbance.

MATERIALS

1. Acetone
2. Petroleum Ether (40-60°C)
3. Anhydrous sodium sulphate
4. 5 per cent sodium sulphate

PROCEDURE

5 g of tomato pulp was extracted with acetone until the residue is colourless. The acetone extracts were pooled and transferred to a separating funnel containing petroleum ether and mixed gently. About 20 ml of 5 per cent sodium sulphate solution was added and the separating funnel shaken gently. Then 20 ml of petroleum ether was added for clear separation of two layers. The two phases were separated and the lower

aqueous phase was reextracted with additional 20 ml petroleum ether until the aqueous phase became colourless and washed once with a little distilled water. Pour the washed petroleum ether extract containing carotenoids into a brown bottle containing about 10 g anhydrous sodium sulphate and kept aside for 30 minutes or longer. The petroleum ether extract was decanted into a 100 ml volumetric flask through a funnel containing cotton wool. Sodium sulphate slurry was washed with petroleum ether until it was colourless and then washings were transferred to volumetric flask. The volume was made up and the absorbance measured in a spectrophotometer at 503 nm using petroleum ether as blank. Milligram of lycopene in 100 g sample was calculated using the following formula,

Absorbance (1 unit) = 3.1206 µg lycopene / ml

$$\text{mg of lycopene in 100 g of sample} = \frac{31.206 \times \text{Absorbance}}{\text{Weight of sample (g)}}$$

APPENDIX - VIII

PROTEIN ESTIMATION

(Lowry *et al.*, 1951)

PRINCIPLE

The blue colour developed by the reduction of the phosphomolybdic - phosphotungstic components in the Folin-Ciocalteau reagent by the amino acids tyrosine and tryptophan present in the protein plus the colour developed by the biuret reaction of the protein with the alkaline cupric tartarate was measured at 660 nm.

MATERIALS

1. 2 per cent sodium carbonate in 0.1 N sodium hydroxide (Reagent A)
2. 0.5 per cent copper sulphate in 1 per cent potassium sodium tartrate (Reagent B)
3. Alkaline copper solution : 50 ml of A and 1 ml of B were mixed prior to use (Reagent C)
4. Folin - Cioacalteau Reagent (Reagent D)

5. Protein standard : 50 mg of bovine serum albumin was dissolved in distilled water and the volume was made up to 50 ml in a standard flask. 10 ml of the stock solution was diluted to 50 ml with distilled water in a standard flask.

PROCEDURE

500 mg of the sample was ground well with 5-10 ml of N/150 phosphate buffer, centrifuged and 0.2 ml of the supernatant was used for protein estimation. A series of protein standard (0.2-1.0 ml) was run. The volume was made up to 1 ml in all the test tubes. 5 ml of reagent C was added, mixed well and allowed to stand for 10 minutes. Then 0.5 ml of reagent D was added, mixed well and incubated at room temperature in the dark for 30 minutes. Blue colour developed. The readings were taken at 660nm. A standard graph was drawn and the amount of protein in the sample was calculated and expressed in grams per 100 g.

APPENDIX - IX

ESTIMATION OF TOTAL FREE AMINO ACIDS

(Moore and Stein, 1948)

PRINCIPLE

Ninhydrin, a powerful oxidizing agent, decarboxylates the alpha-aminoacids and yields an intensely colored bluish purple product which is colorimetrically measured at 570 nm.

Ninhydrin + alpha-amino acid $\longrightarrow$ Hydrindantin + Decarboxylated aminoacid + carbondioxide + Ammonia.

Hydrindantin + Ninhydrin + Ammonia $\longrightarrow$ Purple coloured product + water

MATERIALS

1. Ninhydrin reagent : 0.8 g stannous chloride was dissolved in 500 ml of 0.2 M citrate buffer (pH 5.0) and added to 20 g of ninhydrin in 500 ml of methyl cellosolve (2-methoxy ethanol)
2. 0.2 M citrate buffer pH 5.0

3. Diluent solvent : Equal volumes of water and n-propanol were mixed and used.
4. Leucine working standard: 100 µg/ml.

PROCEDURE

500 mg of the sample was homogenised. To this 5-10 ml of 80 per cent ethanol was added. It was centrifuged and the supernatant was saved. Repeated this until the residue was colourless. To 0.1 ml of the extract, 1 ml of ninhydrin solution was added and the volume made up to 2 ml with distilled water. The tube was heated in a boiling water bath for 20 minutes. 5 ml of the diluent was added and the contents mixed. After 15 minutes the intensity of the purple colour was read against a reagent blank in a colorimeter at 570 nm. A series of standards using leucine (0.2 - 1.0 ml) was run and a graph plotted. From the standard graph the amount of total free aminoacids present in the sample was calculated and expressed in grams per 100 g.

APPENDIX - X
TOTAL KJELDAHL NITROGEN
(APHA, 1976)

PRINCIPLE

Total kjeldahl nitrogen is the sum of ammonia nitrogen and organic nitrogen. This does not include nitrite nitrogen and nitrate nitrogen. The classical kjeldahl method is used to determine the total N content.

Nitrogen of organic matter is converted to ammonium sulphate when treated with sulphuric acid in presence of copper sulphate catalyst. An excess of alkali is then added to liberate ammonia from ammonium sulphate and distilled. The distillate is titrated with standard sulphuric acid after absorption in boric acid solution.

MATERIALS

1. Concentrated sulphuric acid
2. Copper sulphate-10 per cent
3. 50 per cent sodium hydroxide
4. 2 per cent boric acid

5. Mixed indicator solution : 200 mg of methyl red indicator was mixed in 100 ml of 95 per cent ethanol and 100 mg of methylene blue in 50 ml of 95 per cent ethanol.

6. Standard sulphuric acid solution 0.02 N.

PROCEDURE

DIGESTION : 20 ml of the sample was taken in a kjeldahl flask. To this 10 ml concentrated Sulphuric acid and 1.0 ml copper sulphate were added and digested till the solution turned clear and colourless, finally allowed to cool.

DISTILLATION : The contents were transferred to the distillation flask and diluted to 50 ml. The solution was made alkaline with 50 per cent sodium hydroxide. The distillation was started after immersing the tip of the condenser about 20 ml of the distillate.

TITRATION : 0.5 ml mixed indicator solution was added to the distillate and titrated against 0.02 N sulphuric acid. End point was the colour change from pale green to lavender. A blank was conducted starting from digestion step to final titration.

CALCULATION

$$\text{Total kjedahl N in mg / l} = \frac{(\text{ml } 0.02 \text{ N } H_2SO_4 \text{ for sample}) - (\text{ml } 0.02 \text{ } NH_2SO_4 \text{ for blank}) \times 0.28 \times 1000}{\text{ml sample taken}}$$

APPENDIX XI

ESTIMATION OF TOTAL PHOSPHORUS

(Stannous chloride method)

(APHA, 1976)

PRINCIPLE

Phosphorus present in the given sample may be orthoposphate, condensed phosphate (meta, pyro and polyphosphates) and organically bound phosphates. By sulphuric acid - nitric acid digestion all the forms of phosphates are converted to orthophosphate.

Ammonium molybdate reacts with orthophosphates to form molybdophosphoric acid which is reduced to a blue coloured complex `molybdenum blue', by the addition of stannons chloride. The colour intensity in the given solution is compared with that of standard phosphorus solution at 690 nm.

MATERIALS

1. Concentrated sulphuric acid
2. Concentrated nitric acid
3. 1.0 N sodium hydroxide
4. Phenolpthalein indicator
5. Sulphuric acid-nitric acid solution : 75 ml concentrated H_2SO_4 was added to 150 ml of distilled water and cooled. Then 1.0 ml of concentrated HNO_3 was added and diluted to 250 ml with distilled water.
6. Ammonium molybdate solution : 2.5 g of ammonium molybdate was dissolved in 200 ml of distilled water. 280 ml of concentrated sulphiruc acid was added separately to 400 ml of distilled water and cooled. The molybdate solution was added to the acid and diluted to one litre.
7. Stannous chloride solution : 2.5 g of fresh stannous chloride was kept in a beaker, added with 100 ml of glycerol, heated in a water bath and mixed by stirring.
8. Phosphate stock solution : 439 mg of potassium dihydrogen phosphate was made upto one litre with distilled water. 1.0 ml contains 100 µg of phosphorus.

Working standard was freshly prepared by pipetting 10 ml of the stock and making up the volume to a litre with water.

1.0 ml contains 1.0 µg of phosphorus

PROCEDURE

Sulphuric acid - nitric acid digestion: 50 ml of the sample was taken in a microkjeldahl flask and 1.0 ml of concentrated sulphuric acid and 5.0 ml of concentrated nitric acid were added. The sample was digested to a volume of 1.0 ml and the digestion continued till the solution became colourless. Then it was cooled and added with 20 ml of distilled water and

neutralised with 1.0 N sodium hydroxide solution using phenolphthalein as indicator. After transferring to a 100 ml volumetric flask, the volume was made upto the mark with distilled water. This solution was used for the estimation of total phosphate.

ESTIMATION OF TOTAL PHOSPHATE

5.0 ml of the acid digested sample was taken in a 100 ml Nessler tube with a drop of phenolphthalein indicator. Any pink colour appeared was destroyed by adding few a drops of sulphuric-nitric acid solution. Into a series of 100 ml Nessler tubes 5,10, 15, 20 and 25 ml of phosphate working solution was pipetted out and diluted to 90 ml. A Nessler tube containing 90 ml of distilled water was included as the blank. To the blank, standards and the sample 4.0 ml of the ammonium molybdate solution and 0.5 ml of stannons chloride solution were added mixed after each addition. The volume was made up to 100 ml in all the tubes with distilled water. After 10 minutes, the colour was read at 690 nm. A standard graph was prepared with different concentrations of phosphorus on x-axis and colorimeter reading on y-axis and the amount of total phosphate in the sample was calculated.

APPENDIX XII

ESTIMATION OF POTASSIUM

(Flame photometer method)

(Dean, 1960)

PRINCIPLE

Flame photometry: The solution under test is passed under carefully controlled conditions as a very fine spray in the air supply to a burner. In the flame, the solution evaporates and the salt dissociates to give neutral atoms. A very small proportion of these move into a high energy state when these excited atoms fall back to the ground state, the light emitted of characteristic wavelength is measured. Potassium was estimated at 770 nm.

MATERIALS

Potassium stock solution : 1.907 g of potassium chloride was dissolved in deionised distilled water and the volume made upto one litre.

1.0 ml = 1.0 mg potassium

WET DIGESTION OF THE SAMPLE (Toth *et al.*, 1948)

The leaves of the plants were dried in an oven at 60°C for 24 hours. To 1.0 g of dried leaves in a 50 ml beaker added 20 ml of concentrated HNO_3 and covered with a watch glass and allowed to stand until initial reactions subsided. It was heated until the solid particles nearly disappeared, cooled and then 10 ml of 72 per cent perchloric acid was added and heated till a colourless solution was obtained. Then it was cooled and transferred to a 100 ml volumetric flask and the volume made upto the mark with distilled water. The contents were mixed and allowed to stand overnight and filtered. The filtrate was used for the analysis of potassium.

ESTIMATION OF POTASSIUM

The flame photometer was standardised before feeding the sample. The reading was set to zero using deionised water. Using the stock solution of potassium the reading was adjusted to 100 at the specific wavelength of 770 nm. Then the sample was fed in the flame photometer and noted the reading which gave the amount of potassium directly.

APPENDIX XIII

ESTIMATION OF MANGANESE, ZINC AND IRON

(Toth *et al.*, 1948)

PROCEDURE

The technique involves determination of concentration of a substance by the measurement of absorption of the characteristic radiation by the atomic vapour of an element. When radiation characteristics of a particular element passes through an atomic vapour of the same element, absorption of radiation occurs in proportion to the concentration of the atoms in the light path. The source of characteristic radiation is a hollow cathode lamp, the cathode being made of the element desired to be estimated.

MATERIALS

Standard solutions (Krishna and Ranjhan, 1991)

Zinc: Dissolved 4.549 g of zinc nitrate or 4.3987 g of zinc sulphate hexahydrate in one litre of water. This represents 0.1 per cent solution.

Iron : Dissolved 0.7022 g of ferrous ammonium sulphate hexahydrate in 100 ml of water, added 5.0 ml of concentrated sulphuric acid, warmed slightly and added potassium permanganate 0.1 N solution drop by drop until the solution showed a slight pink colouration. Made up the volume to one litre in a graduated flask. Pipetted out 10 ml of this solution into a one litre graduated flask, added 100 ml of hydrogen peroxide solution and made up the volume with water. This solution contains 1 μg of iron per ml.

Manganese : Dissolved 0.575 g of dry potassium permanganate in 50 ml of water in a 250 ml beaker. Added 40 ml of concentrated sulphuric acid and reduced the permanganate by careful addition of sodium metabisulphite solution until the manganese solution just became colourless. Oxidised the excess sulphurous acid in the hot solution by the addition of a little nitric acid. Cooled and transferred the solution quantitatively to a two litre graduated flask. Made up the volume and stored the solution in a glass stoppered reagent bottle. The solution contains 0.1 mg of manganese per ml.

WET DIGESTION OF THE WHOLE PLANT

MATERIALS

1. Reagent A - Concentrated Nitric acid
2. Reagent B - 72 per cent perchloric acid

PROCEDURE

The plant samples were dried in an oven at 60°C for 24 hours. The dried samples were wet digested.

One gram of dried plant material was transferred to a 50 ml beaker and was added with 20 ml of reagent A. It was covered with a watch glass and remained until initial reactions subsided. It was then heated until the solid particles were nearly disappeared. The contents then were cooled, 10 ml of reagent B was added and heated gently at first, then heated more vigorously until a clear, colourless solution has resulted. When the volume was reduced to approximately 3.0 ml heating was discontinued cooled and transferred quantitatively to a 100 ml volumetric flask. The volume was made up to the mark with water, mixed, allowed to stand overnight and then filtered through a dry filter paper without washing. The solution was used for analysis.